Research Article
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Physico–chemical attributes and bacterial diversity of river water at Rudraprayag, Garhwal Himalaya

Abstract

Rudraprayag district is located on the confluence of River Alaknanda and Mandakini in the newly constructed state Uttarakhand. Three different sites were identified S_1 (Mandakini), S_2 (Alaknanda) and S_3 (Confluence) for the collection of water samples. The air temperature was ranged between 8.6 to 19.7 ^{0}C during the study period whereas the pH was recorded from a minimum of 7.53 to a maximum of 8.93. Dissolved Oxygen was ranged between 7.6 to 10.0 mg.l^{-1}. However, the BOD was ranged between 2.0 to 2.6 mg.l^{-1}. Conductivity was found to be minimum (188µS/cm) and maximum (284µS/cm). Salinity was ranged between 39 ppm to 73 ppm. TDS was recorded between 78 to 96 mg.l^{-1}. Free CO_2 was ranged between 4.4 to 8.8 mg.l^{-1}. Chloride was recorded from 3.42 to 4.82 mg.l^{-1}. However, total alkalinity was recorded between 168 to 210 mg.l^{-1}. Nitrate was recorded between 0.03 to 0.086 mg.l^{-1} whereas, sulphates was ranged between 0.274 to 0.344 mg.l^{-1}. However, the concentration of phosphates was ranged from 0.234 to 0.559 mg.l^{-1}. The total hardness was recorded between 76.0 to 84.0 mg.l^{-1}. Calcium was recorded between 15.78 to 18.75 mg.l^{-1} and magnesium was recorded between 8.53 to 9.56 mg.l^{-1}. A total of seven bacterial species were identified from the water samples with the help of MALDI–TOF MS. These bacterial species are *Pseudomonas extremoriental, Bacillus licheniformis, Paenibacillus glucanolyticus, Bacillus badius, Pseudomas fulva, Pseudomonas azotoforman, Paenibacillus thiaminolyticus*. The total coliform density is also very dense which was represented by the MPN method. The presented data will help the government officials to take care of the water quality and also force them to take some necessary steps to improve the water quality of the river.

Keywords: bacterial diversity, Garhwal Himalaya, physico–chemical parameters, Rudraprayag, Uttarakhand

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Introduction

On the planet Earth, mountains cover approximately one quarter land surface, which provides home for about 12% of the global human population. According to the United Nations Conference on Sustainable Development (UNCSD 2012) which was held in Rio de Janeiro, Brazil, ‘‘mountain ecosystem play a crucial role in providing water resources to a large population of the world’’. The rivers provide water for various activities such as industrial, agricultural, aquaculture, commercial and domestic usage. Unfortunately, some rivers are being polluted by indiscriminate disposal of sewage, industrial and human waste. River pollution has already acquired serious dimensions in India. Pollution of rivers first affects its water quality in terms of its physico–chemical properties, and then systematically destroys the community disrupting the delicate food web. Aquatic organisms need a healthy environment to live and adequate nutrients for their growth and development; the productivity depends on the physico–chemical characteristics of the water body. The maximum productivity can be obtained only when the physico–chemical characteristics are present at optimum level. Water for human consumption must be free from any type of contamination including the chemical contamination. The water pollution is increased due to human population, industrialization, the use of fertilizers in agriculture and other anthropogenic activities. Parameters such as temperature, turbidity, nutrients, hardness, alkalinity, dissolved oxygen, etc. are some of the important factors that determines the growth of living organisms in the water body. Hence, water quality assessment involves the analysis of physico–chemical and microbiological parameters that reflect the biotic and abiotic status of the ecosystem.[1] As compared to the organic chemicals, the inorganic chemicals hold a greater portion as contaminants in drinking water.

Bacteria constitute a large domain of prokaryotic microorganisms. Bacteria were among the first life forms to appear on the Earth, and are present in almost everywhere. Bacteria grow in soil, acidic hot springs, radioactive waste, water and even deep in the Earth’s crust. A lot of work has been done so far by various workers on the different aspects of the river that includes the work of Joshi et al.,[2] on the physico–chemical parameters of water of River Ganga in Haridwar, Uttarakhand; Matta et al,[3] on the assessment of physico–chemical characteristics of Ganga Canal, India; Singh et al.,[4] on the assessment of physico–chemical parameters of mountain River Baldi of Garhwal Himalaya; Sharma et al.,[5] on the water quality assessment of Satluj River, Himachal Pradesh; Jain et al.,[6] on seasonal variations in physico–chemical and phytoplankton diversity of Alaknanda River at Garhwal region of Uttrakhand; Sood et al.,[7] on assessment of bacterial diversity in the Gangetic river system of Uttarakhand; Gupta et al.,[8] on physico–chemical analysis and microbial diversity of Yamuna Water and Industrial Effluents and Haritash et al.,[9] on the assessment of water quality and suitability analysis of River Ganga in Rishikesh, India**.** But, no work has been done so far on the physico–chemical characteristics and bacterial diversity of river system at Rudraprayag. Therefore, this study was carried out to provide the basic data on the physico–chemical properties and bacterial diversity of river system at Rudraprayag as reference for further studies.

Materials and methods

Study area

Rudraprayag district is located on the confluence of River Alaknanda and Mandakini (Both Tributaries of River Ganga) in the state of Uttarakhand. Rudraprayag is of immense significance for the pilgrims of Char DhamYatra, as it is the junction for visiting Badrinath and Kedarnath Dham. Rudraprayag is dotted with temples which are significant from archeological as well as religious point of view. Globally Known Shri Kedarnath Temple is at North, Madmaheshwar temple at east, and Nagrasu temple at southern east. The holy River Mandakini originated from Kedarnath is the main river of the district. Rudraprayag district of Uttrakhand was established on September 16, 1997. The district occupies an area of 2439 km². Rudraprayag town is the administrative headquarter of the district. It is located between latitude 30°16′N to 30°28′N and longitude 78°58′E to 78°98′E. Three sites S_1 (Mandakini), S_2 (Alaknanda) and S_3 (Confluence) were identified for the sample collection (Figure 1). Site 1 (S_1) is located at an altitude of 642 m a.s.l. between latitude 30^{0}17.270'N and longitude 78^{0}58.746'E. Site 2 (S_2) is located at an altitude of 634 m a.s.l. between Latitude 30^{0}17.272'N, Longitude 78^{0}58.942'E. Site 3 (S_3) is located at an altitude of 620 m a.s.l. between latitude 30^{0}17.259'N and longitude 78^{0}58.681'E.

Figure 1 Google map of the study area.

Sample collection and analysis of physico–chemical parameters

Water samples were collected from the sites in the morning time between 08:00 A.M to 10:00 A.M during December, 2017 to May, 2018 by dipping the autoclaved thermo steel flask and closing the cap under water surface to prevent any atmospheric exposure and contamination to assess the water quality. Physico–chemical parameters of the water samples were analyzed by following the standard method outlined in Wetzel et al.,[10] APHA[11] and bacterial isolation and identification by following the standard method outlined in Harley et al.,[12] Some of the physico–chemical parameters such as pH, DO, free CO_2, temperature were measured at the sampling site. However, for the remaining physico–chemical parameters and bacterial isolation the water samples were transferred to the Laboratory of Environmental Microbiology, Department of Environmental Sciences, H.N.B. Garhwal University, Srinagar–Garhwal, and Uttarakhand at its earliest possible. A total of seventeen physico–chemical parameters (air temperature, water temperature, Dissolved oxygen, Biochemical oxygen demand, free CO_2, hardness, chlorides, pH, calcium, total alkalinity, TDS, salinity, nitrates, phosphates, sulphates, magnesium and conductivity) were followed by coliform test, bacterial isolation and identification with MALDI–TOF MS during the study period.

Bacterial isolation

Nutrient Agar media (HiMEDIA) was used for the isolation of bacteria from the water samples. The pH of media for bacterial isolation was set according to the pH of water at the sampling sites. Eosin Methylene Blue Agar (EMB) medium was used for the detection of the members of the family *Enterobacteriaceae* and culture plates were incubated at 37^{0}C for 48 hrs. The total number coliforms were determined by using the Most Probable Number (MPN) method. Statistical tables were used to interpret the results of Most Probable Number (MPN) of the total coliform. From each dilution 1ml was added to each of triplicate tubes containing 5 ml of MacConkey broth. The tubes were incubated at 37^{0}C for 48 hrs for total coliforms. The positive tubes were streaked on the Eosin Methylene Blue (EMB) agar plates and incubated at 37^{0}C for 48 hrs.[11]

Bacterial identification

To study the morphological characteristics, the selected bacterial isolates were Gram stained and observed under Phase Contrast Microscope (Nikon Eclipse TS100). Moreover, detailed biochemical characterizations were carried out to identify the bacterial isolates up to possible genus or species level. All the bacterial cultures isolated from the sampling sites were sent to National Centre for Microbial Resources, National Centre for Cell Sciences and Pune for identification by using the MALDI–TOF MS. Statistical treatment (mean; standard deviation) of the physico–chemical parameters of water was carried out for presenting the mean seasonal variations of the river water.

Results and discussion

Physico–chemical characteristics

The water samples collected from three different sites Alaknanda (S_1), Mandakini (S_2) and confluence (S_3) of both rivers were assessed for a period of six months (during December 2017 to May 2018). A total of seventeen physico–chemical parameters (Air temperature, Water temperature, pH, Dissolved Oxygen, BOD, Free CO_2, Total Dissolved solid (TDS), Total alkalinity, Calcium, Magnesium, Conductivity, Sulphate, Nitrate, Phosphate, Chloride, Salinity and total Hardness) were recorded. The recorded data of physico–chemical environmental variables (minimum, maximum, mean and S.D.) are presented in Table 1.

The air temperature was recorded between 9.8^{0}C to 25.5^{0}C at the study area during the study period. It was minimum (9.8^{0}C) and maximum (25.5^{0}C) at Site S_3. The water temperature was recorded minimum (8.6^{0}C) at site S_2 and S_3 and maximum (19.7^{0}C) at S_2. Kansal et al.,[13] was recorded same range of water temperature (17.47 to 19.33^{0}C) for Himalayan Rivers in India. Tiwari et al.,[14] was also recorded similar range of temperature (17 to 28^{0}C) for the Ganga River, India. The pH was ranged from minimum (7.37) to maximum (7.93) at site S_1. This high pH may be due to the high amount of pollution and waste dissolved in the water. Similar range was reported by Sharma and Walia[5] for Satluj River (Himachal Pradesh, India).

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Dissolved Oxygen was ranged between minimum (7.6 $mg.l^{-1}$) at site S_3 to maximum (10.0 $mg.l^{-1}$) at site S_3. This high amount of dissolved oxygen was due to the mixing of river water at the site S_3, which is a confluence. Tiwari et al.,[14] reported the range of DO from 4.0 to 4.8 $mg.l^{-1}$ in the water samples of Ganga River. Biochemical Oxygen Demand was recorded between 2.0 $mg.l^{-1}$ to 2.6 $mg.l^{-1}$. This range of BOD was due to the presence of large number of microbes in the water. Matta et al.,[15] was reported 0.90–2.39 $mg.l^{-1}$ during 2013–2014 for Ganga Canal System in Himalayan Region. Conductivity was recorded between minimum (188.0 µS/cm) at Site S_2 and maximum (284 µS/cm) Site S_3. The high conductivity of water is due to the presence of high amount of free ions. Matta et al.,[15] recorded 97.51 to 138.2µS/cm, for water quality of Ganga Canal System in Himalayan Region.

Salinity was recorded between minimum (39 ppm) to maximum (73.0 ppm) at site S_1. Total Dissolved Solids was ranged between 78 $mg.l^{-1}$ at site S_1 to 96.0 $mg.l^{-1}$ at site S_2 and S_3. Similar range of TDS was reported by Arora et al.,[16] Matta et al.,[15] was reported 138.2 to 161.40 $mg.l^{-1}$. The Free CO_2 was recorded a minimum (4.4 $mg.l^{-1}$) to maximum (8.8 $mg.l^{-1}$) at all the three sites with $\bar{X}$±S.D. (7.33±2.07). Every time, the free CO_2 was recorded at the sampling sites. It resembles the amount or concentration of free carbon dioxide present in the water. Similar range of free CO_2 was reported by Kumar et al.,[17] from Kali River in Pithoragarh, Uttarakhand. Chloride was ranged between minimum (3.4 $mg.l^{-1}$) at site S_2 to maximum (4.82 $mg.l^{-1}$) at site S_1. Similar range was reported by Semwal et al.,[18] for River Alaknanda. Total Alkalinity was recorded within a range of minimum (168 $mg.l^{-1}$) at site S_1 to maximum (210 $mg.l^{-1}$) at site S_3. Chandra et al.,[19] was reported the range between 89.5 to 107.8 $mg.l^{-1}$ from River Ramganga at Bareilly. Nitrate was ranged between minimum (0.03 $mg.l^{-1}$) at site S_1 to maximum (0.1 $mg.l^{-1}$) at site S_2. Tiwari et al.,[14] was reported the nitrates from 2.1 to 2.2 $mg.l^{-1}$ for Ganga River water at Varanasi. Sulphates was recorded from a minimum (0.274 $mg.l^{-1}$) at site S_1 to a maximum (0.345 $mg.l^{-1}$) at site S_1. Phosphates was ranged between minimum (0.033 $mg.l^{-1}$) at site S_3 to maximum (0.559 $mg.l^{-1}$) at site S_1. Tiwari et al.,[14] was reported the phosphate range between 0.82 to 1.58 $mg.l^{-1}$ for Ganga River water at Kanpur. Total Hardness was ranged from a minimum (76 $mg.l^{-1}$) at Site S_1 to a maximum (84.0 $mg.l^{-1}$ at site S_2 and S_3. Hardness of water is due to major cations and Magnesium ions in the river. The concentration of calcium was recorded within a range of minimum (15.78 $mg.l^{-1}$) at site S_1 and maximum (18.8 $mg.l^{-1}$) at site S_2. Similar range of calcium was reported by Sharma et al.,[5] from Sutlaj River. However, the concentration of magnesium was recorded within a range of minimum (8.09 $mg.l^{-1}$) at site S_3 to maximum (18.925 $mg.l^{-1}$) at site S_1.

Table 1 Statistical Mean and standard deviation of physico– chemical parameters

Parameters	S_1			S_2			S_3		
	Min	Max	$\bar{X}$±S.D	Min	Max	$\bar{X}$±S.D	Min	Max	$\bar{X}$±S.D
Air Temperature (^{0}C)	10.7	22.8	17.43±4.56	10.1	23.1	17.6±5.0	9.8	25.5	17.65±5.88
Water Temperature (^{0}C)	8.7	18.5	13.13±3.94	8.6	19.7	13.4±4.3	8.6	18.6	13.03±4.00
pH	7.37	7.93	7.58±0.19	7.7	7.9	7.8±0.1	7.45	7.91	7.60±0.16
DO ($mg.l^{-1}$)	8	9.8	8.57±0.60	8	9.8	8.8±0.6	7.6	10	8.77±0.86
BOD ($mg.l^{-1}$)	2	2.4	2.20±0.16	2.2	2.6	2.4±0.2	2	2.6	2.30±.019
Conductivity (uS/cm)	189	279	243.33±35.84	188	282	243.0±35.4	190	284	247.17±35.47
Salinity (ppm)	39	73	56.83±12.01	39	70	55.8±11.2	40	72	58.00±11.62
TDS (mg/l)	78	96	87.83±6.12	80	96	89.0±5.5	80	96	90.00±5.13
Free CO_2 ($mg.l^{-1}$)	4.4	8.8	7.33±2.07	4.4	8.8	7.3±2.1	4.4	8.8	7.33±2.07
Chlorides ($mg.l^{-1}$)	4.26	4.82	4.48±0.23	3.4	4.8	4.4±0.5	3.42	4.7	4.26±0.42
Total Alkalinity ($mg.l^{-1}$)	168	191	181.00±7.46	172	192	184.5±6.3	168	210	189.00±13.89
Nitrate ($mg.l^{-1}$)	0.031	0.077	0.06±0.02	0	0.1	0.1±0.0	0.03	0.086	0.06±0.02
Sulphate ($mg.l^{-1}$)	0.274	0.345	0.31±0.02	0.3	0.3	0.3±0.0	0.276	0.344	0.32±0.03
Phosphate ($mg.l^{-1}$)	0.501	0.559	0.53±0.02	0.5	0.5	0.5±0.0	0.033	0.449	0.29±0.14
Hardness ($mg.l^{-1}$)	76	82	79.00±2.24	78	84	80.3±2.1	77	84	80.17±2.34
Calcium ($mg.l^{-1}$)	15.78	18.75	17.33±0.96	16.8	18.8	17.8±0.8	16.78	18.75	17.67±0.77
Magnesium ($mg.l^{-1}$)	8.53	8.92	8.76±0.17	8.1	9.6	8.7±0.6	8.09	9.38	8.79±0.47

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Bacterial diversity

Bacteria can use any habitat for harbor because of having tiny size. Microorganisms are having great tolerance towards pH, temperature, pressure, salinity and water availability. Thus, environmental factor greatly influence the survival of microorganisms. A total of nine species of bacteria were found at three different sites (S_1, S_2 and S_3). At site S_1 (*Pseudomonas extremoriental, Paenibacillus glucanolyticus), Bacillus licheniformis*), at site S_2 (*Bacillus badius*). However, at site S_3 (*Pseudomonas extremoriental, Paenibacillus glucanolyticus, Bacillus badius, Pseudomonas azotoformans, Pseudomas fulva, Paenibacillus thiaminolyticus*) were isolated and identified. However, two species of bacteria were not identified. The list of identified bacteria is given in Table 2 along with the morphological and biochemical test results in Table 3.

Table 2 Bacterial Diversity of Mandakini, Alaknanda and Confluence at Rudraprayag

Code	Name of Bacteria	S_1	S_2	S_3
SBRB– S_1:1	*Pseudomonaextremoriental*	Present	Absent	Present
SBRB– S_1:2	*Bacillus licheniformis*	Present	Absent	Present
SBRB– S_1:3	*Paenibacillusglucanolyticus*	Present	Absent	Present
SBRB– S_2:1	*Bacillus badius*	Absent	Present	Present
SBRB– S_2–3	*Not identified*	Absent	Present	Present
SBRB– S_2–4	*Not identified*	Absent	Present	Present
SBRB– S_3:1	*Pseudomasfulva*	Absent	Absent	Present
SBRB– S_3:2	*Pseudomonas azotoforman*	Absent	Absent	Present
SBRB– S_3:3	*Paenibacillusthiaminolyticus*	Absent	Absent	Present

Table 3 Characteristics of Bacterial species of Mandakini, Alaknanda and Confluence at Rudraprayag

Characteristics	*Pseudomonas extremoriental*	*Bacillus licheniformis*	*Paenibacillusra-dioresistens*	*Bacillus badius*	*Paenibacillus-thiaminolyticus*	*Pseudomas-fulva*	*Pseudomonas azotoforman*
Shape	Circular	Undulate	Circular	Circular	Circular	Circular	Circular
Size	3.0–5.0mm	2–4mm	2–3mm	2– 4mm	1–2mm	1.5–3.0mm	2.0–4.0mm
Cell Shape	Rod	Rod	Rod	Rod	Rod	Rod	Rod
Spore formation	–	–	+	+	+	–	–
Motility	+	+	+	+	+	+	+
Grams Staining	–	+	+	+	+	–	–
Flagella	Monotrichous	Peritrichous	Monotrichous	Peritrichous	Monotrichous	Lophotrichous	Monotrichous
Catalase	+	+	+	+	+	+	+
Citrate	+	+	v	–	v	+	+
Pigmentation	+	+	–	–	–	+	+
Urease	+	–	–	–	–	+	+
Fructose	+	+	+	–	+	+	+
Glucose	–	–	+	–	+	+	–
Mannose	+	+	+	–	+	+	+
Mannitol	+	–	v	–	v	–	+
Maltose	V	v	+	–	+	–	+

Abbreviations +: positive; – : negative; v: variable

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Table 4 MPN values per 100ml of sample and 95% confidence limits for various combinations of positive and negative results (when three 10– ml, three 1– ml, and three 0.1– ml test portions were used)

December, 2017

No. of tubes giving a positive reaction			MPN (Per 100 ml)	95% confidence limits	
3 of 10 ml	3 of 1.0 ml	3 of 0.1 ml		Lower	Upper
Site S_1					
2	1	2	28	10	149
Site S_2					
2	2	1	28	10	149
Site S_3					
2	2	0	21	4	47

May, 18

No. of tubes giving a positive reaction			MPN (Per 100 ml)	95% confidence limits	
3 of 10 ml	3 of 1.0 ml	3 of 0.1 ml		Lower	Upper
Site S_1					
3	2	2	210	35	470
Site S_2					
3	3	2	1100	150	4800
Site S_3					
2	3	3	1100	150	4800

Coliform density

During the sampling period of December, 2017 the MPN value of total coliform per 100 ml was recorded to be 28 for site S_1; 28 for site S_2 and 21 for site S_3. However, for the month of May, 2018 it was recorded to be 210 for site S_1; 1100 for site S_2 and 1100 for site S_3 (Table 4).

Conclusion

A variation was observed in the water temperature of river between 9.8 to 25.5^{0}C. It was minimum in the month of December, 2017 and maximum in the month of May, 2018. pH was recorded within a range between 7.53 to 7.93. DO was ranged between 7.6 to 10.0 mg.l^{-1}. BOD was recorded within a range between 2.0 to 2.6 mg.l^{-1}. Conductivity was recorded from 189 to 284 µS/cm. TDS was recorded between 78 to 96 mg.l^{-1}. Free CO_2 was ranged between 4.4 to 8.8 mg.l^{-1}. Chloride was ranged between 3.4 to 4.82 mg.l^{-1} during study period. Total Alkalinity was recorded between 168 to 210 mg.l^{-1}. Nitrate was ranged between 0.0 to 0.1 mg.l^{-1}. However, the concentration of phosphate was recorded between 0.033 to 0.501 mg.l^{-1}. Total Hardness was recorded between 76 to 84 mg.l^{-1} and the concentration of calcium was ranged between 15.78 to 18.75 mg.l^{-1}. A total of nine species of bacteria were isolated from the water samples collected from the study area. Out of which only seven bacterial species were identified. These bacterial species are *Pseudomonas extremoriental, Paenibacillus glucanolyticus, Bacillus licheniformis*, *Bacillus badius, Pseudomonas azotoformans, Pseudomonas fulva* and *Paenibacillus thiaminolyticus*.

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Conflict of interest

The author declares that there is no conflict of interest.

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