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ORIGINAL ARTICLE

Microbial Evaluation of Groundwater and its Implications on Redox Condition of a Multi-Layer Sedimentary Aquifer System

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Abstract A synoptic microbial assessment of groundwater has been carried to describe the groundwater quality and its geochemical environment in the Pondicherry region located in the southeast part of India. Spatial and vertical variability in dissolved oxygen (DO) and chemical species clearly explain that regional geological settings associated with human pressure are controlling the groundwater quality in this region. Results show that the abundance of bacteria is positively related to DO, total organic carbon (TOC) and inversely related to well depth, whereas sulphate reducing bacteria (SRB) present at some locations do not show any systematic trend with the above parameters. About half of the measured groundwater samples showed presence of *E. coli*. Some suboxic deep aquifers (DO≤2 mg/L) showed presence of E.coli which are not commonly observed. SRB are found mostly in deeper Tertiary and Cretaceous aquifers reflecting presence of reducing conditions. The rapid accumulation of organic matter associated with heterotrophic conditions has increased the prevalence of redox sensitive SRB in coastal zones of Pondicherry region. Reducing conditions in deeper aquifers of Cretaceous and Tertiary formations and availability of Marcasite in these formations may lead to possible arsenic contamination in groundwater. Climate change induced sea level rise

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and human pollution may exacerbate the nutrient and microbial pollution in this coastal region, and this warrants a detailed investigation so that proper protection strategies for sustainable management of the groundwater resources along the Indian coastal zone can be proposed.

Keywords Microbial contamination · Dissolved oxygen · Sulphate reducing bacteria · Groundwater · Pondicherry

1 Introduction

Groundwater investigations have mainly focused on supplies with little emphasis given to its microbial quality. In the past, the assumption prevailed that much of the bacteria that contaminated surface water could be absorbed or retained by soils through filtration and leave groundwater free from contamination. This notion has been refuted by continuing outbreaks of waterborne diseases in India and abroad (e.g., Vignesh et al. 2015; Paruch et al. 2014; Anderson and Bohan 2001; Craun et al. 1997; Pathak et al. 1991; Vaidya et al. 2001; Walsh 1990).

Groundwater contains different types of micro-organisms, including viruses, bacteria and parasites. Fecal pollution of waters is of special concern since the most important bacterial gastrointestinal infections in humans, viz., cholera, salmonellosis and shigellosis, are primarily transmitted by water polluted with feces of infected persons (Grabow 1996; Scott et al. 2003). The most important aspect of water quality is its lack from contamination with fecal matter. The higher the level of fecal contamination, the greater is the risk of water-borne diseases (Pipes 1981). Microbiological assessment of the groundwater can significantly contribute to identify microbial contamination of the water, so that potential danger of health risks can be averted by taking proper remedial actions such as water treatment and decontamination (Stein et al. 2010). Besides addressing water quality issues, microbial evaluation of groundwater is also crucial in understanding the geochemical behavior of the aquifer. In recent times, it is becoming increasingly evident that microbial evaluation plays a significant role in characterizing the inorganic processes affecting the leaching and transport of toxic elements in deeper subsurface horizons (Hesham et al. 2009; Mohamed and Sonja 2008). Proper evaluation of the microorganisms is also important for any geochemical modeling (Lotta and Karsten 2008) to address the fate of pollutants.

In India, despite several laws and regulations endorsed by agencies like Environmental Protection Agency, Central & State Groundwater Boards and the Pollution Control Boards, sewer outflows and discharges of the partially treated and untreated wastewater from septic systems and storm water runoff from urban and rural areas are still major sources of pollution of drinking waters. Investigations carried out in some parts of the country pointed out contamination by fecal matter (Kumar and Dube 1985; Mohan et al. 2000; Venkateswaran and Natarajan 1987). According to a report by Planning Commission, India (2002), the risk of water contamination resulting in water-borne disease is higher in rural areas due to ill-maintained water pipelines and sewer lines, lack of disposal of human, animal and household wastes, and lack of awareness of good sanitation and personal hygienic practices. Statistics show that the poor sanitation and hygiene practices in India have led to increased diarrheal problems in the order of 22 million annually (Shankar et al. 2011). Like many other coastal urban regions, Pondicherry has undergone rapid growth in the last few decades and is endowed with substantial water resources due to a wide network of ponds and two rivers (Gingee and Pennaiyar) that flow into the Bay of Bengal through this region. About 90 % of the Pondicherry region comprises alluvial aquifers with fairly good water quality and shallow water levels in the wells ranging between 12 and 14 m below ground level (bgl). Agriculture is the dominant land-use and groundwater contributes 80 % of water requirement. The groundwater exploitation has been increased exponentially in the recent years to meet increased population demands (Thangarajan and Thyagarajan 2003), which has resulted in a substantial decline in groundwater table over the last decade in the range of 15 to 30 m in the west and about 7 m in the eastern part of Pondicherry (D'ozouville et al. 2006).

Recent reports suggest that global warming has resulted in extreme climate events, which coupled with sea level rise synergistically will influence the quantity and quality of both surface and sub-surface water in coming years. Climate change induced global sea level rise is projected to increase from 9 to 88 cm (Church 2001) and 50 to 140 cm (Rahmstorf 2007) by 2100 under a wide variety of greenhouse gas scenarios. Similarly, in-situ and remote sensing analyses exemplify that anthropogenic warming increase sea level rise along the Indian coast (Han et al. 2010), at the rate of 1.06–1.75 mm/year (Unnikrishnan and Shankar 2007). It is also projected that human population density along the coastal zone will be increased by ~50 % by the end of 21st century and indeed degrade both the surface and sub-surface water by high nutrient loads (NRC 2000). The regional ecological models predict that terrestrial nutrient inputs will be increased by several folds over the next several years due to drastic changes in land-use (Foley et al. 2005) and will have remarkable effects on the coastal water quality (Seitzinger et al. 2010). Similar observations were also noticed in the study area. Climate change induced sea level rise and salt water intrusion have also significantly impacted the groundwater quality in coastal Pondicherry region (Gilles 2005). Increasing human pressure alters the hydro-chemical and microbial properties of water, which in turn can be used to determine the impact of anthropogenic activities on the groundwater systems in the Pondicherry region. Hence, an integrated hydrochemical and microbial assessment of groundwater has been carried out to evaluate the microbial quality and the possible sources in different aquifers of coastal Pondicherry region.

The coliform bacteria (Escherichia coli, *E. coli*) were enumerated for the assessment of the fecal contamination of groundwater from three major aquifers of the region viz., Quaternary Alluvium, Tertiary and Cretaceous sedimentary formations in the Pondicherry region. In addition, an assay of total microbial loads (total viable counts- TVC) in different aquifers, distribution of *E. coli* and TVC laterally and vertically, correlations with dissolved oxygen, total organic carbon were also carried out. An attempt was also made to characterize the geochemical condition of the deeper aquifers of this region using sulphate reducing bacteria (SRB). The hydrochemical and microbial data from this study would be highly useful to evaluate the anthropogenic perturbations on the regional water resources that would further facilitate planning of various schemes for better management and restoration of groundwater systems in coastal parts of India.

2 Study Area

The Pondicherry Region is located on the east coast of India forming enclaves within the Cuddalore District of Tamil Nadu. It is bounded by north latitudes 11° 45′ and 12° 03′ and east longitudes 79° 37′ and 79° 53′ (Fig. 1). The Bay of Bengal forms the eastern boundary of this region and, on the remaining sides, it is covered by lands of Cuddalore district. The region is divided into seven communes for administrative convenience, where a commune is the smallest administrative unit. The total area of this region is 293 km^2 and comprises 179 villages. Agriculture, vegetation and plantation together cover ~76 % of the total area, followed by settlement areas (~15 %), water bodies (~7.5 %), and the remaining part is barren

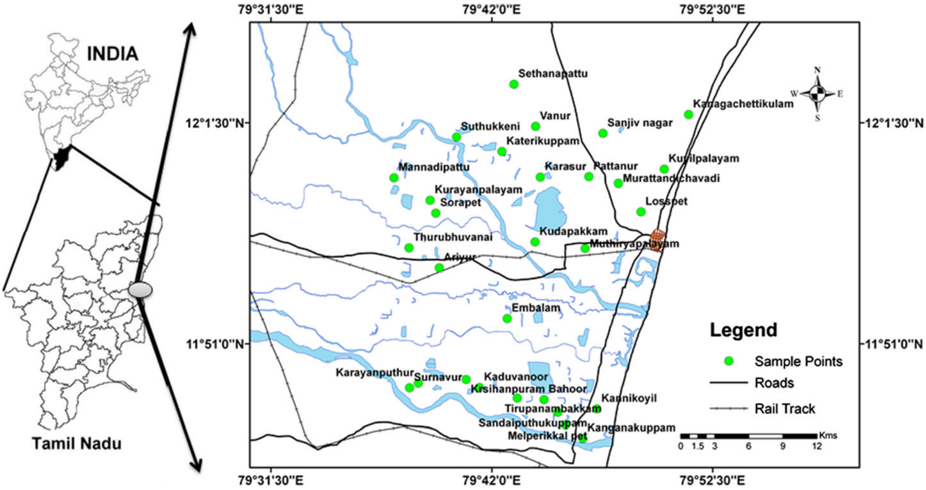

Fig. 1 Location of the study area with sampling points

land (~1.5 %). Industrial units are concentrated in and around Mettupalayam and Keerumampakkam areas. Due to lack of proper maintenance of irrigation tanks in Pondicherry region the entire load of irrigation relies on groundwater resources. Apart from this, the requirement of drinking water and industries is also being met by the groundwater resources.

This region is mostly covered by sedimentary formations ranging in age from Cretaceous to Recent. Among the seven different sedimentary formations in this region, three formations are found to be potential in terms of groundwater development, viz., Alluvium (Quaternary), Cuddalore sandstone (Tertiary) and Vanur-Ramanathapuram sandstone (Cretaceous). Quaternary formations in the region are represented by laterites and alluvium (CGWB 1993). Laterite occurs as thin cap over these formations. The thickness of the aquifer ranges between 5 and 34 m. Groundwater in this aquifer occurs under un-confined to semi-confined condition. The depth of wells tapping this aquifer ranges between 25 and 50 m bgl. Tertiary formations are predominantly calcareous sandstones, yellowish grey to dirty white in color, with thin lenses of clay and shale and bands of shell limestone. Thin seams of lignite also occur in these formations (CGWB 1993; Sukhija et al. 1987). Groundwater occurs mainly under confined condition and the piezometric levels range between 10 and 25 m bgl. Cretaceous formations comprise alternate layers of grey sandstone and carbonaceous-clay stone with thin seams of lignite and abundant marcassites. Groundwater in these formations occurs under confined conditions and the piezometric head at present is about 20 to 60 m bgl.

The topography of the area is a flat plain with an average elevation of about 15 m above mean sea level (amsl). The climate of Pondicherry region is humid and tropical. The mean monthly temperature ranges between 22 and 33 °C. The relative humidity varies between 70 and 80 % and is highest during December - January and lowest during June. The average annual rainfall in Pondicherry is 1254 mm. The region receives rainfall from monsoons spreading over a period of 7–8 months. The southwest monsoon brings 29 % of the annual rainfall from June to September and the North East Monsoon brings 63 % from October to December.

3 Sampling and Measurement

Water sampling was carried out during post monsoon period of 2009 from 29 wells tapping different depths from 10 to 185 m bgl and covering major aquifers. Prior to collection of samples, wells were purged until constant temperature was obtained. The water sample for microbiological analyses was collected in a sterile glass bottle, kept in airtight ice-cold containers and transported to laboratory within 6–8 h of their collection for further processing. For determination of reducing bacteria, sample bottles were flushed with nitrogen and sealed using Teflon to prevent contact with atmospheric oxygen. Acid-washed polyethylene containers were used for total organic carbon analysis. The location of the sampling sites is shown in Fig. 1.

3.1 Physical Parameters and Total Organic Carbon (TOC)

Physical parameters, like temperature, pH and DO, were measured in situ using portable pH/Temp – DO meter (Corning, model 313) with a precision of ±0.02 units and ±0.1 % for pH and DO, respectively. Total Dissolved Solids (TDS) measurements in mg/L were calculated by multiplying a factor 0.64 with electrical conductivity obtained from conductivity meter (Orion model 130) with a precision of ±0.5 %. Alkalinity was measured in the field by titration of 10 mL of water sample with 0.02 N H_2SO_4. A mixed indicator (Bromocresol green - Methyl red) was used to mark the end point of the reaction at pH 4.3. For total organic carbon measurements samples were acidified with HCl to a pH value of 3 immediately upon collection and stored at 4 °C in the dark until analysis. The measurements were performed with a SGE TOC analyzer (ANATOC II series). This is a closed loop technique and works on the principle of photo catalytic oxidation process occurring at room temperature in near UV region at 400 nm using atmospheric oxygen. The catalyst used is TiO_2 in the form of a packed column. The carbon dioxide thus formed is measured by non-dispersive infrared detection. The precision and the detection limit of the measurement were ±2 % and 0.025 mg/L, respectively.

3.2 Microbial Assays

3.2.1 Total Viable Bacteria (TVB)

The enumeration of microbial populations was accomplished by using the Total Viable Count (TVC) method. TVC was performed on a nutrient agar media by means of spread plate method. Dilution of the sample was made in the order of 10^{-3} and 10^{-4} using sterile saline, and one milliliter of diluted sample was transferred in to petriplates containing a molten agar medium (45 °C). The plates were incubated at 37 °C for 24 h to obtain viable colonies. Each test was duplicated and comparable results were averaged to reduce any errors related to the measurement. The viable colonies were counted and converted to represent colony forming units per mL (CFU /mL). A typical plate of bacterial colonies is shown in Fig. 2a.

3.2.2 Escherichia coli (E.coli)

A media containing violet red blue bile agar (VRBA) was used to detect the coliforms. One mL aliquot of undiluted sample was transferred on VRBA by pour plate method and incubated at 35 °C for 24 h. The positive colonies (purple red colonies with diameter 0.5 mm or more) were counted and expressed as CFU 100/mL. Figure 2b shows the presence of E.coli bacteria by characteristic purple colonies. The presence of *E. coli* was confirmed with an indole test.

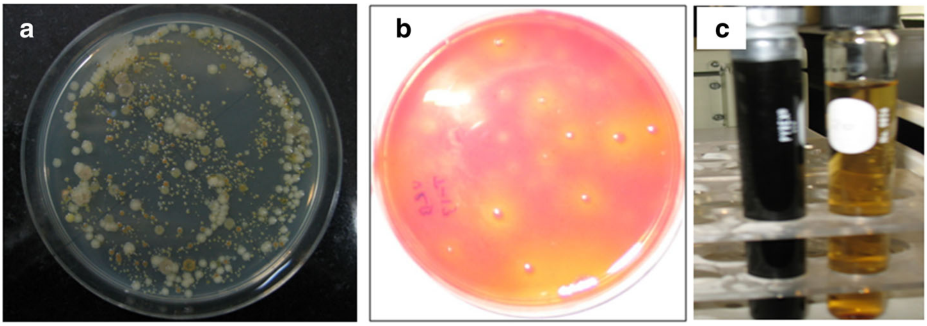

Fig. 2 Colony plates showing: **a** the diverse nature of bacterial communities; **b** *E. coli* with characteristic purple colored colonies; and **c** black coloration of FeS indicating presence of sulphate reducing bacteria

3.2.3 Sulphate Reducing Bacteria (SRB)

Sulphate reducing bacteria were grown in long necked bottles with the medium composition of tryptone (30 g), sodium sulphate (5 g), sodium sulphite (5 g) and ferric citrate (2 g) in 1 L distilled water (Ministry of Health 1939). The growth of a sulphate reducer is recognized by the appearance of the black color of ferrous sulphide (Fig. 2c). The medium used for the enumeration of SRB is prepared with the following composition: Yeast Extract (1), Ascorbic acid (1 g/L), $MgSO_4$ (0.2 g/L), K_2HPO_4 (0.01 g/L), Ferrous Ammonium Sulphate (0.1 g/L), NaCl (10 g/L), Agar-agar (25 g/L), Sodium Lactate (4 g/L). The pH of the medium was maintained at 7.0 by 1 N NaOH. The above medium was autoclaved at 15 lbs for 15 min. The medium was cooled to 40 °C and the samples were diluted ten times. Pour plate method was employed and the plates were allowed to solidify. Once the medium was set, the plates were stacked in an anaerobic jar and the jar was subjected to vacuum to remove the residual oxygen (−15 lbs). The jar was then filled with a mixture of gases (10 % CO_2, 5 % O_2 and 85 % N_2) till the gauge read zero. The jar was then incubated at 37 °C for 5 days. Black colonies displaying sulphur reduction were further subcultured on the medium mentioned above in slants and incubated in a similar manner. Plates where the count was prevalent but did not exhibit any black colonies were not considered.

4 Results

4.1 Chemistry of Groundwater

Physicochemical parameters along with microbiological data are given in Table 1. pH varied from 6.0 to 8.8 and most of the samples showed pH values within allowed limits of 6.5 to 8.5 (BIS 1995). A spatial variation map of pH is shown by Fig. 3a. Alkaline pH is observed in two samples (~pH 8.8) that are collected from shallow wells (depth ~15 m bgl); this high pH could be due to man-made activities. In contrast, a few deep zone samples show slightly acidic nature of water (pH 6.0–6.5). This acidic nature of water can result from subsurface chemical processes like oxidation of sulphide minerals or biological processes. The mineralization of the groundwater is generally represented by TDS. From Table 1, it is noticed that groundwater quality is good as the TDS values fall between 160 and 1000 mg/L and are within drinking water limits set by Bureau of Indian Standards (BIS 1995). There are two deep wells (depths

Table 1 Microbiological data of groundwater from Pondicherry area along with physical parameters and geological formation

Location	Well Depth	Temp.	pH	DO	TDS	TOC	TVC	*E.coli*	SRB	Formation
Ariyur	18	30.9	7.36	4.0	1100	1.62	3650	300	nd	Quaternary
Bahoor	180	31.7	6.66	1.9	401	bdl	3760	nd	560	Tertiary
Embalam	10	31.6	7.41	5.1	669	1.20	33000	110	nd	Quaternary
Kaduvanoor	50	30.4	8.12	4.9	993	0.62	18000	15	nd	Quaternary
Kanagachettikulam	91	31.1	7.31	2.2	1108	bdl	4300	25	nd	Cretaceous
Kanganakuppam	140	32.9	6.32	2.0	230	bdl	1800	20	nd	Tertiary
Kannikoyil	180	32.3	6.27	1.6	257	bdl	10800	nd	nd	Tertiary
Karasur	185	32.1	6.87	2.5	1404	1.31	4500	30	2300	Cretaceous
Karayanputhur	15	31.2	8.83	4.6	553	0.38	3100	60	nd	Quaternary
Katerikuppam	180	30.5	7.05	2.5	449	0.56	17600	nd	610	Cretaceous
Krsihanpuram	71	31.2	7.20	2.6	767	bdl	4800	nd	250	Tertiary
Kudapakkam	13	31.4	8.30	5.4	488	1.37	22000	nd	nd	Quaternary
Kurayanpalayam	91	31.4	7.22	2.0	797	1.25	2800	5	3100	Cretaceous
Kuyilpalayam	37	31.5	6.02	2.8	180	bdl	8000	nd	360	Tertiary
Losspet	100	31.1	6.35	5.0	694	bdl	9100	nd	nd	Tertiary
Mannadipattu	10	31.7	7.97	5.8	438	1.03	18000	nd	nd	Quaternary
Melperikkal pet	20	31.1	8.48	4.2	1235	1.89	3600	300	nd	Quaternary
Murattandichavadi	80	32.1	6.73	3.5	164	bdl	4500	15	nd	Tertiary
Muthiryapalayam	95	31.0	6.59	1.0	472	1.73	3950	180	nd	Tertiary
Pattanur	24	30.9	7.24	3.7	736	3.24	18800	nd	nd	Tertiary
Sandaiputhukuppam	16	31.1	7.65	4.3	767	0.15	4000	50	nd	Quaternary
Sanjivnagar	152	32.5	7.31	2.3	852	bdl	8000	nd	nd	Cretaceous
Sethanapattu	100	31.6	7.32	2.2	794	0.83	24600	10	580	Cretaceous
Sorapet	13	31.5	8.80	4.9	590	0.22	21600	10	nd	Quaternary
Surnavur	61	29.5	7.30	5.5	511	bdl	25400	75	nd	Tertiary
Suthukkeni	120	30.8	7.87	4.8	325	0.73	2700	nd	nd	Quaternary
Thurubhuvanai	20	31.2	8.19	5.3	662	0.86	2400	nd	nd	Quaternary
Tirupanambakkam	120	30.8	7.12	2.6	460	bdl	5600	50	1100	Tertiary
Vanur	180	31.1	7.34	4.0	625	2.05	30000	nd	260	Cretaceous

nd not detected, *bdl* below detection limits, well depth (m bgl), Temp. (°C), DO, TDS, TOC (mg /L), TVC, SRB (CFU/mL), *E. coli* (CFU/100 mL)

90 and 185 m bgl) and one shallow well (depth 20 m bgl) showing TDS values more than 1000 mg/L. This may be possibly due to leaching of salts from the formation in the case of deep wells and influence of lagoon water in the shallow well. From the spatial distribution of TDS (Fig. 3b), it can be observed that there is no systematic increase in TDS towards the seaside, indicating absence of seawater intrusion in these locations.

The dissolved oxygen concentration in water at equilibrium with air at 25 °C at 1 atm is 8.3 mg/L (Fifield and Haines 2000). Generally, in alluvial shallow groundwaters, the DO values are close to 8.3 mg/L. However, several biological and inorganic processes taking place in the subsurface may consume dissolved oxygen and deplete the DO levels. The water samples collected in the study area had DO between 1 and 6 mg/L and a spatial variation is

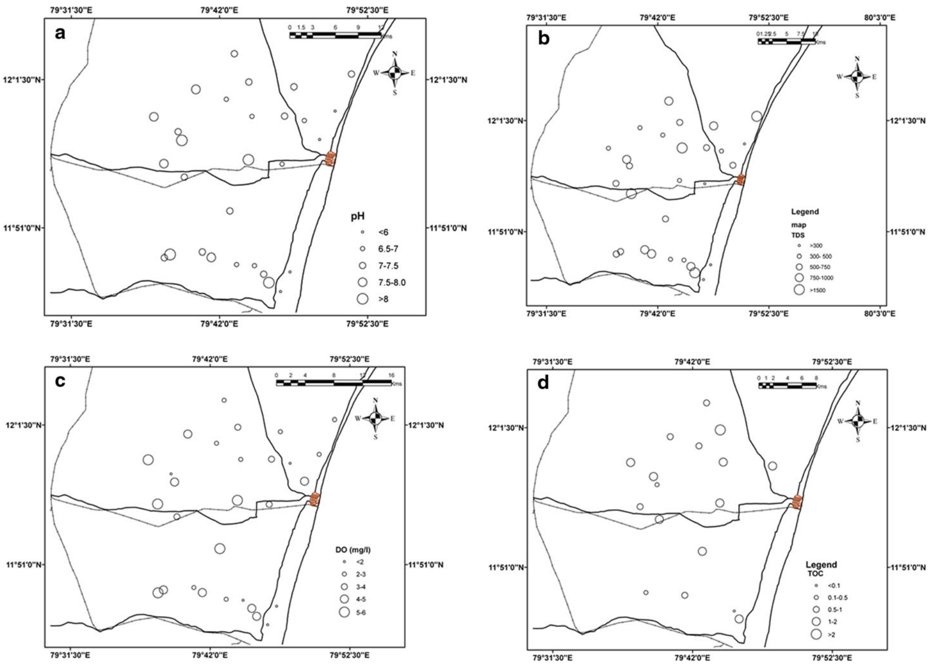

Fig. 3 Spatial distribution of: **a** pH; **b** TDS (mg/L); **c** Dissolved Oxygen (mg/L); and **d** total organic carbon (mg/L)

shown in Fig. 3c. It is surprising to note that even alluvial shallow wells that are supposingly at equilibrium with atmosphere contain DO levels between 3 and 6 mg/L. This lowering of DO can be attributed to the presence of ferruginous / lateritic soils in this region that consume oxygen during the conversion of ferrous to ferric ion. The equilibrium reaction can be represented by the following equation:

$$Fe^{2+} + 1/2\, O_2 + H^+ \leftrightarrow Fe^{3+} + 1/2 H_2O$$

Deep wells show depleted DO levels, which can be attributed to heterotrophic biological respiration. This depletion coincides with the high TOC concentrations in groundwater. Most of the groundwater tapping deep waters have DO levels close to 2.0 mg/L and can be considered to be under sub-oxic condition (Tyson and Pearson 1991). Although H_2S gas odor was very prominently felt during the field sampling, none of the samples showed complete depletion of DO (i.e., DO of 0 mg/L). This might be mostly due to contribution of water from the shallow zone through vertical leakage. Since these formations shows presence of Lignite lenses and Marcasite in the study area, it can be presumed that deposition of the sediments occurred during reducing condition (CGWB 1993). Stratigraphic studies also point to reducing condition of the sedimentary formation (Kennedy and Henderson 1992; Sundaram et al. 2001).

Organic carbon is generally used as an indicator of susceptibility of groundwater for bacterial contamination since it serves as a nutrient source for microbes (Katzenelson 1978). Organic substances originating either from the active soil zone or from the aquifer material dissolve in groundwater and lead to growth of fungi and algae. This is not a desired condition for groundwater being used for drinking. The organic substances generally include humic acids, hydrocarbons, fatty acids, etc. The dissolved organic matter is also contributed by the anthropogenic activities such as petroleum products or organic industrial wastes. All the well waters show TOC levels between 0.1 to 3.2 mg/L and relatively higher levels are observed in shallow wells. A general trend of TOC is shown in Fig. 3d. The low amounts indicate either absence of anthropogenic inputs to the groundwater system or consumption of the organic matter by microbes.

4.2 Microbes in Groundwater

The standard plate count (TVC) in the groundwater was in the range between 1.8×10^3 and 3.3×10^4 CFU/mL in different aquifers. The colonies were identified on the basis of their cultural characteristics on agar plates and microscopic observations. Five to ten different varieties of microbial communities with different morphology and strains of bacteria were observed. The distribution of TVC varied greatly among different wells, shown in Fig. 4a, which can be attributed to factors like chemical characteristics of the catchment area, extent and range of human activities and animal sources in the vicinity of the wells.

About half of the samples (16 out of 29) measured showed presence of *E. coli*, and four samples showed high values between 100 and 300 CFU/100 mL, i.e., they were severely contaminated. The limits set by Bureau of Indian Standards (BIS 1995) and World Health Organizations (WHO 2004) are given in Table 2. According to the WHO guidelines for drinking water quality there should be no trace of total or fecal coliforms present in 100 mL of water. Therefore, half of the groundwater samples measured can be considered as unfit for drinking. Spatial distribution of *E. coli* in the study area is shown in Fig. 4b. The presence of *E. coli* can be attributed to pollution sources such as waste dumps and sewages.

Some samples (9 out of 29) showed the presence of SRB in the range of 250–3100 CFU/mL. It was observed that these samples were mostly collected from wells tapping deeper horizons (>100 m bgl) with low DO levels. The presence of SRB indicates that groundwater is under low oxic or reducing condition in these locations. A spatial distribution map of SRB is shown in Fig. 4c. No sample from Quaternary alluvium showed the presence of SRB. All the samples tested positive for SRB belong to either Tertiary or Cretaceous sedimentary aquifers. Variation of SRB can be due to varying amounts of organic matter, dissolved sulphate and other sediment properties (Kirsten and Donald 1997; Westrich and Berner 1984). In marine sediments, organic acids derived primarily from fermentation serve as the SRB's main carbon sources (Tranvik 1992). These deeper formations (Cretaceous and Tertiary) were formed under marine influence (CGWB 1993; Kennedy and Henderson 1992; Sundaram et al. 2001), therefore, it is possible that the sediments may contain organic matter and could serve as energy sources for SRB.

5 Discussion

Increasing population density coupled with inadequate sewage treatment and disposal facilities in the urban parts of Pondicherry have put groundwaters of this region in a vulnerable position with respect to microbial contamination. On the other hand, improper management of tanks, unplanned dumping of agricultural and domestic wastes, lack of awareness of good sanitation and personal hygienic practices in the rural areas of this region made groundwater resources susceptible to microbial pathogens. Communicable diseases such as typhoid, paratyphoid, amoebic dysentery, bacillary dysentery, cholera and infective hepatitis were noticed in this region which can be attributed to fecal contamination of potable water. A wide range of pathogenic microorganisms can be transmitted via fecal contamination that include enteropathogenic agents (salmonellas, shigellas, enteroviruses, and multicellular parasites) as well as pathogens such as *Pseudomonas aeroginosa*, *Klebsiella*, *Vibrio parahaemolyticus* and

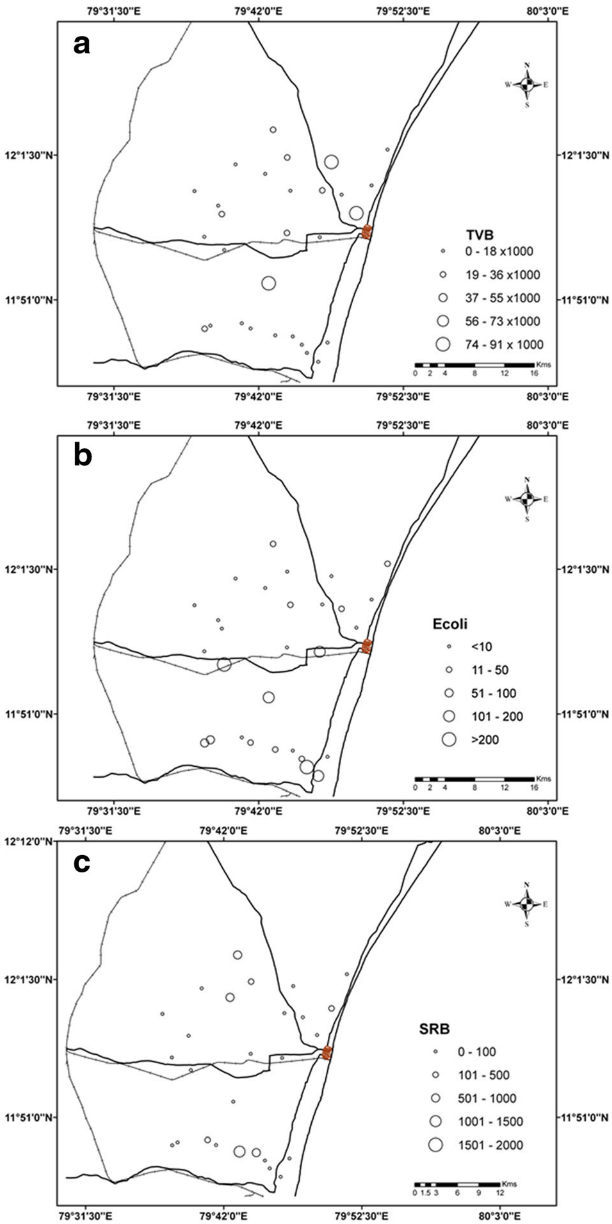

Fig. 4 Spatial distribution of: **a** TVB (CFU/mL); **b** *E. coli* (CFU/100 mL); and **c** SRB (CFU/mL)

Aeromonashydrophila (Hodegkiss 1988). The abundance and diversity of microorganisms are useful to evaluate the suitability of water for drinking purposes (Okpokwasili and Akujobi 1996). However, it is not practicable to test water for all these organisms as the isolation and identification of many of these organisms are extremely complicated (Cairneross et al. 1980). An indirect approach is to assess the coliform bacteria, which are most widely used as indicators of fecal contamination (Kistemann et al. 2002; Pathak and Gopal 2001; Vaidya

Table 2 Contamination status of drinking water on the basis of *E. coli*

E. coli CFU/L	Water pollution status-WHO 2004	Water pollution status-BIS 1995
10,000	Heavily polluted	Polluted
1000	Polluted	Polluted
100	Slightly polluted	Polluted
10	Satisfactory	Acceptable
3 or less	Potable	Excellent

et al. 2001). Thus, the presence of *Escherichia coli* in water provides evidence of excreta of fecal pollution of human or animal origin.

In the study area, it is found that the non-disinfected groundwater has high bacterial loads (TVC: 1.8×10^3 to 3.3×10^4 CFU/mL) and presence of *E. coli* in half of the analyzed samples. No correlation is found between TVC and *E. coli* amounts, indicating that *E. coli* are not always present as a component of bacterial load, even when total bacterial numbers were high for a given sample. This would suggest that the observed total bacterial may have originated from environmental sources, such as soil/organic debris, and not from direct fecal contamination. However, some samples showed *E. coli* counts more than 100 CFU/100 mL, indicating severe contamination. High variation in *E. coli* could be due to the presence of varying amounts of organic content or clayey nature of the sediments or longer residence times of the groundwater, which can be envisaged from the highly heterogeneous nature of sediments in this region as mentioned in the geology of the region (section 2). It has been reported by several researchers that bacteria, especially fecal indicator bacteria, attach to finer particles in the aquifer and could lead to bacterial contamination (Pronk et al. 2007). Organic matter and other nutrients (NH_4^+, NO_3^- and PO_4^{3-}) present in water can act as potential energy source for bacterial growth. Dissolved organic matter in water has also been considered as energy source for bacteria. Clark et al. (2003) also suggest that the high organic content and the clayey nature of sediments favor bacterial growth. Despite the low fecal contamination observed, continuous consumption of contaminated water may pose serious health risks, such as urinary tract infections, pulmonary infections, abscesses and skin-wound infections, to local residents of this areas, especially children.

The production and proliferation of microbes depend on the favorable conditions prevailing in the nearby surroundings, e.g., the presence of dissolved oxygen (Jansons et al. 1989), water physicochemical characteristics such as pH, temperature, salt content and organic carbon contents (Katzenelson 1978), and water source (Melnick and Gerba 1980). In the present case, effects of DO and TOC on bacterial contents were evaluated. *E. coli* and SRB amounts were plotted against corresponding DO levels. It can be seen from Fig. 5 that, in general, *E. coli* show an increasing trend with DO whereas SRB do not show any particular trend with DO. SRB do not degrade polysaccharides, proteins or lipids, but depend on the activity of fermentative bacteria for the supply of energy sources. A number of complex organic carbon sources have been considered as energy sources for biological sulphate reduction, such as, organic effluents (Boshoff et al. 2004), micro-algal biomass (Nedergaard et al. 2002). Therefore, it is possible that the SRB trends are impacted either by the presence of fermentative bacteria or by the availability of energy sources or by combined factors.

One deep well sample showing high DO with presence of SRB might be due to vertical leakage in the well. *E. coli* were also found to be present in low DO groundwater, which indicates that they are anaerobic or microaerophilic in nature. This condition of low dissolved oxygen can considerably extend the survival of these microorganisms in water, which can act as potential reservoirs for pathogens of fecal origin (Roslev et al. 2004).

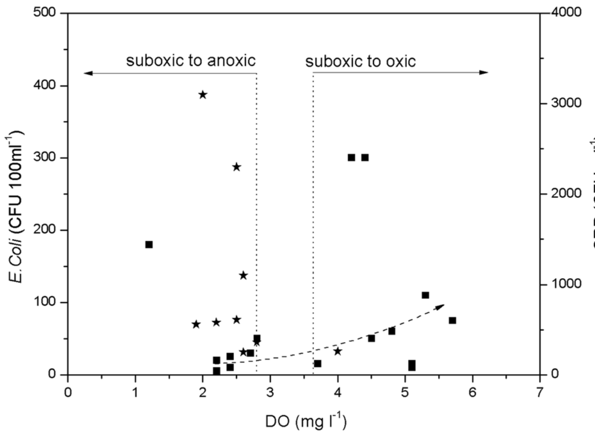

Fig. 5 Scatter plot of DO versus *E. coli* and SRB. The *dashed arrow* is a trend line to *E. coli* data

The *E. coli* show a positive correlation with TOC, whereas SRB do not show any particular trend, they are mostly present in samples with low TOC and are also spatially variable (Fig. 6). Association of coliform contaminants with organic matter was also noted by other researchers (Percival et al. 2000). In general, it is observed that the diversity and numbers of microorganisms diminish with depth from the surface of the soil to the rock strata below the aquifer. This is because, with increasing depth, the environment becomes increasingly hostile for life (Bitton and Harvey 1992). A decreasing trend was seen in the case of *E. coli* with well depth, whereas no such trend was observed in the case of SRB (Fig. 7).

The geochemical environment of the aquifer can be assessed by parameters like redox potential and dissolved oxygen; however, these parameters may be affected by sampling errors due to exposure to the atmosphere leading to erroneous numbers. Bacterial sulphate reduction is one of the most important respiration processes in anoxic habitats; therefore, the presence of SRB can be considered as a tool to indicate the redox state of the aquifer (Muna et al. 2007). Reducing bacteria, such as SRB, has been used very commonly by researchers to understand

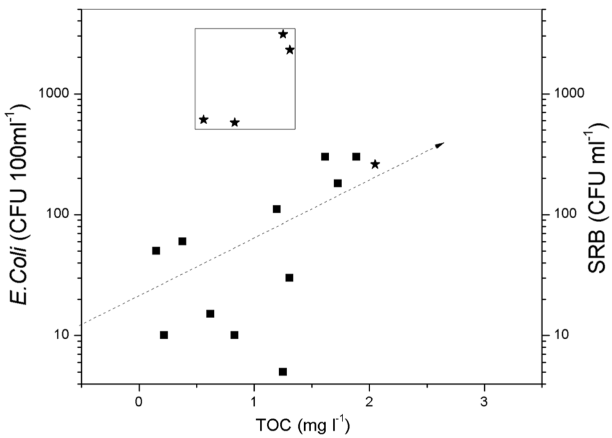

Fig. 6 Scatter plot of TOC versus *E. coli* and SRB. The *box* indicates the cluster of SRB data and the *dashed arrow* is a trend line to *E. coli* data

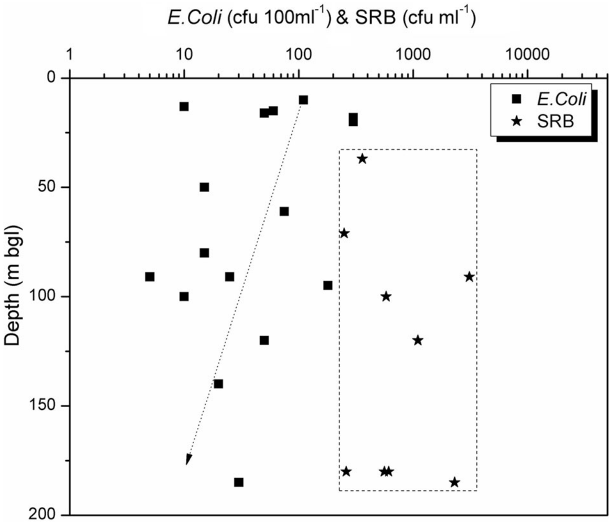

Fig. 7 Depth profiles of *E. coli* and SRB. The *box* indicates the cluster of SRB data, and the *dotted arrow* is a trend line to *E. coli* data

the fate of toxic metals and long lived radio nuclei in deep geological strata (Lotta and Karsten 2008). Presence of marcasite, which is a polymorph of pyrite, and reducing conditions in the deeper horizons of this coastal aquifer render the groundwater system vulnerable to arsenic contamination. However, it has also been reported that SRB can act as decontaminant by precipitating the dissolved metals as metal sulphides, or by incorporating the toxic metals into sulphide minerals, or by adsorption onto mineral surfaces (O'Day et al. 1998). For example, it is found that the SRB-produced iron sulphide had a considerably higher specific uptake capacity for different metal ions from solution than other adsorbents, such as activated carbon (Watson et al. 1995). Under anaerobic conditions, SRB can transform sulphate to hydrogen sulphide using simple organic substrates that can have importance in bioremediation of pollutants. Based on this, SRB use in water purification has now been commercialized (Chang et al. 2000). Assessment of reducing bacteria is also of great significance for abatement of metal pollution caused naturally or by manmade activities like mining, effluent discharge etc. This region being industrially well developed renders surface water bodies vulnerable by dumping high loads of toxic compounds, which subsequently infiltrates and pollute the groundwater. In addition, increase in sea-level also has a direct bearing on submarine groundwater discharge (SGD) dynamics and can strongly disturb the natural SGD flows, and hence, the proportion of nutrients and organic matter loadings going into the sea. This imbalance can further exacerbate the microbial pollution in groundwater (Paytan et al. 2004). All these observations and inferences obtained from this study stress the need to conduct investigations on quantification of SGD and its nutrient and organic matter loads to elucidate the responses of this coastal Pondicherry groundwater system to both natural and anthropogenic perturbations.

6 Conclusions

Compared to the surface water systems, the groundwater aquifer systems received less attention from an ecological point of view. Similar to surface water, the groundwater aquifer systems are highly vulnerable to both climate change and anthropogenic influences. Indiscriminate management of agricultural and industrial effluents increases the vertical percolation of chemical contaminants and organic matter to the deep aquifers. This study attempted to describe the microbial pollution in relation to other chemical properties of groundwater from the coastal Pondicherry region, India. The study indicates that the groundwater being used for potable purposes contains a wide variety of micro-organisms with loads varying from 1.8×10^3 to 3.3×10^4 CFU/100 mL in various aquifers. Even though all the microbes do not fall under fecal origin, half of the samples analyzed indicated presence of *E. coli* which is of fecal origin. *E. coli* showed positive correlations with DO and TOC while an inverse trend was noticed with the well depth. Sulphate reducing bacteria were found in few locations of deeper Tertiary and Cretaceous formations and DO levels were found to be very low. Presence of SRB is indicative of reducing condition of groundwater in the aquifer. Unlike *E. coli*, SRB do not show any systematic trend with DO, TOC and well depth. This study stresses the need for microbial evaluation of groundwater which can provide crucial information on the suitability for drinking source identification of fecal contamination and most importantly the redox condition of the aquifer. The possibility of in situ trace metal decontamination by selective reducing bacteria can also be inferred from this kind of study.

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