

Research article

Insights to bioprocess and treatment competence of urban wetlands

Durga Madhab Mahapatra^{a, b, c}, N.V. Joshi^b, T.V. Ramachandra^{b, c, d, *}^a Biological and Ecological Engineering (BEE), Oregon State University, Corvallis, OR, United States^b Energy and Wetlands Research Group (EWRG), Center for Ecological Sciences (CES), Indian Institute of Science, Bangalore, India^c Centre for Sustainable Technologies (CST), Indian Institute of Science (IISc), Bangalore, India^d Centre for Infrastructure, Sustainable Transportation and Urban Planning (CISTUP), IISc, Bangalore, India

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ABSTRACT

Wetlands play a major role in the recharge of groundwater resources, maintenance of water quality (remediation), moderate microclimate while supporting local livelihood through provision of fish, fodder, etc. The present study aims to investigate algal-bacterial consortium as a function of residence time with the water quality dynamics in two major wetlands in Bangalore city, India. Over thirty-two genera of algae were recorded with more than 40 species in the lakes and two dominant bacterial assemblages. The higher Ammonium-N content favoured the growth of these members. Significant correlation was observed between the nutrient concentrations and the community structure at the inflows and the outflows. The algal community showed negative correlation to filterable COD and high nutrients levels while bacterial abundance was observed under high loadings. The green algae Chlorophyceae (*Chlorella* blooms), which are indicators of nutrient enrichment were observed predominantly, that needs an immediate attention. Higher overall treatment efficiency was observed in terms of CNP removal during the Pre-monsoon season attributed to absence of macrophytes cover and rapid growth of algal assemblage's due to higher temperature regimes with adequate solar insolation.

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1. Introduction

Unplanned rapid urbanization due to globalisation and subsequent push for the industry-economic development in nineties have resulted in the increased stress on natural resources evident from the significant deterioration of urban wetlands/lakes in terms of quality and quantity, aquatic biodiversity, eco-aquatic processes, land use land cover (LULC) and micro-hydrological regimes (Ramachandra et al., 2006; Ramachandra, 2008; Martinuzzi et al., 2014). Untreated or partially treated industrial and domestic wastewater generated in urban locality taking advantage of the existing natural drains in the system of interconnected lakes. Sustained inflow of untreated sewage, though aided in maintaining the water levels, but has contributed to the nutrient enrichment rendering lakes vulnerable to blooms, frothing, foul odour with influx of wide spectrum of organics and inorganics, heavy metals (Timothy, 2000) and xenobiotics. The prime source of pollution is

untreated domestic and industrial wastewater in the urban catchment (Ramachandra et al., 2006, 2013; 2017).

The interaction among the various physico-chemical components of abiotic environment has profound impact on the local microflora and on the primary productivity and treatment capability (Carta-Escobar et al., 2004) of urban wetlands. Bacteria (Paerl et al., 2003), algae (Mahapatra et al., 2011a) and the aquatic plants (Mahapatra et al., 2011b,c) play a major role in remediation and aid as bio-indicators indicating the health of wetlands. The physico-chemical environment together with the abundance of the biological organisms decides the utility of the wetlands (lakes), whether it is fit for recreational, irrigational or potable purposes (Figueiredo et al., 2010). The presence of organics in water, due to inflow of untreated sewage and other high impact industrial toxicants alters the biotic community composition as the micro and macrophytes are sensitive to nutrients loads, various pollutants and alterations of the microenvironment. The changes in the community structure also affect the treatment potential (Kayombo et al., 2002; Tarlan et al., 2002; Kirkwood et al., 2003). The most tolerant and resistant micro flora constitute community assemblage, in urban wetlands with high influx of untreated domestic wastewaters loads (Timothy, 2000; Mahapatra et al., 2011a/

* Corresponding author. Energy & Wetlands Research Group, CES TE15, Centre for Ecological Sciences, Indian Institute of Science, Bangalore, 560019, India.

E-mail address: tvr@iisc.ac.in (T.V. Ramachandra).

URL: <http://ces.iisc.ernet.in/energy>

treatment plant effluents (Mahapatra et al., 2013a,b; Mahapatra et al., 2014; Ramachandra et al., 2015), landfill leachate (Naveen et al., 2017) and industrial effluents (Veeresh et al., 2009; Ramachandra et al., 2012).

The changes in the microbial community structure have altered the metabolic flux flow, which alter the integrity of aquatic ecosystem impairing its functions. This necessitates understanding of all major ecological entities and their dynamics with the nutrient influx, mobilization and accumulations to address sustainability of the ecological processes. Advanced metagenomic techniques are presently being used for a detailed characterization of the eukaryotic and the prokaryotic community structures (Zarrao-naindia et al. 2013). Such analyses provide insight to the bio-processes that help in identifying the pollution pattern and impacts (Singh et al., 2009). However, these techniques are expensive and do not reveal either causal factors or the pollution status. The physico-chemical parameters and the microflora community structure form a complex data structure, and require multivariate analysis and data treatment for the evaluation of water quality and the impact on micro flora (Bernal et al., 2008) with changes in the spatiotemporal patterns. Evaluation of the ecological structure and status of wetlands, pattern recognitions for variability in species abundance and their turnover, and development of an easy assessment tool for management have been done for Dianchi Lake (Yang et al., 2010) and Baiyangdian Lake (Zhao et al., 2012) in China; Navisha Lake, Kenya, (Ndungu et al., 2014), Curtin Lake in Malaysia (Prasanna et al., 2012). The objective of this study is to understand the linkages of the seasonal dynamics in physico-chemical and microflora bioprocesses in two major lakes in Bangalore region and evaluate the treatment competency of wastewater fed lakes.

2. Material and methods

2.1. Study area and its characteristics

The study was undertaken in two major wetlands in the Bangalore city namely Bellandur and Varthur wetlands (Fig. 1). Bangalore has the distinction of having interconnected wetlands due to undulating terrains with three major watersheds (Ramachandra et al., 2016). Bellandur Lake is the largest Lake in the Bangalore city and spreads across an area of 365 ha (mean depth 1.8 m). It is situated 5 km upstream of Varthur Lake. These lakes receive ~500 million litres per day (MLD) of untreated or partially treated wastewater, which include municipal wastewater from i) residential areas near the old Bangalore Airport, and ii) regions around Challaghata and Koramangla that directly flows to these lakes through connected drains. Varthur wetlands/lake is also situated in the south of Bangalore, covers a water-spread area of 220 ha (mean depth 1.1 m) built for catering the decentralised demand of water for domestic and agricultural uses.

The Varthur-Bellandur Lake catchment has seen large-scale land use changes after 2000, consequent to the rapid unplanned urbanization process in the region. The characteristics of both the water bodies are provided in Table 1.

2.2. Water sampling and analysis

Water samples from inlets, middle and outlets were collected across different seasons to evaluate the influent and the effluent water quality. These lakes had a varying extent of floating macrophytes during different seasons, which impeded the use of boats for sampling. Only samples closer to the shore could be reliably sampled at specific times of a day as the wind induced drift of floating macrophytes on the lake made time-specific sampling of all the points unfeasible.

The average annual precipitation of Bangalore is about 700–850 mm and temperatures vary from 14 °C (December to January) to 33 °C (maximum during March to May). There are two rainy periods, i.e. south-west monsoon (June to September) and north-east monsoon (November to December) (Mahapatra et al., 2011a; Ramachandra et al., 2016). During these periods, fresh water enters the lake as runoff. Water samples were collected regularly every month from five predetermined sampling points (locations were recorded using a hand-held pre-calibrated GPS (Global Positioning System, Garmin 48 and 60) to represent inlets, outlets and midpoints (Fig. 1). Physico-chemical parameters - pH, air temperature, water temperature, TDS, EC, turbidity, transparency and DO were measured at site following the standard protocol. Water samples were collected in 1 litre disinfected containers for estimation of chemical parameters in the laboratory (APHA AWWA WEF, 1998). Table S1 in the Supplementary material lists parameters and method adopted for analysis.

2.2.1. Water and key biota analysis

Water samples were also collected seasonally for 15 months from inflows, middle reaches and outflows of Bellandur and Varthur Lakes (Fig. 1) to examine the influent and the effluent water quality together with capturing the water quality in the middle reaches. Nutrient removal was calculated as per equation (1), for COD, BOD, N-species and P-species to assess the treatment efficiencies (in %).

$$\% \text{Removal} = \left\{ \frac{(C_{in} - C_{eff})}{C_{in}} \right\} \times 100 \quad (1)$$

where, C_{in} is the concentration of the influent, C_{eff} is concentration of the effluent.

2.2.2. Bacterial, algal and macrophyte analysis

100 ml of sample were collected from the select locations and were fixed with 70% alcohol. Species were identified using light microscope (Lawrence and Mayo) at 40× with the help of morphological keys as per literatures (Prescott, 1954, 1962; Desikachary, 1959). The algal members were enumerated by following earlier protocols (Mahapatra et al., 2013a,b). The bacterial count and morphology assessments were done through flow cytometry (FACS Caliber) following Gasol and Giorgio (2000) and with Electron Microscopy. These results were compared with the results of light microscope observations. Flow cytometry technique was adopted for rapid enumeration apart from exploration of different bacteria population, which is superior to plating as it is prone to contamination. Bacterio-plankton population was analysed by first filtering the samples with 2.5 µm sieve and then were analysed by the FSC (forward scatter) plots. Macrophytes were collected from the sampling locations and were identified following standard keys for freshwater plants in India by CDK Cook, 1996.

2.2.3. Bacterial analysis through scanning electron microscopy (SEM) and flow cytometry (FC)

At a stable pH, the cells were fixed in 2.5% glutaraldehyde. Samples were dried after dehydration through a series of ethanol in buffer of increasing strength (30, 50, 70, 80, 90, and absolute - 10 min each). Specimen were mounted, gold sputtered and examined through electron microscopy following earlier protocols (Mahapatra et al., 2014). 1.5 ml of 2 micron filtered samples (wastewater/sludge supernatant) were fixed with 1% paraformaldehyde + 0.05% glutaraldehyde (final), allowed in the dark, deep frozen in liquid nitrogen for 10 min to fix and then

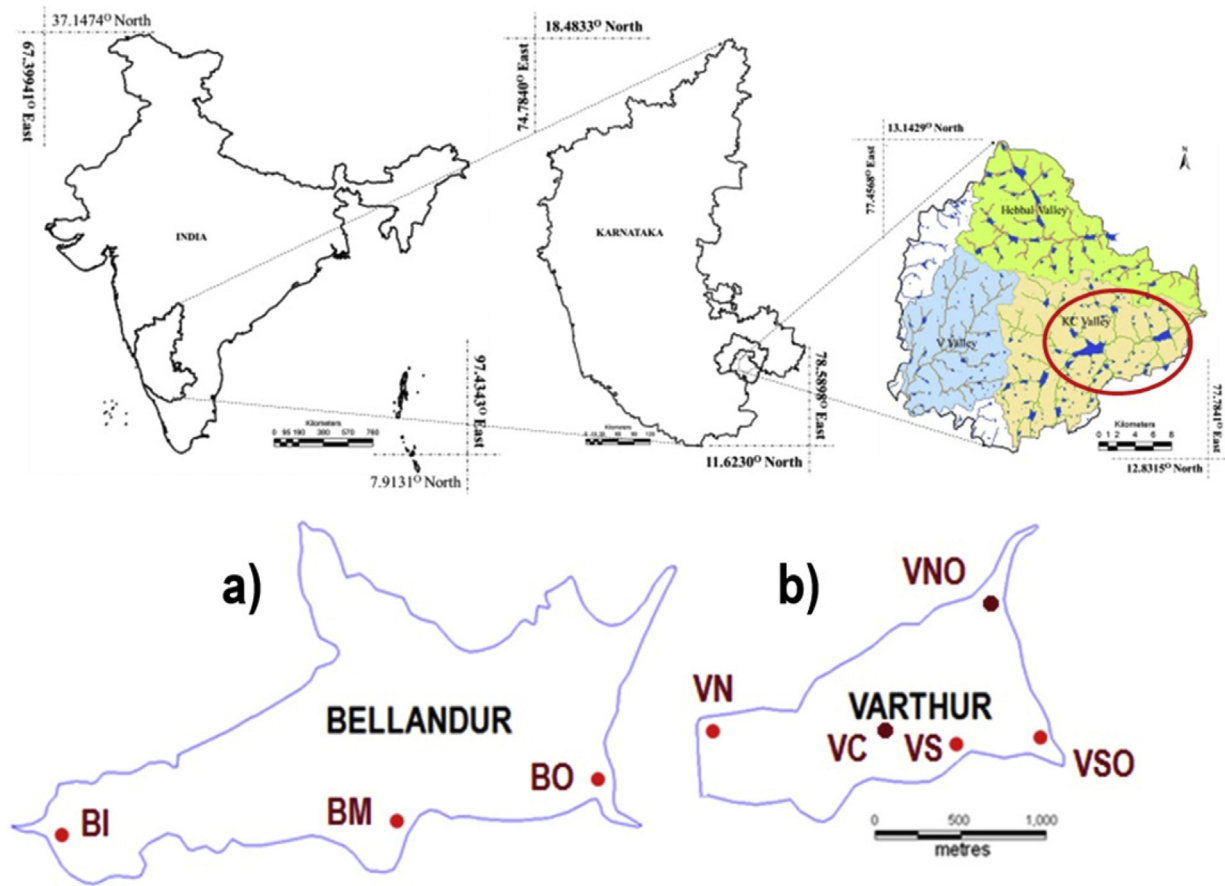


Fig. 1. Study Area - Greater Bangalore; Two large lakes of Bangalore a) Bellandur Lake and b) Varthur Lake. Sampling period (July–June, one year) - Sampling locations in Bellandur Lake: BI (Bellandur Inlet), BM (Bellandur Middle) and BO (Bellandur Outlet) along with Inlet (VN), Middle (VS) and Outlet regions (VSO) of Varthur Lake.

Table 1
Characteristics of the study area.

Characteristics	Bellandur Lake	Varthur Lake
Location	SE of Bangalore	SE of Bangalore
Coordinates	12.943917°–12.927959° N 77.638344°–77.680167° E	12.956683°–12.941499° N 77.745378°–77.72805° E
Primary inflows	Sewage from Bangalore	Bellandur
Primary outflows	Varthur Lake	To river Pennar
Catchment area (sq km)	148	166
Max. length (km)	3.6	2
Max. width (km)	1.4	1.1
Surface area (sq km)	3.6	2.2
Mean depth (m)	2.1	1.1
Surface elevation (m)	921	919
Water colour	Greenish	Greenish (intense)
Odour	Yes	Yes
Macrophyte cover	<i>Eichornia</i> , <i>Alternanthera</i> , <i>Cyperus</i>	<i>Eichornia</i> , <i>Alternanthera</i> <i>Typha</i> , <i>Lemna</i>

stored frozen at -70°C for estimating the bacterial abundance. FACS Caliber of Becton and Dickinson with a laser emitting at 488 nm, samples were treated at low speed (approx. $18\ \mu\text{l}/\text{min}$) with acquisition of data in log mode until 10000 events. Samples were diluted when the sample acquisition rate is higher than 800 cells/s. Usually $10\ \mu\text{l}$ per $200\text{-}\mu\text{l}$ sample is added of a $10^6/\text{ml}$ solution of yellow-green $0.92\ \mu\text{m}$ Polysciences latex beads as an internal standard. Bacteria were detected by their signature in a plot of Side scatter (SSC) vs. Forward scatter (FSC). Adjustments were made in the settings so that the beads fall in channels $\sim 10^3$ for SSC and FL1/

FSC as per standard protocol (Giorgio et al., 1996; Gasol and Giorgio, 2000).

2.3. Data analysis

Relationship between changes in physico-chemical variables and biological variables (relative abundance of algal classes and algal/bacterial abundance) was assessed through the computation of nonparametric Spearman's rank correlation coefficient (r). Multivariate analysis - canonical correspondence analysis (CCA)

was carried out to understand interplays between biological communities with varying physicochemical variables and seasons and to know relationships among them (Martin-Cereceda et al., 2001; Iscen et al., 2008). Higher variability in abundance was handled through \log_{10} transformation of the values. The spatial similarity and patterns across sites was assessed through Bray – Curtis cluster analysis (CA). An open source statistical package PAST 2.14 was used to implement statistical analyses.

3. Results and discussion

3.1. Role of microphyte (algae and bacteria) and macrophyte abundance and distribution in the nutrient dynamics

The physico-chemical alterations occurring spatio-temporally in lakes influence the incidence and activities of algal-bacterial systems in aquatic ecosystems, which has been observed in the present study. Urban lakes with the sustained inflow of untreated wastewater behaves as wastewater lagoons, with an initial anaerobic phase followed by an aerobic process (algal-bacterial symbiosis) where, the macromolecular complex biomolecules as polysaccharides, protein and lipids are first degraded by bacteria (Roche, 1998; Carta-Escobar et al., 2004). Subsequently, the algal systems are involved in key processes of i) reducing carbon (Mahapatra et al., 2013a) ii) inorganic nutrient (N and P) uptake (Mahapatra et al., 2013b) ii) storage of lipids (Ramachandra et al., 2009; Mahapatra and Ramachandra, 2013), proteins (Mahapatra et al., 2016) and carbohydrates (Ramachandra and Mahapatra, 2015) iv) pathogen removal by creating a high pH environment through algal photosynthesis (Mahapatra, 2015) and v) maintain the aerobic environment for the functioning of the treatment processes (Hosetti and Frost, 1998; Kirkwood et al., 2003; Chanakya et al., 2012, 2013; Mahapatra et al., 2013b). Higher species diversity, seasonal variations and species succession were noticed as these characteristics are a function of organic load, retention time, photoperiod, light intensities and predation by zooplanktons (Tharavathy and Hosetti, 2003; Ahmadi et al., 2005). The treatment levels and status of such kind of wastewater fed system have been assessed through investigations of the type of algae, their densities in terms of abundance; biomass and community structure (Sukias et al., 2001).

3.2. Seasonal variability's in nutrients and other parameters

At each sampling locations (Fig. 1), multiple samples were collected. Physico-chemical parameters were estimated for each sample and Tables 2 and 3 list season-wise water quality parameters. Monsoon corresponds to June to November months of South-west and North-east monsoon period. pH values ranged from 7.4 – 8.2 in Bellandur Lake and the highest pH was observed in the outlet reaches during the pre-monsoon period (Table 2). Varthur Lake recorded the maximum pH of 7.9 at the inflows during the pre-monsoon period and pH range from 7.5 to 7.9 (Table 3). Higher values of EC were observed during the pre-monsoon periods in both Bellandur and Varthur Lakes inflows and outflows and lowest were observed during monsoon.

Highest turbidity values (386 NTU) were recorded in the Bellandur lake inflows during the post-monsoon, and the minimum values were recorded during the monsoon (71–79 NTU). Varthur Lake recorded maximum turbidity (325 NTU) values in the inflows during the pre-monsoon period and lowest values during the post-monsoon period. Complete anoxic conditions were observed in Bellandur and Varthur Lakes owing to macrophyte cover and higher organic loads. Higher COD and BOD values were also recorded during the pre-monsoon period in both lakes.

Table 2
Seasonal variations in treatment parameters in Bellandur Lake.

Parameters	Bellandur Lake					
	Pre-monsoon		Monsoon		Post-monsoon	
	Inlet	Outlet	Inlet	Outlet	Inlet	Outlet
pH	7.9	8.2	7.4	7.8	7.7	7.8
Temperature (°C)	27.8	27.3	24.1	23.7	24	21
Electrical Conductivity ($\mu\text{S}/\text{cm}$)	1098	1057	661	735	980	1009
Total Dissolved Solids (mg/l)	868	840	526	592	770	808
Turbidity (NTU)	216.	96.5	71.4	79.2	386	98.9
Dissolved Oxygen (mg/l)	0.0	0.0	0.0	0.0	0.0	0.0
Free CO ₂ (mg/l)	69	21.84	48.4	61.6	64	120
COD (mg/l)	340	153	266	140	253	122
Filtrable COD (mg/l)	182	102	123	93	133	88
BOD (mg/l)	198	74	120	78	170	132
Nitrates (mg/l)	0.05	0.04	0.76	1.63	0.98	0.67
Amm-N (mg/l)	54	29	32	19	65	24
TN (mg/l)	62	44	64	31	69	27
Ortho-Phosphates (mg/l)	2.10	1.8	3.28	3.36	2.97	3.76
TP (mg/l)	16	12	37	19	28	11
Alkalinity (mg/l)	520	300	360	400	260	300
Total Hardness (mg/l)	196	204	268	232	292	276
Chlorides (mg/l)	84	73	82.36	85.2	86	93
Sodium (mg/l)	54	43.82	39.7	40.4	68	62
Potassium (mg/l)	10.4	9.72	9.1	10	9.8	11.2
ORP (mV)	–180	–150	–96	–165	–245	–220

During the entire sampling period, low nitrification was observed as evident from the nitrate values ranging from 0.04 to 1.5 mg/l compared to Amm.-N that ranged from 14 to 65 mg/l. Higher values of Amm.-N (~30 mg/l) were reported by the earlier studies (Chanakya and Sharatchandra, 2008). Nitrate and nitrite concentration constituted an insignificant portion of the dissolved nitrogen pool (<2.0%). Higher Amm.-N were consistently observed in the inflow regions especially in Bellandur Lake during the post monsoon period indicating an inflow of already decomposing wastewater. TN values were higher during the post monsoon period at the inflows in Bellandur Lake and during the pre-monsoon periods in the inflows of Varthur Lake and are elucidated in Tables 2 and 3 respectively. Ortho phosphates levels ranged from 1.8 – 3.76 mg/l in Bellandur Lake and 1.32–3.51 mg/l in Varthur Lake and were comparatively higher in the outlets owing to P re-suspension from anoxic bottom. However, TP values were higher in the inflows compared to the outflows and were ranging from 11 to 37 mg/l in Bellandur Lake and 9–19 mg/l in Varthur Lake.

3.3. Treatment aspects of lakes

These lakes have been functioning as lagoons or functionally comparable to stabilisation ponds with the thriving algal-bacterial communities that helps in maintaining the overall quality of the water. In the current study, a maximum removal of total COD (~55%); filterable COD (~44%); Net COD (67.7%) and BOD (62.5%) was observed during the pre-monsoon season in Bellandur lake where as filterable COD and BOD removal was better during the post-monsoon period (Table 4 and Fig. S1, Supplementary material) attributable to rapid C uptake and transformations at high temperature with higher insolation through the algal C sequestration route. In the case of Varthur Lake, highest total COD removal (52%) was recorded in monsoon period, where as maximum BOD (~62%); filterable COD (44%) and Net COD (70%) removal took place during the pre-monsoon period and the reasons are similar to the observations in Bellandur lake.

High TN removal was observed during post-monsoon period (~61%) in Bellandur Lake attributed to anoxic environments due to

Table 3
Seasonal variations in treatment parameters in Varthur Lake.

Parameters	Varthur Lake					
	Pre-monsoon		Monsoon		Post-monsoon	
	Inlet	Outlet	Inlet	Outlet	Inlet	Outlet
pH	7.6	7.76	7.85	7.53	7.9	7.5
Temperature (°C)	28.6	26	27.7	29.1	22	23
Electrical Conductivity (µS/cm)	1420	1114	910	897	977	952
Total Dissolved Solids (mg/l)	994	792	720	712	768	755
Turbidity (NTU)	325	108	216	102	161	65
Dissolved Oxygen (mg/l)	0.0	0.98	0.54	0.13	0.78	0.88
Free CO ₂ (mg/l)	55	12.4	40.2	21.8	14.96	8.8
COD (mg/l)	224	192	220	133	188	120
Filtrable COD (mg/l)	146	102	113	86	146	93
BOD (mg/l)	172	135	125	78	130.47	74.9
Nitrates (mg/l)	0.26	0.38	1.39	1.43	0.36	0.6
Amm-N (mg/l)	44	14	37.5	19	42	22
TN (mg/l)	49	21	39	22	47	23
Ortho-Phosphates (mg/l)	2.66	3.51	1.72	3.04	1.32	1.72
TP (mg/l)	18	14	16.5	9	19	13.7
Alkalinity (mg/l)	400	360	360	380	380	330
Total Hardness (mg/l)	508	272	248	256	264	288
Chlorides (mg/l)	446	149	85.2	90.88	99.4	85.2
Sodium (mg/l)	1100	810	42.43	42.32	220	221
Potassium (mg/l)	80	80	9.72	8.67	41.5	47
ORP (mV)	-145	-210	-13	-8	-49	-178

Table 4
Physico-chemical characteristics of Bellandur and Varthur Lakes at inflow and outflow during pre-monsoon (April–May), Monsoon (June–November) and post monsoon (December–March).

Water body	Period	Percentage Removal									
		BOD	Total COD	Fil. COD	Net .COD (Tot-Filt)	NO ₃ -N	NH ₄ -N	TN	Ortho Phos.	TP	Bacterial cells
Bellandur	Pre-Mon	62.5	55	44	67.7	20	46.2	29.03	14.28	25	47.72
	Mon	35	47.3	24.5	67.1	-53.37	40.6	51.56	-2.38	48.64	42.3
	Post-Mon	22.4	51.7	34	71.7	31.63	63.07	60.86	-21.01	60.71	25
Varthur	Pre-Mon	21	14	30.1	-15.3	-31.5	68.18	57.14	-24.21	22.22	57.14
	Mon	37.6	39.5	23.9	56	-2.79	49.33	43.58	-43.42	45.45	31.57
	Post-Mon	42.7	36.2	36.3	35.7	-40	47.61	51.06	-23.25	27.89	18.18

large floating macrophytes debris and floating islands comprising of macrophytes and resuspended sludge on the surface of the lake that drifts towards the outlets. Where as Varthur showed higher N removal during the pre-monsoon period (~57%) due to rapid algal uptake. Similarly, the Amm.-N removal was maximum during post-monsoon in Bellandur Lake (~63%) and pre-monsoon in Varthur Lake (~68%) attributable to Amm.-N losses due to increase pH consequent to algal photosynthesis at high light intensities (Weatherell et al., 2003). Higher TP removal was observed in case of Bellandur Lake (~61%) during the post-monsoon period, but Varthur Lake showed higher TP removal (~45%) during monsoon. Samples at outlets were having higher values of orthophosphate than inflows attributable to higher mineralisation of organic phosphates as a function of residence time and due to resuspension of P from the lake bottom (Mahapatra et al., 2011a). A higher bacterial removal was observed during the pre-monsoon period in both the lakes due to higher solar illumination, high photosynthesis and consequent development of an increased pH value.

Enormous quantities of terrestrial nutrients gets immobilised in the physico-chemical environment and within the biological organisms present in these lakes. Approximately, 100 tonnes BOD/d, 31 tonnes TN/d and 8 tonnes TP/d enters during pre-monsoon and ~37 tonnes BOD/d, 22 tonnes TN/d and 6 tonnes TP/d of leaves from Bellandur Lake. In monsoon, ~60 tonnes BOD/d, 32 tonnes TN/d and 18.5 tonnes TP/d enters and 39 tonnes COD/d, 15.5 tonnes TN/d and 9.5 tonnes TP/d leaves from Bellandur lake and 85

tonnes BOD/d, 35 tonnes TN/d and 14 tonnes TP/d entered during post-monsoon period while 66 tonnes BOD/d, 13.5 tonnes TN/d and 5.5 tonnes TP/d leaves the lake.

In the case of Varthur lake, 95 tonnes BOD/d, 27 tonnes TN/d and ~10 tonnes TP/d enters during the pre-monsoon period and ~74 tonnes BOD/d, 11.5 tonnes TN/d and 7.7 tonnes TP/d. During the monsoon period, ~69 tonnes BOD/d, 21.4 tonnes TN/d, ~9 tonnes/d enters Varthur Lake and ~43 tonnes BOD/d, ~12 tonnes TN/d and ~5 tonnes TP/d leaves the system. ~72 tonnes BOD/d, ~26 tonnes TN/d and ~10 tonnes TP/d enters Varthur lake during the post-monsoon period and ~41 tonnes BOD/d, ~13 tonnes TN/d and ~7.6 tonnes TP/d leaves the system daily. This provides vital insights for devising methods for recovering nutrients trapped in wastewater fed lakes and further engineer the system for optimal resource recovery and management.

3.4. Algal community dynamics

Over 32 genera of algae were recorded with more than 40 species. Chlorophyceae (13) dominated the algal community, followed by Bacillariophyceae (7), Cynophyceae (6) and Euglenophyceae (4). Bellandur Lake inlets were dominated by the members of Cynophyceae (~45%; *Merismopedia* sp. and *Microcystis*) followed by Bacillariophyceae (~35%; *Gomphonema parvulum*) as these species can grow in low light conditions, during the pre-monsoon period. In the middle reaches, when the water is clearer and is

free from organic suspenoids, the Chlorophycean members like *Chlorella* sp. dominated (75%). However, Chlorophyceae (45%) such as *Chlorococcum* sp. and *Monoraphidium* sp. dominated outlets. This indicated high photosynthesis by green algal members and requirement of light conditions for rapid photosynthesis (Weatherell et al., 2003). During the monsoon, the Bacillariophycean members dominated the inflows (78%) and Chlorophycean members dominated the middle (78%) and outlets (46%). However, the post monsoon period recorded the highest abundance in the Chlorophycean members (*Chlorella* sp., *Chlorococcum* sp. and *Monoraphidium* sp.) members (62% in middle; 88% in outlets). The inlet region was dominated by diatoms (72%). Biofilms collected from the rock surface, plant surface and sediment layers showed dominance of filamentous algae (*Oedogonium* sp.) and diatom sp. (*Gomphonema* sp. and *Navicula* sp.). The distribution of the different members of phytoplankton at various sampling locations and seasons are presented in Fig. 2. Spatiotemporal variations in algal community's composition are dependent on the nutrient loads, physico-chemical environment and the micro-climatic variables. Solar insolation, precipitation and the wind velocity are crucial in partitioning of the nutrient regimes in the lakes spatially and vertically (Mahapatra et al., 2011b,c).

During pre-monsoon period, in Varthur Lake, diatoms (~90%) dominated the inlet reaches followed by the Chlorophycean members in the middle (75%) and the outfalls (45%). However, during the monsoon period, the Chlorophycean members dominated both at inflow (32%) and middle (66%), while relative

abundance of Cyanophyceae (47%) were high near outlets. During the post monsoon, the Chlorophycean members dominated (~80%) in all these three locations. The results showed *Scenedesmus* sp., *Anabaena* sp. and *Anacystis* sp. were predominant near the shorelines while *Chlorococcum* sp. and *Monoraphidium* sp. (<10 μm) were present during the monsoon season (80%). Algal samples at inlets revealed predominance of *Gomphonema* sp. and *Nitzschia* sp. (10–38 μm) i.e. diatoms during the monsoon, that are succeeded by euglenoides such as *Euglena* sp. and *Phacus* sp. (>20 μm) in the pre monsoon period. Euglenoides are indicators of organic matter accumulation (Veeresh et al., 2009). During monsoon and post monsoon periods, the algal blooms at Bellandur Lake with higher abundance of Chlorophycean members act as inoculum for Varthur Lake.

Filamentous algae such as *Phormidium* sp., *Oedogonium* sp. and *Oscillatoria* sp. (>30 μm) were present near the outlets of the Varthur Lake during monsoon season. Comparative analysis of algal populations in the biofilms revealed a noticeable difference in the community structures at various zones of the Lake. The epilithic (over solid surfaces) algal biofilm consisted of colonies of *Stigeoclonium* sp. Diatom species such as *Gomphonema* sp. and *Nitzschia* sp. were near the inlet regions and species of Chlorophyceae and Euglenophyceae were near outlet regions. These distributions are dependent on the environmental variables such as organic load, nutrients (N and P) and light availability (Mahapatra et al., 2011a). Season-wise investigations indicate that depending upon the wind direction, the extent of growth and movement of the macrophytes

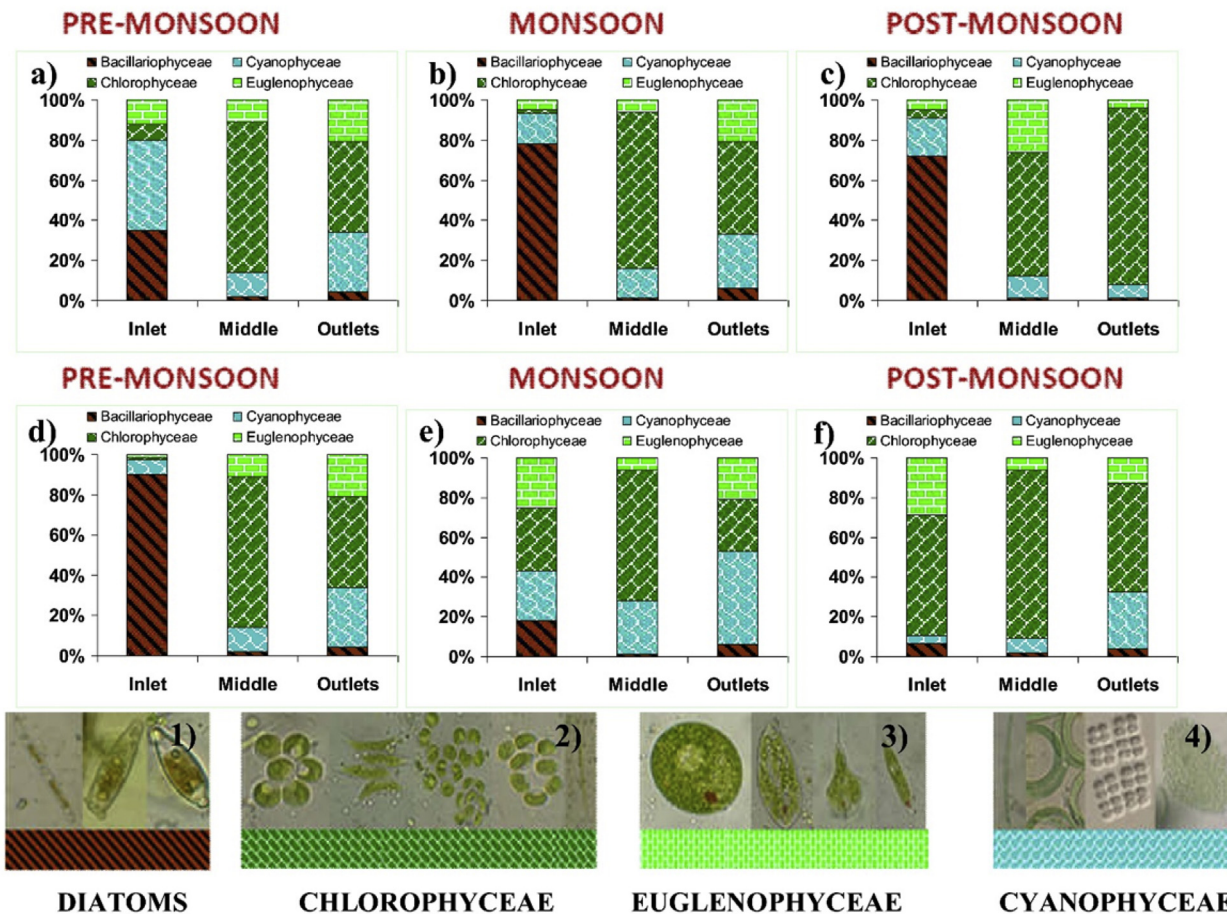


Fig. 2. a), b) and c) represents Bellandur Lake Algal densities at inlet, middle and outlets in various seasons, and d), e) and f) represents Varthur Lake Algal densities at Inlet, middle and Outlets in various seasons. The collage of the algal micrographs below represents: 1) Bacillariophyceae (diatoms); 2) Chlorophyceae; 3) Euglenophyceae and 4) Cyanophyceae.

together with the nutrient influx, there is a periodic transition from the anaerobic-aerobic (in monsoon) to anaerobic (in summer) and aerobic-anaerobic system (winter/pre monsoon) in the lake. Table S2 (in the Supplementary material) lists the algal composition in studied lakes. Fig. 2 elucidates the season-wise transitions in the algal groups at the various sampling locations. The inflow regions of Varthur Lake suffered from higher anoxia during the pre-monsoon period with only *Gomphonema* sp. and *Nitzschia* sp. in shallow regions of the Lake. Inlet regions samples consistently showed a very low count of algal cells in the order of 10^2 – 10^3 cells/ml. However, middle portion of the Lake showed an average cell count of 10^6 – 10^7 cells/ml. The algal cell counts were lower of 10^3 – 10^5 cells/ml in outlet samples, owing to decreased algal abundance with poor interception of sunlight with dense macrophyte cover.

3.5. Bacterio-plankton abundance and distribution

Bacterial growth and community structure are the key biological parameters in wastewater systems. Until recently, bacterial biomass were analysed through either measurements of cell size, usually with image analysis (Blackburn et al., 1998) or epifluorescence microscopy DAPI/Acridine orange (Marie et al., 2017). These are time-consuming techniques and have constraints for continuous and large-scale estimation of bacterial abundance and biomass involving sampling and analysis that needs preservation (Gasol and Giorgio, 2000). In such systems, there is a need to differentially quantify the bacterial and algal biomass to understand the trophic contribution to the purification/treatment process. Flow cytometry (FC) techniques have been used in wastewater microorganisms monitoring especially bacteria for devising and evolving optimal treatment options for urban wastewater treatment. FC has its own advantages in terms of accuracy in cell counting, detecting capability of bacterial species and assessment of live and dead cells (Gasol and Giorgio, 2000). FC aids in the analyses of a large number of cells and recording variability's in cellular characteristics for each cell through several parameters (Shapiro, 2005). In FC, typically 200 to 2000 cells per second circulate (sheath fluid) across beam of a laser and captures the light scattered by each of the particles and the fluorescence emission at different wavelengths generated by the excitation of each particle. FC reduces the time needed for the determination of bacterial abundance, size and activity. In FC usage of various DNA based fluorescent stains, nucleic acid and immunofluorescence probes provide the technique with abilities to discriminate cells on the basis of extent and type of nucleic acids, respiratory enzyme and many other characteristics.

In flow cytometric analysis, three clusters of bacteria were distinguished through normal laser encounter (Fig. 3A–F) with respect to standard beads that were run for dimensionality (Fig. S2, supplementary material). These synthetic beads are used for identifying the potentials that act as markers for the dimension's/size analysis of bacterio-planktons. Samples from the inlet regions (Fig. S3A) of Bellandur Lake showed a high cell density (10^7 cells/ml). However, three different peaks were observed (Fig. S3B) in the middle zone. In samples collected from outlets, only one dominant type of bacteria (Fig. S3C) was observed.

Higher bacterial abundance was observed in the samples of Varthur Lake inlets (shown as single peak in Fig. S3D). Middle regions comprised of large groups of bacteria (Fig. S3E) as highlighted from two peaks indicated by the arrows. Outlet samples comprised mainly several medium sized bacteria represented by a prominent peak depicted in Fig. S3F. The corresponding flow-cytogram (SSC vs. FSC plots) for bacterial abundance and major groups are given in Fig. 3. The scanning electron microscope studies showed different types of bacteria i.e. coccoid, rod-shaped, and curved bacteria

(Fig. 4). Among them, the bacillus i.e. rod shaped bacteria were dominant and are indicator organism in wastewater fed systems.

3.6. Changes in macrophyte communities and their distribution

The most dominant macrophytes observed were the free-floating water hyacinth (*Eichhornia crassipes*) and the rooted alligator weed (*Alternanthera philoxeroides*). Water hyacinth was observed to bloom in the pre monsoon periods during the start of winter mostly in the month of January and then, they grow exponentially in the next three months (Jan–April) and cover almost ~75% of the lake surface. In case of Bellandur Lake, the initial reaches are very shallow and are mostly occupied by the emergent *Typha* sp. At deeper regions, it is observed the predominance of free floating macrophytes, which are depicted in Fig. S4 (Supplementary material).

As the outlets of Bellandur are clogged with floating islands comprising of rooted macrophytes (water hyacinth is not removed from the Lake), and finally dies and settles down into the water after its growth and death (Battie et al., 2000). New macrophytes utilize this floating mass to form large islands. These floating islands mostly comprise of the lighter weight plants and matured sludge materials that accumulate and adhere to floating mass, because of sludge suspension that progressively compacts, dries and remain as huge floating masses that houses a wide range of macrophytes as *Alternanthera philoxeroides*, *Colocassia*, sedges as *Cyperus* spp. etc. Due to a permanent cover of the floating islands, lakes suffer anoxia due to which no DO was found in the water emerging from the outlets of Bellandur Lake (Mahapatra et al., 2011b,c). These outlet regions therefore, have a highly reducing environment and are associated with odour of H_2S .

However, in case of Varthur, the water hyacinth is periodically washed out from the system during monsoon due to high wind velocity and large quantum of water. Sometimes, the local people help in cleaning the clogged outlets to free up water movement during monsoon. Thus, Varthur Lake sheds most of macrophyte islands during monsoon and help to maintain open water surface. During summer, rapidly growing water hyacinth almost entirely covers the surface of the Lake during summer. Upon death, decay and compaction these floating macrophytes are then out-competed by *Alternanthera* sp. This species is a potential nutrient up-taker grows very rapidly after the death of the water hyacinth plants due to ageing and the weevil attacks. Species like *Lemna* and *Pistia* were mostly observed in the Lake edges and in the still and shallow regions. *Typha* sp. and sedges were observed in inflow regions of the water bodies. Fig. S4 (Supplementary material) shows the different macrophyte species found in these lakes based on nutrient affinity and prevailing environment. It was found that the presence of the floating macrophyte cover resulted in temporary anoxic zones and reduced algal growth, resulting in reduced conditions. Dense macrophyte cover in Varthur Lake has resulted in the absence of light penetration resulting in relatively low algal densities compared to the periods when the outlets are free of macrophytes with a reducing condition of low redox potential (–145 to –210 mV).

3.7. Multivariate analysis

Algal abundance with other physico-chemical parameters linkages were assessed through multivariate statistics. Significant positive correlations ($r = 0.85$; $p < 0.01$) were found between the biomass of Chlorophyceae members with total phosphates (TP). However, the other group of algae as Cyanophyceae were negatively correlated with TP ($r = -0.699$; $p < 0.05$) which could be due to higher P accumulation capacity of cyanophytes that they use for

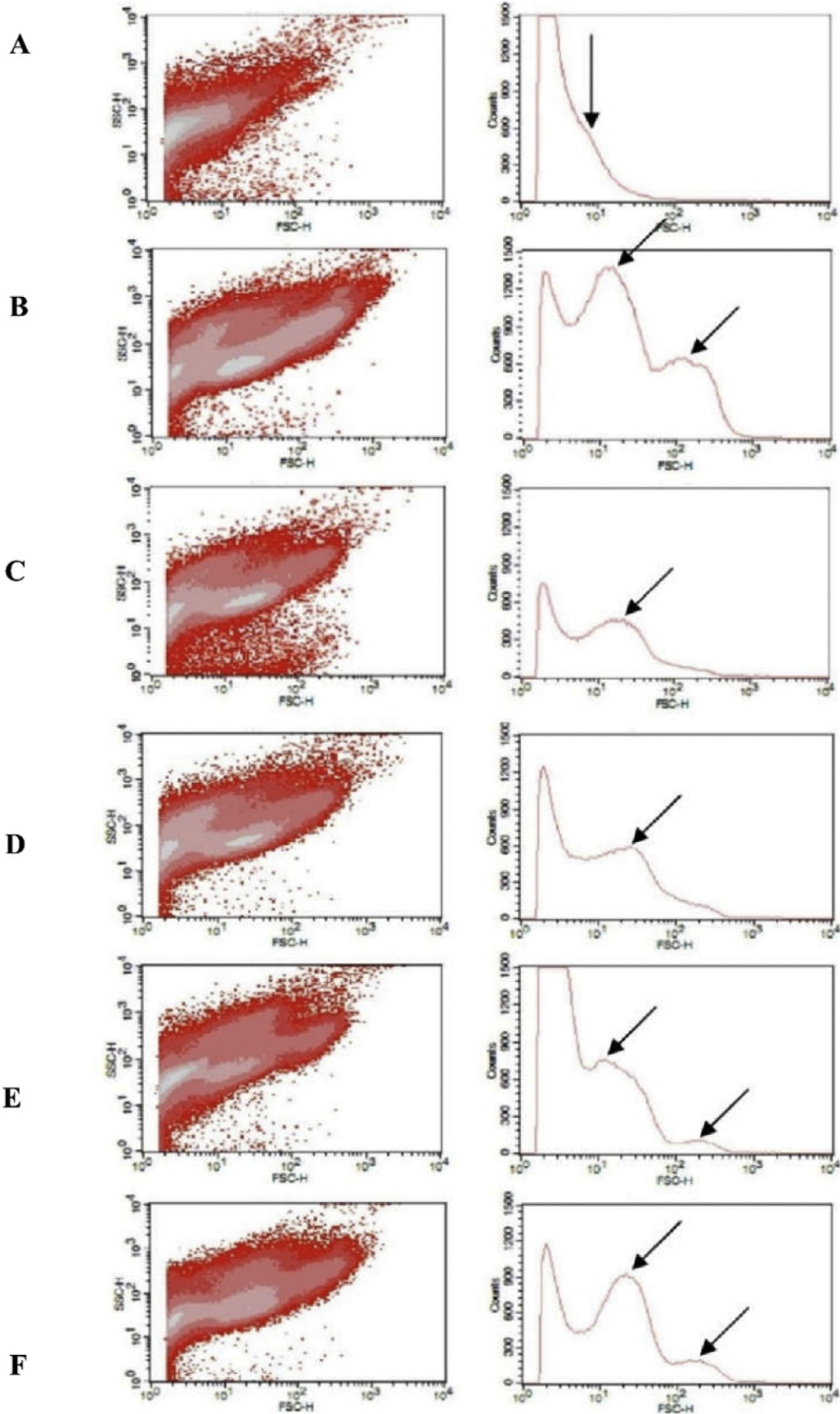


Fig. 3. Flow cytograms for Bellandur Lake (A, B and C) and Varthur Lake (D, E and F). **Note:** The x axis refers to SSC i.e. side scatter that indicates extent of granularity or cellular complexity and y axis represents FSC i.e. forward scatter that refers to size of the cells. Arrows indicate different class/groups of cells gated together on the basis of sizes and complexities. *A and D represents Inlet; B and E – Middle and C and F – Outlet.

growth at low P conditions (Kromkamp, 1987). Euglenophyceae members were negatively correlated with total nitrogen (TN) ($r = -0.64$; $p < 0.05$) whereas Bacillariophyceae members are significantly negative correlated with filterable COD ($r = -0.58$; $p < 0.05$) and BOD ($r = -0.61$; $p < 0.05$) and are known to prefer nutrient stress environment. On the other hand, if the total algal count (TAC) is examined, the rank correlation results show negative correlations with filterable COD ($r = -0.65$; $p < 0.05$), Amm. -N ($r = -0.66$; $p < 0.05$) and TN ($r = -0.73$; $p < 0.01$). Similar results were observed in other studies that showed a bacterial dominance with high concentration of C and N and progressive change as the concentration recedes (Veenstra et al., 1995). Total bacterial counts (TBC) were positively correlated with EC ($r = -0.66$; $p < 0.05$), free CO_2 ($r = -0.68$; $p < 0.05$), COD ($r = -0.64$; $p < 0.05$), filterable COD ($r = -0.69$; $p < 0.05$), Amm. -N ($r = -0.88$; $p < 0.01$) and total N ($r = -0.87$; $p < 0.01$) as provided in Table 5. The bacterial predominance at high COD, N and electrolyte concentration are in accordance to the studies by Mahapatra et al. (2013b) at high C and N loads. This shows higher bacterial growth associated with abundance of C and nutrients with high production of CO_2 . However, the correlation with EC might be because of higher mineralisation of organics due to faster decomposition with high abundance of bacteria.

Canonical Correspondence Analysis (CCA) showed four distinct regions in the ordinate space characterized by different groups of algae being impacted by specific physicochemical conditions and seasons (Fig. 5). Axis 1 accounted for 60.94% and axis 2 explained 31.73% of the total variation, which jointly represents 92.67% of the total variance. Fig. 5 highlights that parameters such as TP, TN, Amm-N, Turbidity, COD affected the inlets of both lakes (i.e. BM-I,

BPOM-I, VPRM-I, VM-I and VPOM-I), showing the abundance of Chlorophycean members. Only the inlets of Bellandur (BPRM-I) were influenced by filterable COD and BOD, Alkalinity, EC and free CO_2 , creating conducive environments for higher bacterial growth and abundance and the highest bacterial counts were observed during the pre-monsoon season. However, the regions near the outlets (BM-O and VPOM-O) were significantly influenced by the inorganic parameters (ionic) as Na, K, Ca and Mg, Chlorides and Nitrate-N where there was predominance of diatom species. Moreover, the outlets (BPOM-O and VM-O) were highly impacted by TDS, pH and temperature pertaining to the growth of Euglenoides.

Bray-Curtis cluster analysis provided the grouping based on spatial similarities of the inlet/outlet seasonality's with different transitions on physico-chemical parameters resulting in changes in biotic activities and communities. The water quality variability in inflows formed four distinct clusters with the outflows especially in the pre-monsoon periods mapping the nutrient concentration with the algal biomass and algal/bacterial abundance (Fig. 6).

Cluster I (87% similarity) comprised of two sub clusters i.e. subcluster-01 (VM-I and VM-O at 93% similarity) with VPOM-I (89.5% similarity) and subcluster-02 (BPRM-O and BPOM-O at 92% similarity) with VPOM-O (89.75% similarity). BM-I and BM-O formed cluster two at a similarity of 90%. Cluster III comprise of BPOM-I and BPRM-I with a similarity of 86% and cluster IV comprised of VPRM-I and VPRM-O at a similarity of 70.25%. The characteristics of VPRM-I and VPRM-O are very different showing significant difference in inlet and outlet characteristics compared to other inflow and outflow characteristics of other seasons as observed by cluster analysis.

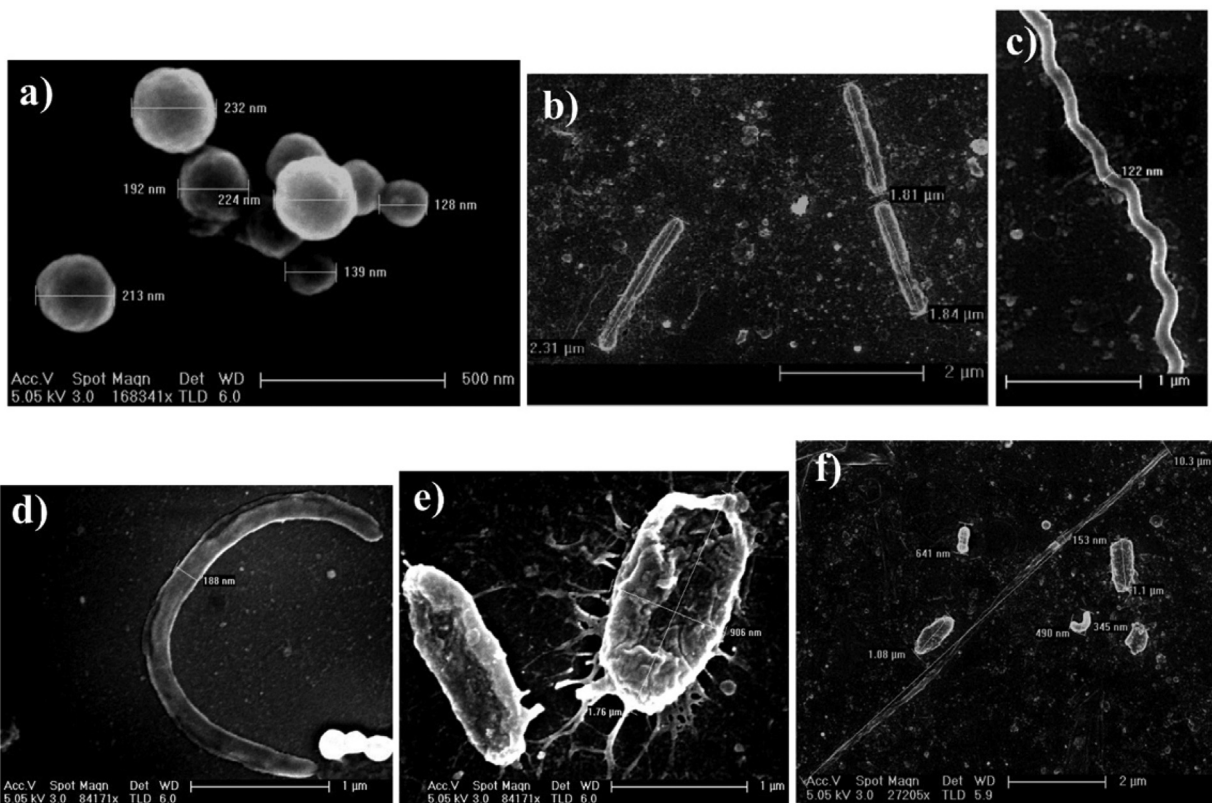


Fig. 4. SEM micrographs of the different shapes of bacteria present in the treatment system a) coccus (avg. size of 200 nm); b) bacillus with numerous lengths (avg. length of 1.8 μm); c) spirillum (width: 120 nm and length $\sim 4 \mu\text{m}$) d) C shaped bacteria (avg. length: 3–4 μm and width: $\sim 180 \text{ nm}$) e) activated flocs: projecting out are the filamentous bacteria (average length: 1.76 μm and width: 900 nm) and f) different varieties of bacteria present [i.e. long straight filamentous bacteria (10 $\mu\text{m} \times 150 \text{ nm}$); various kinds of bacillus (sizes: 640 nm to 1.1 μm); comma shaped bacteria (length: $\sim 500 \text{ nm}$).

Table 5
Spearman's rank correlation between physico-chemical variables and groups of algae growing in the Lakes during the study period.

Parameters	Chlorophyceae	Euglenophyceae	Cyanophyceae	Bacillariophyceae	Algal Count	Bacterial Count
pH	-0.53697	-0.25773	0.27434	-0.30773	0.038664	0.12281
Temperature	-0.30877	0.057471	0.13052	-0.02853	0.15762	0.055944
EC	-0.24211	0.23707	0.36686	-0.10341	0.1296	0.66434*
TDS	-0.47018	0.38793	0.38802	-0.17829	0.32224	0.48252
Turbidity	-0.26714	-0.03238	-0.11838	-0.81263	-0.2807	0.43783
DO	-0.17047	0.27887	-0.11838	0.37149	0.36491	-0.1366
Free CO ₂	-0.18246	-0.21552	-0.10935	-0.14976	-0.22067	0.68531*
COD	0.059649	-0.54598	-0.40919	-0.39223	-0.56743	0.64336*
Filt. COD	0.082893	-0.51817	-0.28901	-0.57712*	0.65141*	0.68542*
BOD	-0.28998	0.023389	-0.0424	-0.6126*	-0.29123	0.55692
Nitrate-N	0.29474	-0.12572	-0.34569	-0.06062	-0.16112	-0.40559
Amm-N	0.11072	-0.4192	-0.23322	-0.56259	-0.65965*	0.87916**
TN	0.31579	-0.63578*	-0.5009	-0.30309	-0.7285**	0.87413**
Phosphates	-0.17047	0.27887	-0.11838	0.37149	0.36491	-0.1366
TP	0.85237**	-0.37962	-0.69966*	0.04465	-0.28772	0.010508
Alkalinity	-0.24513	-0.28185	0.073215	-0.25091	-0.26775	-0.02832
Tot. Hardness	0.44562	0.43463	-0.29631	-0.11054	0.014011	0.12587
Chlorides	-0.18375	0.46664	-0.03197	-0.42373	0.13404	-0.21127
Na	-0.03158	0.5388	0.17285	-0.36014	0.20315	0.06993
K	-0.01056	0.38209	0.14336	-0.29163	0.17223	-0.02456
ORP	0.035088	-0.3592	-0.10935	0.032092	-0.21016	-0.29371

Bold figures indicate statistically significant.

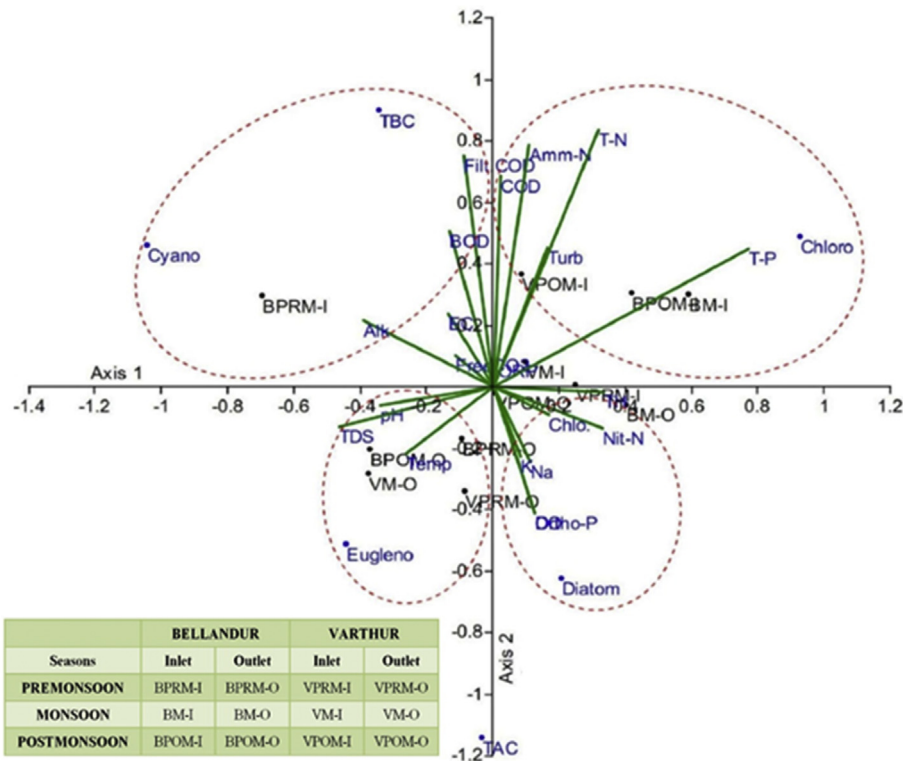


Fig. 5. CCA ordination obtained from algal/bacterial counts and algal relative abundance data and physicochemical variables explaining seasonal variability's (PRM – Pre Monsoon; M – Monsoon and POM – Post Monsoon) with sampling locations in Bellandur (B) and Varthur (V) Lakes. *Illustration show the ordination of lakes inlet(I)/outlets(O) in different seasons (represented by points with black dots and black legends as BPRM-I; BM-I; BPOM-I and BPRM-O; BM-O; BPOM-O and VPRM-I; VM-I; VPOM-I and VPRM-O; VM-O; VPOM-O) and physicochemical variables (denoted by green lines and blue legends), and micro-algal genera indicated by blue points and blue legends. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

3.8. Nutrient dynamics and budgeting

The monsoon and the post-monsoon witnessed a significant quantity of BOD removal inferring higher organic matter degradation and uptake. High BOD removal is noticed at locations with open lake surface (without macrophytes cover) with active aerobic environments, which is also evident from high DO concentrations

in the Lake. Higher DO levels were due to algal photosynthesises and surface aeration that lowered BOD. Compared to this, thick macrophyte cover during late winter and summer months resulted in poor BOD removal. The lake has been functioning in tandem as an anaerobic–aerobic lagoon imparting a satisfactory treatment in the lake. Therefore, understanding the biotic community succession with the wetland dynamics is essential to arrive at appropriate

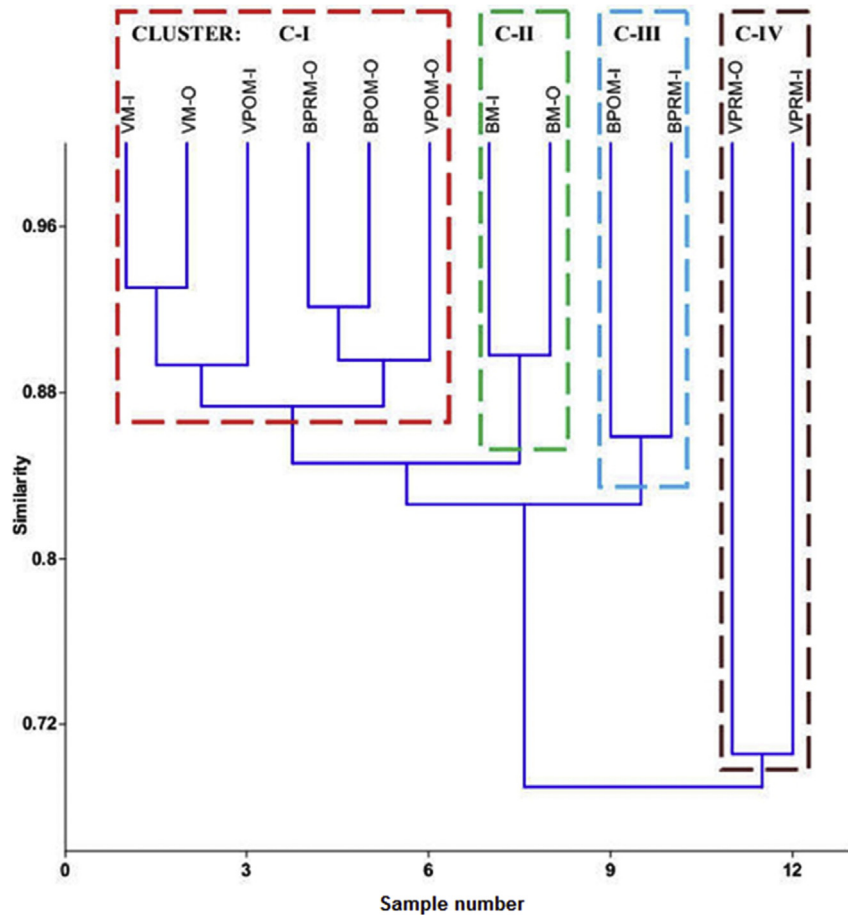


Fig. 6. Dendrogram depicting the similarity in site-seasonality through clustering based on physico-chemical parameters, algal/bacterial abundance and distribution in the studied lakes.

treatment strategies and devising location specific constructed wetland that enhances the sustainable water treatment and nutrient bioremediation. This also helps in replicating similar strategies in the emerging cities and towns in India and many developing nations across tropics in addressing the water scarce situations.

Approximately ~200 tons of BOD enters the lake daily. Considering higher efficiencies of anaerobic-aerobic lagoon systems, a larger loading and conversion are plausible. De-silting would increase of storage volume, while enhancing the water residence time and treatment. Matching the influent concentrations to the assimilative capacity of the lakes will enhance efficacy of the lake systems with a lively biodiversity that maintains the integrity of the lake. The differential activity of the various communities in the lakes depends upon the nature and type of the wastewater fed into the systems and is mostly driven by various environmental factors such as solar insolation, temperature, pressure, wind speed and rainfall.

About 82 tonnes BOD/d and 78 tonnes, BOD/d enters Bellandur and Varthur Lakes respectively (comparable to typical lagoons). On the other hand, when one considers the maximum potential of the anaerobic-aerobic systems, higher loading rates and higher conversion rates are possible. There is, thus, a need to further examine the potential for higher quality of water at the outlet to enable the recycling and reuse of water in the future upon de-silting and increasing the residence time or reducing the loading. In order to make this more sustainable, the extent of the harvest and the reuse of plant nutrients for the system need to be examined. The

contribution of macrophytes and phytoplankton in removing nutrients in these sewage-enriched systems varies with the nature of the effluent and the age of the wetland, in addition to other environmental factors like sunlight, temperature, wind and precipitation.

3.9. Nutrient-integrated treatment efficiencies

Crucial treatment parameters are indicative of the remediation performance of urban wetlands. The important factors that influence the treatment ability of the surface water bodies are influent wastewater concentrations, loading rates, microbial community, and the water residence time. The treatment parameters at different phases of treatment determine the degradation of organic matter and transformations, nutrients uptake, and changes in the physico-chemical environment on microbial growth. Concentrations of influent wastewater, volumetric loading, microbial composition, water residence time are the key factors that drive treatment competence. Thus, formulating appropriate treatment efficiency indices are key to the overall efficiency, which helps in evolving future management plans for effective decisions making. Many treatment parameters such as suspended solids, COD, BOD and $\text{NH}_4\text{-N}$ removal efficiencies have been considered for assessing the efficiency of the treatment systems (Colmenarejo et al., 2006). Integrated treatment efficiency in the present case has been devised taking into consideration all the crucial parameters from the treatment perspectives that focus on clarity of the water, C, nutrient content with bacterial counts and equal weightages have been

considered for these parameters. Such Indices help in assessing the treatment levels to cater to an accepted water quality level to be used for any purpose. This helps in deciding additional treatment options for complete treatment of wastewater. Pond based systems in tropics have been used for polishing and maintaining water quality for the end use after treatment and also cleans of bacteria (Jimenez, 2007). Such systems on an average show efficiency close to 90%. In the present study the treatment interacted efficiency is computed as per equation (2).

$$\text{TIE} = [\text{E}_{\text{TUR}} + \text{E}_{\text{COD}} + \text{E}_{\text{N}} + \text{E}_{\text{P}} + \text{E}_{\text{BAC}}]/5 \quad (2)$$

where

TIE: Treatment Integrated Efficiency (in percentage),
E_{TUR}: turbidity removal efficiency (in percentage),
E_{COD}: COD removal efficiency (in percentage),
E_N: BOD5 removal efficiency (in percentage),
E_P: Nitrogen removal efficiency (in percentage), and
E_{BAC}: Phosphorus removal efficiency (in percentage).

TIE values for Bellandur and Varthur Lakes were 64.85 and 63.19% respectively indicating poor treatment efficiencies due to enormous C and nutrients loading beyond their assimilative capabilities and lower water residence time. This emphasises the need for checking the volume of C and nutrient loads to cope up to their treatment potential. This necessitates a) increasing the oxidative surfaces by regular clearing invasive floating macrophytes (except functionally active floating islands of macrophytes) that hinders sunlight penetration and natural air diffusion in the system, b) provisions for increasing the water residence time by wet dredging for better treatment (which also increases the storage capacity), c) regulating the quality and quantity of wastewater loads by allowing treatments in the upstream of respective wetlands.

4. Conclusion

Regular monitoring of water quality (Physico-chemical and biological parameters) at select locations across all seasons in Bellandur and Varthur wetlands, reveal of functioning of wetlands through algal-bacterial treatment as an anaerobic–aerobic stabilisation pond (for two seasons) with a water residence of 5 days and 70% removal of filterable BOD. Higher proliferation of floating macrophytes reduced the algal photosynthesis and stabilisation of organic matter. Open surfaces facilitate algal growth and thus helps in achieving higher treatment levels appropriate reuse of water in small towns. Urban wetlands in Bangalore have been receiving higher nutrient concentrations from partially or untreated municipal wastewater. This has transformed the physico-chemical integrity and biological entities. Chlorophyceae (*Chlorella* blooms) were dominant due higher Ammonium-N content and were predominant in anoxic-aerobic conditions. Cyanophycean members were mostly observed in the partial anaerobic regions. Seasonal succession is evident with Chlorophycean members dominating the wet periods, while Cyanophycea are dominant during dry periods. Phytoplankton density correlated with the lake's transparency/turbidity. The CCA analysis showed TP, TN, Turbidity, COD Alkalinity, EC and free CO₂ as dominant parameters governing the microflora distribution in the inlets and inorganic parameters (ionic) as Na, K, Ca and Mg, Chlorides and Nitrate-N governing the distribution of other algal members in the outlets. The Cluster analysis revealed grouping of sites/seasons with similar nutrient status and showed broadly four different clusters. The present study reveals that Bellandur and Varthur wetlands have been functioning as wastewater fed treatment systems and

sustained nutrient enrichments have led to the growth of bacterioplankton, phytoplankton and macrophytes. These two wetlands function differently because of flushing out of the macrophytes during the monsoon and enabling clear water surfaces that functions as high rate algal ponds.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at <https://doi.org/10.1016/j.jenvman.2017.10.054>.

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