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Full Length Research Paper

Temporal variations in the abundance of heterotrophic bacteria in ground water according to land use patterns in Mysore district, India

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Temporal variations in the abundance of heterotrophic bacteria were studied from February 2005 to January 2007, in ground waters from Agricultural, Domestic and Industrial land use areas. The lowest mean abundance (AODG ml^{-1}) of Free Living Bacteria (2.2×10^5), Particle Bound Bacteria (0.07×10^5) and Total Bacteria (2.28×10^5) was recorded in ground water of Agricultural area; and the highest (1.07×10^6, 1.13×10^5 and 1.70×10^6, respectively) in that of Domestic area. In this investigation, 2.79% of the total bacteria determined by Acridine Orange staining in the ground water of Agricultural area could be grown on the artificial nutrient agar media and the comparative proportion in groundwater under the industrial area was 3.60 and 4.69% in the domestic area. Statistical analysis of the data revealed that several Physico-chemical parameters (Lab pH, air temperature, water temperature, conductivity, rainfall, BOD, CO_2, alkalinity, hardness, ca, Mg, PO_4, CL_2, NO_3, SO_4, Total Anion of Strong Acid, Total Solids, Total Dissolved Solids and Total Suspended Solids) ($p < .05$) were potentially responsible for some of the temporal variations in heterotrophic bacterial abundance, suggesting the influence of landuse-specific environmental stressors on the biota in these ecosystems.

Key words: Temporal variation, heterotrophic bacterial abundance, colony forming units, Acridine Orange Direct Count, Direct Epifluroscence Microscopic Technique, ground water, land use patterns.

INTRODUCTION

Human activities leading to the depletion of groundwater reserves include anthropogenic activities such as the use of fertilizers and pesticides in agricultural practices, landscape alteration, urbanization, and demand for domestic and public drinking water, various industrial activities, and the rise of tourism in coastal areas. Climate changes too are contributing to the water crisis especially in areas with arid climate and/or in some humid countries. Aquifers are overloaded with pollutants derived from agricultural and industrial operations, domestic wastes and industrial waters, infiltration of pollutants from surface, and intrusion of saline water, and all these affect groundwater quality. The dangerous increase in contaminated subsurface with chemicals and microbial pathogens brings with it, health risks to humans (Dan et al., 2003).

Aquatic ecosystems play an important ecological role. When flowing waters are used as waste water transport devices indirectly they contaminate soils and hence the ground water is also polluted. Planktonic heterotrophic bacteria are important in aquatic ecosystem as mineralisers of organic matter and hence in bio-purification of water, which receives organic pollution. Degradation of this organic matter contributes to the purification of the ecosystem and is, therefore, a major process controlling water-quality. Planktonic bacteria are responsible for much of the respiration in large rivers, and they may affect the quantity and quality of carbon transported by large rivers to the oceans (Richey et al., 1990; Findly et al., 1991; Benner et al., 1995; Castillo et al., 2004). They are also known to be responsible for key processes regulating the function and productivity of ecosystem through the "microbial loop" (Azam et al., 1983). Thus, it can be concluded that bacterioplankton not only are numerous, but also are impor-

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tant component of aquatic ecosystem (Lindström, 2001). Bacterioplankton may also constitute a significant fraction of total Planktonic biomass (Cho and Azam, 1990; Jonas and Tuttle, 1990; Anderson and Rudehäll, 1993). An understanding of the quantitative importance of bacteria in microbial food webs requires reliable estimates of their biomass. Generally, in the aquatic environment, much information has been compiled on taxonomic classify-cation and abundance of producers (photoautotrophs) and their immediate consumers (micro and macro zooplanktons) but less is known of the variables concern-ing decomposers (heterotrophic bacteria). This may be due to the fact that even an accurate determination of such simple variables, abundance and growth rate of these organisms was not possible until about three de-cades ago, and only quite recently a few methods have been developed for the identification of non-cultivable bacteria. However, to the best of our knowledge, there are no reports on temporal variation in heterotrophic bacterial abundance in ground water in India. Hence, the present investigation was under taken.

The main objectives of this investigation were: 1) to compare and contrast the abundance of heterotrophic bacteria in the ground water of Agricultural, Domestic and Industrial areas. 2) To test the hypothesis that the ground water samples might have caused pollution and thus might be responsible for deterioration of water quality. 3) To investigate the relationship between heterotrophic bacteria and physic-chemical parameters in all samples studied.

MATERIAL AND METHODS

Sampling sites

Sampling site (A1) - in agricultural area: This ground water sampling site is located at Naganahalli (12° 22‘14.4“N) (76° 22‘14.4“E), Mysore and it is about 10 km away from Mysore city, India. The groundwater is mainly used for agriculture and also for drinking purpose by farmers. The depth of ground water availability is 105 feet.

Sampling site (A2) - in agricultural area: This ground water sampling site is located at Geekaliundi (12° 07‘19.2“N, 76° 42‘57.8“E), Nanjangude taluk of Mysore district. The ground water is mainly used for cultivation of rice crops. The ground water is at a depth of 200 feet.

Sampling site (D1): Is in a Domestic area called Kumbargiri (12° 19‘23.4“ N, 76° 39‘ 20.8“ E) at Mysore city behind Mysore central Jail. Sewage and different solid and liquid wastes are released into the open canal, which is less than one meter away from the location of the hand pump used to draw the ground water used by the locals for drinking, bathing and cleaning of utensils and washing clothes. The depth of ground water source is 192 feet dow.

Sampling site (D2): Is in a Domestic slum area located at Kalimarahalli (12° 19‘ 8.0“ N, 76° 40‘ 42.0“ E) at Mysore city and the hand pump is installed just 40 cm away from the open canal, where the locals dump solid waste and sewage effluents. They use this ground water for drinking, bathing, cleaning of utensils and washing clothes. The depth of this ground water is 110 feet.

Sampling site (I1): Located in an Industrial area at Halagancheepura (12° 06‘12.7“ N, 76° 45‘ 54.7“ E), Nanjangud taluk of Mysore district, behind Bannaria Amman Sugar Limited is about 40 m away from the factory wall. This ground water is used only for cultivation of annual crops l. The depth of this ground water is 250 feet.

Sampling site (I2): Is also located in an Industrial area at Geekhalli (12° 06‘00.3“N) (76° 43‘00.6“ E), Nanjangud taluk of Mysore district, 50 m away from the Gemini Distillery factory. This ground water is used only for making bricks. The depth of ground water is 212 feet.

Thus, six sampling sites, two each from agricultural, domestic and industrial areas were selected and the location of these sources was done with the help of Global Positioning System (Garmin, USA).

Sampling

From February 2005 to January 2007, ground water samples were collected between 9.00 am and 2.30 pm every month, using clean and well rinsed, 5 -L capacity polythene bottles for the deter-mination of physico-chemical variables; and in 1-L capacity sterile glass bottles, (Schott-Duran, England) for the determination of bacterial variables. Ground water samples for bacterial determi-nation kept in icebox, and samples for other analysis kept in a wooden box were transported back to the laboratory.

Physico-chemical parameters

Twenty-four physico-chemical parameters were analyzed by fol-lowing standard methods given in Trivedy and Goel (1986), APHA (1992) and as described in Yamakanamardi and Goulder (1995). Lab *p*H and Field *p*H of the samples were measured using Griph-D-pH meter model 327 with glass electrode. The electrode was calibrated against pH 7.0 buffer each time before measurements. The ground water temperature and air temperature were measured with a hand-held mercury-in-glass thermometer. The conductivity of ground water was measured in the laboratory using a micro-processor controlled Conductivity meter, model 306. The instrument probe was previously calibrated with 0.1 M KCl solution at 25^{o}C. Turbidity of ground water was measured in the laboratory using a Digital-Nephelo-Turbdity meter. The instrument was setup using ultra pure water as zero and respective range (0-1, 1-10, 10-100 and 100-1000 NTU) of Farmazine solution. The daily rainfall data was obtained from Karnataka State Statistical Department, Mysore District. The total rainfall over the 30 days prior to the sampling day was used to calculate the mean rainfall in all sampling areas. The DO in the sample was immediately fixed with 2 ml potassium Iodide and 2 ml of manganous sulphate in the field itself. The Dissolved Oxygen (DO) content was determined by Winkler's method as described in Trivedy and Goel (1986). BOD was determined as described in Mackereth (1963). The physico-chemical variables were determined by various methods (mentioned within brackets for the respective variable) as described in Trivedy and Goel (1986) : Alkalinity was determined by titrating the water samples with a strong acid (HCl or H_2SO_4), hardness and calcium by EDTA titrometric, magnesium was obtained by subtracting the value of calcium from the total of Ca^{2+} and Mg^{2+} (Total hardness), phosphate (Stannous Chloride), chloride (argentometric), nitrate (Brucine), sulphate (Turbidimetric), and TS, TDS and TSS were determined gravimetrically. Total Anions of Strong Acids (TASA) was calculated by adding the concentrations of chloride, nitrate and sulphate. The remaining Physico-chemical variables were determined by various methods (mentioned within brackets for the respective variable) as described in (APHA (1992): Chemical Oxygen Demand (COD) (closed reflux titrimetric), free Carbon-dioxide (CO_2) (titrometric),

and Fluoride (colorimetric method using SPADNS Solution [Sodium -2 (para sulphophenylazo) -1, 8 dihydroxy 3, 16 naphtnalene disulphonate] (Ranbaxy, Mumbai).

Bacterial parameters

The concentration of directly counted aquatic bacteria was determined by the Acridine Orange Direct Count (AODC) method (Jones and Simon, 1975: Daley, 1979 and as described in Yamakanamardi and Goulder, 1995). On every sampling day within 5 to 6 h of sampling, two 10ml sub -samples from each site were fixed with 2% final concentration of neutral 0.22 µm-filtered formalin (Hobbie et al., 1977). Enumeration of the abundance of aquatic bacteria present in all the samples was carried out within 3 to 5 days of sampling. Aquatic bacteria were stained with Acridine Orange (BDH, Gurr, England) (10 mg l^{-1} final concentration for 10 minutes) and concentrated onto black 0.2 µm pore size polycarbonate membrane filters (Millipore (India) Ltd., Bangalore). Free Living Bacteria (FLB) and also the Particle Bound Bacteria (PBB) in the same fields were separately counted using a Olympus, BX-40 Epifluorescence microscope fitted with a 100 W Hg lamp and type B filter cassette (BP 470 ~ 490 nm excitation filter, DM 500 dichroic mirror and BA 515 Barrier filter) at 1000X magnification. The concentration of CFUs were determined by spreading 0.1ml of 100×diluted sample onto each of 15 CPS agar plates for each water sample as described in (Yamakanamardi and Goulder, 1995). The dilutions were made using sterile bacteria free water. The plating was carried out within 3 - 4 h of sampling. Bacterial colonies were counted after incubation for 10 days at 37°C. The variance of the counts relative to the means tended to be high, hence 95% confidence intervals were calculated after $\log_{10}$ transformation (Elliott, 1977); thus strictly they apply to the geometric means, and are asymmetric when applied to the arithmetic means. The Chromogenic (pigmented) bacterial colonies were also counted separately on each plate. The mean percentage of CCFUs was calculated by dividing the mean CCFU by the total mean CFUs and then multiplying by 100. CFUs as percentage of AODC, which represent the percentage of culturable bacteria, were obtained by dividing the concentration of CFUs in the water sample by the abundance of AODCs determined in the same water sample, and then multiplying by 100.

Statistical analysis

All the statistical analyses were carried out using SPSS for Windows release 10.0 (Norusis, 1993). The Kolmogorov-Smirnov test was used to test for agreement with the normal distribution. Distribution of many variables was found to differ significantly ($p<0.05$) from the normal distribution. Therefore, values for all variables were scaled, if necessary and then $\log_{10}$ transformed. Transformation was necessary because parametric methods require normally distributed data. After transformation, Kolmogorov-Smirnov test was again used to confirm that the transformed variables were generally not significantly different from normal ($p>0.05$).

One-way ANOVA post hoc test was applied for making multiple comparisons among the means. Multiple regression analysis was used with bacterial variables as dependent variables and physico-chemical parameters as independent variables. Variables were entered in to the equation using the stepwise entry method, with p in set at 0.05 and p out set at 0.1.

RESULTS

Abundance of Free Living Bacteria and Total Bacteria showed slight increase during summer season when compared to winter season in the ground water of Agriculture area in the second year of study and in the ground water of Domestic area during both first and second year of study, whereas, TB were more in Industrial area in the first year of study. However there were no significant changes in the abundance of FLB and TB in the ground water of Industrial areas of the second year of study period (Tables 2 - 3). It is noteworthy that the lowest mean abundance of FLB (2.2×10^5 ml^{-1}), PBB (0.07×10^5 ml^{-1}) and TB (2.28×10^5 ml^{-1}) were recorded in the ground water of Agriculture area and the highest abundance of FLB (1.07×10^6 ml^{-1}), PBB (1.13×10^5 ml^{-1}) and TB (1.70×10^6 ml^{-1}) in Domestic area (Table 1). There was no much variation in the abundance of PBB in the ground water except in the ground water of Agriculture and Domestic areas (in the second year of study). Some samples showed increased abundance of PBB during rainy season.

The abundance of Colony Forming Units (CFUs) was more in the ground water of Domestic area followed by Industrial area. Interestingly, no seasonal variations were observed in CFUs of the ground water in all sampling sites during the study period (Tables 2 - 3).

There was no growth of CCFUs in the ground water from Agriculture, Domestic and Industrial areas during study the period (Table 1).

It is noteworthy that the lowest CFUs as percentage of AODCs (1.85%) recorded in the ground water of Agriculture area and the highest value of 6.44% recorded in the ground water of Domestic area these were the lowest and the highest recorded values of CFUs as percentage of AODCs during the study (Table 1) period.

The calculation of Pearson's correlation coefficients between physico- chemical and bacterial parameters is given in the Tables 4a – 4c. These were not necessarily casual relationships. However, the correlation between these groups did not establish uniform pattern. Highest number (25) of correlations were found in the ground water of Domestic area, out of which 24 were positive and 1 was negative (Table 4b); moderate number (14) of correlations were recorded in the ground water of agriculture area, out of which 11 were positive and 3 were negative (Table 4a) and the least number (8) of correlations in the ground water of Industrial area, out of which 3 were positive and 5 were negative (Table 4c) correlations. The interrelationships among heterotrophic bacteria parameters shown in Table 5.

The extent of potential dependence of bacterial variables on physico- chemical parameters was further Investigated by stepwise multiple regression analysis. The results are given in tables from 6a to 6c. There was much multicollinearity amongst the independent physico-chemical parameters. It is possible, therefore, that some physico-chemical parameters, which are biologically relevant, were excluded from the regression equations be-cause of their collinearity with variables, which entered equations at earlier steps. Also included in Tables 6a to 6.c, therefore, are excluded physico-chemical para-meters, which were

Table 1. Summary of Microbial (Bacterial) parameters in the ground water of agriculture, domestic and industrial areas, February 2005 to January 2007.

Sl. No	Parameters	Sampling areas					
		Agriculture		Domestic		Industrial	
		Mean (range)	CV %	Mean (range)	CV%	Mean (range)	CV%
1	FLB $X10^6$ ml^{-1}	00.27^a (00.22 -00.34)	11	00.73^b (00.57 - 01.07)	18	00.30^A (00.23 -00.56)	26
2	PBB $X10^6$ ml^{-1}	00.010^a (00.007 - 00.014)	19	00.085^b (00.059 - 00.113)	17	00.014^a (00.010 - 00.022)	22
3	TB $X10^6$ ml^{-1}	00.283^a (00.228 - 00.351)	11	00.815^b (00.637 - 01.170)	17	00.310^a (00.242 - 00.574)	25
4	CFUs $X10^5$ ml^{-1}	00.08^a (00.06 - 00.100)	14	00.39^b (00.23 - 00.51)	15	00.11^c (00.07 - 00.14)	16
5	-	-	-	-	-	-	-
6	CFUs as % of AODCs	02.79^a (01.85 - 03.73)	16	04.96^b (02.99 - 06.44)	17	03.60^c (02.14 - 04.75)	21

Mean values with different superscripts area significantly different (P<0.05, Student-Newman-Kules test, after log10 transformation).
CV = Coefficient of Variation, FLB = free Living Bacteria, PBB = Particle Bound Bacteria, TB = Total Bacteria, CFUs = Colony Forming Units, CCFUs = Chromogenic Colony Forming Units, CFUs as % AODCs = Colony Forming Units as Percentage of Acridine Orange Direct Counts, N = 24.

Table 2a. Seasonal variation in the heterotrophic bacterial parameters in the groundwater of agricultural area, 1st year of seasonal study, February 2005 to January 2006.

Sl. No	Micribial parameters	Pre-Monsoon (Summer)	Monsoon (Rainy)	Post-Monsoon (Winter)	[1]F-value	[1]P-Value
1	PBB	$0.008^a \pm 0.002$	$0.012^b \pm 0.001$	$0.011^b \pm 0.003$	4.5581	0.0429*

Table 2b. Seasonal variation in the Heterotrophic Bacteria parameters in the groundwater of Agricultural area, 2nd year of seasonal study, February 2006 to January 2007.

Sl. No	Micribial parameters	Pre-Monsoon (Summer)	Monsoon (Rainy)	Post-Monsoon (Winter)	[1]F-value	[1]P-Value
1	**FLB**	$0.31^a \pm 0.02$	$0.27^b \pm 0.01$	$0.28^b \pm 0.01$	6.8621	0.0155*
2	**PBB**	$0.010^a \pm 0.001$	$0.011^a \pm 0.001$	$0.009^b \pm 0.001$	8.2174	0.0094*
3	**TB**	$0.32^a \pm 0.02$	$0.28^b \pm 0.01$	$0.28^b \pm 0.010$	6.6139	0.0171*

Table 3a. Seasonal variation in the Heterotrophic Bacterial parameters in the groundwater of Domestic area, 1st year of seasonal study, February 2005 to January 2006.

Sl. No	Micribial parameters	Pre-Monsoon (Summer)	Monsoon (Rainy)	Post-Monsoon (Winter)	[1]F-value	[1]P-Value
1	FLB	$0.75^a \pm 0.10$	$0.61^b \pm 0.05$	$0.63^b \pm 0.01$	5.1156	0.0329*

Table 3b. Seasonal variation in the Heterotrophic Bacterial Parameters in the groundwater of Domestic area, 2nd year of seasonal study, February 2006 to January 2007.

Sl. No	Micribial parameters	Pre-Monsoon (Summer)	Monsoon (Rainy)	Post-Monsoon (Winter)	[1]F-value	[1]P-Value
1	FLB	$0.69^a \pm 0.07$	$0.74^b \pm 0.05$	$0.69^a \pm 0.02$	30.7131	0.0001*
2	PBB	$0.11^a \pm 0.005$	$0.09^b \pm 0.003$	$0.08^b \pm 0.003$	51.5976	0.0000*
3	TB	$1.07^a \pm 0.07$	$0.84^b \pm 0.05$	$0.77^b \pm 0.2$	37.3885	0.0000*

Values are Mean ± SD,
[1]Values obtained from ANOVA pot hoc nonparametric test, * = Significant, P<0.05, NS = Non Significant, P>0.05.
Mean values with different superscripts are significantly different (p< 0.05, Student-Newman-Keuls test).
Note: only those parameters, which have significant correlations, are given in the tables.

Table 4a. Interrelationship between Physico-chemical and Heterotrophic Bacterial Parameters in the ground water of Agriculture area, February 2005 to January 2007.

Parameters	F pH	L pH	A Tem	W Tem	Cond	Turb	R fall	DO	BOD	COD	CO_2	ALK	HD	Ca	Mg	PO_4	Cl_2	NO_3	SO_4	TASA	TS	TDS	TSS	F
FLB	NS	NS	NS	0.45*	0.45*	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	0.49*	NS	0.50*	NS	NS	NS	NS	NS	NS
PBB	NS	0.45*	NS	NS	NS	NS	0.70*	NS	NS	NS	NS	NS	-0.43*	-0.42*	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
TB	NS	NS	NS	0.50*	0.41*	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	0.45*	NS	0.46*	0.51*	NS	NS	NS	NS	NS
CFUs	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	-0.42*	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS

Table 4b. Interrelationship between Physico-chemical and Heterotrophic Bacterial Parameters in the ground water of Domestic area, February 2005 to January 2007.

Parameters	F pH	L pH	A Tem	W Tem	Cond	Turb	R fall	DO	BOD	COD	CO_2	ALK	HD	Ca	Mg	PO_4	Cl_2	NO_3	SO_4	TASA	TS	TDS	TSS	F
FLB	NS	NS	NS	NS	0.56**	NS	NS	NS	NS	NS	NS	NS	NS	0.42*	0.55**	0.56**	0.48*	0.68**	0.65**	0.61**	0.49*	0.52**	NS	NS
PBB	NS	NS	0.42*	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
TB	NS	NS	NS	NS	0.53**	NS	NS	NS	NS	NS	NS	NS	NS	NS	0.53**	0.55**	0.45*	0.65**	0.62**	0.58**	0.47*	0.48*	NS	NS
CFUs	NS	NS	NS	NS	NS	-0.45*	NS	NS	NS	NS	0.42*	NS	NS	NS	NS	NS	NS	0.45*	0.50*	0.43*	NS	NS	NS	NS

Table 4c Interrelationship between Physico-chemical and Microbial parameters in the ground water of Industrial area, February 2005 to January 2007.

Parameters	F pH	L pH	A Tem	W Tem	Cond	Turb	R fall	DO	BOD	COD	CO2	ALK	HD	Ca	Mg	PO_4	Cl_2	NO_3	SO_4	TASA	TS	TDS	TSS	F
FLB	NS	NS	0.53*	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
PBB	NS	NS	NS	NS	NS	NS	NS	NS	-0.41*	NS	NS	NS	-0.40*	-0.40*	NS	NS	NS	NS	NS	-0.42*	NS	-0.41*	0.43*	NS
TB	NS	NS	NS	0.53*	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS

1.Values are Pearson's correlation coefficient, a 2- tailed test was applied and calculated after $\log_{10}$ transformation of all variables after scaling so that all values were > 1, n= 24, * Correlation is significant at the 0.05 level, ** Correlation is significant at the 0.01 level and NS= Non Significant.
2. F pH = pH measured in the field, L pH = pH measured in the laboratory, A Temp= Air temperature, W Temp = Water temperature, Cond = Conductivity, Turb = Turbidity, R.fall = Rainfall, DO= Dissolved Oxygen, BOD= Biological Oxygen Demand, COD= Chemical Oxygen Demand, CO_2 = free Carbon di-Oxide, ALK= Alkalinity, HD = Hardness, Ca = Calcium, Mg = Magnesium, PO_4 = In-Organic Phosphate, Cl_2 = Chloride, NO_3 = Nitrate, SO_4 = Sulphate, TASA = Total Anions of Strong Acids, TS = Total Solids, TDS = Total Dissolved Solids, TSS = Total Suspended Solids, F = Fluoride.
3. FLB = Free Living Bacteria, PBB = Particle Bound Bacteria, TB = Total Bacteria, CFUs = Colony Forming Units, CCFUs = Chromogenic Colony Forming Units, CFUs as % AODCs = Colony Forming Units as Percentage of Acridine Orange Direct Counts.
Note: only those parameters, which have significant correlations, are given in the tables

Table 5a. Interrelationship between Heterotrophic Bacterial parameters in the ground water of Agriculture area, February 2005 to January 2007.

Heterotrophic Bacterial parameters	FLB	PBB	TB	CFUs	% CCFUs	CFUs as % AODCs
CFUs as % AODCs	-0.52**	NS	-0.52**	0.73**	NS	
% CCFUs	NS	NS	NS	NS		
CFUs	NS	NS	NS			
TB	0.10**	NS				
PBB	NS					
FLB						

Table 5b. Interrelationship between Heterotrophic Bacterial parameters in the ground water of Domestic area, February 2005 to January 2007.

Heterotrophic Bacterial parameters	FLB	PBB	TB	CFUs	% CCFUs	CFUs as % AODCs
CFUs as % AODCs	NS	-0.53**	NS	0.71**	NS	
% CCFUs	NS	NS	NS	NS		
CFUs	NS	NS	NS			
TB	0.10**	0.80**				
PBB	0.75**					
FLB						

Table 5c. Interrelationship between Heterotrophic parameters Bacterial in the ground water of Industrial area, February 2005 to January 2007.

Heterotrophic Bacterial parameters	FLB	PBB	TB	CFUs	% CCFUs	CFUs as % AODCs
CFUs as % AODCs	-0.70**	NS	-0.70**	0.46*	NS	
% CCFUs	NS	NS	NS	NS		
CFUs	NS	NS	NS			
TB	0.10**	NS				
PBB	0.42*					
FLB						

Values are Pearson's correlation coefficient, a 2- tailed test was applied and calculated after $\log_{10}$ transformation of all variables after scaling so that all values were > 1, n = 24, * Correlation is significant at the 0.05 level, ** Correlation is significant at the 0.01 level and NS = Non Significant. FLB = free Living Bacteria, PBB = Particle Bound Bacteria, TB = Total Bacteria, CFUs = Colony Forming Units, CCFUs = Chromogenic Colony Forming Units, CFUs as % AODCs = Colony Forming Units as Percentages of Acridine Orange Direct Counts.

correlated ($p<0.01$) with each bacterial variable. The regression analysis reveled that 16% of the variation in the abundance of Direct Count
Free Living

Bacteria was due to NO_3 (+) in the ground water of Agri-culture area, 18% due to NO_3 (+) in the ground water of Domestic area and 6% due to Air temperature (+) in the ground water of Industrial area, whereas, 19% variation in the abundance of Direct Count Particle Bound Bacteria was due to TSS(+) in the ground water of Agriculture area, 10% due to Air temperature in Domestic area and 10% was due to TSS (+) in the ground water of Industrial area. The 15% of the variation in abundance of total bacteria was due to water temperature (+) in ground water of Agriculture area, 17% due to NO_3(+) in the ground water in Domestic area and 9% in Industrial area due to water Temperature(+). The abundance of TB and FLB were affected by more number of many physico-chemical parameters and the extent to which they affected it was also more, when compared to the abundance of PBB. Furthermore, 9% variation in the concentration of CFUs was due to Alkalinity in the ground water of Agriculture area, 13% due to SO_4 (+) in the ground water of Domestic area and no physico-chemical

Table 6a. Results of stepwise multiple regression between Heterotrophic Bacterial and Physico-chemical parameters in the groundwater of Agriculture area, February 2005 to January 2007.

Heterotrophic Bacterial parameters	Physico-chemical parameters
FLB	NO_3 (+), (R^2= 0.16, F= 4.77, P<0.05), PO_4(+) , W Temp(+), Cond(+)
PBB	Rainfall (+), (R^2= 0.19, F= 8.34.85, P<0.05), L PH(+), HD(+),Ca(+)
TB	W Temp(+), (R^2= 0.15, F= 4.02, P<0.05), NO_3 (+),PO_4(+), Cond(+)
CFUs	ALK(-), (R^2= 0.09, F= 4.81, P<0.05)

Table 6b. Results of stepwise multiple regression between Heterotrophic Bacterial and Physico-chemical parameters in the groundwater of Domestic area, February 2005 to January 2007.

Heterotrophic Bacterial parameters	Physico-chemical parameters
FLB	NO_3 (+), (R^2= 0.18, F= 7.07, P<0.05), SO_4(+), TASA(+),PO_4(+), Cond(+), Mg(+),TDS(+),TS(+),Cl_2(+), Ca(+)
PBB	A Temp(+), (R^2= 0.10, F= 5.11, P<0.05)
TB	NO_3(+),R^2= 0.17, F= 6.42, P<0.05), SO_4(+), TASA(+), PO_4(+), Mg(+), Cond(+),TDS(+),TS(+), Cl_2(+), Turb(-), CO2(+)
CFUs	SO_4 (+), (R^2= 0.13, F= 4.19, P<0.05), Turb(-), NO_3(+), TASA(+)

Table 6c. Results of stepwise multiple regression between Heterotrophic Bacterial and Physico-chemical parameters in the groundwater of Industrial area, February 2005 to January 2007.

Heterotrophic Bacterial parameters	Physico-chemical parameters
FLB	A Temp(+), (R^2= 0.06, F= 3.41, P<0.05)
PBB	TSS(+), (R^2= 0.10, F= 5.21, P<0.05) TASA(-), BOD(-), HD(-),Ca(-), TDS(-)
TB	W Temp(+), (R^2= 0.09, F= 4.11, P<0.05)

Physico-chemical parameters (independent) in the final regression equation (P in = 0.05, P out 0.1) are shown: multiple coefficients of determinations (R^2) and over F and P values for each equation are given in the parenthesis. Physico-chemical parameters which were not in the final equation but are correlated (P<0.05) with the relevant microbial variable listed in order of decreasing magnitude of correlation coefficient; the sign of the correlation is indicate in the parenthesis. * Correlation is significant at the 0.05 level, **Correlation is significant at the 0.01 level.

Physico-chemical parameters are F pH = pH measured in the field, L pH= pH measured in the laboratory, A Temp = Air temperature, W Temp= Water temperature, Cond = Conductivity, Turb = Turbidity, R.fall = Rainfall, DO = Dissolved Oxygen, BOD = Biological Oxygen Demand, COD = Chemical Oxygen Demand, CO_2 = free Carbon dioxide, ALK = Alkalinity, HD = Hardness, Ca = Calcium, Mg = Magnesium, PO_4 = In-Organic Phosphate, Cl_2= Chloride, NO_3= Nitrate, SO_4= Sulphate, TASA = Total Anions of Strong Acids, TS = Total Solids, TDS= Total Dissolved Solids, TSS= Total Suspended Solids, F = Fluoride.

Mirobial (Bacterial) parameters are FLB = free Living Bacteria, PBB= Particle Bound Bacteria, TB = Total Bacteria, CFUs = Colony Forming Units, CCFUs = Chromogenic Colony Forming Units, CFUs as % AODCs = Colony Forming Units as Percentage of Acridine Orange Direct Counts.

Note: only those parameters, which have significant correlations, are given in the tables.

parameters showed correlation and regression with CFUs in the ground water of Industrial area. There was no CCFUs growth in any of the ground water from the three Agriculture, Domestic and Industrial areas in both the years. Furthermore, no physico-chemical parameters showed correlation and regression with in CFUs as AODCs in the ground water of Agriculture Domestic and % Industrial areas during study period.

DISCUSSION

Bacteria are the most abundant and the most important biological components involved in the transformation and mineralization of organic matter in the Biosphere. Heterotrophic bacteria contribute to the cycles of nutrients and carbon in two major ways: by the production of new bacterial biomass (secondary production) and by the remineralization of organic carbon and nutrients. The increased bacterial abundance during summer season may be due to the increased temperature - similar observation was reported by Jonas and Tuttle (1990). High bacterial abundance in the ground water of Domestic area when compared to other areas in this study, my be due to the less depth of the ground wa-

ter in the study area, where the accumulated material is more due to less effectiveness of filtration process to remove them through soil layers as reported by Strayer (1994) or may be due to the availability of nutrients (Wright et al., 1983, 1984; Shiah, 1993; Wommack and Colwel, 2000) and also due to the effect of the distance of this sampling site from the pollutants source. However, less bacterial abundance in Industrial and Agriculture areas may be due to the location of these sampling sites far away from the pollutants source similar findings were reported by Marxsen (1981), Harvey et al. (1984), Harvey and George (1987) and Godsy et al. (1992).

The increased abundance of PBB in the ground water, especially in rainy season may be due to increase in the turbidity which carried more abundance of PBB during rainy season and this is in agreement with Byron et al. (1998) who studied the dominance of PBB in the Columbia River estuary, USA,.

Colony Forming Units (CFUs) represent the number of microbes that can replicate to form colonies on artificial nutrient media, determined by the number of colonies developed. It is an indirect approach used for enumerating microorganisms (Atlas and Bartha, 1998). It does not, however, give an indication of the types of organisms present or their sources. The abundance of Colony Forming Units (CFUs) was more in the ground water of Domestic area followed by Industrial area, suggesting, probably, that the natural habitat in both these areas is not favorable for normal replication of microbes.

The proportion of Chromogenic CFUs (% of CCFUs) was assayed as a potential indicator of stress, since increase in the proportion of pigmented colonies has been related to unfavorable acid conditions in upland streams (Goulder, 1989; Simon and Jones, 1992). The absence of CCFUs in all samples may be due to the non acidic condition of these ground waters. Similar findings have been reported by Goulder (1988); Simon and Jones (1992), Yamakanamardi (1995) and Harsha et al. (2006). CFUs as the percentage of AODCs or percentage of culturable bacteria represent the ability of directly counted bacteria to cultivate on the artificial nutrient medium. As reported by Perry and Staley (1997) in oligotrophic and mesotrophic aquatic habitats, only less than 1 percent of the total bacteria can grow on the best artificial media (Maki et al., 1986; McCoy and Olson, 1986; Servais et al., 1992; Wagner et al., 1993). It should also be noted that the results obtained using this test are not an accurate assessment of total heterotrophic concentrations, instead, are indications of culturable organisms present. However, in this investigation, as much as 2.79% (in Agriculture area) and 3.60% (in Industrial area) of total bacteria could be grown on the artificial nutrient agar media, whereas, the bacterial found growing more (4.96%) in the artificial nutrient agar media (Table 1).

Apart from this, the other physico-chemical parameters such as F pH, L pH, Turbidity, CO_2, Chloride, TASA, Sulphate, Hardness, Calcium, magnesium, Alkalinity, TDS, TS, TSS, BOD, Conductivity, Air and water Temperature, Rainfall and Nitrate also entered in the regression equation and thus participated in deciding the abundance of bacterioplankton. Similar observations were reported by Anesio et al. (1997), Mohamed et al. (1998); Kirschner et al. (1999), Mitsuru et al. (2000); Lindstrom, (2001); Heidelberg et al. (2002) and Castillo et al. (2004). However, this study revealed that a more number of environmental factors in the ground water of Agriculture and Domestic areas and less number of environmental factors affected the bacterial abundance in the ground water of Industrial area. Hence, it can be concluded that abundance of bacterioplankton in all these ground waters studied may have been largely controlled by Physico-chemical parameters, which is in agreement with numerous other such studies (Baker and Farr, 1977; Goulder, 1980; Goulder, 1986; Marxsen, 1980; Nuttall, 1982a, 1982b; Roszak and Colwell, 1987; Gocke and Rheinheimer, 1988; Toolan et al., 1991; Unanue et al., 1992; Madsen and Ghiorse, 1993; Yamakanamardi and Goulder, 1995; Mohamed et al., 1998; Ludvigsen et al., 1999; Pedersen, 2001; Castillo et al., 2004).

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