



Heavy metal and Bacteriological analysis in spring water of North Sikkim with reference to seasonal variation

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Abstract

Background/Objectives: The natural spring (dhara) is the main source of drinking water in North Sikkim. Though it is pure water for drinking and other domestic purposes, it gets polluted with various environmental factors. **Methods/Statistical analysis:** The study was performed to assess the quality of spring water. A total of nine sampling sites were selected at an interval of four months, i.e. monsoon, pre-monsoon and post-monsoon during August 2015-July2016. **Findings:** All the spring water samples were analyzed in terms of physical properties, heavy metals like copper, lead, iron, chromium, cadmium and bacterial characteristics such as Escherichia coli, Klebsiella sp, Salmonella sp, Shigella sp, Proteus sp, Citrobacter sp, Enterobacterclocae etc. In the study all the observed results were compared with Bureau of Indian Standard. **Improvements/Applications:** All the results of physical parameters and heavy metals were found within the permissible limit except the range of pH and iron shows slightly higher concentration than permissible limit. During monsoon, isolation of organisms was recorded in significant percentage followed by post-monsoon and pre-monsoon.

Index Terms

Bacteria, Physico-Chemical Parameters, Spring Water, Seasons

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I. INTRODUCTION

Sikkim is the 22nd state of India and very small hilly state in the eastern Himalayas, extending approximately 114 kms from north to south and 64 kms from east to west. The state being a part of inner ranges of the mountain of Himalaya and its elevation is ranging from 300 to 8583 mtrs [1].

The total population of Sikkim is 6,07,688 as per 2011 census. North Sikkim is the largest (4,226 sq. km.) in area but in term of population it is the smallest with (43,709) out of this, the rural population is 39065 (89.37%) and the urban population is 4644 (10.62%)[2]. Maximum number of the population resides in rural areas than urban who fulfill their basic needs with the spring water sources (untreated) as their main source of drinking, irrigation and other domestic purposes. Springs occur where inclined ground and impermeable strata intersect with ground water table [3]. In Sikkim, weather condition also plays a vital role in the amount of water flow during the months from early May to October. Spring (dhara) water said to be pure and natural water, however it contain various minerals, salts and micro-organism which comes from rain water, sediment soil through landslides, soil erosion and anthropogenic activities. Since, the water is in continuous contact with the surface and thereby increasing the risk of microbial presence and elemental contamination from country rocks[4]. Contamination of water in rural areas can cause by different factors like runoff water, poor sanitations, human and animal excreta and waste etc. The water that comes from the rainfall and the mountains results in small streams, springs and kholas [5] which are further tapped into pipelines and distributed with or without any treatment for drinking and domestic purpose. In the rural areas people depend on untreated water sources due to lack of awareness about the quality and contamination of water which needs at least basic treatment before consumption. Testing of water quality on a regular basis is, therefore an important part of maintaining a safe and reliable source [6]. Water pollution results in transmission of infectious diseases such as dysentery, cholera, diarrhea, typhoid, shigellosis, salmonellosis and varieties of other bacteria as well as fungi, viral and parasitic infection [7]. Reliable information on the qualities of raw water is necessary to guide its suitability for use [8]. Therefore, the present study intend to assess the quality of spring water used for drinking, irrigation and domestic purposes in rural areas of North Sikkim by determining the physico-chemical parameters and bacterial characteristics, as the quality of water plays a crucial role for preventing diseases and enlightening better quality of life for the population, animals and irrigation purposes.

II. MATERIALS And Methods

The study was conducted in rural areas of North Sikkim, during the period from August 2015 - July 2016. A total of twenty seven spring water samples, nine samples from each site were collected at an interval of four months that is, monsoon, post-monsoon and pre-monsoon. A liter of spring water sample for physico-chemical and 500 ml for bacterial analysis were collected in air tight container from the selected sources. Sample bottle was labeled with full details of the selected source at the time of sample collection. The value of pH and temperature were measured at the sampling site using a pocket-sized pH meter (Hanna Instrument) and mercury thermometer. The collected samples for bacterial analysis were transported to the laboratory using ice box. The physical parameters were measured in the laboratory within 24 hours of sample collection using conductivity meter, nephelometric method, azide modification method, titrimetry method and heavy metals were determined within six months of sample collection using concentrated HNO₃ as preservative and analysed with the help of inductively coupled plasma mass spectrometry (ICPMS) as per the standard guidelines of the American Public Health Association (APHA)[9]. For bacteriological analysis, the water samples were analysed within 6 hours of collection using membrane filtration method. The membrane apparatus were autoclaved for sterilization at 115°C for 10 minutes and 250 ml of each sample was filtered through a cellulose acetate grided membrane filter (47 mm diameter, 0.45 µm pore size, Millipore) with the help of vacuum. The filtered membrane was transferred on MacConkey agar plate (Himedia, Mumbai) and the plate was incubated at 37°C overnight. Further identification of the organism in collected water samples was performed on the basis of biochemical characteristics, conventional method of morphological and cultural characteristics of the isolated colony using standard guidelines of Mackie and McCartney [10].

III. RESULT AND DISCUSSION

The study has been conducted in different areas of North Sikkim on the basis of seasonal variation. The analysis for the quality of different spring water has been carried out which includes physical parameters, heavy metals and bacterial characteristics are shown in the tables 1, 2 and 3. The average temperatures were measured with a minimum range of 11°C during post-monsoon with a mean of 17.3 °C to maximum range of 25 °C during monsoon with a mean 22°C. The variation in temperature may be due

to various factors such as climate of geographical area, extend of shade from direct sunlight and depth of water and high population rates [11], pH were measured with a minimum range of 6 during monsoon with a mean 6.9 to maximum range of 8.86 during pre- monsoon with a mean 8. The pH value showed slightly alkaline during pre-monsoon due to the presence of carbonates and bicarbonates [6]. Similarly, the pH value of fresh spring water of Kashmir Himalaya also showed in alkaline range [12]. The alkaline range may be due to mixing up of the alkaline chemicals, soap and detergents etc. produce due to industrial, commercial and residential activities. The ISI permissible pH values containing water is suitable for domestic use and irrigation purposes [13]. Total dissolved solids (TDS) were measured with a minimum range of 10.57 mg/l during pre-monsoon with a mean 21.82 mg/l to maximum range of 69 mg/l during monsoon with a mean 58.3 mg/l. All the studied sample showed the TDS values within the permissible limit which can be considered for drinking [14,15]. High value of TDS in water sample signify high quantity of dissolved solids and less values be a sign of less pollution in water. Conductivity was measured with a minimum range of 23.51 μ s during pre-monsoon with a mean of 44.52 μ s to a maximum range of 94.75 μ s during post-monsoon with a mean 54.8 μ s. In our study conductivity value shows fluctuations may be due to the contamination from domestic sewage and inorganic fertilizer inputs [16], it may be due to bicarbonate and calcium ions present in the rocks [12], turbidity were measured with a minimum range of 0.01 NTU during post-monsoon with a mean 0.13 NTU to a maximum range of 1.28 NTU during monsoon with a mean 0.85 NTU. Turbidity values in all the selected sites were found within the permissible limit of Bureau of Indian Standard [17]. However, most of the water sample was observed high turbid during monsoon due heavy rain, agricultural runoff and soil erosion around studied areas. It might be due to human activities, decrease in the water level and presence of suspended particulate matter [18]. Turbidity can interfere with disinfection and provide a medium for microbial growth [19]. These organisms include bacteria, viruses and parasites that can cause symptoms such as nausea, cramps, diarrhea and associated headaches [20]. Salinity were measured with a minimum range of 24.63 mg/l during pre-monsoon with a mean 42.53 mg/l to maximum range of 86 mg/l during monsoon with a mean 60.9 mg/l. The range of salinity in all the water sample were found within the limit. The reason for the high amount of salinity

might be the dissolution of organic waste due to discharge of industrial effluents containing high concentration of chlorides [13]. Presence of dissolved oxygen (DO) is a significant sign of water contamination. In our study dissolved oxygen were measured with a minimum range of 1.2mg/l during pre-monsoon with a mean 1.69 mg/l to maximum range of 3.2mg/l during post-monsoon with a mean 2.23 mg/l. All the observed ranges were within the limit. In compare to our study, several authors also observed high range of dissolved oxygen in winter and summer [21,22]. Hardness were measured with a minimum range of 28mg/l during post-monsoon with a mean 49.4 mg/l to maximum range of 240 mg/l during monsoon with a mean 128 mg/l. Hardness in water of all the selected areas were found within the permissible limit. The highest range of hardness was observed in monsoon. In the present study, the higher concentration of copper (Cu) (0.179 mg/l) and cadmium (Cd) (0.005mg/l) were determined which is within the permissible limit and the concentration of lead (Pb) and chromium (Cr) in water samples was found below detection limit in all the three season. However, the concentration of iron (Fe) were determined with a range of 0.126 mg/l - 10.997 mg/l, exceeds the permissible limit of Bureau of Indian Standard [17]. The high concentration of iron was observed during pre-monsoon followed by monsoon and post monsoon. The higher concentration may be due to the anthropogenic activities, sedimentary rocks etc. around the study sites. Higher concentration of iron in water sample may be due to the nature of the metal that is strongly absorbed to soil and more easily dissolved in minutely negligible amounts [24]. Similarly, several authors also observed nearly same concentration of heavy metals in their studies [25,26]. The bacteriological analysis of water plays a vital role as it helps to determine potability of water for drinking and other domestic purposes. In the study highest percentage of bacteria were isolated in monsoon (43.9%) followed by post-monsoon (29.2%) and pre-monsoon (26.8%). Isolation of organisms such as *Escherichia coli* and *Klebsiella sp* was in significant number as compared to other organisms like *Citrobacter sp*, *Salmonella sp*, *Shigella sp*, *Proteus sp* etc. These pathogenic microorganisms lead to the public health significance, such as gastrointestinal infections, diarrhoea, dysentery, typhoid and other infections [17]. Contamination with various bacteria in water may be due to the presence of bushes and shrubs around the water bodies, rapid growth of population, poor sanitation and lack of cleanliness around the spring bodies [24].

TABLE 1. COMPARATIVE ANALYSIS OF PHYSICAL PARAMETERS

Parameter	Unit	Monsoon			Post-monsoon			Pre-monsoon		
		Min	Max	Mean	Min	Max	Mean	Min	Max	Mean
Temperature	°C	20	25	22	11	21	17.3	13	22	18.2
pH	-	6	7.7	6.9	7.24	7.42	7.32	7.75	8.86	8
TDS	mg/l	48	69	58.3	17.8	42.7	26.5	10.57	37.47	21.82
Conductivity	µs	32	60	41.3	39.62	94.75	54.8	23.51	78.53	44.52
Turbidity	NTU	0.23	1.28	0.85	0.01	0.22	0.13	0.46	0.92	0.70
Salinity	mg/l	42	86	60.9	36.63	77.68	50.81	24.63	67.89	42.53
Dissolved Oxygen	mg/l	1.68	2.32	1.96	1.6	3.2	2.23	1.2	2.24	1.69
Hardness	mg/l	76	240	128	28	76	49.4	36	80	60.5

All the collected samples were analysed during monsoon, post- monsoon and pre- monsoon at an interval of four months and the obtained results shows seasonal variation. Values were expressed in mg/l=milligram per liter, NTU= Nephelometric Turbidity Unit, µs=microsiemens and °C=degree centigrade.

TABLE 2. COMPARATIVE ANALYSIS OF HEAVY METAL IN WATER SAMPLE.

Parameter	Unit	Monsoon		Post-monsoon		Pre-monsoon	
		Min	Max	Min	Max	Min	Max
Copper	mg/l	BDL*	0.072	BDL	0.058	BDL	0.179
Lead	mg/l	BDL	BDL	BDL	BDL	BDL	BDL
Iron	mg/l	0.324	8.509	0.126	3.225	0.596	10.997
Chromium	mg/l	BDL	BDL	BDL	BDL	BDL	BDL
Cadmium	mg/l	BDL	0.003	BDL	0.003	BDL	0.005

*BDL: Below Detection Limit.Heavy metal in water sample were analysed for three season and all the observed values expressed in mg/l= milligram per liter.

TABLE 3. TOTAL PERCENTAGE OF BACTERIAL ORGANISMS ISOLATED IN SELECTED SPRING WATER.

ORGANISM	Monsoon	Post-monsoon	Pre-monsoon
E.coli	9 (25%)	7 (29.1%)	3 (13.6%)
Klebsiella aerogenes	0%	0%	0%
Klebsiella pneumonia	4 (11.2%)	3 (12.5%)	3(13.6%)
Klebsiella oxytoca	2 (5.6%)	2(8.3%)	1 (4.5%)
Klebsiella ozania	3 (8.4%)	2 (8.3%)	2 (9%)
Klebsiella ornitholytica	0%	0%	0%
Klebsiella planticola	0%	0%	0%
Shigella dysenteria	0%	1 (4.1%)	0%
Shigella flexneri	0%	0%	0%
Shigella boydii	0%	2 (8.3%)	1 (4.5%)
Salmonella typhi	0%	1 (4.1%)	0%
Salmonella paratyphi A	0%	1 (4.1%)	0%
Proteus vulgaris	0%	0%	0%
Proteus mirrabilis	2 (5.6%)	1 (4.1%)	0%
Citrobacter freundii	0%	0%	0%
Citrobacter koseri	0%	1 (4.1%)	1 (4.5%)
Hafnia alevi	0%	0%	1 (4.5%)
Morganella morganii	2 (5.6%)	0%	1 (4.5%)
Providencia rettgeri	2 (5.6%)	0%	0%
Providencia stuartii	1 (2.8%)	1 (4.1%)	0%
Serratia marcescens	3 (8.4%)	0%	0%

<i>Pseudomonas oleovorans</i>	1 (2.8%)	0%	0%
<i>Pseudomonas alcaligenes</i>	2 (5.6%)	0%	0%
<i>Pseudomonas aeruginosa</i>	0%	1 (4.1%)	0%
<i>Pseudomonas putida</i>	2 (5.6%)	0%	0%
<i>Enterobacter cloacae</i>	1 (2.8%)	1 (4.1%)	1 (4.5%)
<i>Alcaligenes faecalis</i>	2 (5.6%)	0%	0%
<i>Acinetobacter lwoffii</i>	0%	0%	2 (9%)
<i>Acinetobacter baumannii</i>	0%	0%	1 (4.5%)
<i>Aeromonas hydrophilla</i>	0%	0%	3 (13.6%)
<i>Escherichia hermannii</i>	0%	0%	1 (4.5%)
Moraxella group	0%	0%	1 (4.5%)
Total	36 (43.9%)	24(29.2%)	22(26.8%)

Isolation of bacterial organisms in selected spring water during monsoon, post-monsoon and pre-monsoon.

IV. CONCLUSION

The present study showed that the physical parameters and heavy metals in spring water of selected areas were found within the permissible limit except the value of pH and iron (Fe) in few areas exceeds the permissible limit. However, water of all the selected areas in North Sikkim were found to be highly contaminated with various pathogenic bacteria which can cause serious health problem. Presence of heavy metal and bacteria in drinking water make serious threat to human health if exposing to long term. Thus, personal hygiene, proper disposal of human and animal waste and maintenance is essential to these water bodies to maintain a quality of water and the treatment or boiling of water is very essential before consumption and other domestic purposes to lead better quality of life.

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REFERENCES

- [1] Geographical information of Sikkim.
- [2] Office of the registrar general census commissioner (2011) census of India 2011, series I, India, paper 2 of 2011, provisional population totals, Registrar general and census commissioner, New Delhi.
- [3] Tripathi, D.K. (2015). Physicochemical Analysis of Selected Springs Water Samples of Dehradun City, Uttarakhand, India. *Int. J. for innovative research in science and technology*. 2(05):99-103.
- [4] Ramteke, P.W. (1992). Evaluation of coliforms as indicators of water quality in India. *72(4):352-6*.
- [5] Starkel, L. (1970): Cause and effects of a heavy rainfall in Darjeeling and in the Sikkim Himalayas. *J. Bombay Nat. Hist. Soc.* 67, 45–50.
- [6] Kumar, R.R & Parita, K. (2014). Physico-chemical and microbial analysis of drinking water in Rajkot district, Gujarat (India). *IJES*, 5 (2), 0976-4402.
- [7] Beniston, M. (2003). Climate change in mountain regions: a review of possible impacts. *Climate Change*
- [8] Nwachu, E. (2013). Bacteriological and physicochemical qualities of drinking water sources in local area of Eastern Nigeria. *J. Env. Sc. And water Res* 2(9): 336-341.
- [9] APHA (2005): Standard methods for examination of water and waste water, 20th edition, American Public Health Association, Washington D.C.
- [10] Collee, J.G. (1996). Mackie and McCartney Practical Medical Microbiology. 14th ed. London: Churchill Livingstone. 889–92.
- [11] Ekhaise, F.O. (2005). Influence of brewery effluent discharge on the microbiological and physicochemical quality of Ikpobar river, Nigeria. *Afri. J. Biotechnol* 4, 1062-1065.
- [12] Sheikh, M.A. (2013). A study of physico-chemical characteristics of three fresh water springs of Kashmir Himalaya. India. *Int. J. of Water Resources and Environmental Engineering*. 5(6):328-331.
- [13] Krishnan, R.R., Dharmaraj, K., Kumari, B.D.R (2007). A comparative study on the physicochemical and bacterial analysis of drinking, borewell and sewage water in the three different places of Sivakasi. *J. of Env. Biology*, 28 (1), 105-108.
- [14] EPA (2003), US: Environmental Protection Agency, Safe Drinking Water Act, EPA 816-F-03-016
- [15] WHO (2006). Guidelines for Drinking Water Quality. First Addendum to 3rd Edition, vol. 1. Geneva.

- [16] Kumar, P.B.A.N. (1996).Phytoextraction: The use of plants to remove heavy metals from soils. *Environ. Sci. Technol.* 29(5):1232-1238.
- [17] Drinking water specifications: (2005)10500 Indian standard (IS),10500 Bureau of Indian standard (BIS).
- [18] Yashoda, T., Byragi, B.T., Ramana, C.V (2014).Pre and postmonsoon variation in physico-chemical characteristics in ground water quality of Mindi industrial area, Visakhapatnam, India. *IJES*,4(5),0976-4402.
- [19] National Primary Drinking Water Regulations (NPDWR) and Massachusetts Drinking Water Regulations,(2002), Standard Health Effects Language for Public Notification, Department of Environmental Protection.
- [20] US EPA (2003), Water: Basic Information about Regulated Drinking Water Contaminants.
- [21] Poonia, S. (2014). Antibiotic susceptibility profile of bacteria isolated from natural sources of water from rural areas of East Sikkim, *Indian J. com. Med.*39(3):156-160.
- [22] Manjare, S.A.,Vhanalakar S.A., Muley,D.V. (2010). Analysis of water quality ushing physico-chemical parameters, Tamdalge tank in Kholapur district, Maharashtra. *Int.J. Adv.Biotec. Res.*1,115-9.
- [23] Parashar, C., Dixit, S., & Srivastava, R. (2006), Seasonal Variations in Physicochemical characteristics in upper lake of Bhopal, *Asian Journal of Experimental Science*, 20(2), pp 297-302
- [24] Sunday, O.E. (2012). Physicochemical andMicrobiological analysis of water bodies in uturu, Abia state- Nigeria, *As. J. of natural and app. Sciences.* 1(4): 58-65.ISSN: 22186-8476.
- [25] Jeelani, G. (2010). Chemical and microbial contamination of Anantnag springs, Kashmir valley. *J.HimalayanEcol.sustain dev.* 5, 0973-7502.
- [26] Afiukwa, & Eboatu (2013). Analysis of spring water quality in Ebonyi south zone and its health impact.2153-649X.