Microbiological quality of drinking water in Amarja reservoir catchment, Aland taluk, Karnataka, India

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Geogenic and anthropogenic activities are the main reasons for the deterioration of groundwater quality. Different kinds of microbial pathogens present in drinking water are responsible for gastrointestinal and waterborne diarrhoeal diseases. The present study estimates the microbial quality of drinking water of Amarja reservoir catchment in Aland taluk, Karnataka, India. Thirty-two water samples were taken from different villages in the study area. Microbiological parameters like *Escherichia coli* and coliform bacteria, *Pseudomonas aeruginosa*, sulphite-reducing anaerobes and aerobic plate count at 37°C was analysed. The results showed that 18 of the 32 samples were positive for *E. coli* and total coliform, 17 samples for *P. aeruginos*a and 18 samples for sulphite-reducing anaerobes. Moreover, six samples were positive for both *E. coli* and sulphite-reducing anaerobes, whereas five samples were positive for sulphite-reducing anaerobes and *P. aeruginosa*.

Keywords: Aerobic plate count, drinking water contamination, *Escherichia coli*, *Pseudomonas aeruginosa*, sulphite-reducing anaerobes.

NOWADAYS, it has become a challenge to provide safe drinking water[1]. Contamination of groundwater resources is mainly responsible for the deterioration of its quality[2,3]. Microbiological pathogens are the most dangerous pollutants in drinking water[4,5]. Most of the waterborne diseases are directly related to microbiological contamination that is largely based on the need to identify the indicators of pollution[4–7]. Therefore, microbiological analysis has become an important step for detecting microbial pollutants in drinking water. Separating the pathogens growing in the intestinal tract of humans and animals in the laboratory is a challenging task. Viruses, bacteria and protozoa are the major causes of harmful diseases. The influence of diarrhoeal disease in human beings can be minimized with good sanitation, fairish dumping of human beings' and other vertebrates' dropping[1]. Enterobacteriaceae is the most common family of microorganisms that contaminates drinking water; it includes common species like *Escherichia coli*[1].

The analysis of physical parameters of water includes determination of pH, total dissolved solids (TDS), electrical conductivity (EC) and turbidity. The pH value represents the hardness of water. Turbidity represents the limpidity of water in terms of individual particles, organic matter, plankton, etc. TDS is a measure of total ions in solution. The ratio between the current density and electric field intensity is known as electrical conductivity.

Coliform bacteria are generally Gram-negative with a rod-shaped structure[5]. They belong to the genera *Klebsiella*, *Enterobacter*, *Citrobacter* and *Escherichia*[2]. According to modern taxonomical methods, this group is heterogeneous in nature[1]. These bacteria have been categorized as total coliform, faecal coliform and *E. coli*. Bacteria which belong to the total coliform family generally exist in soil or vegetation. The source of total coliform bacteria could be faecal or environmental befoul. Faecal coliform is a subgroup of the total coliform group of bacteria that exist in the intestines and faeces of humans and animals[6]. *E. coli* is a subgroup of faecal coliform bacteria. The existence of these bacteria in drinking water are very harmful. They grow in a timespan of 12–72 h with temperature variation between 30°C and 37°C under favourable conditions.

P. aeruginosa is also a Gram-negative bacterium, which can be distinguished by its Gram-morphology with the capability to grow at 42°C. Fluorescence under ultraviolet light is helpful in early identification of *P. aeruginosa* colonies. The incubation period of *Pseudomonas* bacteria is noticed from 24 to 72 h. The nature of *P. aeruginosa* infection can be identified by isolation and laboratory investigation of pathogens. The treatment of infections due to *P. aeruginosa* has become difficult, as it resists the common antibiotics. However, the intermixture of gentamicin and carbenicillin sometimes reduces *Pseudomonas* infections.

Sulphite-reducing anaerobes are Gram-positive and free-living bacteria. They may be found in the intestinal tract of humans, decaying vegetation, marine sediments and also in a few other vertebrates. They can live in the water for a longer time[8]. Sulphite-reducing anaerobes can reduce down sulphite to sulphide at 37°C within 24 h. The most common species of sulphite-reducing anaerobes is *Clostridium perfringens* that includes several significant pathogens.

Aerobic plate count (APC) is a method used to determine the level of the microorganisms (bacterial populations) in drinking water. APC serve as a good indicator in the efficacy of different processes responsible for the treatment of drinking water like disinfection, coagulation and filtration[1]. APC are used in the measurement of level of sanitary quality and organoleptic properties. The acceptable limit of aerobic colony count is 20 cfu/ml at 37°C. The microorganisms occur in drinking water as single-cell structures, pair colonies, group structures, chain structures and dense bunches. Each individual free-living bacterium does not grow in a different colony on incubation. Therefore, the number of colonies which

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Table 1. Norms for microbiological parameters according to BIS (IS-14543)

Parameters	BIS code	ISI unit
Escherichia coli	IS 5887–1976 (Part 1)	Absent
Coliform bacteria	IS 5401–2002 (Part 1)	Absent
Sulphite-reducing anaerobes	Annex C of IS-13428	Absent
Pseudomonas aeruginosa	Annex D of IS-13428	Absent
Aerobic microbial count	IS 5402–2002	20 cfu/ml at 37°C in 24 h
Turbidity (NTU)	IS-3025 (Part 10)	< 2 units
pH	IS-3025 (Part 11)	6.5–8.5
Total dissolved solids (TDS)	IS 3025 (Part 16)	<500 mg/L

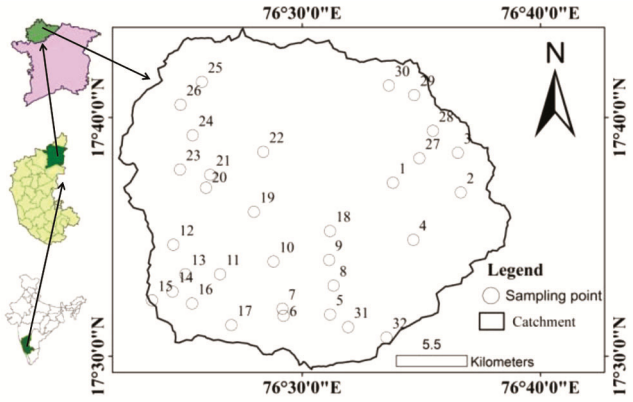

Figure 1. Sampling location of Amarja Reservoir Catchment.

occur on the plate does not indicates the total number of bacteria in the test volume of a sample[1].

The Amarja reservoir catchment covering an area of 544.76 sq. km in Aland taluk, Karnataka lies between 17°50′9″–17°72′20″N lat and 76°45′8″–76°55′20″E long (Figure 1). The area falls under semiarid region of the Deccan plateau (basalts). The rocks are composed of soft and hard lava flows whose weathering has produced flat-topped hills and terrace-like features. Ridges, pediments, flat and undulating landscape are the main structures in the area. The highest contour of 600 m elevation is at the northern part, while the lowest contour of 475 m is at the southern part of the catchment. The area contains deep black soils generally derived from Deccan traps. The summer season is from February and June. The southwest monsoon is from June to September. December is the coldest month having a temperature variation from 29.5°C to 10°C. In the peak of summer, temperature can rise up to 45°C. The relative humidity is about 26% in summer and increases up to 62% in winter. The area receives an average annual rainfall of about 777 mm while the annual minimum rainfall is 342 mm and the annual maximum rainfall is 1270 mm (ref. 9).

A total of 32 samples were collected from different locations in the study area. These samples were further analysed according to the norms suggested by BIS-14543 (Table 1) for the different microbiological and physical parameters.

For sample collection, narrow-mouth, high-density, stainless steel bottles of 1 litre capacity were utilized. The bottles were sterilized using an autoclave. Sterile rubber gloves and face mask were used while collecting the samples. The sampling point (tap, pipe, etc.) was sprayed 4–5 times with 70% alcohol and after 2 min the water samples were collected in the sterile bottles[10,11].

E. coli and total coliform bacteria were detected using MacConkey broth, eosin methylene blue (EMB) agar and violet–red bile (VRB) agar. *P. aeruginosa* was detected using asparagine proline broth and cetrimide agar. Moreover, sulphite-reducing anaerobes test was done using differential reinforced clostridial broth (DCRB), and aerobic microbial count test was done to check the microbial load and hygiene status of the water samples.

For the detection of coliform bacteria and *E. coli*, 250 ml of water sample was filtered through 0.2 µm membrane filter, inoculated and transferred into 30 ml MacConkey broth. The broth was prepared using 30 ml screw-cap bottles in which a Durham tube was kept in an inverted position. After inoculation, the bottles were incubated at 37°C for 48 h. Positive results were indicated by a colour change of the broth and presence of air bubble in the Durham tube. The positive samples were further inoculated onto EMB agar plates for *E. coli* and simultaneously onto VRB agar plates for coliform bacteria. The plates were kept under incubation at 37°C for 48 h (ref. 6). *E. coli* strain showed green metallic sheen on EMB agar, whereas coliform bacteria showed green metallic sheen on VRB broth.

For the identification of *P. aeruginosa*, 250 ml of water sample was filtered through the 0.45 µm membrane and transferred into asparagine–proline broth tubes. The tubes were incubated at 37°C for 48 h. The growth and fluorescence were examined under UV light. The presence of *P. aeruginosa* was recorded by a subculture of a loopful of culture medium on Cetrimide Agar plate that was kept for incubation at 37° ± 0.5°C for 48 h. The presence of *P. aeruginosa* was marked by pigmentation and fluorescence under UV light.

Differential reinforced clostridial medium (DRCM) was used to detect the sulphite-reducing bacteria. The water sample has been heated in a water bath at 75° ± 5°C for 15 min. Then 50 ml of double-strength

DRCM medium was prepared in two separate 100 ml screw-cap bottles that were sterilized. Next 50 ml of water sample was added into one bottle with medium and the other bottle was kept as control. Further, single-strength DRCM medium was added up to the neck of the bottles ensuring that some air remains inside then the bottles were sealed and incubated at 37°C for 48 h. The confirmation of sulphite-reducing anaerobes was done by observing blackening as a result of the reduction of sulphite and precipitation of iron sulphide.

Plate count agar (PCA) medium was used in this study. The water sample was mixed thoroughly and then 1 ml of the sample was transferred into sterile petri dishes. The sterilized PCA media was transferred aseptically into the same petri dishes and was kept for solidification. While transferring the media to petri dishes, the temperature was set around 40°C to 45°C. After solidification of the media, the petri dishes were incubated at 37°C for 24 h. Results were analysed on the basis of colony count and reported as Total Plate Count of cfu/ml of the water sample.

Total coliform and *E. coli*, *P. aeruginosa* and sulphite-reducing anaerobes were recorded as absent or present. The northwest (NW) and southwest (SW) parts of the study area were more affected by coliform bacteria and *E. coli* while the south–southeast (S–SE) part was less affected (Figure 2). In the case of *P. aeruginosa* the NW part was more affected, while the east and S–SE parts were less affected (Figure 3). Furthermore, sulphite-reducing anaerobes affected the NE, SW and southern parts of the catchment (Figure 4). APC at 37°C, showed that the east–northeast (E–NE) and east–southeast (E–SE) regions of the catchment were the only in safe zones, whereas rest of regions was highly affected (Figure 5). Microbiological pathogens in the study area may due to a higher level of organic load which enhances microbial activity with high BOD (biochemical or biological oxygen demand) and TDS. Dissolved oxygen concentration provides useful information about water quality, regarding the stability of many organic and inorganic contaminants in groundwater.

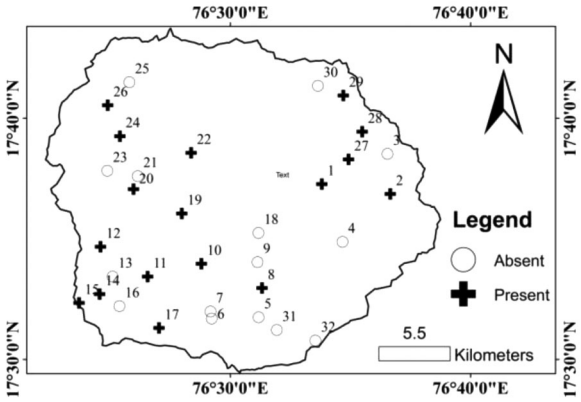

Figure 2. Total caliform and *Escherichia coli* in Amarja Reservoir Catchment.

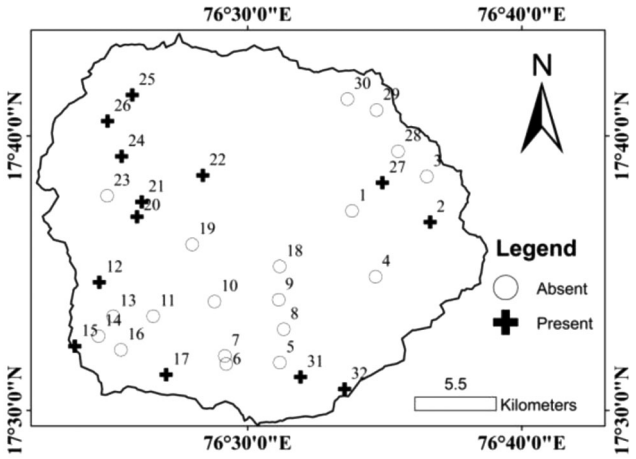

Figure 3. *Pseudomonas aeruginosa* in Amarja Reservoir Catchment.

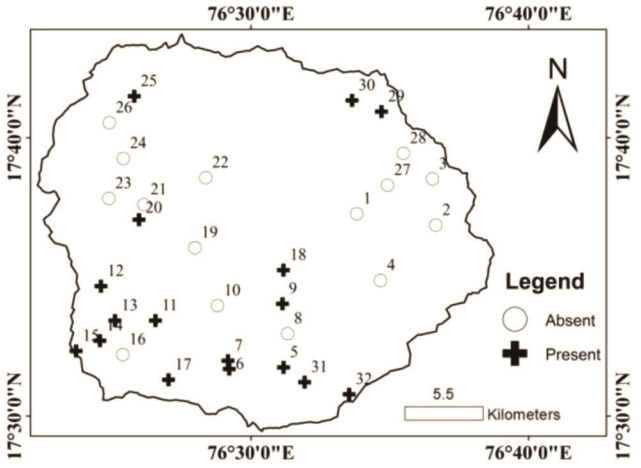

Figure 4. Sulphite reducing anaerobes in Amarja Reservoir Catchment.

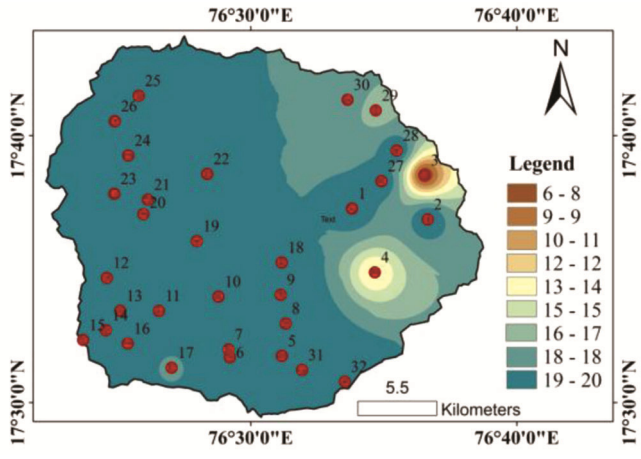

Figure 5. Aerobic plate count (APC) at 37°C in Amarja Reservoir Catchment.

The conductivity values were in the range from 161 µs/cm to 1413 µs/cm (Figure 6)[12]. The electric conductivity was calculated using TDS[12]. Lower values were observed in the north–northeast, north–northwest and some parts of the west–southeast region of the catchment, whereas in the southern and eastern regions of higher conductivity was recorded. Conductivity always increases as the concentration of ions increases[8]. TDS values in the analysed water sample were in the range 108–947 mg/l (Figure 7). The highest value of the TDS was recorded in the southern side of the catchment. The overall TDS of water in the study area showed an increasing tendency towards the lower part of the catchment, which may be due to the loading and accumulation of ionic compounds and contamination through surface water run-off. The pH value in the study area was in range 7.1–8.9. The SE and SW parts of the catchment showed higher pH (Figure 8). The lower pH of drinking water can cause gastrointestinal disorder, while higher values (alkaline nature) can affect functioning of the kidney. The turbidity of the catchment was recorded from 1.0 to 2.0 NTU (Figure 9). The highest turbidity value was recorded in the south–southeast part of the study area. Figure 10 represents the growth of bacteria at 37°C with respect to different location, indicating that almost all the villages have higher APC values.

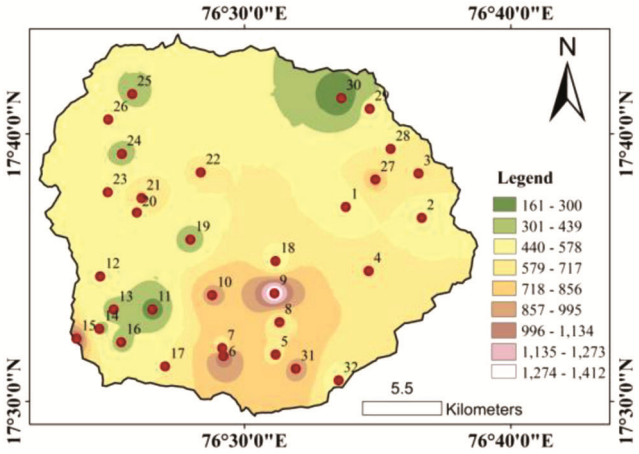

Figure 6. Electrical conductivity in Amarja Reservoir Catchment.

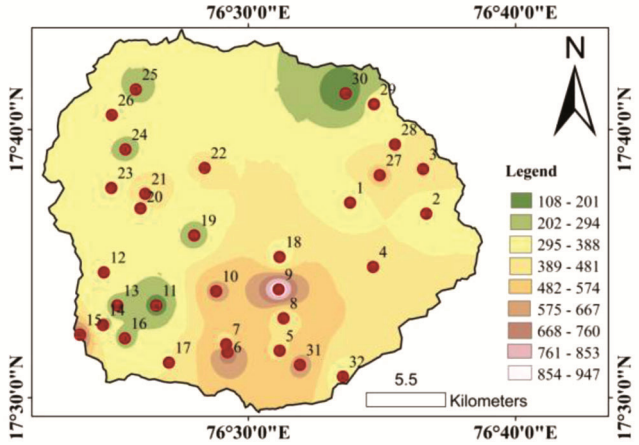

Figure 7. Total dissolved solids (TDS) in Amarja Reservoir Catchment.

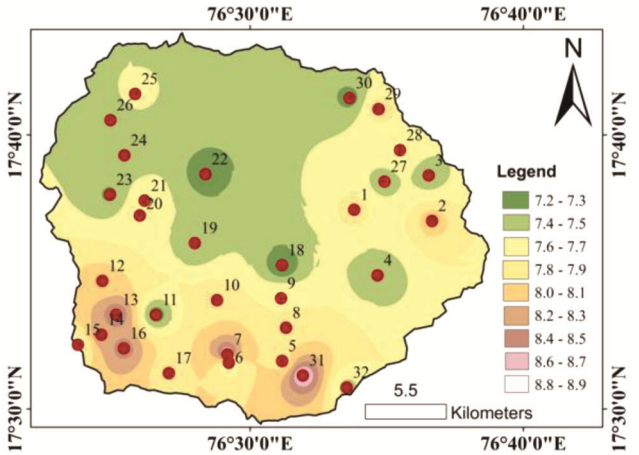

Figure 8. pH concentration in Amarja Reservoir Catchment.

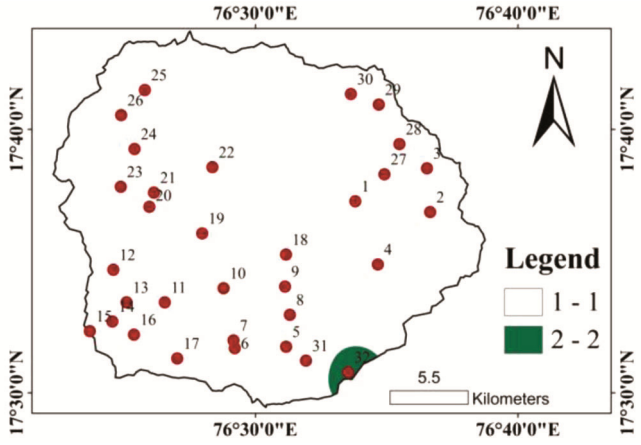

Figure 9. Turbidity in Amarja Reservoir Catchment.

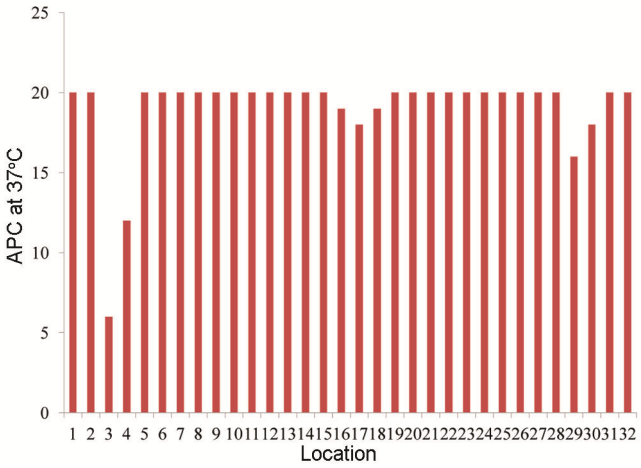

Figure 10. Growth of APC in different location.

Table 2. Physical and microbiological parameters of water in Amarja reservoir catchment, Aland taluk, Karnataka

Location	Turbidity (NTU)	pH	Electrical conductivity (EC) (μS/cm)	TDS (mg/l)	Total coliform and *E. coli*	*Pseudomonas aeruginosa*	Sulphite-reducing anaerobes	Aerobic plate count at 37°C (cfu/ml)
Telikuni	<1	7.82	444	298	Present	Absent	Absent	>20
Kenisultan	<1	8.03	461	309	Present	Present	Absent	>20
Jamga (R)	<1	7.42	673	451	Absent	Absent	Absent	6
Aland	<1	7.34	631	423	Absent	Absent	Absent	12
Gulahalli	<1	7.84	668	448	Absent	Absent	Present	>20
Jidga	<1	7.20	1229	824	Absent	Absent	Present	>20
Jamga Khandala	<1	8.88	528	354	Absent	Absent	Present	>20
Shakhapur	<1	7.76	570	382	Present	Absent	Absent	>20
Jheerahalli	<1	7.71	1413	947	Absent	Absent	Present	>20
Khanapur	<1	7.89	905	607	Present	Absent	Absent	>20
Swaleshwar	<1	7.23	216	145	Present	Absent	Present	>20
Sarasambha	<1	7.98	531	356	Present	Present	Present	>20
Nagelagaon	<1	8.52	397	266	Absent	Absent	Present	>20
Sakkarga	<1	8.27	402	270	Present	Absent	Present	>20
Kamanalli	<1	7.50	1132	759	Present	Present	Present	>20
Kinnihabas	<1	8.42	401	269	Absent	Absent	Absent	19
Mogha (B)	<1	7.81	574	385	Present	Present	Present	18
Hebli	<1	7.16	453	304	Absent	Absent	Present	19
Padsawli	<1	7.34	373	250	Present	Absent	Absent	>20
Chincholi (K)	<1	7.71	510	342	Present	Present	Present	>20
Chincholi(B)	<1	7.70	761	510	Absent	Present	Absent	>20
Nirgudi	<1	7.24	601	403	Present	Present	Absent	>20
Khairat	<1	7.31	492	330	Absent	Absent	Absent	>20
Varnalwadi	<1	7.42	385	258	Present	Present	Absent	>20
Aloor	<1	7.61	404	271	Absent	Present	Present	>20
Bolegaon	<1	7.34	562	377	Present	Present	Absent	>20
Chitali	<1	7.45	794	532	Present	Present	Absent	>20
Bangerga	<1	7.75	571	383	Present	Absent	Absent	>20
Bableshwar	<1	7.82	465	312	Present	Absent	Present	16
Khajuri	<1	7.29	161	108	Absent	Absent	Present	18
Tadola	<1	8.72	932	625	Absent	Present	Present	>20
Korahalli	<2	7.49	458	307	Absent	Present	Present	>20

Table 3. Correlation between microbiological and physical parameters

Coefficient	TDS	Turbidity	pH	EC	APC at 37°C
TDS	1				
Turbidity	–0.09	1			
pH	–0.004	–0.09	1		
EC	0.99	–0.09	–0.004	1	
APC at 37°C	–0.06	0.03	0.13	–0.06	1

A correlation matrix has been used to describe whether significant correlation exists between the appearance and growth of microbiological pathogens, and measured physical parameters. Pearson's correlation coefficient measures the degree to which two variables are linearly related. If the linear relationship between two variables is perfect with a positive slope, the Pearson's correlation coefficient becomes 1 (ref. 13). If the linear relationship between the two variables is negative, the Pearson's correlation coefficient becomes –1 (ref. 13). The coefficient having a value of '0' indicates that there is no relationship between the two variables[13]. Table 2 shows the analysed physical and microbiological parameters, whereas Table 3 shows the correlation matrix obtained using these parameters.

The colony count at 37°C represents a positive relationship with respect to pH (Tables 3 and 4). The result is consistent with that of a study carried out by Habuda-Stanic *et al.*[13]. Figure 11 gives a clear representation of the growth of APC with an increasing pH. The other values of correlation coefficients (Table 3) between APC and EC, TDS, turbidity suggest that correlation does not exist among these parameters. The results also reveal that there is a strong relationship between TDS and EC. The scatter plots between the growth of APC at 37°C and TDS, EC, turbidity are shown in Figures 12–14 respectively.

Table 4. Standard of correlation coefficient[13]

Coefficient	Strong relation	Moderate relation	Weak relation	None
Positive correlation	0.5 to 1.0	0.3 to 0.49	0.1 to 0.29	0 to 0.09
Negative correlation	–1.0 to –0.5	–0.49 to –0.3	–0.29 to –0.1	–0.09 to 0

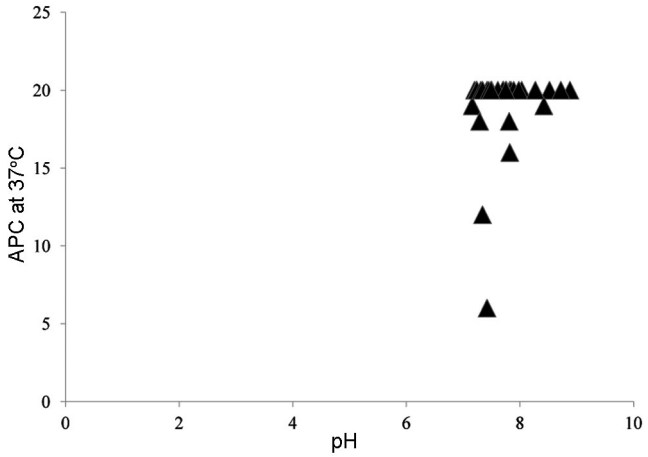

Figure 11. Relationship between total plate count and pH.

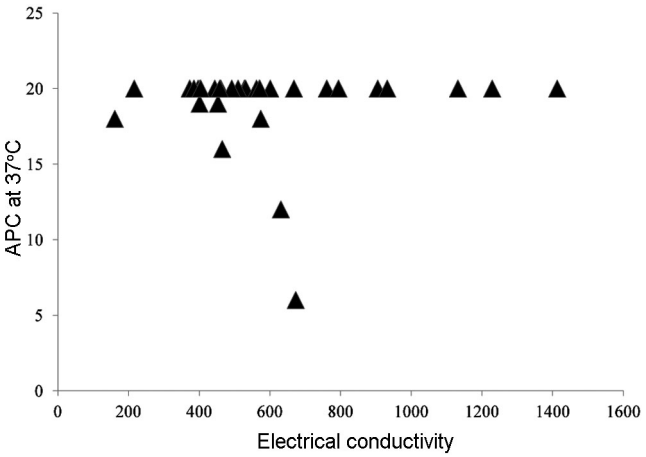

Figure 13. Relationship between total plate count and electrical conductivity.

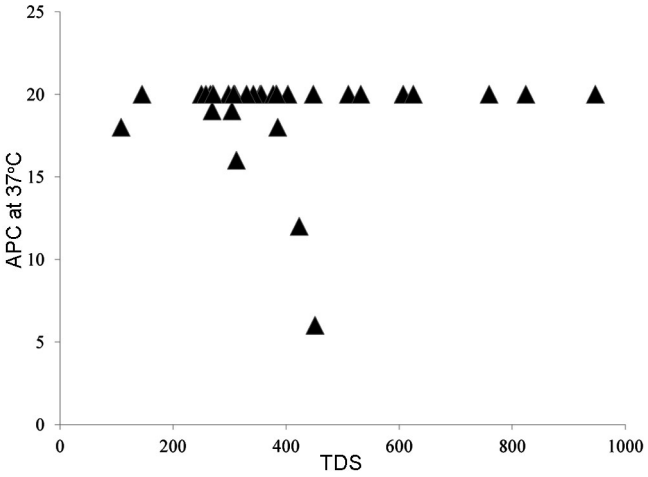

Figure 12. Relationship between total plate count and TDS.

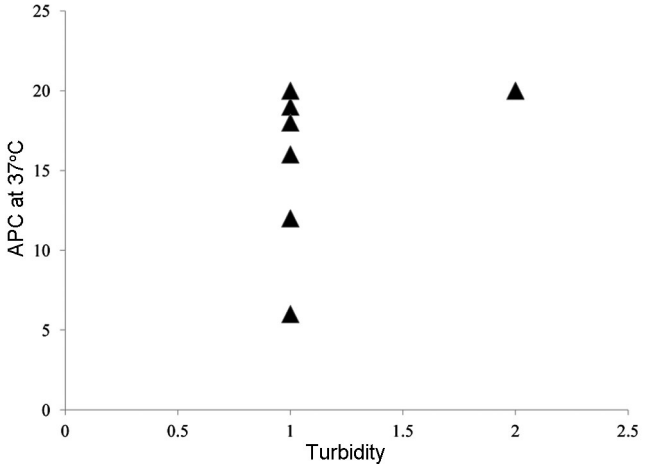

Figure 14. Relationship between total plate count and turbidity.

The confirmation of *E. coli* indicates that the contamination of media exist near the source of these pathogens[14]. One of the most dangerous species of *E. coli*, *E. coli O157:H7* (ref. 15) several disease to outbreaks[1]. Moreover, it is also responsible for kidney failure, gastroenteric infection, pneumonia, urinary tract infection[14] and respiratory illnesses. The most common symptoms of these diseases are vomiting, nausea, diarrhoea, fever and stomach cramps.

P. aeruginosa is considered as an expedient pathogen. Several diseases occur due to *P. aeruginosa*, like urinary infection, dermatitis, soft tissue infection, bacteraemia, respiratory system infection, joint and bone infection, gastrointestinal infection and a diverseness of systemic infections[13,16,17]. In addition, most of the outbreaks have been recorded in swimming pools and water slides due to exposure to *P. aueroginosa* pathogens. Skin exposure in hot tubs and lung exposure from inhaling aerosols are the most common infections occur due to water infected by *P. aeruginosa*[18]. To reduce the infections and colonization caused by *P. aeruginosa*, it is recommended to use filters on water taps.

Clostridium perfringens, *Clostridium botulinum* and *Clostridium tetani* are the most common types of sulphite-reducing anaerobes. *C. perfringens* causes food poisoning, fasciitis and gas gangrene; *C. botulinum* causes botulism,

Table 5. Types of water exposure and related diseases[20]

Classification	Description	Type of water exposure	Sub-class	Examples
Waterborne microbial diseases	Diseases due to consumption of water contaminated with bacteria; most of them due to animal and human faecal contamination of water.	Drinking water	Treated or untreated water; public or private supply.	Viral, typhoid, cholera, gastroenteritis, e.g. due to the effect of Norovirus
Waterborne chemical diseases	Disease due to ingestion of toxic material in the water.	Drinking water	Treated or untreated water; public or private supply.	Arsenicosis
Water contact diseases	Caused by skin contact with pathogen-infested water or with chemical-contaminated water.	Recreational water	Freshwater sources; marine waters.	Schistosomiasis (bilharzia); cyanobacteria.
Water hygiene diseases	Diseases whose incidence, prevalence or severity can be reduced using safe (clean) water to improve personal and domestic hygiene.	Any water used for washing/personal hygiene.	Diseases related to variations in water quality; diseases related to water shortage.	Scabies, shigellosis; trachoma.
Water vector habitat diseases	Diseases where the vector lives all or part of its life in or adjacent to a water habitat.	Untreated freshwater sources.	Rivers, streams; small collections of stagnant water, e.g. water butts.	Malaria, filiariasis, onchocerciasis (aquatic flies); schistosomiasis (snails); trypanosomiasis (tsetse flies).
Water aerosol diseases	Diseases due to respiratory transmission, where a water aerosol containing suspended pathogens enters the airway.	Drinking or raw water sources.	Water used in industrial/ residential buildings Raw water sources.	Legionellosis (Legionnaires' disease; humidifier fever); Norwalk-like viral gastroenteritis.
Excreta disposal diseases	Diseases related to unsanitary disposal of human waste (faeces and urine).	Drinking water and untreated water sources.	Diseases related to human/ animal waste in drinking water; disease related to direct/indirect contact with faeces/urine.	Ascariasis; faecal-oral infections, e.g. shigellosis; schistosomiasis, trachoma.

while *C. tetani* causes tetanus infections, *Clostridium sordellii* is another important species of sulphite-reducing anaerobes which is responsible for fatal infections.

APC is a valuable parameter for evaluating water and food quality. A higher count of colonies can also be an indication of poor sanitation. Therefore, it is important to assess the water and food for specific pathogens before ruling on their quality. APC are the poor indicators of safety in most cases. They do not make any direct correlation to the presence of pathogens or toxins. A low colony count does not indicate that the ingredient is free from bacteria. Moreover, some pathogens show excessively high colony count which can result in a potential health hazard[19]. Table 5 lists some water exposure diseases that have been classified by Stanwell-Smith[20].

In this study, microbial analysis was conducted at the Amarja reservoir catchment. Most of the analysed water samples were found to be contaminated. The lower part of the catchment was alkaline in nature, whereas the southeastern part showed higher TDS concentration. The total coliform and *E. coli* and sulphite-reducing anaerobes clearly indicated increasing tendency towards the downstream parts of the catchment. The northern and southern parts of the catchment were highly affected by *Pseudomonas*. Many villages of the study area have been affected with bacterial growth at 37°C and this may due to the slightly alkaline nature of drinking water (pH 6.5–7.5). The study revealed that the catchment has been highly affected with microbial pathogens, giving rise to an alarming situation from the public health point of view. GIS-based spatial distribution maps are useful in the better understanding of vulnerable zones of the catchment for adopting a better management strategy, as done in this study. The results suggest that groundwater in the lower part of the catchment needs appropriate treatments before use for domestic consumption.

1. Rajendra, S., Rubin, D. and Abhishek, M., Microbiological quality of potable water in Dehradun city. *Int. Res. J. Pharma*., 2012, **3**(6), 130–137.
2. Palamuleni, L. and Akoth, M., Physico-chemical and microbial analysis of selected borehole water in Mahikeng, South Africa. *Int. J. Environ. Res. Public Health*, 2015, **12**(8), 8619–8630.

3. Macy, J. T. and Quick, R. E., Transmission and prevention of water–diseases. In Encyclopedia of Life Support System, Eolss Publishers Co Ltd, Oxford, UK, 2009, ISBN: 978-1-84826-182-2.
4. Mena, K. D. and Gerba, C. P., Risk assessment of *Pseudomonas* bacteria in water. *Rev. Environ. Contam. Toxicol.*, 2009, **201**, 71–115.
5. LeChevallier, M. W., Conditions favouring coliform and HPC bacterial growth in drinking water and on water contact surfaces. In *Heterotrophic Plate Counts and Drinking–Water Safety*, IWA Publishing, London, UK, 2003; http://www.who.int/water_sanitation_health/dwq/HPC10.pdf
6. Smith, D. P., Cason, J. A., Fletcher, D. L. and Hannah, J. F., Evaluation of carcass scraping to enumerate bacteria on prechill broiler carcasses. *Polut. Sci.*, 2007, **86**(7), 1436–1439.
7. Barrel, R. A. E., Hunter, P. R. and Nichols, G., Microbiological standards for water and their relationship to health risk. *Commun. Dis. Public Health*, 2000, **3**(1), 8–13.
8. Majagi, S., Vijaykumar, K., Rajshekhar, M. and Vasanthkumar, B., Chemistry of groundwater in Gulbarga district, Karnataka, India. *Environ. Monit. Assess.*, 2008, **136**, 347–354.
9. Groundwater Information Booklet, Gulbarga District, Karnataka. South-Western Region, Bangalore, 2012; http://cgwb.gov.in/District_Profile/karnataka/2012/Gulbarga-2012.pdf
10. Chaidez, C., Soto, M., Martinez, C. and Keswick, B., Drinking water microbiological survey of the Northwestern State of Sinaloa, Mexico. *J. Water Health*, 2008, **6**(1), 125–129.
11. Collins, C. H., Lyne, P. M. and Grange, J. M., *Microbiological Methods*, Oxford University Press Inc, New York, USA, 2004.
12. Atekwanaa, E. A., Atekwanaa, E. A., Roweb, R. S., Werkema, D. D. and Legalld, F. D., The relationship of total dissolved solids measurements to bulk electrical conductivity in an aquifer contaminated with hydrocarbon. *J. Appl. Geophys*., 2004, **56**(4), 281–294.
13. Habuda-Stanic, M., Santo, V, Sikora, M. and Benkotic, S., Microbiological quality of drinking water in public and municipal drinking water supply systems in Osijek–Baranja County, Croatia. *Croat. J. Food Sci. Technol*., 2013, **5**(2), 61–69.
14. Amenu, D., Bacteriological and physicochemical quality of well water sources. *Res. J. Chem. Environ. Sci.*, 2014, **2**(5), 13–27.
15. Hunter, P. R., Colford, J. M., LeChevallier, M. W., Binder, S. and Berger, P. S., Waterborne diseases. *Emerg. Infect. Dis.*, 2001, **7**(3), 544–545.
16. Al Moosa, M. E., Khan, M. A., Alalami, U. and Husaain, A., Microbiological quality of drinking water from water dispenser machines. *Int. J. Environ. Sci. Develop*., 2015, **6**(9), 710–713.
17. Ashbolt, J. N., Microbial contamination of drinking water and disease outcomes in developing regions. *Toxicology*, 2004, **198**, 229–238.
18. Shakya, P., Joshi, T. P., Joshi, D. R. and Bhatta, D. R., Evaluation of physico-chemical and microbiological parameters of drinking water supplied from distribution systems of Kathmandu Municipality. *Nepal J. Sci. Technol*., 2012, **13**(2), 179–184.
19. Medha, A. and Hysko, M., Assessment of water quality of Buna River using microbiological analysis. *Albanian J. Agric. Sci*., 2014, **13**, 113–116.
20. Stanwell-Smith, R., Classification of water-related disease. In *Encyclopedia of Life Support System*, Eolss Publishers Co. Ltd, Oxford, UK, 2009; http://www.eolss.net/sample-chapters/c03/e2-20a-01-01.pdf

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Production of novel bio-flocculants from *Klebsiella variicola* BF1 using cassava starch wastewater and its application

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In this study, *Klebsiella variicola* BF1 that uses cassava starch wastewater to produce flocculants was identified using 16S rDNA gene sequencing. The pure flocculants of strain BF1 could be easily extracted by ethanol precipitation with a high yield of 7.5 g/l. It was mainly composed of 83.1% carbohydrates and 10.6% proteins. The flocculating activity revealed 97.6 ± 0.6% for kaolin suspension at 12.8 mg/l extracted flocculants from strain BF1 and 2.5 g/l $CaCl_2$. Interestingly, the flocculating activity was 78% without the addition of metal ions. Furthermore, flocculants of strain BF1 can be effectively applied in the treatment of cassava starch wastewater and municipal wastewater.

Keywords: Cassava starch, exopolysaccharide, flocculants, *Klebsiella variicola*, wastewater treatment.

FLOCCULANTS are widely used in industrial processes, including wastewater treatment, textiles, detergents, adhesives and oil recovery[1,2]. Flocculants consist of two main classes: (1) chemical flocculants such as polyacrylamide, polyelectrolytes, polyethyleneimine, polyaluminium chloride and aluminium sulphate, and (2) natural flocculants such as cellulose, microbial flocculants, gelatin, chitosan, gum and mucilage, sodium alginate and tannin[1,2]. The chemical flocculants have been widely used in various applications due to their effectiveness and low cost. However, chemical flocculants can negatively affect ecosystems. Therefore, it is important to replace chemical flocculants by biodegradable flocculants.

Microbial flocculants have attracted research interest[3–14]. Bacteria, fungi and algae are known to be responsible for the production of flocculants[4–11,13,14]. The large-scale production and recovery of bio-flocculants has been studied[4–11,13,14]. Therefore, they are widely applied in many industrial sectors. For the aim of commercialization, a considerable effort has gone into reducing the production cost through using some wastes rich in organic matter,

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