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ORIGINAL ARTICLE

Physico-Chemical and Bacteriological Study Of Hot Water Spring From Aravali (Dist-Ratnagiri), Maharashtra, India

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ABSTRACT

Numerous hot springs are found in the area of 300 kms along the West coast of India, One of them is Aravali hot water spring located at Aravali village in Ratnagiri district of Maharashtra, India. It is in the vicinity of the Gad river to the south of the bridge over the river. The temperature of the said hot water spring is 42°C. The hot spring shows extreme environmental conditions such as high temperature, alkalinity, high sulphur concentration , metal concentration, etc. Physico-chemical analysis of the hot spring water was carried out on the seasonal basis(pre and post-monsoons) from the main source during years 2015 and 2016.The physical parameters like Temperature, Total Solids (TS), and Total dissolved solids (TDS), Total suspended solids (TSS), Electrical Conductivity (EC) were analyzed. The chemical parameters included pH, total hardness, total alkalinity and turbidity. Ionic parameters such as chloride, phosphate, sulphate, calcium, magnesium, sodium, potassium, fluoride, iron and nitrate and bicarbonate were observed. Trace elements like manganese, lead, copper, and lithium were analyzed. Biological Oxygen Demand (BOD), Chemical Oxygen Demand (COD), Dissolved Oxygen (DO) were also analyzed by standard APHA methods. Biological parameters like Standard Plate Count (SPC),Most Probable Number (MPN), Presumptive Test, Confirmed Test, Completed Test, Test for fecal coliforms andI MViC test were performed. These parameters were analyzed with the help of APHA standard methods. Present investigation highlights the physicochemical and bacteriological parameters of Aravali hot water spring. These parameters were compared with WHO potability parameters and it was observed that the water was non-potable in nature. However, it could be used for domestic sanitation purposes.

Keywords: *Physico-chemical parameters, World Health Organization (WHO),Permissible Limit, Bacteriological parameters, potability.*

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INTRODUCTION

The west coast geothermal province is considered as one of the important geothermal prospects in India. Aravali hot spring is located in Ratnagiri district of Maharashtra and lies between 17° 08'N Latitude and 73° 19' E longitude. The area for study is covered by the Deccan traps. According to Vouk [7] the discharged temperature of the hot water spring ranges from 30-50°C is considered as "Euthermal" spring. Hot spring water gives out bubbles of CO_2 and H_2S gases. Hot springs provide natural habitat for thermophilic organisms. Generally hot springs are considered to have medicinal values and cure skin diseases [2-4].

The objective of the present work was to analyze the physico-chemical and bacteriological parameters of hot spring water in pre and post monsoon seasons in the year 2015-16 and to highlight the suitability of water for sanitation, domestic as well as for human consumption. Hot water springs provide stable environment to ecosystem which may have remained unchanged. [5].

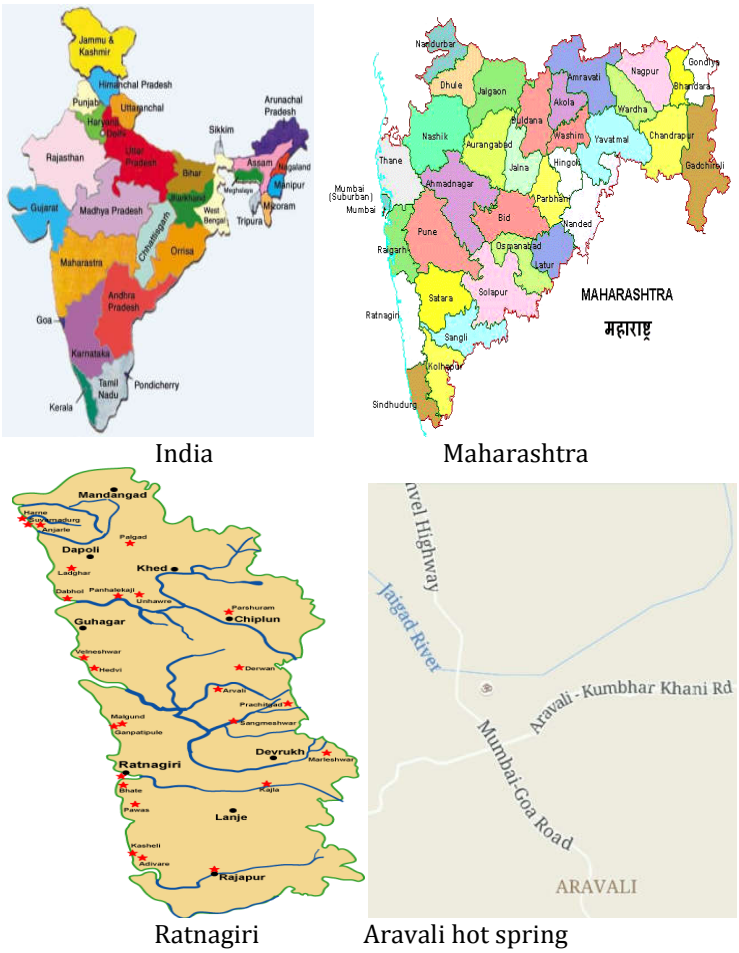


Figure.1-The location map of the study area of Aravali hot water spring in Ratnagiri district in Maharashtra, India.

MATERIALS AND METHODS

The hot water samples were collected in one liter sterile thermos stainless steel container at depth 0.6 to 1.5 meter from the water surface with the help of extendable metal sampler from Aravali hot water spring of Maharashtra(Fig-1). The water samples as well as sediment samples were collected during May and October 2015. The samples were stored in a refrigerator and processed later on. The temperature and pH of the water samples were measured *in situ* with the help of ordinary thermometer accurate at 0.1°C and portable pH meter.The geographical location was measured on the site using portable GPS unit.

All physico-chemical and ionic parameters were analyzed by using standard procedures described by APHA [1] and Trivedy and Goel [8] in the laboratory.

Standard qualitative Bacteriological analysis of hot spring water was carried out to check potability of hot spring water by biological methods [5].

Standard Plate Count (SPC): The bacteriological analysis of water samples was carried out on Thermus agar (ATCC medium 697) plate. Plates were incubated at 40ºC for 48hrs for direct measurement of viable aerobic and facultative anaerobic bacteria species.

Most Probable Number (MPN): Bacteriological analysis for potability of hot spring water samples were done by Multiple tube fermentation method. The Most Probable Number (MPN) of coliforms present in water samples were monitored and results were expressed in MPN/100mlfrom the MacCrady's table [6].

Presumptive test: Presumptive test performed by using lactose fermentation broth followed by multiple tube fermentation technique shows positive results by formation of acid and gas.

Confirmed test: The samples from positive presumptive test was transferred in Brilliant Green Lactose Bile broth and streaked on Eosin Methylene Blue (EMB) agar and then incubated at 37ºC for 24-48 hours and observed for gas production and typical colonies.

Completed test: The samples from positive confirmed test were inoculated in lactose fermentation brothand streaked on nutrient agar slant. Colonies of nutrient agar slant were then incubated at 37ºC for 24-48 hours. Selected and young cultures were used for Gram staining Hucker's method [5].

Test for fecal coliform: The samples were analyzed for presence of fecal coliform by using Eijkman's test. The samples were inoculated in Sodium azide broth and incubated at 44ºC and observed for gas production.

IMViC test: Indole test:1% tryptone broth in test tube were incubated with hot spring water samples.After incubation of 48hrs at 37°C, 1ml of chloroform and then 2ml of Kovac's reagent were added and incubated for 20min's and results were noted down.

Methyl Red- and Voges Proskauer test: 5ml glucose phosphate broth tubes were inoculated with water samples, incubated for 48hrs at 37°C.After incubation 2-3 drops of methyl red indicator was added. In remaining broth, few drops of 4% potassium hydroxide and few drops of 5% naphthol in ethanol were added and observed for results.

Citrate Utilization test: The water samples were inoculated on Simmon's citrate agar and incubated at 37°C for 24hrs and results were noted down.

RESULTS AND DISCUSSION

Table-1: Physico-chemical analysis of Aravali hot spring water

S.N	Water parameters	Methods used	unit	Pre monsoon	Post monsoon	Permissible limit by WHO (1993)	Mean±S.D
Physical parameters							
1.	Temperature	Mercury Thermometer	-	42ºC	40°C	-	-
2.	Taste	Human sensory receptor	-	Tasteless	Tasteless	-	-
3.	Odour	Human sensory receptor	-	Hydrogen sulphide odour	Hydrogen sulphide odour	-	-
4.	Total solids	Gravimetric method	mg/L	1341	1290	1500	1315.5±494.24
5.	Total dissolved solids	Gravimetric method	mg/L	664	563	500	613.5±80.25
6.	Total suspended solids	Evaporation method	mg/L	254	221	NS	237.5±23.33
7.	Electrical conductivity	Conductometry	(uS/cm)	1038	994	250	1016±31.11
Chemical parameters							
6.	pH	pH meter		7.5	7.8	6.5-8.5	7.65±0.21
8.	Total hardness	EDTA method	mg/L	36	30	150-500	33±4.2
9.	Total alkalinity	Acid titration method	mg/L	24	51	200	37.5±19.09
10.	Turbidity	Nephelometry method	NTU	0.1	0.096	6.0	0.098±0.0028
Ionic parameters							
11.	chloride	Silver nitrate method	mg/L	188	170	250	179±12.7
12.	phosphate	Spectrophotometry	mg/L	0.42	0.56	---	0.49±0.098
13.	Sulphate	Turbidometry method	mg/L	68	26	500	47±29
14.	Calcium	EDTA method	mg/L	9	3	75	6±4.24
15.	Magnesium	EDTA method	mg/L	4.6	7.5	30	6.05±2.05
16.	Sodium	Flame photometry	mg/L	140	98	200	119±29.69
17.	Potassium	Flame photometry	mg/L	33.2	10.8	---	22±15.83
18.	Fluoride	SPANDS method	mg/L	1.8	0.85	0.65-1.5	1.325±0.67
19.	Iron	Thiocynate method	mg/L	0.09	0.06	0.3	0.075±0.021
20.	Nitrate	Spectrophotometry	mg/L	1	0.9	---	0.95±0.07
21.	Lead	Atomic absorption spectrophotometry	ppm	0.0056	0.00446	0.01	0.0055±0.0007
22.	Copper	Atomic absorption spectrophotometry	ppm	0.0000139	0.000069	0.01	0.000004±0.005
23.	Lithium	Atomic absorption spectrophotometry	ppm	0.000878	0.000529	0.01	0.00070±0.00024
24.	Manganese	Atomic absorption spectrophotometry	ppm	0.002397	0.001867	0.01	0.00213±0.00037
25.	Bicarbonate	Titration method	mg/L	22.93	20.65	0.01	21.79±1.61
26.	BOD	Sodium thiosulphate titration	mg/L	26.5	30	30	28.25±2.47
27.	COD	Open reflux	mg/L	85	98	250	91.5±9.19
28.	Dissolved O_2	Modified wrinkler's method	mg/L	3.2	5.1	4.6	4.15±1.34

The present study focused on finding out the physico-chemical and bacteriological parameters of Aravali hot spring water and its potability. Aravali hot spring water was tasteless, colorless and the hot spring water had a characteristic Hydrogen sulphide odour. Physico-chemical and bacteriological characters of water samples in pre and post monsoon seasons are shown in Table-1 and Graph-1.It was observed that the average temperature of the hot spring water and sediment were 42°C, so it is considered as Euthermal Aravali hot spring.The mean value of Total solids is 1315mg/L which is within the WHO permissible limit.The average value of TDS samples are 613.5 mg/L which were excess than the WHO acceptable unit i.e. 500mg/L .There can be slight amount of variation in the TDS measurement. Total suspended solids (TSS) are measure of quality of water. The observation showed that TSS of 237.5mg/L is within the permissible range prescribed by WHO. The average electric conductivity is the level of dissolved ionic molecules in the water was found to be 1016uS/cm, WHO limit is 250 uS/cm which is less than the obtained mean value.

Chemical parameters (Graph-2) were analyzed and compared with WHO permissible limit.The pH recorded at 42°Cwas 7.5 (pre monsoon) and at 40°C it was 7.8 (post monsoon)and was found in alkaline range.Physico-chemical analysis of water from Aravali hot spring had total hardness 33mg/L is considered as soft and moderate water when compared with tap water having total hardness of 0-100 mg/L as soft water and 100-200 mg/L as moderate water.[6].Alkalinity is the capacity of water to neutralize acids.The mean total alkalinity 37.5mg/L is below the permissible WHO limit 200 mg/L.

Ionic parameters were also analyzed (Graph-3). The average value of Chloride is 179mg/L which falls within WHO permissible limit i.e. 250 mg/L. The average value of Sulphate obtained was 47mg/L which is less than the WHO permissible limit 500mg/L. The average value of calcium was found to be 6mg/L which falls below the standard WHO limit that is 75mg/L. The obtained mean value of magnesium was 6.05 mg/L which was found much less than the standard WHO permissible limit 30mg/L. The sodium content obtained was 119mg/L which falls within the WHO permissible limit 200mg/L.Iron obtained was 0.075mg/L which is less than WHO permissible limit 0.3 mg/L.Nitrate, phosphate, potassium content in water sample was 0.95mg/L, 0.49 mg/L, 22mg/L which falls within the standard permissible limit.

Fluorides are salts of inorganic compounds found in minerals, rocks. The average value of fluoride was recorded as 1.325 mg/L which is found to be above WHO permissible limit 0.65 mg/L which is a serious concern. The excess fluoride presence can cause major health problems and hence the hot spring water is non-potable.

Lead, Copper, Lithium, Manganese are trace elements found in very small quantity in hot spring water. The mean values of Lead was 0.0055 ppm, copper with 0.00004 ppm, lithium was found to be 0.00070 ppm and manganese was obtained 0.00213 ppm which was almost negligible and falls within the WHO permissible limit that is0.01 ppm.

The average values of Biological Oxygen Demand (BOD) obtained was 28.25mg/L which is less than that of the standard WHO permissible limit 30 mg/L. Chemical Oxygen Demand (COD) obtained was 91.5 mg/L which is almost less than WHO permissible limit 250 mg/Land Dissolved Oxygen (DO) was found to be 4.15 mg/L which also falls within WHO permissible limit 4.6 mg/L. But overall hot spring water is considered as non-potable in nature.

Table.2: Analysis of Bacteriological parameters of hot spring water,Aravali.

S.no	**Water parameters**	**Medium used**	**Pre monsoon**		**Post monsoon**		**Result**
1.	Standard Plate Count (SPC)	Serial dilution and spread plate method	23×10^6 CFU/ml		45×10^3 CFU/ml		Positive test
2.	Most Probable Number MPN Index/100ml	Double strength and single strength Mac Conkey's broth	1600		920		Positive test
			Lower limit	Upper limit	Lower limit	Upper limit	
			640	3800	300	3200	
3.	Presumptive test	MacConkey broth	Acid & gas production		Acid & gas production		Positive test
4.	Confirmed test	Brilliant Green Lactose Bile broth	Acid & gas production		Acid & gas production		Presence of coliform
		EMB agar	Typical colonies with dark center and green metallic sheen		Typical colonies with dark center and green metallic sheen		Presence of coliform
5.	Completed test	Lactose fermentation broth	Acid & gas production		Acid & gas production		Positive test

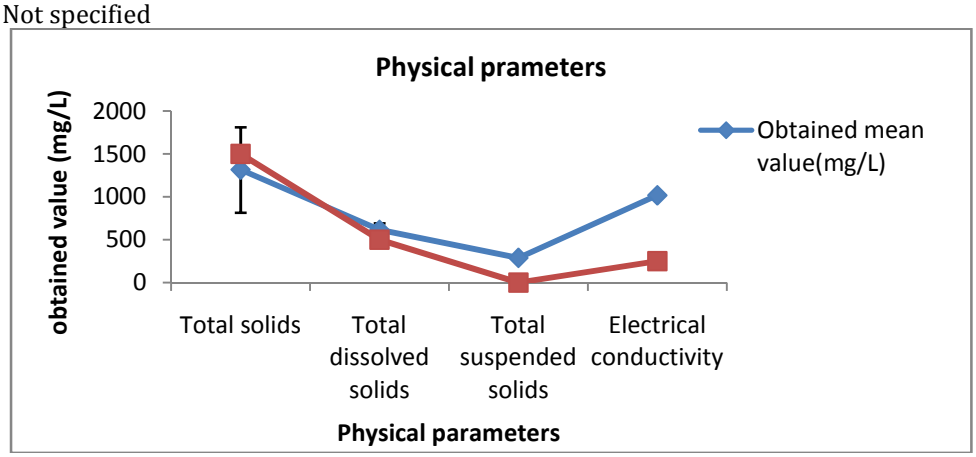
		Growth on nutrient agar slant & Gram stain	Gram negative rods	Gram negative rods	Positive test
6.	Test for fecal coliform/ Eijkman's test	Sodium azide broth	Gas production at 44°C	Gas production at 44°C	Positive test
7.	**IMViC test** i. Indole test	Tryptone broth	Red color ring	Red color ring	Positive test
	ii. Methyl red test	Glucose phosphate broth	Red color	Red color	Positive test
	iii. Voges-Proskauer test	Glucose phosphate broth	No rose red color	No rose red color	Negative test
	iv. Citrate utilization test	Simmon's agar slant	Green color	Green color	Negative test

The biological parameters were analyzed to check the potability of water. Results of the bacteriological analysis of Aravali hot spring water sample are presented in Table-2.The total viable count Standard Plate Count (SPC)for pre monsoon was 23×10^6 CFU/ml and for post monsoon45×10^3 CFU/ml.The Most Probable Number (MPN) for total coliform count of the pre monsoon water sample was 1600 MPN/100ml. Post monsoon water sample had lowest total coliform count of 920 MPN/100ml.In bacteriological examination of hot spring water sample presumptive test and confirmed coliform tests shows acid and gas production indicated tests were positive for presence of coliform bacteria. Confirmatory test using Brilliant Green Lactose Bile broth confirmed the presence of coliform by acid and gas production and showed typical colonies with dark center and green metallic sheen on Eosin Methylene Blue (EMB) agar (HiMediaPvt.ltd) plates. The completed test using lactose fermentation broth showed acid and gas production and growth on nutrient agar slant followed by observation of Gram negative pink color rods under 100X oil emersion lens indicated positive completed test. Isolates from nutrient agar slant were identified as Gram-negative rods. Sodiumazide broth showed gas production which indicated the presence of fecal coliform in water sample.IMViC tests were analyzed for detection of presence of *E.coli* which is indicator of fecal pollution. The formation of red color ring at the top indicated positive indole test and presence of red color indicated positive methyl red test. Absence of red color indicated negative Voges Proskauer test and the green color of Simmon's citrate agar indicated negative citrate utilization test.

Table.3: Comparison of mean values of physical prameters of hot spring water with standard values .

Parameters	Obtained mean value(mg/L)	Standard value (mg/L)	Standard deviation
Total solids	1315.5	1500	494.2463
Total dissolved solids	613.5	500	80.25
Total suspended solids	287.5	NS	23.33
Electrical conductivity	1016	250	1.11

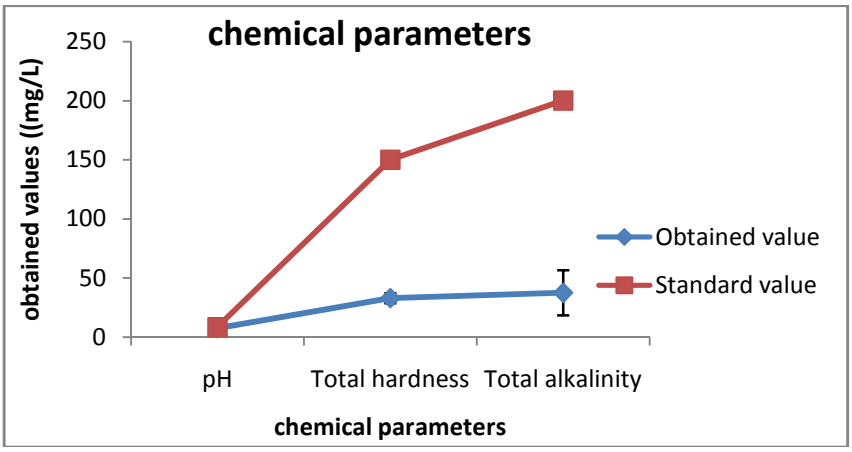
Note: NS- Not specified



Graph.1: Comparison of standard values with obtained mean values of physical prameters of hot spring water

Table.4: Comparison of standard values with obtained mean values of Chemical parameters of hot spring water

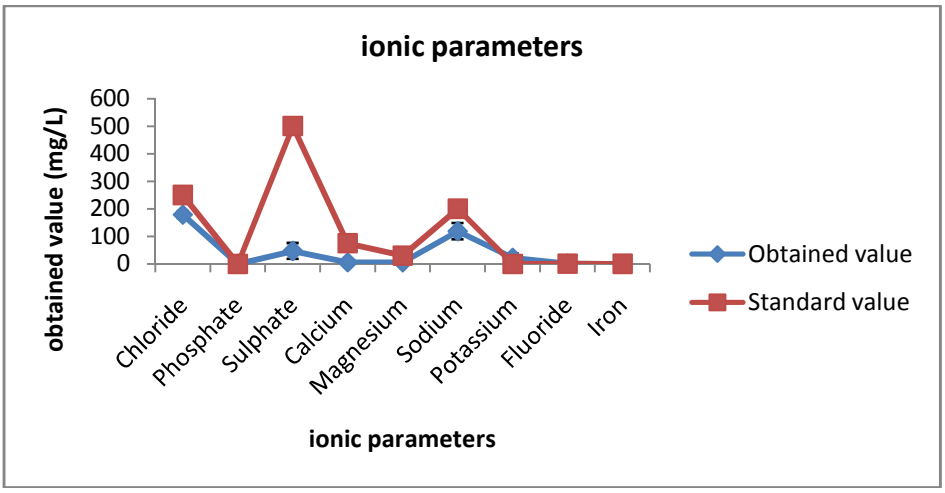
parameters	Obtained mean value(mg/L)	Standard value(mg/L)	Standard deviation
pH	7.65	8.5	0.21
Total hardness	33	150	4.2
Total alkalinity	37.5	200	19.09



Graph.2:Comparison of standard values with obtained mean values ofChemical parameters of hot spring water

Table.5: Comparison of standard values with obtained mean values of Ionic parameters of hot spring water

parameters	Obtained mean value (mg/L)	Standard value (mg/L)	Standard deviation
Chloride	179	250	12.7
Phosphate	0.49	0	0.09
Sulphate	47	500	29
Calcium	6	75	4.24
Magnesium	6.05	30	2.05
Sodium	119	200	29.69
Potassium	22	0	15.8
Fluoride	1.325	0.65	0.67
Iron	0.075	0.3	0.021



Graph.3: Comparison of standard values with obtained mean values of Ionic parameters of hot spring water

The water quality of Aravali hot spring water was assessed for physico-chemical characteristics of water in pre monsoon and post monsoon season and its potability for human consumption. Potential health effects of long term exposure to elevate fluoride include dental and skeletal fluorosis. The water is non-potable for human beings because of high concentration of Fluoride and sulphur. Human beings may get affected and infected due to the presence of high concentration of metal like fluoride and possible presence of pathogenic bacteria which may cause gastrointestinal infections, diarrhea, urinary tract infection, etc. Microbiological studies confirmed the presence of coliform bacteria(*E coli*) in water sample making water resource unfit for consumption.

CONCLUSION

The present study has provided data for physiological and bacteriological analysis of Aravali hot water spring. The obtained data was compared with WHO standards for drinking water. By considering the results and observation of present study, the following conclusions are drawn:

1) Total coliform count met above WHO standards.
2) All trace elements analyzed for hot water spring samples fell within the WHO limit.
3) The study of hot spring water is an important aspect to find new renewable source of energy and it is beneficial for medical tourism in India.
4) Maintenance of proper sanitary conditions, protection of water source, hot spring is recommended.

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