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Biogeochemical variables influence the abundance of sulfate-reducing bacteria in Vembanad estuarine sediment

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ABSTRACT

The investigation explored the intricate relationship between biogeochemical variables and the population dynamics of sulfate-reducing bacteria (SRB) in the sediments of a tropical estuary in South India. The study spanned three seasons – monsoon, post-monsoon, and pre-monsoon – revealing pronounced fluctuations in environmental parameters across both temporal and spatial dimensions. Using the roll tube technique, SRB enumeration was conducted using two different carbon substrates: lactate and acetate. This method comprehensively understood how these bacteria responded to varying conditions throughout the year. The sulfate concentration, a crucial component in the biogeochemical makeup, was pivotal in shaping the SRB population. Additionally, the labile fraction of organic matter, particularly its protein content, emerged as a critical factor influencing the microbial community. Notably, the study highlighted significant seasonal and spatial variations in the environmental variables, indicating the dynamic nature of the estuarine ecosystem. A high protein factor in the labile organic matter during all three sampling seasons suggested the continual deposition of fresh organic material, signifying both natural processes and human-induced impacts on the estuarine environment. The positive correlation between the protein factor of labile organic matter and the SRB population reinforced the conception that these bacteria thrive in conditions characterized by specific biochemical constituents. This correlation, backed by statistical analyses, underlined the importance of understanding the interaction between environmental variables and microbial populations in estuarine ecosystems. Furthermore, the study hinted at potential anthropogenic interventions in the estuary, as evidenced by the deposition of labile organic matter. The findings underscored the need for comprehensive monitoring and management strategies to mitigate the impact of human activities on estuarine ecosystems, particularly concerning the microbial communities that play a vital role in biogeochemical cycling. The research provided valuable insights into the seasonal and spatial dynamics of sulfate-reducing bacteria in a tropical estuary, shedding light on the significant relationships between biogeochemical variables and microbial populations.

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1. Introduction

Estuarine sediments contain high levels of organic matter and sulfates and frequently experience anoxic conditions, making sulfur transformation one of the most active cycles in this ecosystem (Lyimo et al., 2002). This anoxic environment can elicit variable and dynamic responses in the biogeochemical cycling of nutrients in sediment(Foster & Fulweiler, 2019). Most sulfur is in the oxidation of organic sulfur from terrestrial sources, burning fossil fuels and discharging wastewater containing the sulfate (Dornblaser et al., 1994). The redox status of estuarine sediment is connected to the remineralization of organic matter. Coastal and estuarine sediments are primary sites for biomass mineralization (Jørgensen, 1982). The nature and quality of organic matter depict the biogeochemical characterization of sedimentary environments in estuaries. The labile fraction of this organic matter is composed of simple or combined organic biopolymer molecules such as carbohydrates, proteins and lipids. These are available for benthic organisms and get mineralized rapidly (Venturini et al., 2012). LOM is a fraction of organic matter potentially available as a benthic food source (Mayer et al., 1995).

The physical and biogeochemical interactions are complex at different spatial scales. Anthropogenic activities and physical/biological processes influence the delivery of water and sediments and the rates of biogeochemical activities (Canuel & Hardison, 2016). It is challenging to find out the influence of organic matter in enhancing the biogeochemical processes in a sedimentary environment of estuaries. This is due to the wide range of source and spatiotemporal variations of the same in sediments. Previous investigations made it clear that the availability of degradable organic matter intensifies microbial sulfate reduction in marine sediments(Pomeroy & Wiebe, 2001). The lack of oxygen and the abundance of organic matter creates an optimal environment for several anaerobic organisms, such as sulfate-reducing bacteria (SRB)and methanogens (Dar et al., 2008). SRB populations appear very complex, with several different distributions in various sites. This complexity reflects their characteristic interaction with the gross environmental factors that distinguish multiple sites (Purdy et al., 2002). The estuaries located on the southwest coast of India undergo biogeochemical changes due to the influence of the southwest monsoon (Araujo et al., 2018). The sulfate-reducing property of the environment is directly linked with the quality of the environmental factors and seasonal changes, which is site-specific. The present study aimed to determine the influence of biogeochemical factors on the abundance of SRB in the sediments of the Vembanad estuary, designated as one of the three Ramsar sites in Kerala, India.

2. Materials and Methods

2.1. Study Area

Vembanad Lake (Latitude 9° 35' 48" N; Longitude 76° 23' 54" E) is the most extensive estuarine-lagoon system in Kerala (designated as a Ramsar site-1214, in 2002).

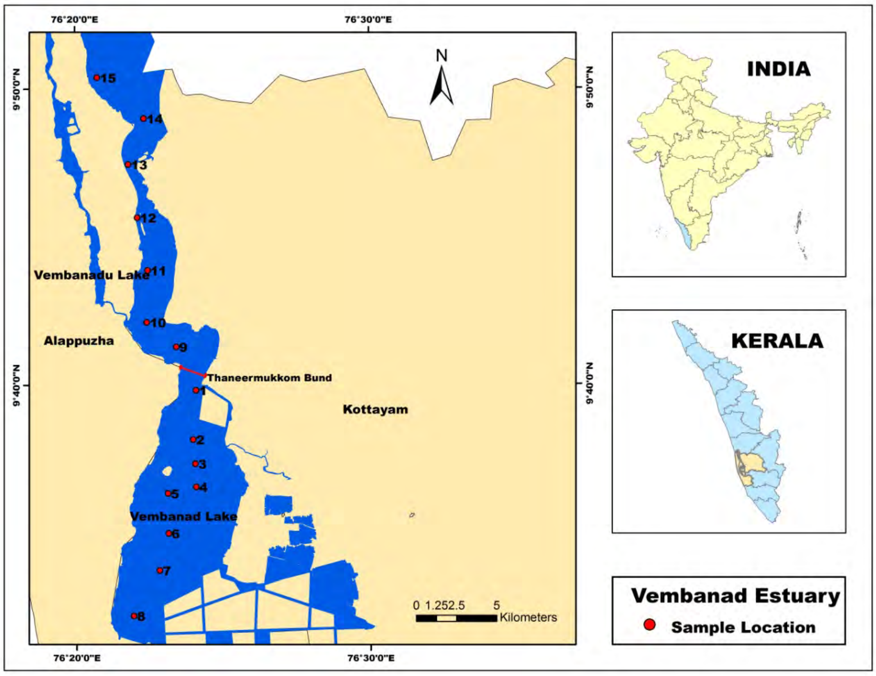

Fig. 1. Map of study area showing sampling stations (S1 to S15)

This oxbow-shaped lake extends 96 km from Azheekode in the north to Alappuzha in the south with a Northwest–Southeast orientation. It spread across three central districts of Kerala, viz, Ernakulam in the north, Kottayam in the east and Alappuzha in the south, and covers an area of 241 km^2 (Verma & Subramanian, 2002). This lake consists of a complex system of backwaters, marshes, small islands, mangrove forests, and a network of canals. In and around this lake is a highly populated coastal zone where people depend directly or indirectly upon this ecosystem for their livelihood. The major rivers discharging into the Vembanad backwater system are Periyar, Muvattupuzha, Meenachil, Manimala, Pamba and Achencoil.

2.2. Sample Collection and Preparation

Sediment samples were collected manually from 15 stations of the wetland (Fig. 1) using Van Veen's grab. The sampling sites were selected from 15 random grid points, concentrating on the lake's central area, after the map was made into 5×5 m² grids. Eight samples were taken from the southern region, south of the mud barrier, where the lake received the rivers Pamba, Meenachil, Manimala and Achencoil. The remaining seven samples were collected from the north zone, north of the bund, where the rivers Muvattupuzha and Periyar entered. Two sets of samples were taken from each station, one for physicochemical analysis and another for microbial distribution studies. Samples were collected during the monsoon (August 2017), post-monsoon (November 2017) and pre-monsoon (May 2018) seasons. Sediment samples for microbial studies were collected in sterilized bottles and kept at 4°C after reaching the lab. These subsamples were immediately used to quantify the SRB colonies. The remaining samples were stored at 4°C in a refrigerator for further physicochemical analyses.

2.3. Biogeochemical variables

Physicochemical and nutrient characteristics of sediment samples such as temperature, pH, sulfate, redox potential (Eh), sediment granulometry and salinity of the overlying water were analyzed using standard methods. The pH was measured with a meter (m pH system 361, Systronics), and the sediment redox potential was measured using an ORP tester (Eutech Instruments, Oakton). Salinity(PSU) was measured using the HANNA water quality kit (HANNA HI 9828). Granulometric analysis was performed using standard sieve and pipette techniques after inorganic and organic carbon removal (Folk, 1974). Organic carbon (C_{org}) and total organic matter (TOM) of the estuarine sediment were determined by Walkley and Black's method (1934). The labile organic matter (LOM) was estimated by calculating the protein, carbohydrates and lipids of the sediment. Protein was estimated by following the modified procedures (Lowry et al., 1951). Carbohydrates were analyzed using phenol-sulfuric acid (Dubois et al., 1956). The lipid content of the sediment samples was measured by the acid-dichromate method (Parsons et al., 2013; Bligh and Dyer, 1959).

2.4. Isolation and estimation of SRB

SRB isolation involved serial diluting sediment samples in anaerobic conditions using sealed serum vials with butyl rubber stoppers and aluminium caps. The vials were purged with ultra-high pure nitrogen gas. Enumeration employed the roll tube technique in the Postgate medium, containing specific components (Ramasamy et al., 1992). Resazurin, a redox-sensitive dye, monitored the medium's redox potential. Acetate or lactate (0.1M) served as substrates. Anaerobic conditions were maintained by purging the headspace with high-purity N2 gas. Bacterial colony counts, expressed as CFU/g of sediment, were determined after one week of incubation.

2.5. Statistical Analyses

A two-way analysis of variance was carried out to test whether there were significant differences in the variables obtained within the seasons and stations and the interaction between seasons and stations. They were conducted using IBM SPSS version 20.0 software. Pearson correlation was performed (n=15) to investigate the relation between the variables and the SRB population.

3. Results and Discussion

3.1. Changes in biogeochemical variables

The investigation encompassed a comprehensive analysis of environmental variables within the sediment and overlying water at 15 study sites across three seasons (monsoon, post-monsoon, and pre-monsoon) in the estuary. Examined variables included pH, redox potential (Eh), overlying water salinity, sediment texture, sulfate content, organic carbon (C_{org}), organic matter (OM), and labile organic matter (LOM), exhibiting notable spatial and temporal variations (Table 1).

The pH of sediment pore water consistently displayed alkalinity across all stations, except for stations 7 and 8, which exhibited slight acidity during the monsoon. Spatial and temporal variations in pH were statistically significant (Table 2), indicating a pH gradient along sampling stations. Marine influences favoured alkaline conditions, particularly during post and pre-monsoon seasons towards the north direction of the estuary. Monsoons, marked by higher fluvial inflow and reduced marine impact, promoted lower alkalinity. The intrusion of seawater, an influx of fresh water, and precipitation processes influence the hydrodynamics in this system(Joseph & Kurup, 1990)

Redox potential values in sediments exhibited a range between -123 ± 4.04 and -320 ± 2.5 RmV during monsoon, -101 ± 1.73 and -216 ± 2 RmV during post-monsoon, and -117 ± 10 and -177 ± 5.5 during pre-monsoon. Highly reducing environments were noted in stations 7 (monsoon) and 14 (post and pre-monsoon). Overall, a reduced environment dominated during monsoon. The spatially and temporally significant negative redox status suggested optimal conditions for sulfate-reducing bacteria (SRB) growth, crucial in anaerobic estuarine sediments. Tropical estuaries show high sedimentation and abundant organic matter (OM), which strongly reduces the estuarine sediments below a thin oxic surface layer (Hopkinson & Vallino, 1995). The lack of oxygen and the abundance of organic matter creates an optimal environment for SRB, whose presence in most coastal sediments is selected by the redox potential (Dar et al., 2008). Likewise, the highly favourable redox conditions (extreme negative) found in all the sampling stations of the estuary were supportive of the growth of SRB.

Overlying water salinity exhibited maximum values during the post- and pre-monsoon periods and minimum values during the monsoon period, marking significant seasonal variation. Spatial variation in sediment pore water salinity was insignificant, with no meaningful interactions between seasons and stations (Table 2). Stations 14 and 15 recorded maximum salinity (Table 1), attributable to marine influence, as the estuary opens towards the northern Arabian Sea, leading to high saline conditions in the last two stations.

Sediment texture varied from loam, silt, sandy loam, and silty loam, with loamy sediment predominant. Seasonal changes were observed in stations 7 and 15. A subtle positive correlation (Table 3) was noted between the labile fraction of organic matter and sediment texture. Finer sediment granules, like silt and clay, exhibited a positive correlation with organic and labile organic percentages, while coarser granules, like sand, showed a negative correlation with organic content throughout the sampling periods. The higher surface area of finer fractions of sediments makes them organic-rich than coarse particles (Rodríguez-Barroso et al., 2010).

Sulfate, a significant seawater constituent, showed spatiotemporal variations and interactions between seasons and stations (Table 1), particularly during the pre-monsoon period, indicating an overall high sulfate concentration in estuarine sediments. Sulfate penetrates deep into sediments (Jorgensen et al., 1990) and is one of the primary electron acceptors in anoxic habitats (Margalef-Marti et al., 2023). The distribution of sulfur species in estuarine sediments is altered by the upbringing of inflated sulfate load by the tidal activities that occur regularly in these ecosystems (Wang et al., 2022). The pre-monsoon period was contented with an overall high sulfate concentration in the estuarine sediments. Furthermore, the mud barrier (Thanneermukkam Bund) was opened during monsoon, and the stations near and north of the barrier exhibited dominant sulfate content during the season, suggesting elevated inland nutrient runoff. Sulfate has unique properties that provide sulfate reducers to grow in niches unavailable to other microorganisms (Rubio-Rincón et al., 2017). Their availability in marine sediments varies due to changes in the amount and natural ability of the organic matter undergoing decomposition (Westrich et al., 1984).

Sulfate concentration correlated positively with LOM, emphasizing the role of labile organic content in SRB activity. A significant portion of organic matter sinks through the water column and is ultimately preserved in sediments by interacting with a series of physical, chemical and biological processes (Liu et al., 2006). In Vembanad estuarine sediments, the sulfate content showed the same trend as that of LOM in the sediment samples of all the stations during monsoon. They showed a positive correlation (Table 3a **).** This indicated that the labile fraction of the organic content in estuarine sediments controls the activity of SRB. Vincent et al., 2017 confirmed a high LOM percentage in Ashtamudi estuarine sediments, a tropical estuary in south India, where sulfate reduction was established to be the predominant terminal electron-accepting process.

C_{org} and organic matter displayed spatiotemporal variations, with an elevated percentage of LOM observed post-monsoon, indicating an influx of fresh organic matter. The lowest LOM percentage occurred during pre-monsoon.

Table 1. Environmental variables of Vembanad estuarine sediment (Mean ± Standard Deviation). Eh - Redox potential; C_{org} - Organic carbon; TOM - Total organic matter; LOM - Labile organic matter; SRB Lactate - Sulfate reducing bacterial colonies in media with lactate as substrate; SRB Acetate - colonies in media with acetate as substrate.

Season	Parameters	Stations														
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Monsoon 2017	pH	8.03 ± 0.15	6.97 ± 0.15	7.1 ± 0.1	7.37 ± 0.15	7.5 ± 0.1	7.63 ± 0.21	6.87 ± 0.15	6.93 ± 0.15	7.58 ± 0.08	7.49 ± 0.09	7.77± 0.16	7.48 ± 0.16	7.51 ± 0.1	7.79 ± 0.17	8.13 ± 0.15
	Eh (RmV)	-123 ± 4.04	-241 ± 3.5	-189 ± 2	-197 ± 4.04	-161 ± 4.6	-304 ± 4	-320 ± 2.5	-316 ± 4	-213 ± 3.1	-154 ± 4	-174 ± 3.5	-141 ± 3	-201 ± 4.04	-282 ± 3.5	-218 ± 3
	Salinity(psu)	0.02 ± 0.01	0.02 ± 0.01	0.02 ± 0.01	0.02 ± 0.01	0.02 ± 0.01	0.02 ± 0.01	0.01 ± 0	0.02 ± 0.01	0.01 ± 0.01	0.03 ± 0.01	0.01 ± 0.01	0.02 ± 0	0.02 ± 0.01	0.01 ± 0	0.01 ± 0.01
	Sediment Texture	Sandy loam	Sandy loam	Loamy	Silt	Silt loam	Silt loam	Silt	Silt	Silt loam	Silt loam	Silt loam	Loamy	Loamy	Loamy	Sandy loam
	C_{org}(%)	2.53 ± 0.03	2.42 ± 0.02	2.45 ± 0.03	1.51 ± 0.02	1.81 ± 0.03	0.7 ± 0.03	1.04 ± 0.03	1.32 ± 0.03	1.35 ± 0.03	0.74 ± 0.03	1.06 ± 0.02	3.2 ± 0.02	2.8 ± 0.03	2.66 ± 0.02	2.71 ± 0.02
	TOM(%)	4.38	4.18	4.23	2.58	3.1	1.19	1.81	2.27	2.32	1.27	1.83	5.5	4.83	4.6	4.65
	% of LOM in TOM	3.8	5.04	4	11.68	6.16	29.5	18.32	21.47	8.95	17.41	15.32	2.33	3	2.68	2.81
	Sulfate(mg 100 g^{-1})	3.17 ± 0.15	2.88 ± 0.11	2.66 ± 0.06	7.54 ± 0.03	4.03 ± 0.03	12.52 ± 0.36	7.48 ± 0.06	11.31 ± 0.07	19.25 ± 0.11	24.65 ± 0.06	13.72 ±0.05	9.87 ± 0.09	10.99 ± 0.09	4.76 ± 0.08	12.83 ± 0.07
	SRBLactate (CFUg^{-1})	1160 ± 20	210 ± 10	920 ± 20	860 ± 20	270 ± 20	490 ± 30	700 ± 30	1110 ± 30	770 ± 20	1140 ± 30	1210 ± 20	540 ± 20	810 ± 20	1060 ± 30	670 ± 20
	SRBAcetate(CFUg^{-1})	230 ± 20	140 ± 20	140 ± 30	260 ± 40	150 ± 30	320 ± 30	270 ± 30	650 ± 30	330± 30	420 ± 20	370 ± 20	360 ± 20	280 ± 20	250 ± 10	290 ± 20
Post Monsoon 2017	pH	8.83 ± 0.03	8.64 ± 0.05	7.94 ± 0.07	8.04 ± 0.04	8.07 ± 0.03	7.86 ± 0.07	7.8 ± 0.09	7.54 ± 0.05	8.46 ± 0.07	8.14 ± 0.04	8.03 ± 0.04	8.03 ± 0.02	7.66 ± 0.09	7.19 ± 0.07	7.2 ± 0.09
	Eh (RmV)	-103 ± 2.31	-163 ± 1.15	-165 ± 1.15	-124 ± 1.15	-116 ± 0.58	-216 ± 2.08	-176 ± 1	-186 ± 0.58	-94 ± 2.52	-205 ± 0.58	-164 ± 1.73	-150 ± 2.31	-101 ± 1.73	-216 ± 2	-205 ± 1
	Salinity(psu)	0.06 ± 0.01	0.02 ± 0.01	0.06 ± 0	0.04 ± 0.02	0.04 ± 0.01	0.04 ± 0	0.05 ± 0.01	0.06 ± 0	0.01 ± 0.01	0.03 ± 0.01	0.03 ± 0.01	0.03± 0.01	0.08 ± 0.01	0.21 ± 0.01	0.23 ± 0.01
	Sediment Texture	Sandy loam	Sandy loam	Loamy	Silt	Silt loam	Silt loam	Silt loam	Silt	Silt loam	Silt loam	Silt loam	Loamy	Loamy	Loamy	Loamy
	Corg(%)	2.74 ± 0.04	2.43 ± 0.02	0.34 ± 0.02	0.2 ± 0.01	1.5 ± 0.25	0.28 ± 0.02	1.04 ± 0.03	0.37 ± 0.02	3.32 ± 0.03	2.67 ± 0.02	2.89± 0.02	3.26 ± 0.01	3.32 ± 0.02	0.75 ± 0.02	0.86 ± 0.01
	TOM(%)	4.74	4.18	0.59	0.33	2.59	0.48	1.78	0.63	5.7	4.59	4.96	5.63	5.74	1.3	1.48
	% of LOM in TOM	4.96	5.77	34.95	45.31	14.22	40.92	11.53	53.53	1.96	3.89	4.58	2.74	2.01	19.19	22.74
	Sulfate(mg 100g^{-1})	2.33 ± 0.17	8.88 ± 0.11	32.31 ± 0.1	30.46 ± 0.12	12.92 ± 0.05	23.89 ± 0.04	13 ± 0.1	25.2 ± 0.03	1.62 ± 0.04	4.67 ± 0.05	9.66 ± 0.1	7.58 ± 0.04	3.74 ± 0.07	7.63 ± 0.06	21.04 ± 0.05
	SRBLactate(CFUg^{-1})	580 ± 40	1080± 20	1440 ± 20	1290 ± 10	1220 ± 20	910 ± 30	1590 ± 20	1160 ± 20	540 ± 20	1350 ± 20	1270 ± 30	640 ± 30	1520 ± 10	1540 ± 40	1120 ± 10
	SRBAcetate (CFUg^{-1})	90 ± 20	250 ± 10	320 ± 30	240 ± 20	190 ± 20	260 ± 20	310 ± 40	430 ± 30	130 ± 20	250 ± 10	370 ± 30	210 ± 20	370 ± 30	320 ± 20	340 ± 30
Pre Monsoon 2018	pH	7.71± 0.12	7.88 ± 0.5	7.9 ± 0.26	7.63 ± 0.5	7.36 ± 0.3	7.43 ± 0.22	7.6 ± 0.31	7.83 ± 0.41	7.89 ± 0.41	7.47 ± 0.18	7.46 ± 0.1	8.08 ± 0.35	7.47 ± 0.31	7.45 ± 0.31	7.55 ± 0.14
	Eh (RmV)	-131 ± 1.4	-130 ± 1.5	-159 ± 2	-145 ± 1.9	-127 ± 1.3	-130 ± 1.3	-117 ± 1.1	-124 ± 1.1	-128 ± 5.1	-127 ± 1.2	-133 ± 9.5	-154 ± 9.6	-151 ± 1.6	-178 ± 5.5	-175 ± 8.5
	Salinity(psu)	0.06 ± 0.01	0.05 ± 0.02	0.06 ± 0.03	0.04 ± 0.02	0.08 ± 0.02	0.06 ± 0.01	0.07 ± 0.02	0.03 ± 0.01	0.08 ± 0.03	0.07 ± 0.02	0.1 ± 0.02	0.09 ± 0.02	0.12 ± 0.01	0.14 ± 0.02	0.19 ± 0.03
	Sediment Texture	Sandy loam	Sandy loam	Loamy	Silt	Silt loam	Silt loam	Silt loam	Silt	Silt loam	Silt loam	Silt loam	Loamy	Loamy	Loamy	Loamy
	Corg(%)	1.85 ± 0.05	2.14 ± 0.13	2.17 ± 0.15	2.36 ± 0.06	2.26 ± 0.05	2.55 ± 0.05	2.66 ± 0.05	2.74 ± 0.04	2.33 ± 0.03	2.13 ± 0.11	2.55 ± 0.05	2.23 ± 0.2	2.42 ± 0.05	2.48 ± 0.07	1.05 ± 0.05
	TOM(%)	3.2	3.78	3.84	4.08	3.91	4.42	4.6	4.73	4.01	3.76	4.42	4	4.17	4.29	1.82
	% of LOM in TOM	6.74	5.2	3.13	6.02	3.15	2.98	7.37	5.97	2.08	2.73	1.52	3.11	3.45	1.5	5.31
	Sulfate(mg 100g^{-1})	4.25 ± 0.05	15.45 ± 0.05	42.46 ± 0.06	45.61 ± 0.06	22.44 ± 0.05	31.25 ± 0.05	19.85 ± 0.06	30.41 ± 0.04	2.44 ± 0.04	8.43 ± 0.04	12.42 ± 0.05	13.61 ± 0.06	6.26 ± 0.06	9.44 ± 0.05	38.92 ± 0.06
	SRBLactate (CFUg^{-1})	600 ± 50	1280 ± 60	1110 ± 50	580 ± 40	640 ± 40	650 ± 80	1140 ± 50	1110 ± 80	690 ± 50	1270 ± 50	1270 ± 50	670 ± 40	1150 ± 30	1320 ± 40	930 ± 40
	SRBAcetate (CFUg^{-1})	2780 ± 40	2570 ± 50	750 ± 40	3070 ± 40	1450 ± 50	940 ± 60	1790 ± 70	1250 ± 60	1040 ± 60	1170 ± 40	710 ± 40	930 ± 50	780 ± 50	640 ± 40	220 ± 40

Table 2. Results of the two-way ANOVA (F) of environmental and microbiological variables between seasons and among stations

Variables	$f_{se}(2,44)$	p-value	$f_{st}(14,44)$	p-value	$f_{se\times st}(14,44)$	p-value
pH	43.191***	.000	5.517***	.000	6.910***	.000
Eh(RmV)	1129.956***	.000	152.945***	.000	78.753***	.000
EC(µS)	1172.005***	.000	682.788***	.000	820.505***	.000
TDS(mgL^{-1})	1203.736***	.000	514.429***	.000	475.243***	.000
Sal(PSU)	298.227***	.000	1.539	.113	1.539	.066
C_{org}(%)	1425.614***	.000	861.999***	.000	896.457***	.000
TOM(%)	1005.857***	.000	959.166***	.000	911.597***	.000
LOM in TOM (%)	34168.272***	.000	8314.958***	.000	4772.589***	.000
Sulfate (mg100g^{-1})	156830.542***	.000	70649.996***	.000	31354.500***	.000
SRB Lactate CFUg^{-1}	972.251***	.000	359.260***	.000	131.013***	.000
SRB Acetate CFUg^{-1}	12714.831***	.000	471.378***	.000	608.271***	.000

Where f(m,n) denotes the f ratio with (m,n) degrees of freedom. *** Shows that p< 0.001, i.e. significant at 0.1% level. p-significance. 'se×st' shows the interaction effect between seasons and stations. SRB: Sulfate Reducing Bacteria

The estuarine biogeochemistry, primarily controlled by benthic OM remineralization, relies on efficient processes. Sedimentary organic matter in the Vembanad estuary is derived from terrigenous and marine inputs, with the northern part influenced by marine in situ biological production and the southern portion dependent on terrestrial origin (Gireeshkumar et al., 2013; Chakraborty et al., 2015).

3.2 Abundance and distribution of SRB

The visualization of sulfate-reducing bacteria (SRB) population was conducted through the observation of distinct black colonies on the sides of roll tubes (Fig. 2). This distinctive black colouration resulted from the formation of FeS precipitates due to bacterial hydrogen sulfide (H_2S) production in the presence of iron minerals. The olfactory evidence of H_2S in the roll tubes further affirmed its existence. The selection of carbon substrates, namely lactate and acetate, was influenced by the microbial community characteristics and the quality of decomposed organic matter, which can vary across diverse environments (Wallenius et al., 2021).

During the monsoon season, the SRB sediment population peaked in station 12 with 1210 ± 20 CFU g^{-1} using lactate and station 8 with 650 ± 30 CFU g^{-1} using acetate as substrate. Post-monsoon witnessed an average SRB population of 1590 ± 20 CFU g^{-1} (lactate) and 430 ± 30 CFU g^{-1} (acetate) at station 8. The pre-monsoon season exhibited SRB abundance with 1320 ± 40 CFU g^{-1} using lactate at station 14 and 3070 ± 40 CFU g^{-1} using acetate at station 4 (Table 1). The predominance of organic-rich sediments favoured anaerobic modes of organic matter mineralization, mainly through sulfate reduction, Fe (III) and Mn (IV) reduction, and methanogenesis (Jørgensen, 1982; Jørgensen et al., 1990; Hoehler et al., 2001). They oxidize organic substrate either by complete oxidation, producing carbon dioxide or by incomplete oxidation, producing acetate (Kaksonen & Puhakka, 2007; Muyzer & Stams, 2008). In natural environments, sulfate reducers will likely use fermentation products such as H_2, alcohol, and organic acids like acetate, propionate, and butyrate. The abundance and distribution of SRB exhibited significant spatiotemporal variations and interacted considerably between seasons and stations (Table 2).

Spatiotemporal variations in SRB abundance displayed considerable interactions between seasons and stations (Table 2). Biogeochemical parameters of estuarine sediment correlated with SRB population during different seasons, as outlined in Table 3. Notably, acetate-utilizing bacteria exhibited significant positive correlations with labile organic matter (LOM), sulfate concentration, clay content, and the protein content of LOM during monsoon. The post-monsoon revealed positive correlations between acetate and lactate-utilizing SRB, while the pre-monsoon season replicated similar correlations observed during the monsoon season, with an additional positive correlation with the lipid content of LOM. Protein emerged as the primary contributor to LOM in estuarine sediments across all seasons, indicating the presence of fresh organic matter and suggesting anthropogenic interventions in the estuary (Danovaro et al., 1993). A considerable amount of waste enters the estuary, contributing to the protein enrichment in the organic matter, and ultimately gets settled in the sediment phase (Balasubramanian, 2012).

The sulfate-reducing bacteria group, known for its varying sensitivity to electron acceptors (Park et al., 2020), exhibited consistent abundance among lactate-utilizing SRB throughout the sampling period, with a slight increase in post-monsoon compared to other seasons (Fig. 3 a and b). Usually, when fed lactate as a carbon source, high bacterial abundance was observed due to the availability of sub-substrates/intermediary compounds formed by lactate fermentation. Lactate is usually incompletely oxidized to acetate and propionate (Taylor & Parkes, 1985; Laanbroek & Pfennig, 1981). In contrast, acetate-utilizing SRB experienced a drastic increase during pre-monsoon. This intriguing observation suggested potential competition dynamics between SRB and methanogens, coexisting in reduced sediments. Previous studies indicated that SRB dominates under high saline conditions, whereas methanogens thrive in freshwater environments (Reshmi et al., 2015). The sulfate concentrations are generally lower in freshwater, elevating the methanogenic activity in freshwater (Margalef-Marti et al., 2023). The type of carbon substrates available in sediment also plays a role in determining their dominance.

The competition between SRB and methanogens is further

Table 3a. Results of correlation analysis of environmental variables in the estuarine sediments on Monsoon

	TOC	TOM	LOM	LOTO	Sulph	Carb	Prot	Lip	Sand	Silt	Clay	SRBLac	SRBAce
TOC	1												
TOM	1.000**	1											
LOM	-.734**	-.734**	1										
LOTO	-.889**	-.889**	.855**	1									
Sulph	-.524*	-.525*	.170	.426	1								
Carb	-.459	-.459	.428	.487	-.226	1							
Prot	-.697**	-.697**	.975**	.812**	.255	.223	1						
Lip	.183	.182	.146	-.005	-.248	-.023	.104	1					
Sand	.853**	.853**	-.890**	-.828**	-.251	-.630*	-.815**	.002	1				
Silt	-.851**	-.852**	.889**	.827**	.249	.632*	.813**	-.002	-1.000**	1			
Clay	-.765**	-.766**	.801**	.762**	.376	.102	.849**	-.056	-.742**	.739**	1		
SRBLac	-.176	-.177	.149	.120	.293	-.302	.219	.279	-.060	.060	.166	1	
SRBAce	-.393	-.394	.615*	.542*	.605*	-.246	.710**	.326	-.425	.423	.618*	.478	1

**. Correlation is significant at the 0.01 level (2-tailed).

*. Correlation is significant at the 0.05 level (2-tailed).

Table 3b. Results of correlation analysis of environmental variables in the estuarine sediments on Post monsoon

	TOC	TOM	LOM	LOTO	Sulph	Carb	Prot	Lip	Sand	Silt	Clay	SRBLac	SRBAce
TOC	1												
TOM	1.000**	1											
LOM	-.450	-.450	1										
LOTO	-.886**	-.887**	.325	1									
Sulph	-.860**	-.860**	.285	.899**	1								
Carb	.066	.065	-.332	.232	.135	1							
Prot	-.370	-.370	.979**	.227	.206	-.475	1						
Lip	-.340	-.340	-.118	.251	.226	.131	-.256	1					
Sand	.429	.430	-.117	-.532*	-.408	-.234	-.023	-.379	1				
Silt	-.425	-.426	.111	.528*	.404	.234	.018	.382	-1.000**	1			
Clay	-.551*	-.551*	.439	.695**	.682**	.205	.389	-.118	-.496	.488	1		
SRBLac	-.404	-.403	.156	.192	.276	.110	.108	-.025	-.069	.067	.055	1	
SRBAce	-.371	-.372	.270	.393	.402	.335	.174	.021	-.104	.100	.240	.688**	1

**. Correlation is significant at the 0.01 level (2-tailed).

*. Correlation is significant at the 0.05 level (2-tailed).

Table 3c. Results of correlation analysis of environmental variables in the estuarine sediments on Pre monsoon

	TOC	TOM	LOM	LOTO	Sulph	Carb	Prot	Lip	Sand	Silt	Clay	SRBLac	SRBAce
TOC	1												
TOM	1.000**	1											
LOM	.265	.262	1										
LOTO	-.180	-.184	.898**	1									
Sulph	-.162	-.163	.258	.317	1								
Carb	.434	.432	.878**	.695**	.261	1							
Prot	.211	.208	.976**	.898**	.175	.780**	1						
Lip	-.150	-.153	.264	.341	.308	-.042	.241	1					
Sand	-.464	-.464	-.544*	-.331	-.344	-.639*	-.455	-.050	1				
Silt	.465	.465	.544*	.331	.343	.639*	.455	.050	-1.000**	1			
Clay	.191	.189	.349	.273	.314	.419	.272	.055	-.712**	.709**	1		
SRBLac	.189	.190	-.082	-.186	-.193	-.039	-.031	-.424	.190	-.190	-.128	1	
SRBAce	.094	.091	.655**	.634*	.036	.318	.682**	.708**	-.343	.343	.152	-.317	1

**. Correlation is significant at the 0.01 level (2-tailed).

*. Correlation is significant at the 0.05 level (2-tailed).

highlighted by the low population of acetate-utilizing SRB during monsoon and post-monsoon, potentially due to competition with methanogenic archaea. When sulfate concentrations are low, the shift from sulfate reduction to methane production in marine sediments occurs during an extended phase of methane cycling by methanogens. Following sulfate depletion, a consistent growth is observed in both sulfate reducers and methanogens (Kevorkian et al., 2022). Acetate is a competitive substrate for SRB and methanogens (Oremland & Polcin, 1982). Acetate, as a substrate, exhibits lower Km values for SRB (0.2 mM) compared to methanogens (3 mM), indicating higher affinity towards the substrate. This enables the sulfate reducers to maintain the pool of these substrates at concentrations too low for the methanogens when sulfate is not limiting (Isa et al., 1986). The pre-monsoon season was characterized by an almost equal coexistence of lactate and acetate-utilizing bacteria populations, which confirmed the dominance of SRB over methanogens. Fukui and Takii 1994, estimated that in the case of lactate as the electron donor, the Km value for free-living and FeS-associated SRB was 0.05 mM and 0.01 mM, respectively, showing its high affinity towards lactate. Cooperative and competitive interactions between microbial metabolisms can result from disturbances in wetland biogeochemistry (Berrier et al., 2022).

4. Conclusion

The biogeochemical variables of the estuarine sediments exhibited significant variance season-wise and at stations, and they showed considerable interaction. This influenced the bacterial population positively. The unique reducing environment in the estuary provided an anaerobic atmosphere, which supported the growth of SRB. Labile organic matter of sediments was a vital component influencing the SRB population. The protein fraction of LOM was abundant throughout the sampling period, which confirmed the deposition of fresh organic matter in the lake, indicating the human interventions. The sulfate content in the sediments strongly interlinked with the SRB population. The SRB count taken using the roll tube technique found that the abundance of lactate utilizing SRB was elevated in all seasons compared to acetate-using ones. The competitive nature of SRB and methanogens for a common substrate (acetate) influenced the count of acetate utilizing SRB. However, during pre-monsoon, a drastic elevation in the acetate utilizers was observed, which indicated the dominance of SRB over methanogens in the lake. The observed effects suggest that alterations in carbon mineralization and other factors influenced the competition between SRB and methanogens for shared electron donors. In summary, this study revealed intricate interactions among environmental variables in the South Indian tropical estuary, highlighting the influence of seasonal dynamics, sediment characteristics, and organic matter on sulfate-reducing bacteria growth. The findings contribute to a comprehensive understanding of estuarine ecosystems and their susceptibility to changing environmental conditions.

Fig 2. Black colonies of SRB in roll tubes

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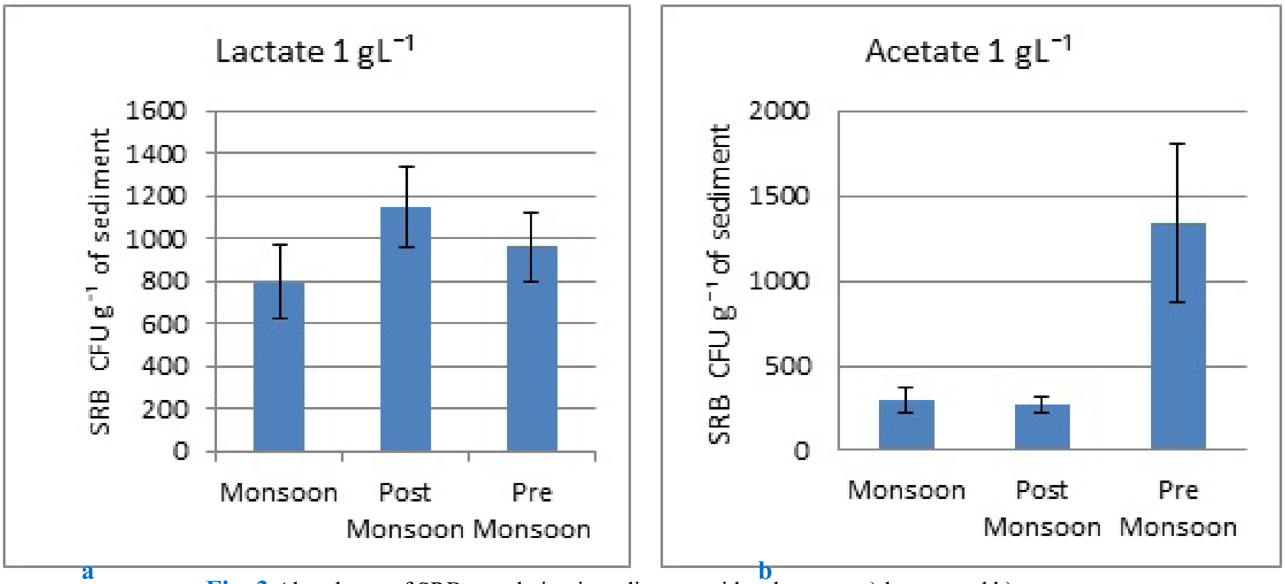

a b

Fig. 3. Abundance of SRB population in sediments with substrates; a) lactate and b) acetate

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