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Research Article

Spatial Distribution and Controlling Factors on the Bacterial Abundance in the Indian Groundwaters

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Abstract

Microbiology plays a vital role in groundwater quality. The bacterial distribution in groundwater mainly depends on various parameters like nutrient concentrations, availability of the organic matter, grazing pressure by protozoans and viral lysis. To examine the spatial distribution and the impact of biogeochemical parameters on bacterial distribution, groundwater samples are collected at 87 locations along the Indian coastal region during the monsoon. This study revealed *E. coli* O157:H7, *Achromobacter* sp., *Pseudomonas aeruginosa* are the most abundant bacteria. East coast groundwaters are more abundant with *E. coli* O157:H7 and west coast with *Achromobacter* sp. The total viable counts of bacteria showed significant linear positive relations with bacterial respiration rates pCO_2 levels, TDCHO, TDFAA, and TDPRO and inverse relation with depth and DO saturation. Suggesting that the bacterial abundance and distribution mainly depending on organic matter availability. *E. coli* O157:H7 consumed more of TDCHO and TDFAA, and *Achromobacter* sp., more depend on DTPRO and *Pseudomonas aeruginosa* on TDCHO and TDPRO.

Keywords: Groundwater; Bacteria; *E. coli;* Monsoon; Carbohydrates; Amino acids; Proteins

Introduction

Groundwater plays a vital role in human life, and it is one of the crucial sources of water for drinking and irrigation purposes. Since the last five decades, there have been drastic changes in the monsoonal pattern, and the precipitation decreases from year to year [1]. Because of this, the usage of groundwater enhanced dramatically. It is essential to know the groundwater's geological, chemical, and biological components, especially microbiology (bacteria, virus, and fungus), vital in groundwater health [2]. According to the world health organization (WHO), nearly 3.4 million people die from water-related diseases [3]. Microbial contamination is also happening at the location where the density of people is more [4]. Some bacteria are noticed in groundwater, such as microbial contaminants, which are harmful to human health such as *Escherichia coli*, *Clostridium*, *Campylobacter*, *Rhodococcus coprophilous*, *Enterococci*, *Arcobacter*, *Fecal streptococci*, *Archromobacter*, Sulphite reducing *Clostridia*, *Klebsiella*, and *Pseudomonas aeruginosa* [5]. In addition to this, other significant sources of pathogenic microorganisms into surface or groundwater are from agricultural lands, mixing of domestic sewage, underground storage tanks, and unauthorized dumpsites [6]. Several factors influence the survival of microorganisms in groundwater, like pH, precipitation, soil moisture content, soil microflora, temperature, nature of organic carbon, amount of nutrients, dissolved oxygen levels [7]. Diarrhoea, Cholera, Typhoid, and Hepatitis A, are the most common diseases found in many developed and developing countries because of groundwater contamination [8]. From the above studies, it is clear that knowing about the microbiology of underground water is essential. However, very few studies are focused on microbial variability in Indian groundwater systems [9,10]. Therefore, we attempted to understand the qualitative structure of microorganisms (bacteria) in coastal groundwaters along the Indian coast and understand the impact of biogeochemical parameters on their distribution.

Materials and Methods

India has a very long coastline covering almost 7571km, including east and west coastal regions. There are nine major coastal states: West Bengal-WB, Odisha-OD, Andhra -Pradesh-AP, Tamil Nadu-TN, Kerala-KL, Karnataka-KA Goa-GA, Maharashtra-MH, and Gujarat-GJ, in which GJ has the highest coastline, 1256km. Groundwater samples have been collected from 87 wells along India's east and west coast during the monsoon season (Figure 1).

Sampling and analytical methods

Groundwater samples were collected from 87 wells along the east and west coast during the monsoon season, and the position of the sample collection locations are given in Table 1. The groundwater temperature was measured using a mercury thermometer, and the conductivity was measured with the help of a conductivity meter (H12003) with an accuracy of ±1%. Groundwater samples were collected with a 5L Niskin sampler and initially dissolved gas (dissolved oxygen-DO) samples were collected in 125ml glass bottles. DO was analyzed by the following method described by Carritt and Capenter [11]. Bacterial Respiration (BR) is measured following the DO incubation method [12]. The groundwater samples were collected in four 125ml bottles for DO analysis. The percentage of DO saturation is computed as

$$\text{DO saturation (\%)} = (\text{DO measured} / \text{DO saturated}) \times 100$$

The total dissolved carbohydrates (TDCHO) were measured at 490nm using the spectrophotometric method described in Dubois

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et al. Dissolved amino acids were measured at 342nm excitation and 452nm emission using the spectrofluorometric method described in Parsons et al. The dissolved proteins were measured at 650nm, using the spectrophotometric method described in Lowry. CO_2 was computed with the data of salinity, pH, alkalinity, silicate, phosphate, and dissociations constants from Millero et al. using the sys program [13]. TVC method is used for the enumeration of pathogenic, indicator, and heterotrophic bacteria. R2A agar medium is used to enumerate heterotrophic bacteria and *E. coli* O157:H7 medium for the computation of pathogenic *E. coli* O157:H7 bacterium McConkey agar for total coliforms, cetrimide agar for enumeration of Pseudomonas sp. were used. 100µl of groundwater sample was transferred on the different agar plates, and then the selection was spread over the surface and allowed to be absorbed by the medium. Petri dishes were inverted and incubated in a BOD incubator at 35±5°C for two days. The colonies were quantified using a colony counter, and the numbers of colonies are represented in the colony-forming unit (CFU/ml).

Data graphical representation and statistical analysis

Statistica program is used for the mathematical study of the rank correlation matrix (Spearman's r) and the Student *t*-test measure. Arc GIS is used to visualize there-dimensional data plots.

Results

Variability in biogeochemical parameters in Indian coastal groundwaters

The temperature in Indian coastal groundwater varied from 24.00 to 34.00 °C, with an average of 30.21±1.23 °C and higher temperatures are noticed in the east coast (30.92±1.35 °C) groundwater than west coast groundwater (29.61±1.19 °C; t = 4.75, p<0.001, n = 85; Table 2). Groundwater conductivity varied from 0.75 to 48 mS/cm, with an average 6.04±8.75 mS/cm and higher conductive waters are noticed along the east coast (8.84±9.74 mS/cm) water than west coast (3.67±7.04 mS/cm) and the conductivity difference between east and west coast groundwater is statistically significant (t = 2.86, p<0.001, n = 85; Table 2). The pH of coastal groundwater varied from 4.71 to 7.44, with an average of 6.53±0.73, and more neutral waters are on the east coast (6.95±0.30) water than on the west coast (6.17±0.80). The pH difference between east and west coast groundwater is statistically significant (t = 5.15, p<0.001, n = 85; Table 2). DO saturation of coastal groundwater varied from 26.5 to 101.2% with an average of 60.76±35.35%, and more saturated water is noticed along the west coast region (64.56±28.54%) than east coast (57.31±26.08%; Table 2). In contrast, to DO saturation, bacterial respiration (BR) rates are found to be high in the east coast region (4.33±3.14 mg/l/h) than the west coast region (2.87±2.93 mg/l/h; Table 2). Along with bacterial

Table 1: Groundwater samples GPS locations of Indian coastal groundwaters during the monsoon period.

State	Lat (Deg)	Long (Deg)	State	Lat (Deg)	Long (Deg)	State	Lat (Deg)	Long (Deg)	State	Lat (Deg)	Long (Deg)
East Coast											
West Bengal			Odessa			Andhra Pradesh			Tamil Nadu		
WB1	22.1889	88.7132	OD1	21.6701	87.2859	AP1	18.941	84.5648	TN1	12.94	80.2401
WB2	22.2271	88.4461	OD2	21.3413	86.7631	AP2	17.728	83.3302	TN2	12.382	80.0894
WB3	22.2181	88.0949	OD3	20.8119	86.7609	AP3	17.317	82.5695	TN3	11.748	79.7687
WB4	22.4632	87.9733	OD4	20.5014	86.3277	AP4	16.957	82.2552	TN4	11.031	79.8503
WB5	22.0428	88.0657	OD5	20.0989	86.1886	AP5	16.397	81.8095	TN5	10.377	79.8497
WB6	22.0129	87.8173	OD6	19.8422	85.9032	AP6	16.178	81.1385	TN6	10.039	79.2333
WB7	21.8712	87.7571	OD7	19.9016	85.3656	AP7	15.781	80.3801	TN7	9.3316	78.9741
WB8	21.7852	87.7441	OD8	19.5972	85.1211	AP8	15.505	80.0365	TN8	9.2353	78.7875
WB9	21.7205	87.6637	OD9	19.3503	84.9882	AP9	14.297	80.0904	TN9	8.7571	78.1566
WB10	21.6234	87.5206	OD10	19.1611	84.7112	AP10	13.589	80.0343	TN10	8.0885	77.5493

West Coast														
Kerala			Karnataka			Goa			Maharashtra			Gujarat		
State	Lat (Deg)	Long (Deg)	State	Lat (Deg)	Long (Deg)	State	Lat (Deg)	Long (Deg)	State	Lat (Deg)	Long (Deg)	State	Lat (Deg)	Long (Deg)
KL1	8.3945	77.0885	KA1	12.7884	74.8593	GA1	14.9111	74.0816	MH1	15.7243	73.6882	GJ1	20.5981	72.9424
KL2	8.8634	76.6935	KA2	12.8748	74.8292	GA2	14.959	74.0551	MH2	16.108	73.4725	GJ2	21.124	72.7861
KL3	9.3183	76.4217	KA3	13.2228	74.7845	GA3	15.002	74.0786	MH3	16.473	73.3943	GJ3	22.251	73.1852
KL4	9.9994	76.2929	KA4	13.3889	74.7431	GA4	15.042	73.9991	MH4	16.994	73.317	GJ4	21.61	72.2693
KL5	10.4192	76.1052	KA5	13.6189	74.6926	GA5	15.126	73.9495	MH5	17.502	73.1855	GJ5	21.059	71.7591
KL6	10.9248	75.9139	KA6	13.8183	74.6343	GA6	15.207	73.9504	MH6	17.976	73.0918	GJ6	20.761	71.0551
KL7	11.4696	75.6548	KA7	14.0271	74.5291	GA7	15.71	73.7257	MH7	18.647	72.8783	GJ7	20.898	70.3932
KL8	11.8847	75.3725	KA8	14.2417	74.4468				MH8	19.375	72.8275	GJ8	21.644	69.6032
KL9	12.2428	75.1449	KA9	14.4308	74.4231				MH9	19.956	72.8231	GJ9	22.24	68.9323
KL10	12.6821	74.9032	KA10	14.6572	74.3121				MH10	20.136	72.8065	GJ10	22.472	70.0786

Table 2: Mean (±SD) of groundwater properties along the Indian coast during the wet period.

Parameter	Wet Period	Coast	Wet period	t	p	df
Temperature (°C)	30.21±1.43	East	30.92±1.37	4.75	0.0001	85
		West	29.61±1.20			
Conductivity (mS/cm)	6.04±8.75	East	8.84±9.7	2.86	0.001	85
		West	3.67±7.04			
pH	6.53±0.73	East	6.95±0.30	5.15	0.001	85
		West	6.17±0.80			
DO Saturation (%)	60.76±27.35	East	57.31±26.08	5.65	0.001	85
		West	64.56±28.54			
BR (mg/l/hr)	3.54±3.10	East	4.33±3.14	5.68	0.001	85
		West	2.87±2.93			
pCO_2 (µatm)	31680±16326	East	36232±19102	4.85	0.001	85
		West	26446±10361			
TDCHO(mg/l)	1.14±0.55	East	1.16±0.10	0.45	0.3	85
		West	1.10±0.39			
TDPRO(mg/l)	0.78±0.36	East	0.90±0.3	1.2	0.1	85
		West	0.70±0.31			
TDFAA(mg/l)	0.26±0.11	East	0.30±0.12	1.22	0.1	85
		West	0.20±0.09			
TVC (cfu/ml)	6252±7510	East	7085±6642	6.35	0.0001	85
		West	5543±8180			

Figure 1: Study area and Sample locations along the east and west coast of India.

respiration rates, pCO_2 concentrations were also found to be high along the east coast groundwaters (36232±19102 µatm) than west coast (26446±10361 µatm; Table 2). The concentrations of labile organic biochemical compounds such as TDCHO, TDFAA and TDPRO were high along with the east coast groundwater than west coast groundwater (Table 2).

Spatial distribution of bacteria in Indian coastal groundwater

The total viable heterotrophic bacterial counts in Indian coastal groundwater varied from $1.6x10^2$ to $3x10^4$ CFU/ml, and this is more in east coast groundwater than west coast (Table 2 and Figure 2a). The most common bacteria isolated from the Indian coastal groundwater are *E. coli* O157:H7 (Figure 2b), *Achromobacter* sp. (Figure 3a), and *Pseudomonas aeruginosa* (Figure 3b), *Klebsiella* sp. (Figure not shown). In the east coast groundwater, *E. coli* O157:H7, followed by *Achromobacter* sp., *Pseudomonas aeruginosa*, are more abundant types in which more dominant bacterium is *E. coli* O157:H7 (37%) followed by *Achromobacter* sp. (26%), and minimum *Pseudomonas aeruginosa* with 7% and other types of unidentified bacteria contribute 30%. In the west coast coastal groundwater, *Achromobacter* sp. with 40%, followed by *E. coli* O157:H7 (30%), *Pseudomonas aeruginosa* (10%), are dominant bacteria and in addition to this *Klebsiella* sp., also noticed with 7% distribution in this region and other unidentified bacteria contributed 13%. The spatial distribution of bacteria from state to state and from north to south in east and west coast varied significantly, the North East state of coastal India (West Bengal-WB) is equally shared with *Achromobacter* sp., and *E. coli* O157:H7 and Odisha state were dominated with *E. coli* O157:H7 and *Pseudomonas aeruginosa*, whereas in Andhra Pradesh *Achromobacter* sp., was dominated after *E. coli* O157:H7, and in Tamil Nadu, *E. coli* O157:H7was dominated next to *Achromobacter* sp., and *Pseudomonas aeruginosa* (Figure 4). Overall from north to south in the east coast region, *E. coli* O157:H7 was the dominant bacterium (Figure 4). However, on the west coast, the North West state (Gujarat-

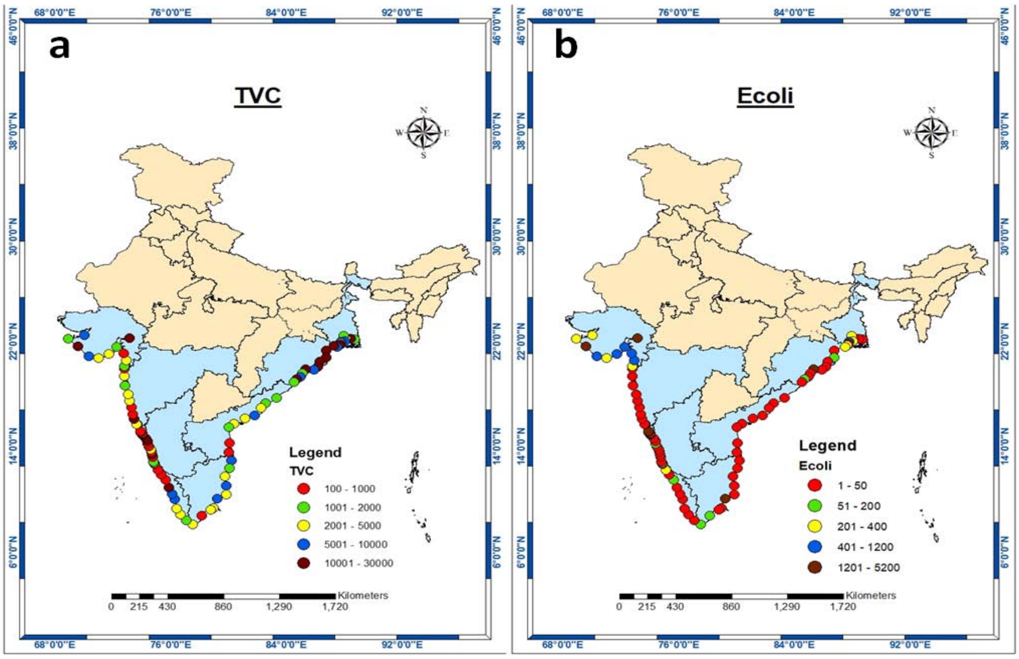

Figure 2: Distribution of a) TVC (CFU/ml) and b) *E. coli* O157:H7 in the east and west coast groundwaters of India.

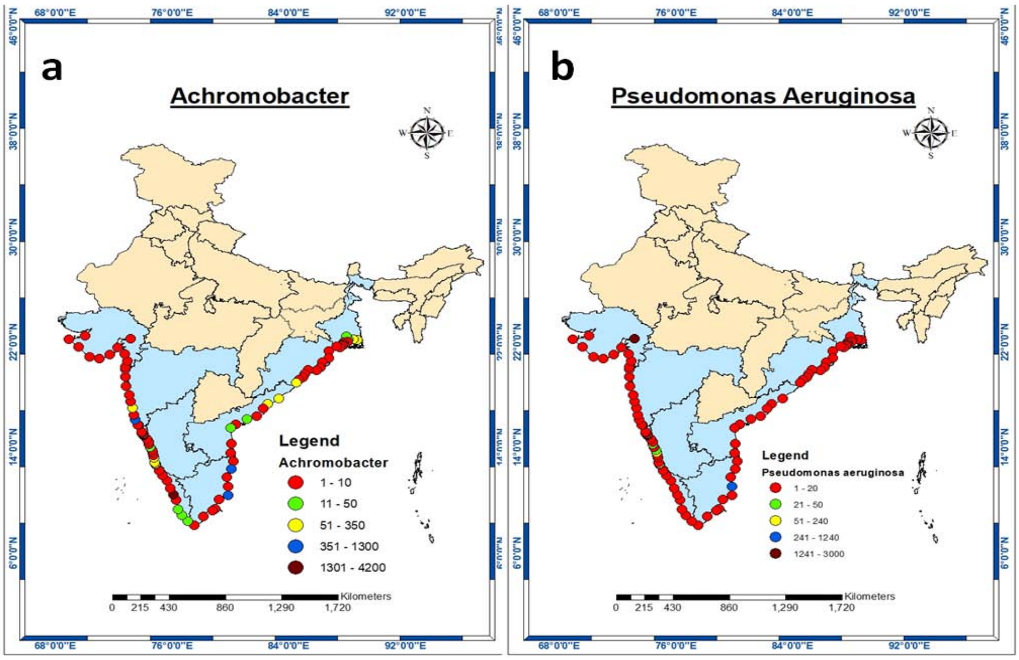

Figure 3: Distribution of a) Achromobacter sp., and b) Pseudomonas aeruginosa in the east and west coast groundwaters of India.

GJ) is dominated with *Achromobacter* sp., bacterium followed by *E. coli* O157:H7, in contrast to GJ, MH contains more number of *E. coli* O157:H7 followed by *Achromobacter* sp., and this state groundwater is contaminated with more unknown bacteria (64%; Figure 4). In GA and KA, *E. coli* O157:H7 is the dominant bacteria in groundwater, whereas in KL, *Achromobacter* sp. was predominant, followed by *E. coli* O157:H7. Overall in west coast groundwater most dominant bacterial type is *Achromobacter* sp., followed by *E. coli* O157:H7 (Figure 4).

During study period TVC showed a significant linear positive relation with temperature (r^2 =0.52, p<0.001; Table 3) and BR (r^2 =0.45, p<0.01; Table 3), pCO_2 (r^2 =0.32, p<0.05; Table 3), and inverse relation with depth (r^2 =0.85, p<0.001; Table 3), DO saturation (r^2 =0.30, p<; Table 3), TDCHO (r^2 =0.45, p< 0.01; Table 3), TDFAA (r^2 =0.42, p<0.01; Table 3), and TDPRO (r^2 =0.35, p<0.05; Table 3). *Achromobacter* sp., also showed a linear significant positive relations

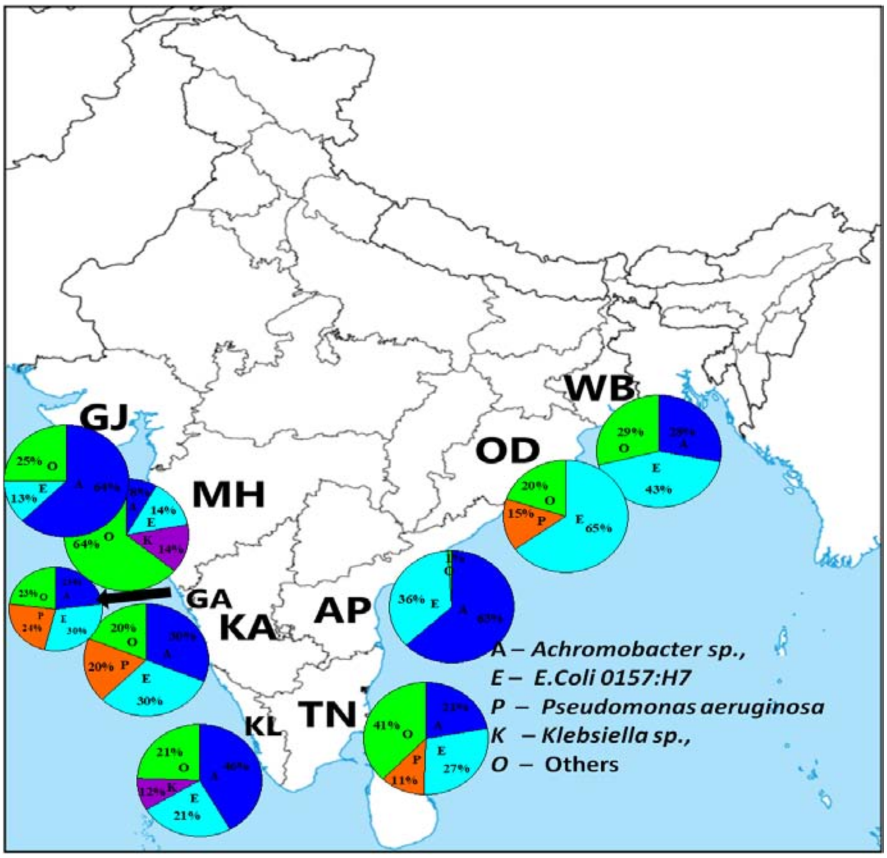

Figure 4: Variability in the percentage of different bacteria in Indian coastal groundwater during the monsoon period.

Table 3: Rank and correlation matrix (Spearman's, r) of different properties in Indian coastal ground waters during monsoon period.

Parameter	Depth	Temp	DO Sat	BR	pCO_2	TDCHO	TDFAA	TDPRO	TVC	A	E	P	K#
Depth	--	-0.57***	-0.51***	-0.16	0.19	-0.12	0.30*	-0.1	-0.85***	-0.86***	-0.58***	-0.67***	-0.45**
Temp		--	0.43**	0.39*	-0.34*	0.49**	-0.22	0.23	0.52***	0.09	0.32*	0.25***	0.21
DO Sat			--	0.16	-0.2	0.06	-0.19	-0.2	-0.30*	-0.19	-0.30*	0.26	-0.57***
BR				--	-0.27	0.17	-0.12	0.34*	0.45**	0.31*	0.35*	0.32*	-0.35*
pCO_2					--	-0.03	0.25	0.42**	0.32*	0.62***	0.30*	0.38*	0.42**
TDCHO						--	0.23	-0.45**	-0.45**	-0.30*	-0.58***	-0.55***	-0.25
DFAA							--	-0.12	-0.42**	-0.35*	-0.54***	-0.31*	-0.61***
TDPRO								--	-0.35*	-0.43**	-0.32*	-0.56***	-0.31*
TVC									--	0.41**	0.35*	0.41**	0.79***
A										--	--	--	--
E											--	--	--
P												--	--
K													--

$^{***}p < 0.001$; $^{**}p < 0.01$; $^{*}p < 0.05$. #indicates the correlations taken for only two states (KL and MH).

with BR (r^2 =0.31, p<0.05; Table 3), pCO_2 (r^2 =0.62, p<0.001; Table 3), and inverse relations with depth (r^2 = 0.86, p<0.001; Table 3), TDCHO (r^2 =0.30 , p<0.05; Table 3), TDFAA (r^2 =0.35, p<0.05; Table 3), TDPRO (r^2=0.43, p<0.01; Table 3). *E. coli* O157:H7 also showed same relations with all these biogeochemical parameters as like above. *E. coli* O157:H7 showed a linear significant positive relations with BR (r^2= 0.35, p<0.05; Table 3), pCO_2 (r^2 = 0.30, p<0.05; Table 3), and inverse relations with depth (r2 = 0.58, p<0.001; Table 3), DO saturation (r^2 = 0.30, p<0.05; Table 3), TDCHO (r^2 = 0.58, p<0.001; Table 3), TDFAA (r^2 = 0.54, p<0.001; Table 3), TDPRO (r^2 = 0.32, p<0.05; Table 3). *Pseudomonas aeruginosa* also showed a linear significant positive relations with BR (r^2 =0.32, p<0.05; Table 3), pCO_2 (r^2 = 0.38, p< 0.05; Table 3), and inverse relations with depth (r^2 = 0.67 , p<0.001; Table 3), DO saturation (r^2 = 0.26; Table 3), TDCHO

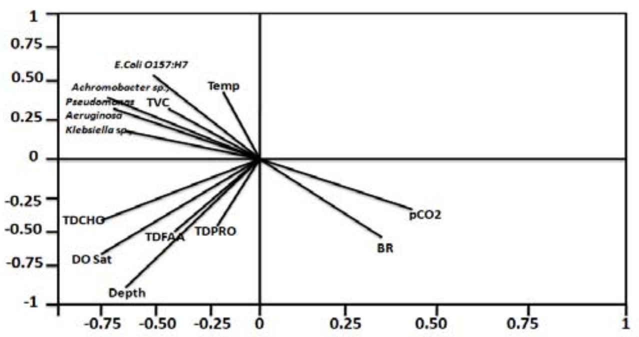

Figure 5: Principal component analysis of different controlling factors on bacterial distribution in Indian coastal ground waters during the monsoon period.

(r^2 = 0.55, $p<0.001$; Table 3), TDFAA (r^2 = 0.31, $p<0.05$; Table 3), TDPRO (r^2 = 0.56, $p<0.001$; Table 3). Even though the *Klebsiella* sp., noticed in only two states (KL and MH), but the relations with various biogeochemical parameters are same as like other bacterial groups. It showed a linear significant positive relations with BR (r^2 = 0.35, $p<0.05$; Table 3), pCO_2 (r^2 = 0.42 $p<0.01$; Table 3), and inverse relations with depth (r^2 = 0.45, $p<0.01$; Table 3), DO saturation (r^2 = 0.57, $p<0.001$; Table 3), TDCHO (r^2 = 0.25, $p<0.1$; Table 3), TDFAA (r^2 = 0.61, $p<0.001$; Table 3), TDPRO (r^2 = 0.31, $p<0.05$; Table 3).

Discussion

Variability in bacterial cells and their possible sources into the groundwater

Groundwater can be contaminated in several ways, natural and anthropogenic. In the natural processes, groundwater is contaminated because of rock weathering, organic matter decomposition, and leaching of inorganic compounds, organic compounds, and microorganisms during the monsoon season. Anthropogenic sources contain mainly septic sewage disposal, agricultural waste, and industrial and domestic waste in open lands. Several studies on groundwater quality noticed that groundwater's biological contamination mostly occurs through septic sewage and open dump yards [14,15]. India is the second-largest country in the population (1,390,508,600) after China. In the last decade, the Indian population has increased from 123.1 crores to 137.5 crores; concerning enhancement in population, the amount of sewage production also increased. Central pollution control board (https://cpcb.nic.in/status-of-stps) reported that sewage treatment plants have increased from 450 to 1760 in the last ten years all over India (http://www.sulabhenvis.nic.in/Database/STST_wastewater_2090.aspx). However, most of the rural and urban regions are still using septic tanks at homes. The sewage produced from these is not included in the plants as mentioned above count. A few research studies reported that the sewage from the septic tank could also be used for recycling of water for irrigation and the sludge used as fertilizer for the crop, in such cases, there are many chances for groundwater to be contaminated in more porous regions [16,17]. In some areas, this septic tank sewage is dumping in the ponds and lagoons, which has a significant effect on the groundwater contamination with microbes [18]. Along with this, septic tanks and soakaways increased drastically in housing, public apartments, and industries as the population increased day by day. This may lead to an increase in the seepage of sewage into groundwater through soil [16], so all these practices are contaminating the groundwater chemically and biologically. Al-Bahry et al. [16] noticed that bacteria in sewage sludge remain viable for several days to weeks if that kind of material is used as fertilizers; consequently, microbial contamination increases in the environment.

E. coli is one of the most commonly found bacteria in any aquatic system, especially in groundwater, and it is a good indicator of the presence of pathogens in groundwater. In Indian coastal groundwater on the east coast, 40% and 30% of groundwater are contaminated with these bacterial groups on the west coast. OD and WB have the highest *E. coil* count on the east coast, and KA and GA have the highest count on the west coast. Not only in Indian groundwater but also there are several countries facing problems with *E. coli* contamination in groundwater, USA [19], UK, Finland) [20], Ireland [21], Canada [22]. *E. coli* or coliform bacteria is not identified in any 100mL of drinking water sample [23] standards for microbiological quality of drinking water [24]. *E. coli* O157:H7 is a faecal pathogen that is regularly isolated from water [25] and identified as the primary cause of gastrointestinal illness outbreaks [26]. Several studies noticed that these bacteria could also survive in warm and cold and nutrient starving conditions [27].

Achromobacter sp. are found in the groundwater of WB, AP, GJ, KA, KL, TN states and these bacteria are well known for oxidation of arsenite to arsenate in the groundwater [28]. This bacterium was found more in the west coast groundwater (40%) than in the east (25%). Fertilizers, pesticides, and herbicides, which are used for agricultural purposes, contain a high percentage of arsenic it may contaminate the groundwater with arsenic in these regions. Shahina et al. [29] noticed that majority of the area in Indian states are significantly contaminated with arsenic (As) pollution and also found the highest contamination in WB (20%), GJ (5%), KA (0.5%), KL (0.5%), and OD (0.4%), AP, TN, and MH with (0.1%). The two most prevalent clinical manifestations of *Achromobacter* sp. infections in non-CF hosts are pneumonia and bacteremia [30]. Skin and soft tissue infections, urinary tract infections, intra-abdominal organ infections, Central Nervous System (CNS), eye and ear infections are less common, with endocarditis and bone infections being extremely rare [31].

Pseudomonas aeruginosa is more common in the west coast region (20%), especially in Karnataka and Goa state groundwater and on the east coast (8%) in Odisha and Tamil Nadu state groundwater. *Pseudomonas aeruginosa* is a prevalent pathogen found in faeces, soil, water, and sewage. It can multiply in water settings and on the surface of appropriate organic materials in contact with water. *Pseudomonas aeruginosa* is a well-known source of hospital-acquired infections that can lead to significant consequences. It was insulated from various wet surroundings, including sinks, water baths, hot water systems, showers, and spa pools [32]. *Pseudomonas aeruginosa* can cause deadly progressive lung infections. Warm, wet surroundings, such as swimming pools and spas, are connected with water-related folliculitis and ear infections. Many strains are resistant to various antimicrobial treatments, which increases the organism's importance in medical settings [33]. Shahina et al. [29] noticed that most southeast coast groundwater is contaminated with *Pseudomonas aeruginosa*. This could be possible due to the open dumping of waste, which causes groundwater contamination with microbes.

Klebsiella sp. is the least abundant bacterial group found in Indian coastal groundwater. This is only noticed in west coast groundwater and two states, Kerala (12%) and Maharashtra (14%). This bacterium is the natural occupant of many aquatic settings. They may reproduce rapidly in nutrient-rich fluids such as pulp mill effluent, textile finishing factories, and sugar-cane processing facilities. They are known to colonize tap washers in drinking-water distribution systems. Organisms can develop in water distribution networks. *Klebsiella* sp. are also found in the faeces of many healthy animals, and they can be found in sewage-contaminated water [34]. Bartram [33] noticed that *Klebsiella* sp. was infecting hospital patients, with dissemination linked to frequent patient handling (e.g., in intensive care units). Patients with compromised immune systems, such as the elderly or very young, patients with burns or extensive wounds, those on immunosuppressive medicine, or those infected with HIV/AIDS, are at the greatest danger.

Factors affecting the distribution of bacteria in coastal groundwater

Several biogeochemical properties significantly impact the distribution of bacterial count in the coastal groundwater along the Indian coast. A significant inverse linear relation between Total Viable Count (TVC) to depth ($r^2 = 0.85$, $p<0.001$; Table 3), and a significant linear positive relation between TVC and temperature ($r^2 =0.52$, $p<0.001$; Table 3), which is suggesting that the bacterial number decreasing with depth and warm groundwater has more bacterial count than low-temperature waters. East coast groundwater has warm temperatures and high TVC (Table 1). TVC also shows a significant positive relations with bacterial respiration ($r^2 = 0.45$, $p<0.01$; Table 3) and pCO_2 ($r^2 =0.32$, $p<0.05$; Table 3), and inverse relation with labile organic compounds such as total dissolved carbohydrates (TDCHO; $r^2 = 0.45$, $p<0.01$), total dissolved amino acids (TDFAA; $r^2 = 0.42$, $p<0.01$; Table 3), total dissolved proteins (TDPRO; $r^2 = 0.35$, $p<0.05$; Table 3). The linear positive relations with BR and pCO_2 and inverse relations with labile organic compounds of TVC, suggesting that bacteria utilized the available labile organic compounds significantly for their growth in coastal groundwater (Figure 5). The presence of high bacteria (TVC (CFU/ml): $7.1x10^3±6.6x10^3$; Table 2) along the east coast groundwater is may be due to the high availability of labile organic compounds such as TDCHO (1.16±0.10 mg/l; Table 2), TDFAA (0.30±0.12 mg/l; Table 2), and TDPRO (0.90±0.3mg/l; Table 2), which also associated with high bacterial respiration rates in the east coast (4.33±3.14mg/l/h; Table 2) than west coast (2.87±2.93 mg/l/h; Table 2) groundwater along the Indian coast. *Achromobacter* sp. showed a significant relation with TDPRO than TDCHO and TDFAA, suggesting that these bacteria utilizing more proteinaceous food than TDCHO and TDFAA (Table 3; Figure 5). *E. coli* O157:H7 showed significant relations with TDCHO and TDFAA among three compounds, indicating that these bacteria may be more dependent on these two compounds for their growth (Table 3; Figure 5). *Pseudomonas aeruginosa* bacteria showed significant relation with TDCHO and TDPRO compounds, suggesting that these bacteria may consume them to meet their growth requirements (Table 3; Figure 5). (Any references supporting this will be useful).

Summary

Groundwater is one of the vital water sources for drinking and irrigation in many countries, especially in India. The quality of groundwater is mainly depending on the composition of the chemical ions and biological groups present. The microbiology of the groundwater is another essential tool to assess the health of the water body. In Indian coastal groundwater, the total viable count of bacteria varied from $1.6x10^2$ to $3.0x10^4$ CFU/ml with a high abundance in groundwater along the east coast of India. However, more bacterial diversity is noticed along with the west coast groundwater. The most dominant bacteria type in east coast groundwater is *E. coli* with 37% and in west coast *Achromobacter* sp., with 40%. All these bacteria are significantly influenced by biogeochemical properties of groundwater, the inverse linear relations with TDCHO, TDFAA, TDPRO, BR, pCO_2 suggesting that the growth of these bacteria depends on the biochemical, organic compounds available in the groundwater along the coastal regions of India. In addition to this, specific bacteria have shown good correlations with specific biochemical compounds, indicating that the growth also depends on the nutrition specificity. Further, more experimental studies are required for better understating the bacteria food selection and chemistry of groundwater.

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