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AN ASSESSMENT OF BACTERIOLOGICAL AND PHYSICO-CHEMICAL PROPERTIES OF THE SPRING WATER FROM SIKKIM, INDIA

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ABSTRACT

The study's aim is to assess drinking water quality in the springs of the state of Sikkim. Physico-chemical properties and pathogenic bacterial presence (including their drug resistance profile) have been studied across seasons. Important findings are (a) the concentration of iron and manganese is above the permissible limit; (b) high contamination with pathogenic bacteria exists. These are *Escherichia coli* (22.4%) followed by *Klebsiella spp*. (20.6%), *Citrobacter spp.* (7.5%) and *Shigella spp.* (7.1%). Isolated samples show that these bacteria have high resistance to common antibiotics.

Keywords: Antibiotics, Bacterial, Chemical, Physical, Spring water, Seasons.

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INTRODUCTION

In the global context, water pollution is a serious health issue.[1] Diarrhea diseases continue to be the second leading cause of death in children under the age of five worldwide.[2] As the water becomes inadequate, residents tend to use local sources without any treatment, which leads to problems with health and sanitation. Over the years, population growth[3] the aggressive impact of urbanization[4], and enormous development of industries had a severe impact on the quality of water. About 88% of diarrheal infections are due to the consumption of hazardous water and poor sanitation maintenance around the globe.[5] waterborne diseases are known to affect 37.7 million Indians and 90% of diarrheal cases are due to contaminated water.[6] Pathogens such as *Escherichia coli, Shigella spp., Salmonella spp.,* and others are the most commonly encountered pathogens in water sources and are responsible for a variety of water-borne diseases such as diarrhea, dysentery, typhoid even enteric fever.[7] The properties of water influence the category and features of drinking water.[8] Sikkim is a hilly region, and inhabitants of rural areas depend on spring water as their main source for drinking and other domestic purposes. Almost all the spring water sources are contaminated with chemical and bacterial characteristics due to the growth of the population, and urbanization. There have been few studies of spring water sources in Sikkim in terms of the physical, chemical, and microbial quality of the water consumed by people in rural areas. As a result, this study was undertaken with the goal of determining the physicochemical and bacterial water quality of various spring sources.

EXPERIMENTAL

Physicochemical Analysis

A liter of spring water was collected for physicochemical analysis. The determination of physical parameters was performed within 24 hours of sample collection and chemical analysis was done within 6 months of adding HNO_3 as a preservative (APHA, 2005).[9] A pocket-sized pH meter (Hanna Instrument) and a mercury thermometer were used to measure pH and temperature on the spot, while total dissolved solids, conductivity, turbidity, salinity, dissolved oxygen, hardness, and alkalinity were measured using a conductivity meter winkler method, nephelometric and titrimetric methods. The concentration level of

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chemicals such as calcium, magnesium, iron, manganese, lead, nickel, and chromium was determined using inductively coupled plasma mass spectrometry (Perkin-Elmer Nexion 300X). All the results were compared with the guidelines given by the Bureau of Indian Standards.

Bacteriological Analysis

About 500 ml of water samples for bacterial analysis were collected and transported maintaining the cold-chain condition and the analysis was done within 6 hours of sample collection. For the detection of bacteria, the water sample has been passed through a 47 mm diameter, 0.45 μm pore size cellulose acetate gridded membrane filter from Millipore and finally transferred onto the surface of a MacConkey agar (HiMedia, Mumbai) plate. All plates were incubated at 37°C overnight. Further identification was carried out on the basis of their colonial, morphological, and biochemical properties following Mackie and McCartney.[10] Kirby Bauer disc diffusion methods on Muller-Hinton agar were used to determine the antibiotic susceptibility of isolated organisms from each sample, as described in Mackie and McCartney.[10] The results were analyzed and interpreted according to the Clinical Laboratories Standards Institute, (CLSI 2015).[11]

RESULTS AND DISCUSSION

Physico-Chemical Analysis

In the study of the 1st phase (May 2018 to April 2019) and in the 2nd phase (May 2019 to March 2020), all the physicochemical parameters are within the limits, except the level of pH, iron, and manganese. Temperature is recognized as an important factor that can influence the physical, chemical, and biological properties of bodies of water.[12] The pH is one of the most important operational water quality parameters. The present study shows all the pH values are within the limit, while a higher level was found in spring water collected from Sikip of West Sikkim during the post-monsoon of phase-I. This may be due to the presence of various chemicals, pollutants, soil or rock composition, and any other contaminants. The pH determines the corrosivity of water, but its association with several other parameters is multifaceted.[13] The study also observed TDS levels within the limit. A high concentration of TDS can have a detrimental effect on household appliances due to unwarranted scaling[14] and a high concentration of TDS can adversely affect patients having kidney and heart diseases.[15] The maximum value of conductivity was 314.53 µs/cm in west Sikkim during the post-monsoon of phase-I. Conductivity is the capacity of the water to conduct an electrical current and is directly associated with the TDS.[16] The highest turbidity value was measured as 1.83NTU during monsoon which is found within the limit. Turbidity increases can have a negative impact on the natural features of water sources such as lakes, springs, and so on, while also harming the environment and posing health risks.[17] The study observes the highest salinity range 246.94 mg/L in west Sikkim during the pre-monsoon of phase II. The solubility of oxygen levels in water is affected by increasing salinity. Another significant feature for evaluating the condition of the water is the dissolved oxygen (DO) concentration. Like temperature, the concentration of dissolved oxygen also depends on the physical, chemical, and biological characteristics of the water body. The dissolved oxygen in the water body decreases as temperature rises.[18] In the present study, DO, hardness, and alkalinity was observed to be within the limit in all the collected water samples in three seasons as per the standard guidelines of the Bureau of Indian Standards.[19] During the study, the concentrations of calcium (18.94 mg/L) and magnesium (30.01 mg/L) were found to be within the limit, while the concentrations of copper, zinc, lead, cadmium, chromium, and nickel were found to be below the detection limit (BDL) in all water samples whereas the level of iron in Pipalay (12.3 mg/l) during the monsoon season of phase-I and manganese in Karung (1.95 mg/l) in the monsoon of phase-II was found beyond to the limit as per the standard guidelines of BIS[19] as shown in Fig.-1 to 2. As the higher concentration of iron can cause deposition in the water tanks, pipelines, and water heaters even their presence can affect the taste and color of the food and water and our results are as expected.

Bacterial Analysis

In the 1st phase (May 2018 to April 2019, a total of 278 organisms were isolated and in the 2nd phase (May 2019 to March 2020), 293 organisms were isolated from the selected areas. *Escherichia coli* (22.4%) was the most common organism isolated from the studies, followed by *Klebsiella spp.* (20.6%), *Citrobacter spp.* (7.5%), *Shigella spp*. (7.1%), *Salmonella spp.* (6.8%), *Proteus spp*. (6.6%), *Pseudomonas spp*. (8.7%), *Enterobacter spp*. (4.7%), *Aeromonas spp*. (2.8%), *Providencia spp*. (2.9%), *Acinetobacter spp*. (2.8%), *Serratia spp*. (4%), *Morganella spp*. (1.2%), *Alcaligenes faecalis* (0.35%), *Hafnia alvei* (0.52%), *Delftiaacidovorans, Moraxella* and *Escherichahermanii* (0.17%). The isolation of organisms had no significance in comparison to the two phases and seasonally.

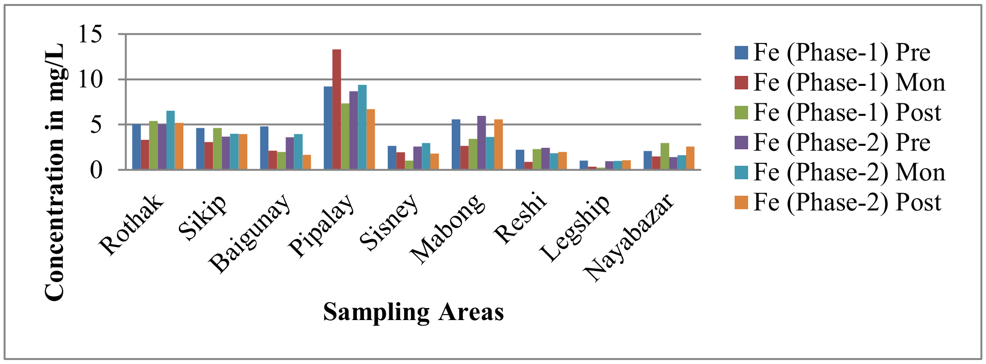

Fig.-1: Concentration of Iron in spring water in selected areas of Sikkim

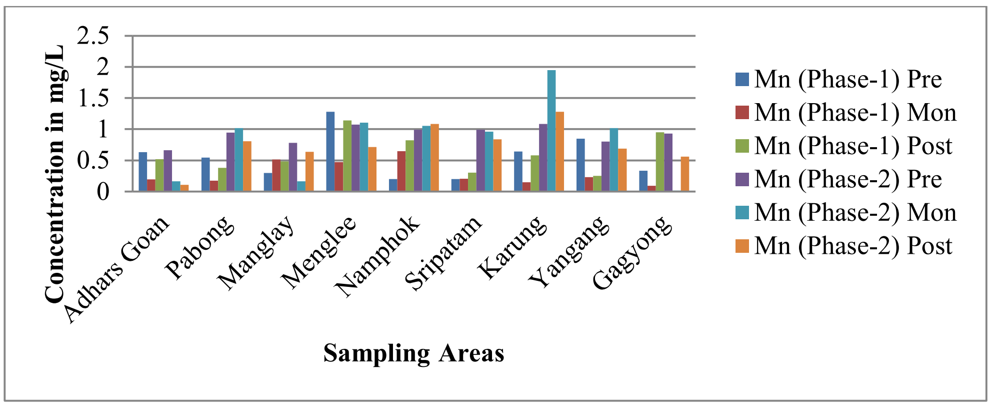

Fig.-2: Concentration of Manganese in spring water in selected areas of Sikkim

The rate of organism isolation varied between districts; south district samples had (phase-I = 54; phase-II = 59); compared to east district samples (phase-I = 80; phase-II = 86). This is because, in comparison to other districts, the southern district has recurrent water scarcity all year, as shown in Fig.-3. The principal bacteriological groups recognized in the water samples were *Enterobacteriaceae, Pseudomonas spp*., and *Bacillus spp*. Likewise, other researchers[20] have also reported similar findings. *E. coli, Klebsiella spp., Citrobacter spp., Shigella spp.,* and *Salmonella spp.* were found the most isolated organism in the study. *Pseudomonas spp.* isolation from water has also been reported elsewhere.[21] Isolation of *Salmonella* and *Shigella* is a matter of concern and has been reported from stream waters of Nigeria by Shittu *et al*.[22] However the present study isolated *Salmonella Typhi* and *Salmonella Paratyphi* and also *Shigella spp. Salmonellae spp*. can endure in water and soil for a long period if the surrounding factors like humidity, pH, etc. are favourable[23] *Aeromonas hydrophila* has been gaining public health importance over the recent years as an opportunistic pathogen. Being ubiquitous in nature, it has recurrently been isolated from various sources like food, water, and aquatic environments.[24]

Antibiotic Sensitivity Result

In the present study, isolated bacteria from different spring water sources in Sikkim have indicated a significant percentage of antibiotic resistance: Ampicillin (100%) followed by Tetracycline (81.2%) Ceftazidime (66.7%), Cotrimoxazole (65%), Gentamicin (62%) and Amikacin (58%), Piperacillin/tazobactam (60%), Imipenem (60%), Amoxiclave (55%), Chloramphenicol (65%), Ofloxacin

(54%), Ciprofloxacin (48%) and Amikacin (2.4%). Antimicrobial resistance in the surface water is influenced by human, animal and environmental factors.[25]

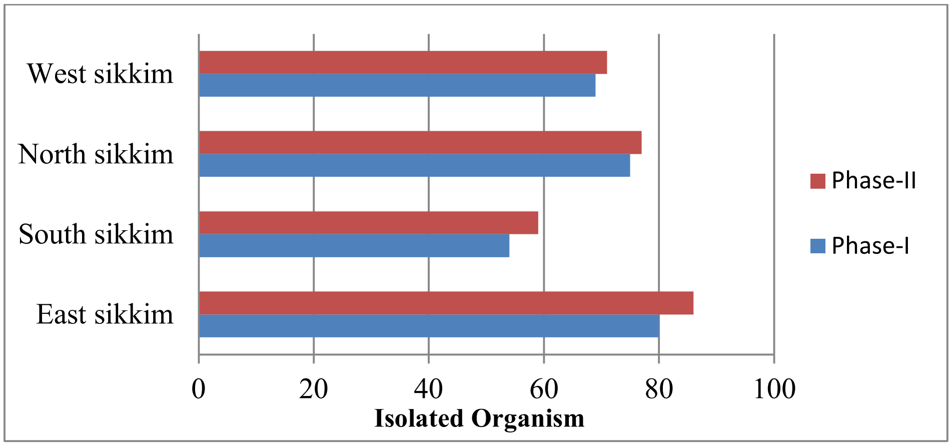

Fig.-3: Isolated Organisms in Four Districts of Sikkim in Phase-I and Phase-II

Many authors said that the high level of antibiotic resistance in water is exhibited where more contact with human and animal activities occurs.[26] The findings of the present study showed that *Escherichia coli* and *Klebsiella spp*. are more than 40% resistant to various selected drugs. Other authors have also reported similar observations.[27] Antibiotic resistance in bacteria is accelerated by uncontrolled antibiotic use.[28] Untreated surface water for drinking and domestic use becomes a threat to public health as it serves as a reservoir for the transfer of many antibiotic-resistant bacteria.[29] The present study brings to attention the fact that the resistance trends between phase-I and phase-II among the antibiotics tested were found to be increasing for various organisms isolated in spring water due to various factors such as genetic mutation, growth of population, and improper use of antibiotics. This is a serious health issue in the state of Sikkim, which relies on surface water. Regulatory authorities need to come up with preventive measures.

CONCLUSION

Water contamination can cause a variety of health problems. Routine basic analysis for drinking water is paramount and cannot be compromised. As the spring water has become more contaminated in recent years, the study recommends further investigation, raising awareness, regular monitoring, and maintaining hygiene systems in and around the sources.

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CONFLICT OF INTERESTS

The authors declare that there is no conflict of interest.

AUTHOR CONTRIBUTIONS

All the authors contributed significantly to this work, participated in reviewing/editing and approved the final draft for publication. The research profile of the authors can be verified from their ORCID ids, which is given below.

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REFERENCES

1. Z. Kılıç, *Journal of Istanbul Sabahattin Zaim University Natural Sciences Institute,* **3(2)**, 129(2021), https://doi.org/10.47769/izufbed.862679
2. Recha, W. Charles, and W.M. Manetu, *Open journal Epidemiology*, **11(3)**, 207(2021), http://dx.doi.org/10.4236/ojepi.2021.113018
3. B. K. Mishra, P. Kumar, C. Saraswat, S. Chakraborty, and A. Gautam, *Water,* **13(4)**, 490(2021), https://doi.org/10.3390/w13040490
4. S.J. Ferreira, S. Pinel, E. A. Ríos-Villamizar, S.A.F. Miranda, D. Pascoaloto, A.R. T.s Vital, M. T. Ferreira Monteiro et al., *Environmental Earth Sciences,* **80(8)**, 1(2021), https://dx.doi.org/10.1007/s12665-021-09621-7
5. A.Z. Kostic, N.D. Pantelic, J.P. Jonas, L.M. Kaluđerovic, B.P. Dojcinovic and J.B. Popovic-Djordjevic, *Exposure & Health,* **8(2)**, 227(2016), http://doi.org/10.1007/s12403-016-0197-7
6. WHO – *World Health Organization Guidelines for Drinking Water Quality*, 3rd ed., *WHO Press Geneva,* 1 (2008).
7. B.C. Sharma, B. Rai, *Indian Journal of Fundamental and Applied Life Science*, **2(2)**, 108(2012), http://www.cibtech.org/jls.htm
8. Y. Liu, B. Moy,Y. Kong, J. H. Ta, *Enzyme and Microbial Technology*, **46(6)**, 520(2010), DOI:10.1016/j.enzmictec.2010.02.001
9. APHA: *Standard Methods for Examination of Water and Waste Water*, 20th edition, *American Public Health Association, Washington, D.C.* (2005).
10. J.G. Collee, A.G. Frasher, B.P. Marmion and A. Simmons, *Mackie and McCartney, Practical Medical Microbiology,* 14th edition (1996).
11. P.A. Wayne, *Clinical and Laboratory Standard Institute,* CLSI (2015).
12. D.R. Khanna, R. Bhutiani, G. Matta, V. Singh and FouziaIshaq, *Environment Conservation Journal,* **13(1&2)**, 145(2012)
13. pH in Drinking-water, Revised Background Document for Development of WHO Guidelines for Drinking-water Quality, WHO/SDE/WSH/ 2007, 07.01/1
14. Total dissolved solids in Drinking Water Background Document for Development of WHO Guidelines for Drinking-water Quality, WHO, SDE, WSH, 2nd, 2, 2003, 03.04/16
15. M. Kumar and A. Puri, *Indian Journal Occupational and Environmental Medicine,* **16(1)**, 40(2012), https://doi.org/10.4103/0019-5278.99696
16. H.M.A. Rossiter, P.A. Owusu, E. Awuah and M.M. Alan, *Science Total Environment*, **408(11)**, 2378(2010), https://doi.org/10.1016/j.scitotenv.2010.01.053
17. H. Zvikomborero, *Physics and Chemistry of the Earth*, **30(11-16)**, 859(2005) https://doi.org/10.1016/j.pce.2005.08.031
18. M. Rajesh and S. Rehana, *Scientific Reports*, **12**, 9222(2022), https://doi.org/10.1038/s41598-022-12996-7
19. *Indian standard,* Drinking Water- Specifications, IS 10500 (2012).
20. C. Zvidzai, T. Mukurtiwa, R. Mundembe, L. Sithole-Niang L, *African Journal Microbiology Research,* **1(6)**, 100(2007), https://doi.org/10.5897/AJMR.9000486
21. E.E. Geldreich, *Microbial Quality of Water Supply in Distribution Systems*, CRC Press, Bioscience, Engineering & Technology, 512 (1996), https://doi.org/10.1201/9781003075622
22. O.B. Shittu, J.O. Olaitan, T.S. Amusa, *African Journal of Biomedical Research,* **11(3)**, 285(2008), https://doi.org/10.4314/ajbr.v11i3.50739
23. M. Dworkin, S. Falkow, E. Rosenberg, E*lectronic Release* 3.14, *3rd ed., Eds. Springer-Verlag,* New York, NY, USA, (2003).

24. Guidelines for Canadian Drinking Water Quality: Guideline Technical Document. Bacterial Waterborne Pathogens- Current and Emerging Organisms of Concern. *Health Canada*: Ottawa, Ontario, Canada, (2006).
25. C. N. Ateba, N. M. Tabi, J. Fri, M. E. A. Bissong, C. C. Bezuidenhout, *Antibiotics*, **9(11)**, 745(2020), https://doi.org/10.3390/antibiotics9110745
26. M. Amato, D. Dasi, A. Gonzalez, M. A. Ferrus, M. A. Casyillo, *Agricultural Water Management,* **256,** 107097(2021), https://doi.org/10.1016/j.agwat.2021.107097
27. S. Poonia, T.S. Singh, D.C. Tsering, *Indian Journal of Community Medicine,* **39(3)**, 156(2014), http://doi.org/10.4103/0970-0218.137152
28. N.C. Onwa, I. C. Uzomaka, A. L. Maduak, E. E. Elom, M.O. Ikeanumba, V.F. Nwode, *International Journal of Pharmaceutical Science Invention*, **8(2)**, 55(2019)
29. J.O. Oluyege, A.C. Dada, A.T. Odeyemi, *Water Science and Technology*, **59(10)**, 1929(2009), https://doi.org/10.2166/wst.2009.219

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