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Environmental factors influencing methanogenic activity in two contrasting tropical lake sediments

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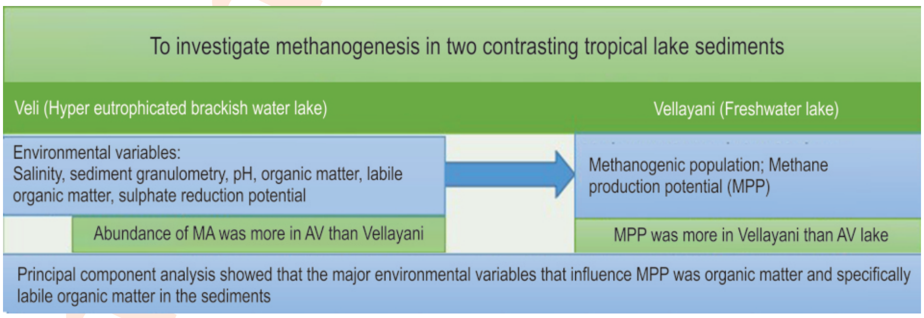
Abstract

Aim: To investigate the influence of environmental variables on the abundance and activity of methanogenic archaea (MA) in Akkulam-Veli and Vellayani Lake sediments.

Methodology: Sediment and overlying water samples (n=5 each) were collected from Veli and Vellayani lakes of Thiruvananthapuram, Kerala. Samples were analysed for environmental variables using standard protocols. Multivariate analysis was done to study the influence of environmental variables on abundance and activity of MA.



Results: Environmental variables of overlying water and sediment showed significant variation between the two lakes. Salinity and sulphate were more in Akkulam-Veli than Vellayani, as Akkulam-Veli is a brackish lake and temporarily connected with Arabian Sea. Highly reduced sediments of Akkulam-Veli favoured more population of methylotrophic and acetoclastic MA than Vellayani. A distributional difference of MA with depth was observed in both lakes, which is attributed to availability of more labile organic matter. The methylotrophic MA activity was not significantly different between the two lakes; however, their abundance was significantly different. Nevertheless, methane production was higher in Vellayani than in Akkulam-Veli Lake. PCA revealed that Corg and labile organic matter (LOM) were the important environmental variables influencing methane production potential.

Interpretation: Anthropogenic activities like sewage and waste disposal results in increased input of organic matter in lake sediments. The labile organic matter fraction in the sediments favours methanogenic activity thereby resulting in methane production and release from the lakes.

Key words: Labile organic matter, Methanogenic archaea, Methane production, Sulphate reduction, Tropical lakes

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Introduction

Lakes provide recreation, fishing, drinking water, irrigation, transportation, energy and as dumping site for wastewaters. Therefore, settlements, agriculture and industry have been developed extensively to the surroundings of lakes. Among the various states in India, Kerala ranks first in having the largest area of wetlands (Nayar and Nayar, 1997), which includes brackish and freshwater lakes. During recent decades, riverine discharges of nutrients and organic matter have increased significantly due to human interventions within watersheds. The quantity and quality of organic carbon reaching the sediments influence the community structure and function of benthic microorganisms. Wetlands differ in microbial community structure and diversity in sediments, which are controlled by hydrological and nutritional factors (Xu *et al.,* 2017). Moreover, microbial activity is aggravated in tropical wetlands due to high temperature and high rates of organic matter decomposition (Verma *et al.,* 2002) and thus, they may act as sources of green house gases like CO_2 and CH_4 (Pighini *et al.,* 2018; Zhang *et al.,* 2020).

In tropical lake sediments, sulphate reduction by sulphate reducing bacteria (SRB) and methanogenesis by methanogenic archaea (MA) are important terminal oxidation processes during organic matter mineralization. Sulphate reduction is reported to be the predominant terminal electron accepting process in Ashtamudi estuary in Kerala, India (Vincent *et al.,* 2017). Nevertheless, the mineralization of organic matter is mainly driven by sulphate reduction in the upper 2-4 cm, below that methanogenesis occurs. Sulphate from seawater inhibits methane production in tidal wetlands and salinity has been used as a general predictor of methane emission (Holm *et al.,* 2016). Methane emissions within individual wetland regime are highly variable both spatially and temporally due to heterogeneity of environmental variables (Verma *et al.,* 2002). Several environmental factors of sediments such as temperature, presence of dissolved oxygen, salinity, pH, supply of organic matter, soil texture and mineralogy, electron acceptors and microbial interactions with other anaerobic metabolic group such as sulphate reducing bacteria may influence methanogenesis (Ramesh *et al.,* 1997; Marinho *et al.,* 2012).

Lakes with different trophic status have sediments with different carbon and nutrient concentration with consequently different microbial communities. In tropical conditions, strong precipitation patterns influence significant fluctuations in river discharge and associated hydrological conditions including salinity. In addition to the physical environmental variables, origin and biochemical composition of organic matter also are key factors, which control sediment bacterial composition and activity (Fabiano *et al.,* 2004). The labile fraction of sedimentary organic matter consists of simple organic biopolymer molecules such as proteins, carbohydrates and lipids, which are available for benthic organisms and are rapidly mineralized (Venturini *et al.,* 2012). Due to fast decomposition and turnover rate, labile soil organic pool in wetlands may accelerate the greenhouse effect. Ultimately, the carbon pool in wetland soils can change from a sink to source, leading to a positive climatic feedback by increasing atmospheric CO_2 and CH_4 concentrations (Cao *et al.,* 2017). Estuaries are potential source of CH_4 into atmosphere (Araujo *et al.,* 2018). Lack of adequate field studies in understanding the source or sinks of CH_4 in lakes hampers the global atmospheric budgeting (Araujo *et al.,* 2017). Studies have been undertaken in India in relation to biogeochemistry of CH_4 (Ramesh *et al.,* 1997). However, the population and activity of MA as well as their competitors, SRB and their role in controlling methane emission has not been studied so far in tropical estuaries. The aim of the study was to compare the factors influencing the population and activity of MA in Akkulam-Veli (AV), a hyper-eutrophicated brackish water lake and Vellayani, a fresh water lake in Thiruvananthapuram district, Kerala, India.

Materials and Methods

Study area and sampling: Akkulam-Veli Lake is located in the north western part of Thiruvananthapuram along the Arabian Sea. The lake experiences marine influence during monsoon and is separated from the sea by a sandbar, which is approximately 150 m long and 20-40 m wide during non-rainy season. Vellayani lake, located in the outskirts of Thiruvananthapuram city is the largest freshwater lake in Kerala. Sediment samples were collected manually, from 5 stations each of Akkulam-Veli and Vellayani lakes, using a core and Van Veen's grab. Samples of overlying water were collected using a Dussart-flask water sampler. Sediment samples for microbiological studies were collected in sterilized sample bottles, brought to laboratory and stored at 4°C. Cores obtained in each sampling station were segmented in two sections (0–5 cm and 5–10 cm) under a nitrogen atmosphere. The remaining sediment samples were maintained under 4°C in a refrigerator to perform physico-chemical analyses. Sediment granulometry was determined by differential settling method (Folk, 1974) and classified according to Picard (1971).

Analyses of environmental variables: Physico-chemical and nutrient characteristics of sediment samples like temperature, pH, electrical conductivity (EC), salinity, total kjeldahl nitrogen (TKN), sulphate, organic carbon (C_{org}), organic matter (OM), redox potential (Eh) and sediment granulometry were analyzed by standard methods (Trivedy *et al.,* 1998; APHA, 2017). Organic carbon and organic matter content in sediments were estimated by wet oxidation method (Walkley and Black, 1934).

Biochemical analyses of sediments: Estimation of protein, carbohydrates and lipids from estuarine sediment samples were carried out for assessing the lability of sedimentary organic matter. Protein estimation was done by following the procedures of Hartree (1972); Fabiano *et al.* (1995) and Lowry *et al.* (1951). Carbohydrates were analyzed by phenol-sulphuric acid method (Dubois *et al.,* 1956; Kochert, 1978). Lipids from sediment samples were analyzed by acid-dichromate method by Bligh and Dyer (1959) and Parsons *et al.* (1984).

Abundance of Methanogenic archaea and sulphate reducing bacteria: Abundance of MA and SRB were enumerated by roll tube method described by Hungate (1950, 1969). Mah media was used for MA and postgate media for SRB. Acetate and methanol (0.1 M) substrates were used for the enumeration of acetoclastic and methylotrophic Methanogenic archaea, respectively. The medium and sediment pore water was dispensed into test tubes, sealed with rubber cork and rolled in a wet sponge until the medium solidified inside the tubes. To maintain anaerobic conditions, tubes were flushed with highly pure nitrogen gas before and after rolling the tubes. Methanogenic archaea and sulphate reducing bacteria colony count was taken after one week of incubation and expressed as sediment (CFU g^{-1}).

Methane production potential (MPP) of lake sediments: Microcosm studies were undertaken to analyze the methane production potential of sediments enriched in basal medium, added with different carbon sources (0.1 M acetate or 0.1 M methanol). Sediment samples (1.0 g) from each station were added to 9.0 ml of the respective media in 40 ml serum vials and covered with rubber stopper and aluminium cap assembly. After a week of incubation, gas samples (0.5 – 1.0 ml) were taken from headspace of the incubation bottles using gas lock syringes and analyzed for the presence of methane in a gas chromatograph (Perkin Elmer, Clarus 580) equipped with Flame Ionization Detector. Isothermal separation was performed at 35°C in a 30 m long 0.53 mm, Elite-PLOT Q column, with nitrogen as carrier gas. Samples that contained high methane concentration, in percentage levels, were analyzed on a Thermal Conductivity Detector equipped gas chromatograph (NUCON 5765) packed with PORAPAK Q, 80/100 mesh size 5 m long column and nitrogen as carrier gas. MPP was expressed in terms of methane (mol m^{-3}) produced per day. For estimating sulphate reduction potential (SRP), sediment samples (1.0 g) were added to 10 ml postgate broth in 40 ml serum vials and incubated for a week. SRP of the sediments was derived from initial and final sulphate concentration in the incubation mixture and expressed in terms of sulphate reduced (mol m^{-3}) per day (Trivedy *et al.* 1998).

Statistical analyses: Two-way analysis of variance (two-way ANOVA) was conducted to test for significant differences in the variables between seasons and stations and the interaction effect of stations. Principal Component Analysis (PCA) was used to standardize environmental variables (Reid and Spencer, 2009). Varimax and Kaiser Normalization were carried out for the extraction and interpretation of components. Statistical analyses were conducted using SPSS version: 17.0; SPSS Inc. (2008) and primer 6 softwares.

Results and Discussion

Akkulam-Veli Vellayani lakes differed from each other significantly with regard to specific environmental variables like water temperature, water and sediment pH, redox potential and clay content. Although Akkulam-Veli and Vellayani lakes were brackish and fresh water lakes respectively, no significant difference were observed in their sediment salinities (Table 1). This was due to the reason that the sampling was done during summer, where Akkulam-Veli lake was detached from the sea by a temporary sand bar and the salinity values indicated freshwater conditions. Nevertheless, the average salinity of Akkulam-Veli lake was 7 fold higher than Vellayani in both water and sediments. The results suggest dominant control of salinity on methanogenesis (Araujo *et al.,* 2018). Although sulphate content of Akkulam-Veli lake was more than Vellayani, the difference was not statistically significant. During sampling period, Akkulam-Veli lake was completely detached from the sea by means of a sand

Table 1: Descriptive statistics of environmental variables and microbial activity in Vellayani and Akkulam-Veli Lake

Parameters	Vellayani	Akkulam-Veli lake	ANOVA (p values)
Water temperature (ºC)	32.00±1.00	29.80±0.84	0.005*
Water pH	6.74±0.08	7.30±0.44	0.02*
Water electrical conductivity (µS cm^{-1})	151.40±22.12	1214±125	0.096
Water salinity (PSU)	0.07±0.01	0.550±0.1	0.10
Sediment temperature (ºC)	30.60±0.55	30.00±0.71	0.17
Sediment pH	6.76±0.12	7.22±0.35	0.02*
Sediment electrical conductivity (µS cm^{-1})	188.68±23.92	1215±1035	0.06
Redox potential (mV)	-186.60±23.27	-286.60±36.49	0.001**
Sediment salinity (PSU)	0.08±0.01	0.55±0.50	0.06
Sulphate (mg g^{-1})	10.72±1.74	13.47±8.00	0.48
Total organic carbon (Corg) (%)	3.96±1.40	2.93±1.32	0.26
Total organic matter (TOM) (%)	6.84±2.41	5.04±2.28	0.26
Sand (%)	9.45±9.97	28.75±18.77	0.08
Silt (%)	54.97±10.14	60.75±13.22	0.46
Clay (%)	31.00±11.12	10.50±8.18	0.011*
Labile organic matter (LOM) (µg g^{-1})	25240±989	22505±9041	0.52
LOM (% in TOM)	41.53±16.41	46.82±9.53	0.55

Values are mean of replicates ± S.D.

Table 2: Descriptive statistics of microbial activity in Vellayani and Akkulam-Veli Lakes

Parameters	Vellayani	Akkulam-Veli	ANOVA (p values)
Methylotrophic methanogens (0-5 cm) CFU g^{-1}	18.00±14.83	2502±1220	0.002*
Methylotrophic methanogens (5-10 cm) CFU g^{-1}	40.00±38.73	2652±1419	0.003*
Acetoclastic methanogens (0-5 cm) (CFU g^{-1})	224.00±211.85	786±682	0.12
Acetoclastic methanogens (5-10 cm) (CFU g^{-1})	90.00±75.17	972±981	0.08
MPP-methanol (0-5 cm) (mol m^{-3})	94.23±4.49	72.51±18.48	0.03*
MPP-methanol (5-10 cm) (mol m^{-3})	90.74±3.66	64.11±19.72	0.02*
MPP-acetate (0-5 cm) (mol m^{-3})	98.93±12.40	83.62±22.48	0.22
MPP-acetate (5-10 cm) (mol m^{-3})	97.04±6.12	86.59±17.88	0.25
SRB-acetate (0-5 cm) (CFU g^{-1})	6.00±8.94	100±154.11	0.21
SRB-acetate (5-10 cm) (CFU g^{-1})	70.00±156.52	0.00±0.00	0.35
SRB-lactate (0-5 cm) (CFU g^{-1})	86.00±143.28	74±15.94	0.90
SRB-lactate (5-10 cm) (CFU g^{-1})	22.00±17.89	28.0000±3.14	0.72
SRP-lactate (0-5 cm) (mol m^{-3})	0.03±0.038	0.05±0.02	0.35
SRP-lactate (5-10 cm) (mol m^{-3})	0.31±0.65	0.05±0.05	0.400

MPP – Methane production potential; SRB – Sulphate reducing bacteria; SRP: Sulphate reducing potential

bar. High sulphate concentration results in low methane efflux (Shiau *et al.,* 2016). The population of both methylotrophic and acetoclastic MA were high in Akkulam-Veli than Vellayani lake. This difference of MA abundance was more pronounced in methanol than acetate, where methylotrophic MA was 139 fold more in Akkulam-Veli in the top layer and 66 fold more in bottom layer than Vellayani. A difference in distribution of MA with depth was observed in Akkulam-Veli and Vellayani lakes (Table 2). In Akkulam-Veli, the abundance of acetoclastic and methylotrophic MA was more in bottom layer; however, in Vellayani, acetoclastic MA was abundant in top and methylotrophic MA were more in bottom layer. The distributional difference of MA with depth is attributed to the availability of organic matter and it is reported that the availability and lability index of organic matter decreases with depth (Nedwell *et al.,* 2004; Gonsalves *et al.,* 2011). The reasons for these uncertainties include complex interactions among various physical, geological and biological factors that define each lake system and control organic matter cycling in their sediment (Goni *et al.,* 2003). Shifts in microbial community function and structure can be indicators of environmental stress and ecosystem change in wetland soils (Chambers *et al.,* 2016).

Although MA abundance was favoured by methanol as substrate in Akkulam-Veli, methane production rate was more with acetate than methanol. The difference between the two lakes was observed particularly with abundance of methylotrophic MA and methane production rate with acetate as substrate. This means that methylotrophic methanogenic activity was not significantly different between the two lakes, although their population was different. In contrast, the abundance of acetoclastic MA was not different between the two lakes; however, the difference in methanogenic activities was statistically significant. Hence, it is understood that number of bacteria necessarily does not reflect microbial activity. Generally, methane production rates are higher in sediments of hypereutrophic lakes than oligotrophic lakes (Huttunen *et al.,* 2003). However, methane production was more in Vellayani lake than in hyper-eutrophicated Akkulam-Veli lake (Fig. 1). This can be explained by electron donor limitations or presence of inhibitors, which can potentially inhibit methanogenesis (Roden and Wetzel, 2003). Moreover, different types of anthropogenic disturbance experienced in Akkulam-Veli lake might have increased microbial diversity, but decreased their metabolic activity (Jin *et al.,* 2017). Akkulam-Veli is a coastal lake and during sampling period it was completely detached from marine influence. Nevertheless, the presence of non-competitve substrates like methanol, methylamine (Winfrey and Ward, 1983) might have favoured the abundance of methylotrophic MA in Akkulam-Veli lake. Hence, it can be conceived that hypereutrophic lakes with higher Corg availability in sediments has highest catabolic diversity, when the microbial communities are able to efficiently use a broader range of substrates (Torres *et al.,* 2011). The presence of MA and SRB in same depth reveals that the distribution of abundant MA and SRB are mutually exclusive. Viable population of SRB corresponds to Corg and sulphate concentration in the sediment (Vincent and Raj, 2018). It is predicted that in low salinity bottom sediments such as fresh water lakes, sulphate reduction is inhibited and methane formation gets elevated. However, it was surprising to note that in Vellayani, despite lower salinity values, sulphate reduction rate was much higher in the bottom sediments than Akkulam-Veli, which had periodic marine influence. In freshwater environments, where available sulphate is usually limited, sulphate reduction still occurs but methanogenesis usually dominates (Nedwell *et al.,* 2004).

The influence of environmental variables on MPP was analysed by multivariate analysis. Principal component analysis (PCA) generated four principal components for both Akkulam-Veli and Vellayani lakes. In Akkulam-Veli lake, the first principal component accounted for 32.84% of the total variance, whereas, PC2, PC3 and PC4 explained 268.08, 25.03 and 14.05% of the total variance, respectively. The first component includes Corg, LOM, CHO, PRT and LIP (Tables 3, 4). In Vellayani lake, the first

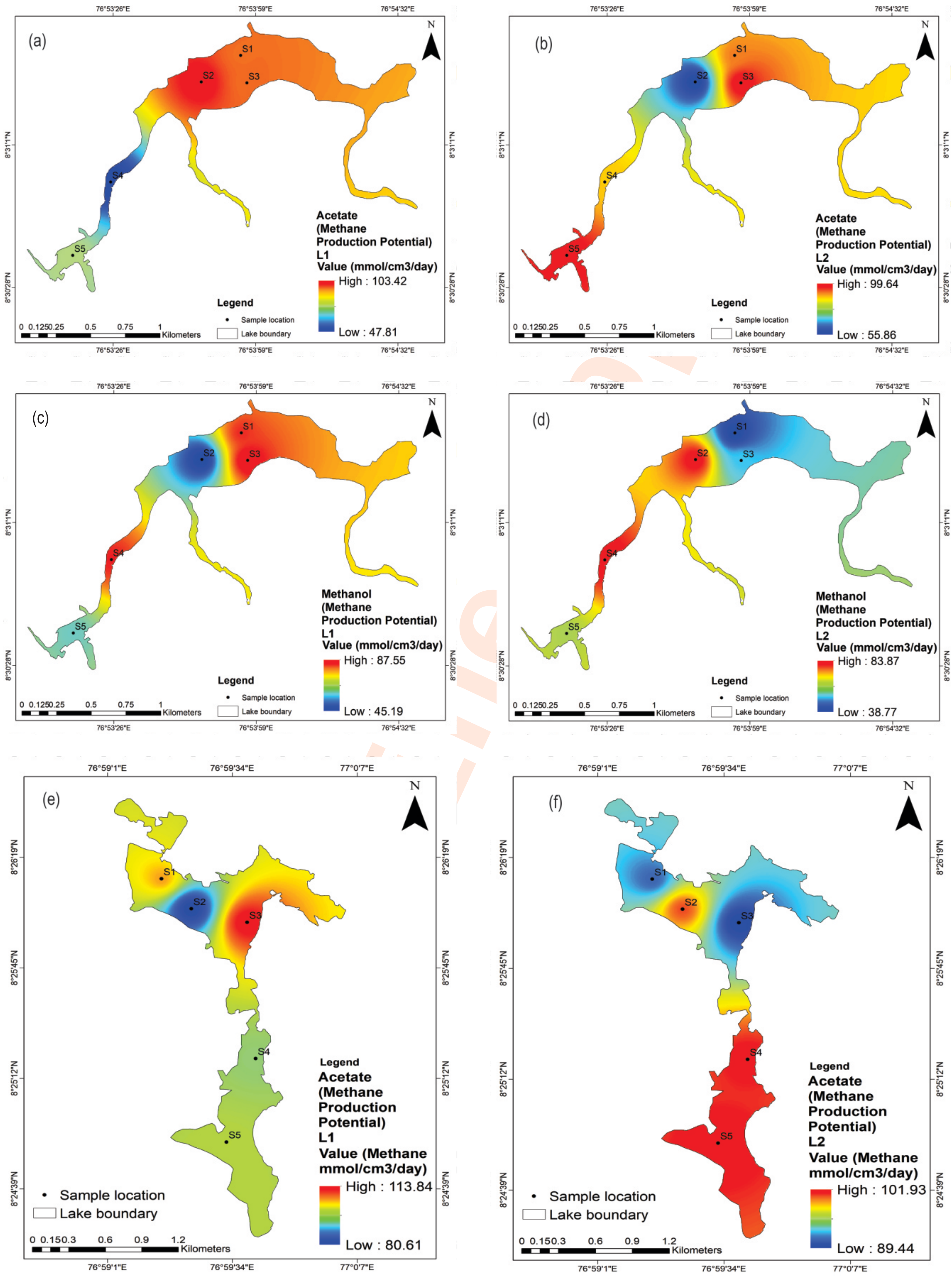
Table 3: Principal Component Analysis for Akkulam-Veli lake

Factor loadings	PC 1	PC 2	PC 3
Temperature	-.022	-.012	.115
pH	.015	-.090	-.010
EC	-.003	-.003	.109
TDS	-.002	-.006	.110
Salinity	-.003	-.003	.109
Sulphate	.007	-.095	.008
Sediment temperature	.064	-.039	.023
Sediment pH	-.019	.104	-.007
Sediment EC	-.063	.009	.078
Sediment redox potential	-.020	.104	-.007
Sediment salinity	-.038	.072	.029
Total organic carbon	.067	-.014	.011
Sand	.042	.088	-.027
Silt	-.025	-.100	.056
Clay	-.055	-.041	-.027
TOM	.067	-.014	.011
Carbohydrate	.058	-.013	.050
Lipid	.040	-.019	.078
Protein	.075	.011	-.045
LOM percentage	-.065	.009	-.035
LOM percentage of TOM	-.027	.009	.014
MA Methanol1	.046	.055	.025
MA Methanol2	.036	.089	.000
MA Acetate1	.027	.048	.065
MA Acetate2	.007	.034	.089
MPP Methanol1	-.075	-.009	.008
MPP Methanol2	.041	.092	-.020
MPP Acetate1	.039	-.076	-.047
MPP Acetate2	-.066	-.049	.083
SRB Acetate1	-.040	.077	-.004
SRB Acetate2	-.048	.050	-.036
SRB Lactate1	.074	.028	-.071
SRB Lactate2	.045	.027	-.099
SRP1	-.077	-.011	.020
SRP2	.041	-.049	.074
Eigen value	13.234	9.304	8.868
% of variance	37.812	26.582	25.336
Cumulative %	37.812	64.395	89.730

Table 4: Principal Component Analysis for Vellayani lake

Factor loadings	PC 1	PC 2	PC 3
Temperature	.308	-.009	-.844
pH	.516	.811	-.200
EC	-.331	.178	.880
TDS	-.010	-1.000	-.002
Salinity	-.260	.416	.776
Sulphate	.906	-.131	-.199
Sediment temperature	.303	.399	-.765
Sediment pH	-.459	-.845	.276
Sediment EC	.909	.216	-.271
Sediment redox potential	.552	.433	.712
Sediment salinity	.925	.091	-.183
Total organic carbon	.916	.288	.107
Sand	-.880	-.420	-.156
Silt	.109	.623	.535
Clay	.991	.025	-.038
TOM	.915	.288	.109
Carbohydrate	.047	-.826	.562
Lipid	.415	.847	.274
Protein	-.655	-.511	.548
LOM percentage	.020	-.216	.971
LOM percentage of TOM	-.895	-.345	-.110
MA Methanol1	.027	.835	-.537
MA Methanol2	-.783	.479	-.107
MA Acetate1	.633	.519	-.566
MA Acetate2	.368	.871	.306
MPP Methanol1	.824	.458	.026
MPP Methanol2	.018	.231	-.877
MPP Acetate1	.462	-.849	-.087
MPP Acetate2	-.887	.391	-.108
SRB Acetate1	.412	.216	.876
SRB Acetate2	.052	.999	.013
SRB Lactate1	.441	-.331	.834
SRB Lactate2	.436	.529	.561
SRP1	-.823	-.099	.139
SRP2	.652	-.272	-.606
Eigen value	12.987	10.162	9.092
% of variance	37.105	29.035	25.978
Cumulative %	37.105	66.140	92.118

principal component accounted for 38.18% of the total variance. However, PC2, PC3 and PC4 accounted for 23.81, 23.63 and 14.39% of the total variance respectively. In Vellayani lake, the first component included salinity, Corg, clay content, sediment EC, water TDS and water pH. The third component was redox potential and EC of overlying water. The fourth component include water salinity and negative loading of sediment sulphate. Among the two lakes, the environmental variables of water in Akkulam-Veli did not influence MPP. However, in case of Vellayani, water TDS and pH had an influence on MPP. Labile carbon is regarded as the most active fraction of organic carbon in soils with rapid turn over rates compared to refractory organic carbon (Zou *et al.,* 2005). LOM% was more in Akkulam-Veli than Vellayani. Higher rates of decomposition of sedimentary organic carbon in the surface sediments contribute to the availability of easily degradable fraction. This is reflected in the abundance of MA and SRB, which was more in Akkulam-Veli than Vellayani. The composition and activities of microbial communities are regulated by the quality and availability of carbon (Torres *et al.,* 2011). The LOM pool was contributed more by carbohydrates in both lakes followed by protein. Carbohydrate fraction was 14 and 20 fold more than protein in Akkulam-Veli and Vellayani, respectively. The dominance of carbohydrates may be because of more refractory composition compared to lipids and proteins, which are rapidly utilized (Danovaro *et al.,* 2000). Generally, higher contribution of labile organic carbon to total organic carbon indicates potential threat from the increasingly speedy global warming (Cao *et al.,* 2017). Release of labile substrates is one important factor controlling microbial production of methane (Wang *et al.,* 2017). However, in both Akkulam-Veli and Vellayani


(a)
S1
S2
S3
S4
S5
Acetate
(Methane
Production Potential)
L1
Value (mmol/cm3/day)
High : 103.42
Low : 47.81
Legend
Sample location
Lake boundary
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76°53'59"E
76°54'32"E
8°31'1"N
8°30'28"N
(b)
Acetate
(Methane
Production Potential)
L2
Value (mmol/cm3/day)
High : 99.64
Low : 55.86
(c)
Methanol
(Methane
Production Potential)
L1
Value (mmol/cm3/day)
High : 87.55
Low : 45.19
(d)
Methanol
(Methane
Production Potential)
L2
Value (mmol/cm3/day)
High : 83.87
Low : 38.77
(e)
Legend
Acetate
(Methane
Production
Potential)
L1
Value (Methane
mmol/cm3/day)
High : 113.84
Low : 80.61
Sample location
Lake boundary
0 0.150.3 0.6 0.9 1.2 Kilometers
76°59'1"E
76°59'34"E
77°0'7"E
8°26'19"N
8°25'45"N
8°25'12"N
8°24'39"N
(f)
Legend
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(Methane
Production
Potential)
L2
Value (Methane
mmol/cm3/day)
High : 101.93
Low : 89.44

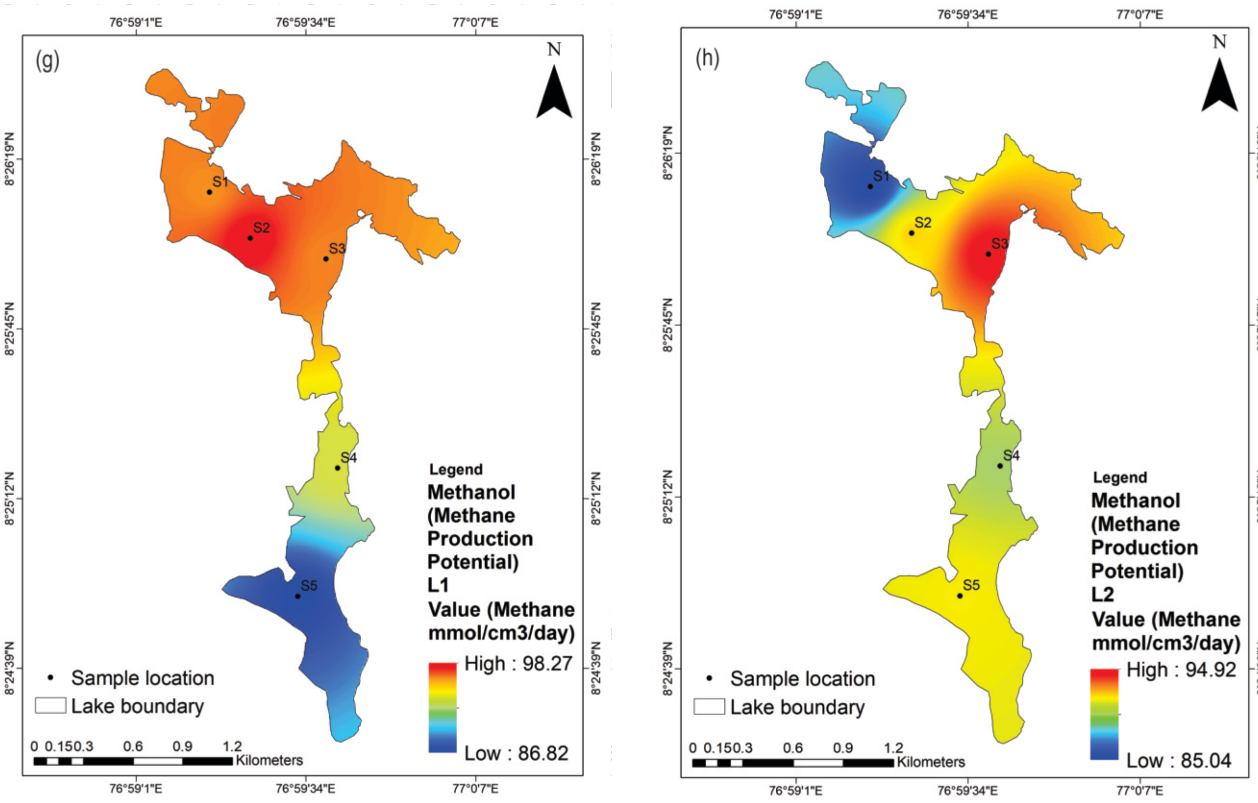

Fig. 1: Methane production potential in AV and Vellayani lake sediments; (a) Acetate (Methane Production Potential L1 value in AV lake sediments (b) Acetate (Mathane Production Potential L2 value in AV lake sediments; (c) Methanol (Methane Production Potential L1 value in AV lake sediments (d) Methanol (Mathane Production Potential L2 value in AV lake sediments; (e) Acetate (Methane Production Potential L1 value in Vellayani lake sediments (f) Acetate (Mathane Production Potential L2 value in Vellayani lake sediments and (g) Methanol (Methane Production Potential L1 value in Vellayani lake sediments (h) Methanol (Mathane Production Potential L2 value in Vellayani lake sediments.

lakes the contribution of labile organic carbon to total carbon pool was less than 50%. Moreover, the anaerobic conditions and high productivity of wetland ecosystems may enhance carbon accumulation in wetland soils (Cao *et al.,* 2015). In Arabian Sea and mangrove sediment of Goa, India, protein fraction of sediments was found to be important factor affecting natural methane production. All three components of LOM, CHO, PRT and LIP were the first PC in Akkulam-Veli lake; however, LOM was not a PC in Vellayani lake. The difference can be attributed to the difference in sedimentary granulometry of the lakes (Gonsalves *et al.,* 2011). Clay content was three fold more in Vellayani than Akkulam-Veli and the difference was statistically different ($p<0.05$). Clay was found to be the first PC in Vellayani. However, in Akkulam-Veli, sediment granulometry was not a PC. However, the percentage sand was three fold more in Akkulam-Veli than Vellayani. Labile organic carbon was higher in sandy sediment, which suggest that high methane production in sandy sediment can be due to increased lability of organic matter.

Lake depth can also affect the quality of organic matter reaching the sediments. In deep lakes, sediment organic matter undergoes intense decomposition in the water column due to prolonged period of settling. Consequently, low amount of labile organic carbon reach the sediments in deep lakes (Suess, 1980). Akkulam-Veli is a shallow and Vellayani a deep lake which explains more of labile organic matter in Akkulam-Veli in addition to its hyper eutrophication status. Anthropogenic activities in the hinterland as well as coastal area alter the physicochemical setting of estuaries (Jennerjahn and Mitchell, 2013). Moreover, riverine input into Akkulam-Veli might have transported decomposed organic matter composing of undegraded sewage sludge and freshwater algae and bacteria (Soetaert and Herman, 1995). Downstream transportation of organic matter undergoes decomposition and is not supplemented with fresh labile organic matter because autochthonous production is limited to marine part of Akkulam-Veli (Middelburg *et al.,* 1996).

Moreover, in the Akkulam-Veli lake, a large amount of city's municipal wastewater enters the wetland untreated through a system of drains due to deficiencies in the sewage collection system (Ghermandi *et al.,* 2016). Leading to high sedimentation rate, which reduce the contact time between organic matter and dissolved oxygen in the water column, and therefore, can contribute to higher concentration of carbon and nutrients in sediment. Although human activities leading to release of untreated sewage promotes methane emission in wetlands (Martinez-Cruz and Gonzalez-Valencia, 2017), restoration of coastal wetlands can convert them into sink of carbon, thus reducing their methane production potential (Yang *et al.,* 2020). Among the various environmental factors, Corg and labile organic matter was found to influence the methanogenic activity in both lakes. The dominance of methanogenic activity over sulphate reduction in Akkulam-Veli lake is attributed to the utilization of non-competitive substrates by MA. Increased methane production potential in Akkulam-Veli than Vellayani is attributed to untreated sewage and waste disposal.

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References

Abhijna, U. G., R. Ratheesh and A.B. Kumar: Distribution and diversity of aquatic insects of Vellayani lake in Kerala. *J. Environ. Biol.,* **34**, 605 (2013).

APHA: Standard Methods for Examination of Water and Wastewater. 23[nd] Edn., APHA, AWWA, WPCF, Washington DC, USA (2017).

Araujo, J., A. Pratihary, R. Naik, H. Naik and S.W.A. Naqvi: Benthic fluxes of methane along the salinity gradient of a tropical monsoonal estuary: Implications for CH_4 supersaturation and emission. *Marine Chemi.,* **202**, 73-85 (2018).

Araujo, J., S.W.A. Naqvi, H. Naik and R. Naik: Biogeochemistry of methane in a tropical monsoonal estuarine system along the west coast of India. *Estuarin.Coastal Shelf Scie.,* **207**, 435-443 (2017).

Bligh, E.G. and W.J. Dyer: A rapid method of total lipid extraction and purification. *Canadian J. Bioche. Physiol.,* **37**, 911-917 (1959).

Cao, L., J. Song, Q. Wang, X. Li, H. Yuan, N. Li and L. Duan: Characterization of labile organic carbon in different coastal wetland soils of Laizhou Bay, Bohai Sea. *Wetlands,* **37**, 163-175 (2017).

Cao, Q., R. Wang, H. Zhang, X. Ge and J. Liu: Distribution of organic carbon in the sediments of Xinxue River and the Xinxue River constructed wetland, China. *PloS ONE,* **10** (2015).

Chambers, L.G., R. Guevara, J.N. Boyer, T.G. Troxler and S.E. Davis: Effects of salinity and inundation on microbial community structure and function in a mangrove peat soil. *Wetlands,* **36**, 361-371 (2016).

Danovaro, R., D. Marrale, A. Dell'Anno, N.D. Croce, A. Tselepides and M. Fabiano: Bacterial response to seasonal changes in labile organic matter composition on the continental shelf and bathyal sediments of the Cretan Sea. *Progress in Oceanography,* **46**, 345-366 (2000).

Dubois, M., A. Kyle, J. K. Gilles, P.A. Hamilton, T. Rebers and F. Smith: Colorimetric method for determination of sugars and related substances. *Analy. Chemi.,* **28**, 350-356 (1956).

Fabiano, M., R. Danovaro and S. Fraschetti: A three-year time series of elemental and biochemical composition of organic matter in subtidal sandy sediments of the Ligurian Sea (Northwestern Mediterranean). *Contin. Shelf Res.,* **15**, 1453-1470 (1995).

Fabiano, M., V. Marin, C. Misic, M. Moreno, V. Salvo and L. Vezzulli: Sedimentary organic matter and bacterial community in microtidal mixed beaches of the Ligurian Sea (NW Mediterranean). *Chemi. Ecol.,* **20**, 423-435 (2004).

Folk, R.L.: Petrology of Sedimentary Rock. Hemphill Publishing Company, Austin, Texas, 182 p. (1974).

Ghermandi, A., A.M. Sheela and J. Joseph: Integrating similarity analysis and ecosystem service value transfer: Results from a tropical coastal wetland in India. *Ecosy. Servi.,* **22**, 73-82 (2016).

Goñi, M.A., M.J. Teixeira and D.W. Perkey: Sources and distribution of organic matter in a river-dominated estuary (Winyah Bay, SC, USA). *Estua. Coas. Shelf Sci.,* **57**, 1023-1048 (2003).

Gonsalves, M., C.E.G. Fernandes, S.O. Fernandes, D.L. Kirchman and P.A.L. Bharathi: Effects of composition of labile organic matter on biogenic production of methane in the coastal sediments of the Arabian Sea. *Environ. Monit. Assess.,* **182**, 385-395 (2011).

Hatree, E.F.: Determination of protein: A modification of the Lowry method that gives a linear photometric response. *Anal. Biochem.,* **48**, 422-427 (1972).

Holm, G.O., B.C. Perez, D.E. McWhorter, K.W. Krauss, D.J. Johnson, R.C. Raynie and C.J. Killebrew: Ecosystem level methane fluxes from tidal freshwater and brackish marshes of the Mississippi River Delta: Implications for coastal wetland carbon projects. *Wetlands*, **36**, 401-413 (2016).

Hungate, R.E.: A roll tube method for cultivation of strict anaerobes. In: Methods in Microbiology. Academic Press, Vol. **3**, pp. 117-132 (1969).

Hungate, R.E.: The anaerobic mesophilic cellulolytic bacteria. *Bacteriol. Rev.,* **14**, 1-49 (1950).

Huttunen, J.T., J. Alm, A. Liikanen, S. Juutinen, T. Larmola, T. Hammar, J. Silvola and P.J. Martikainen: Fluxes of methane, carbon dioxide and nitrous oxide in boreal lakes and potential anthropogenic effects on the aquatic greenhouse gas emissions. *Chemosphere,* **52**, 609-621 (2003).

Jennerjahn, T.C. and S.B. Mitchell: Pressures, stresses, shocks and trends in estuarine ecosystems–An introduction and synthesis. *Estua. Coas. Shelf Sci.,* **130**, 1-8 (2013).

Jin, X., Y. Ma, Z. Kong, W. Kou and L. Wu: The variation of sediment bacterial community in response to anthropogenic disturbances of Poyang Lake, China. *Wetlands*, **39**, 63-73 (2017).

Kochert, G.: Protein determination by dye binding. In: Handbook of Phycological Methods: Physiological and Biochemical Methods, Cambridge University Press London, pp. 91-93 (1978).

Lowry, O.H., N.J. Rosebrough, A.L. Farr and R.J. Randall: Protein measurement with the Folin phenol reagent. *J. Biol. Chem.,* **193**, 265-275 (1951).

Marinho, C.C., E. A. Campos, J.R.D. Guimarães and F. A. Esteves: Effect of sediment composition on methane composition and production in the transition zone of a mangrove (Septiba Bay, Rio de Janaeiro, Brazil). *Brazilian J. Biol.,* **72**, 429-436 (2012).

Martinez-Cruz, K., R. Gonzalez-Valencia, A. Sepulveda-Jauregui, F. Plascencia-Hernandez, Y. Belmonte-Izquierdo and F. Thalasso: Methane emission from aquatic ecosystems of Mexico City. *Aqua. Sci.,* **79**,159-169 (2017).

Middelburg, J.J., G. Klaver, J. Nieuwenhuize, A. Wielemaker, W. de Haas, T. Vlug and J.F.W.A van der Nat: Organic matter mineralization in intertidal sediments along an estuarine gradient. *Marine Ecol. Prog. Ser.,* **132**, 157-168 (1996).

Nayar, S. and N.M. Nayar: Wetlands In: The Natural Resources of Kerala (Eds.: K.B. Thampi, N.M. Nayar, C.S. Nayar). WWF State Office, Trivandrum, Kerala (1997).

Nedwell, D.B., T.M. Embley and K.J. Purdy: Sulphate reduction, methanogenesis and phylogenetics of the sulphate reducing bacterial communities along an estuarine gradient. *Aqua. Micro. Ecol.,* **37**, 209-217 (2004).

Parsons, T.R., Y. Maita and C.M. Lalli: A manual of chemical and biological methods for seawater analysis. Pergamon, Oxford sized algae and natural seston size fractions. *Marine Ecol. Prog. Ser.,* **199**, 43-53 (1984).

Picard, M.D.: Classification of fine-grained sedimentary rocks. *J. Sedime. Res.,* **41**, 179-195 (1971).

Pighini, S., M. Ventura, F. Miglietta and G. Wohlfahrt: Dissolved greenhouse gas concentrations in 40 lakes in the Alpine area. *Aqua. Sci.,* **80**, 32 (2018).

Ramesh, R., G.R. Purvaja, D.C. Parashar, P.K. Gupta and A.P. Mitra: Anthropogenic forcing on methane efflux from polluted wetlands (Adyar River) of Madras City, India. *Ambio*, **26**, 369-374 (1997).

Reid, M.K. and K.L. Spencer: Use of principal components analysis (PCA) on estuarine sediment datasets: The effect of data pre-treatment. *Environ. Pollu.,* **157**, 2275-2281 (2009).

Roden, E.E. and R.G. Wetzel: Competition between Fe (III)-reducing and methanogenic bacteria for acetate in iron-rich freshwater sediments. *Micro. Ecol.,* **45**, 252-258 (2003).

Shiau, Y., M.R. Burchell, K.W. Krauss, F. Birgand and S.W. Broome: Greenhouse gas emissions from a created brackish marsh in eastern North Carolina. *Wetlands*, **36**,1009-1024 (2016).

Soetaert, K. and P.M.J Herman: Carbon flows in the Westerschelde estuary (The Netherlands) evaluated by means of an ecosystem model (MOSES). *Hydrobiologia,* **311**, 247-266 (1995).

SPSS, Statistical Package for the Social Sciences, version 17.0, Chicago: SPSS Inc (2008).

Suess, E.: Particulate organic carbon flux in the oceans—Surface productivity and oxygen utilization. *Nature*, **288**, 260-263 (1980).

Torres, I.C., K.S. Inglett and K. R. Reddy: Heterotrophic microbial activity in lake sediments: Effects of organic electron donors. *Biogeochemistry,* **104**, 165-181 (2011).

Trivedy, R.K., P.K. Goel and C.L. Trisal: Practical Methods in Ecology and Environmental Science. Enviromedia Publications, Karad, pp. 40-110 (1998).

Venturini, N., A.L. Pita, E. Brugnoli, F. García-Rodríguez, L. Burone, N. Kandratavicius, M. Hutton and P. Muniz: Benthic trophic status of sediments in a metropolitan area (Rio de la Plata estuary): Linkages with natural and human pressures. *Estua. Coas. Shelf Sci.,* **112**, 139-152 (2012).

Verma, A., V. Subramanian and R. Ramesh: Methane emissions from a coastal lagoon: Vembanad Lake, West Coast, India. *Chemosphere,* **47**, 883-889 (2002).

Vincent, S.G.T. and R. Raj: Occurrence and activity of sulphate reducing bacteria in selected estuarine sediments of South Kerala, India. *Int. J. Ecol. Environ. Sci.,* **44**, 191-198 (2018).

Vincent, S.G.T., R.R. Reshmi, S.J. Hassan, K.D. Nair and A. Varma: Predominant terminal electron accepting processes during organic matter degradation: Spatio-temporal changes in Ashtamudi estuary, Kerala, India. *Estua. Coas. Shelf Sci.,* **198**, 508-517 (2017).

Walkley, A. and I.A. Black: An examination of the Degtjareff method for determining soil organic matter, and a proposed modification of the chromic acid titration method. *Soil Sci.,* **37**, 29-38 (1934).

Wang, J., C. Song, A. Hou and F. Xi: Methane emission potential from freshwater marsh soils of Northeast China: Response to simulated freezing-thawing cycles. *Wetlands,* **37**, 437-445 (2017).

Winfrey, M.R. and D.M. Ward: Substrates for sulfate reduction and methane production in intertidal sediments. *Appli. Environ. Microbio.,* **45**, 193-199 (1983).

Xu, S., Y. Wang, C. Guo, Z. Zhang, Y. Shang, Q. Chen and Z. Wang: Comparison of microbial community composition and diversity in native coastal wetlands and wetlands that have undergone long-term agricultural reclamation. *Wetlands*, **37**, 99-108 (2017).

Yang, H., J. Tang, C. Zhang, Y. Dai, C. Zhou, P. Xu, D.C. Perry and X. Chen: Enhanced carbon uptake and reduced methane emissions in a newly restored wetland. *J. Geophy. Res. Biogeosci.,* **125**, e2019JG00522 (2020)

Zhang,S., F. Zhang, Z. Shi, A. Qin, H. Wang, Z. Sun, Z. Yang, Y. Zhu, S. Pang and P. Wang: Sources of seasonal wetland methane emissions in permafrost regions of the Qinghai-Tibet Plateu. *Scient. Rep.,* **10**, 7520 (2020).

Zou, X.M., H.H. Ruan, Y. Fu, X.D. Yang and L.Q. Sha: Estimating soil labile organic carbon and potential turnover rates using a sequential fumigation–Incubation procedure. *Soil Biol. Bioche.,* **37**, 1923-1928 (2005).