JETIR.ORG ISSN: 2349-5162 | ESTD Year : 2014 | Monthly Issue
JOURNAL OF EMERGING TECHNOLOGIES AND INNOVATIVE RESEARCH (JETIR)
An International Scholarly Open Access, Peer-reviewed, Refereed Journal

Microbiological Study and Diseases Caused from Water Sample of Machagora Dam Reservoir, Chhindwara

Shareen Bano Ahmed[1] , Mamta Prajapati[2]

[1,2]**Department of Chemistry Pt. S.N. Shukla University, Shahdol (M.P.)**

Abstract

The objective to estimate the impact of the reservoir on various microbiological parameters of the water. months and early monsoon due to the higher phyto planktonic production. The microbiological characteristics included green algae, Blue green algae, Diatoms, Total Coliforms, Faecal Coliform and Total plate count. Microbiological properties of the dam reservoir water at different sites with an objective to indicate changes in the quality of water at the beginning and lower end of the reservoir. The study is important to the present situation and can be applied in similar environmental compartments in the future to access the pollution status of other reservoir in the country.

Key words: Mircobes, Machgora Dam, Bacterial count, MPN, Coliform etc.

Introduction

Water plays a pivotal role in human life. It is indispensable and one of the precious natural resources of this planet. The urbanization and industrialization leads to spoil the water. Ground water is explored in rural areas especially for agriculture in those areas where other sources of water like dam, river or canal is not available. This is observed that the ground water getting polluted drastically because of increased human activities. Waterborne diseases cause health hazards. Therefore, monitoring of water quality is necessary to observe the pollution level in water bodies. Water is essential for life on planet and in life for different purposes i.e., for drinking, industries, irrigation, swimming, and fishing etc. Water for different purposes requires different composition and purity; it is also an essential part of protoplasm and creates a state for metabolic activities to occur smoothly. The quality of water is typically determined by monitoring microbial presence, especially fecal coliform bacteria (FC). These parameters could be affected by external and internal factors. Many potential pathogens could be associated with water; it is thus impractical to screen samples for all possible pathogens. Instead, various indicator organisms have been used as surrogate markers of risk. Most waterborne disease is related to faecal pollution of water sources (Jiang SC et al., 2022), therefore, water microbiology is largely based on the need to identify indicators of faecal

pollution such as coliforms and *E.coli,* but the use of enterococci and *Clostridium perfringens* is increasing. In addition, the less specific term 'faecal coliforms' (which includes species of *Klebsiella*, *Enterobacter*, and *Citrobacter*) is used in recreational water testing because the examination of large numbers of colonies to identify *E. coli* is labor sensitive. (Wassie, S.B, 2020)

A large number of microorganisms both saprophytes and pathogens and also belonging to various groups like Bacteria, Fungi, Algae, Protozoa and Nematodes are found in water. The majority of bacteria found in water belong to groups like fluorescent bacteria (*Pseudomonas*), chromogenic rods (*Xanthomonas*), coliforms groups (*E.coli* and *Arthrobacter*), *proteus*, non-spore forming rods, spore forming rods (*Bacillus*), pigmented and non-pigmented cocci (*Micrococci*) (Dubey and Maheshwari, 2000; Sivakumar N et a., 2020). The presence of microorganisms may contaminate or pollute the water and when this contaminated water is used for drinking purposes it may become a very severe biological hazard.(EPA, 2021; Ashbolt NJ, 2015)

Pollution in water causes the following water-borne diseases. Cholera is considered to be one of the major diseases caused by water pollution. If the small intestine is infected by unclean and polluted water, then it causes diarrhea. (Coelho, L. M.,2015) This condition finally results in dehydration, which decreases the level of water in body. This adverse condition may even cause deaths. Diarrhea most frequently occurs in infants and seniors as their immune system is not strong enough. When the contaminated and polluted water is consumed; the pathogenic bacteria found in water grow and multiply in the human body. This may lead to kidney failure and intestinal haemorrhage if diagnosis and treatment are not done at the earliest.(WHO, 2022) This water borne disease is known as typhoid. Viral infection infectious Hepatitis (Jaundice), Poliomyelitis and Protozoal infections Amoebic dysentery also caused by Water Pollution.Worms are present in the human intestines, this may adversely affect your lungs. These intestinal worms are called hookworms. Inadequate protein and lack of essential nutrients can obstruct the immune system's activities; worms also caused Giardiasis, Ascariasis etc. Some types of algae are toxic and may overgrow due to the presence of nitrates and phosphates in runoff water (especially agricultural runoff), such overgrowth is usually referred to as "red tide" or "brown tides". Their toxin may affect the food chain including fish and birds, and ultimately humans. Oxygen depletion in polluted water is another serious problem responsible for killing fishes all over the world. (Shanko, Kebede, Jelalu Kemal, and Dufera Kenea, 2015)

Water-borne diseases are among the most recent emerging and re-emerging infectious diseases. These diseases continue to be a major cause of human mortality and morbidity. Diarrhoeal diseases remain a leading cause of illness and death in the developing world which alone causes 2.2 million out of the 3.4 million water related deaths per year, 90% of these deaths involve children less than five years of age. (Bernadeta Dadonaite, Hannah Ritchie and Max Roser et al.,2019)

The degradation of both surface and ground water resources had adverse impact on the quality of drinking water for the human use and aquatic life.Water is one of the important sources to sustain life and has long been suspected of being the source of much human illness. (Nada Sasokova, 2018)Source of surface water and ground water have

become increasingly contaminated due to increased industrial and agricultural activity. Water is known to contain large number of chemical elements, the interaction of both physical and chemical properties of water play a significant role in composition, distribution and abundance of aquatic community. (Himshikha, Dobhal, S., Ayate, D., Lal, P., 2022)

Water is a growing problem of urban cities and cities under urbanisation. To meet the demands of growing population & modern life style, water is depleting day by day and most of the available ground and other water sources are polluted. Machgora Dam Chhindwara region is at the threshold of speedy urbanization and industrialization. Along with other pollutions *viz.* air, soil etc. Water pollution in these areas is gradually increasing.

Figure 1. Machagora dam reservoir, Chhindwara (M.P.)

Material and Method

Microbiological analysis of water

All the experiment was performed under aseptic conditions in laminar air flow. All the culture media and glassware used during the experiments were sterlized by autoclaving for 15 psi at 121^0c for 15 minutes and in hot air oven, respectively. (Zahra Ghaemmaghamian et al., 2022)

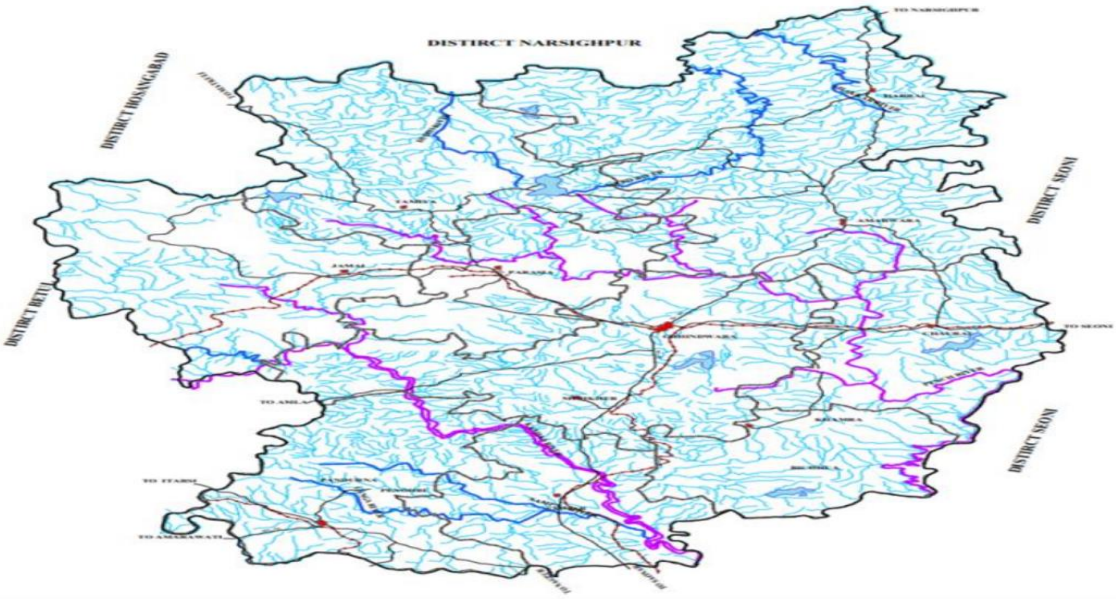


Figure 2. Study area Chhindwara (M.P.)

Most probable number (MPN)

Presumtive coliform test Determine the MPN Index with the help of MPN Index

Table 1. MPN Test

os. Tubes			MPN/g	Conf. lim.		Pos. tubes			MPN/g	Conf. lim.	
0.10	0.01	0.001		Low	High	0.10	0.01	0.001		Low	High
0	0	0	<>	–	9.5	2	2	0	21	4.5	42
0	0	1	3.0	0.15	9.6	2	2	1	28	8.7	94
0	1	0	3.0	0.15	11	2	2	2	35	8.7	94
0	1	1	6.1	1.2	18	2	3	0	29	8.7	94
0	2	0	6.2	1.2	18	2	3	1	36	8.7	94
0	3	0	9.4	3.6	38	3	0	0	23	4.6	94
1	0	0	3.6	0.17	18	3	0	1	38	8.7	110
1	0	1	7.2	1.3	18	3	0	2	64	17	180
1	0	2	11	3.6	38	3	1	0	43	9	180
1	1	0	7.4	1.3	20	3	1	1	75	17	200
1	1	1	11	3.6	38	3	1	2	120	37	420
1	2	0	11	3.6	42	3	1	3	160	40	420
1	2	1	15	4.5	42	3	2	0	93	18	420
1	3	0	16	4.5	42	3	2	1	150	37	420
2	0	0	9.2	1.4	38	3	2	2	210	40	430
2	0	1	14	3.6	42	3	2	3	290	90	1,000
2	0	2	20	4.5	42	3	3	0	240	42	1,000
2	1	0	15	3.7	42	3	3	1	460	90	2,000
2	1	1	20	4.5	42	3	3	2	1100	180	4,100
2	1	2	27	8.7	94	3	3	3	>1100	420	–

Confirmed coliform test

Label the covers of the three EMB plates and three endo agar plates with the source of the water samples using a positive 24-hour lactose broth culture from the Madhav Lake water series from the presumptive test, streak the surface of one EMB and one Endo agar plate. Repeat Step using the positive lactose broth cultures from Machgora

dam water series to inoculate the remaining plates. Incubate all plate cultures in an inverted position for 24 hours at 37°c. (Hassan Mahmoudi et al., 2017)

Completed test

Label each tube with the source of its water sample. Inoculate one lactose broth and one nutrient agar slant from the same isolated E.coli colony obtained from an EMB or an Endo plate from each of the experimental water samples. Incubate all tubes for 24 hours at 37°c. After growth of slant make gram stain from slant. (Sanders ER, 2012)

Gram Staining

Crystal violet solution for up to one minute. Iodine solution, for mordant, alcohol used for decolorization is not diluted. 95% alcohol for 10 seconds, safranin solution and allow to counterstain for 30 seconds. All slides of bacteria must be examined under the oil immersion lens (100x). (American Society for Microbiology © 2016)

Membrane Filter Method

Label the four 90 ml water blanks with the source of the water sample and dilute (10^{-1}, 10^{-2}, 10^{-3} and 10^{-4}). Using 10 ml pipettes, aseptically perform a 10 fold serial dilution of the assinged undiluted water sample, using the four 90-ml water banks to effect the 10^{-1}, 10^{-2}, 10^{-3} and 10^{-4} dilution. Arrange the 15 petri dishes into three sets of five plates

TCC and FSC plates for 24 hours at 37^0c. FCC plates sealed with waterproof tape and placed in a weighted watertight plastic bag, which is then submerged in 44.5^0c water bath for 24 hours. (APHA, 2020)

Observation

Remove the filter discs from the petri dishes and allow to dry on absorbent paper for 1 hour. Examine all filter discs under a dissecting microscope and perform colony counts on each set of discs as folows. (Dubey and Maheshwari, 2010)

Total plate count (TPC)

Aseptically 10 ml sample was taken in to sterile flask. 9.0 ml diluent (normal saline) was added and shake vigorously to obtain 10^{-1} dilution. From this dilution 1 ml was transfered to 9 ml diluents (normal saline), 10^{-2}, All decimal dilutions (10^{-2}, 10^{-3}, 10^{-4}, 10^{-5}, 10^{-6}) were prepared by this method. 1 ml of each dilution was separately pipetted into appropriately marked petri dishes in duplicates. Immediately within 15 minutes. 15 ml plate count agar was added to each plate and mixed thoroughly and uniformly. Plates were incubated for 48 hrs at 37 ^{O}C ± 2^{O}C. Colonies were counted by colony counter. (Dubey and Maheshwari, 2010)

IMViC Test

Inoculated on tryptone broth and incubated for 24 hrs at 37^{O}C ± 2^{O}C. 0.5 ml kovac's reagent was added and observation was recorded. MR-VP broth and incubated for 24-48 hrs at 35 ± 2^{O}C. 5 drops of methyl red solution was added and observation was recorded. MR-VP broth and incubated for 24-48 hrs at 37^{O}C ± 2^{O}C. 0.6 ml alpha-naphthol and 0.2 ml 40% KOH solution were added in tubes, and shake. Few crystals of creatine were added and

shake it. Results were recorded after 2hrs. simmon citrate medium and incubated for 96 hrs at $37^{O}C \pm 2^{O}C$. Observation was recorded after 96 hrs. (Sushmita, 2018; Jain, A., Jain, R., Jain, S, 2020)

Result and Discussion

Microbiological analysis of water

Bacterial flora of the water samples was evaluated by MPN method. The MPN of water samples collected from three sampling sites were analyzed for its bacterial population. The coliform bacteria is the primary bacterial indicator for fecal pollution in water. The purpose of study was to make people aware about the bacterial load in water bodies. Based on this, categorical representation of water was unacceptable for the purpose of swimming and bathing. (Bhumbla U, 2020)

The MPN of machagora dam water ranged between 17 - 70 cfu/100ml in pre monsoon and in post monsoon it ranged between 31 - 240 cfu/100ml. Minimum value of MPN was observed to be 17 cfu/100ml and maximum value of MPN was observed 240 cfu/100ml. 30 (±7.81), 36.2 (±18.67) & 41.4 (±23.58) in years 2009, 10 & 11 respectively in pre monsoon and in post monsoon 60.8 (±23.97), 150.8 (±59.44) & 131.8 (±72.18) were observed. Other study suggested that Microbial confirmatory testing indicated severe fecal contamination of water sources with high counts of total coliform (TC), *Escherichia coli* (EC) and *Enterococcus* (EN). The highest level of TC was recorded from West Sikkim (37.26 cfu/100 ml) and the lowest in North Sikkim (22.13 cfu/100 ml). The highest level of contamination of *E. coli* and *Enterococcus* was found in East Sikkim (EC = 8.7 cfu/100 ml; EN = 2.08 cfu/100 ml) followed by South Sikkim (EC = 8.4 cfu/100 ml; EN = 2.05 cfu/100 ml). There was a significant positive correlation between the contamination levels of the spring water and the community reservoir tank. As far as the seasonal variation is concerned, the rainy season showed the most contamination with coliform correlating with a high incidence of different water-borne diseases (East = 86%; West = 100%; South = 100%; North = 80%). (Singh AK, 2019)

Table 2. Gwoth of Coliform Bacteria

Sample No.	LB +/-	Gram reaction	Result	Sample	LB +/-	Gram reaction	Result
01	+	Gram (-), short rod	*E. coli* confirmed	48	+	Gram (-), short rod	*E. coli* confirmed
11	+	Gram (-), short rod	*E. coli* confirmed	51	+	Gram (-), short rod	*E. coli* confirmed
13	+	Gram (-), short rod	*E. coli* confirmed	52	+	Gram (-), short rod	*E. coli* confirmed
17	+	Gram (-), short rod	*E. coli* confirmed	53	+	Gram (-), short rod	*E. coli* confirmed
18	+	Gram (-), short rod	*E. coli* confirmed	54	+	Gram (-), short rod	*E. coli* confirmed
21	+	Gram (-), short rod	*E. coli* confirmed	55	+	Gram (-), short rod	*E. coli* confirmed
24	+	Gram (-), short rod	*E. coli* confirmed	56	+	Gram (-), short rod	*E. coli* confirmed
28	+	Gram (-), short rod	*E. coli* confirmed	57	+	Gram (-), short rod	*E. coli* confirmed
36	+	Gram (-), short rod	*E. coli* confirmed	58	+	Gram (-), short rod	*E. coli* confirmed
46	+	Gram (-), short rod	*E. coli* confirmed	59	+	Gram (-), short rod	*E. coli* confirmed
47	+	Gram (-), short rod	*E. coli* confirmed				

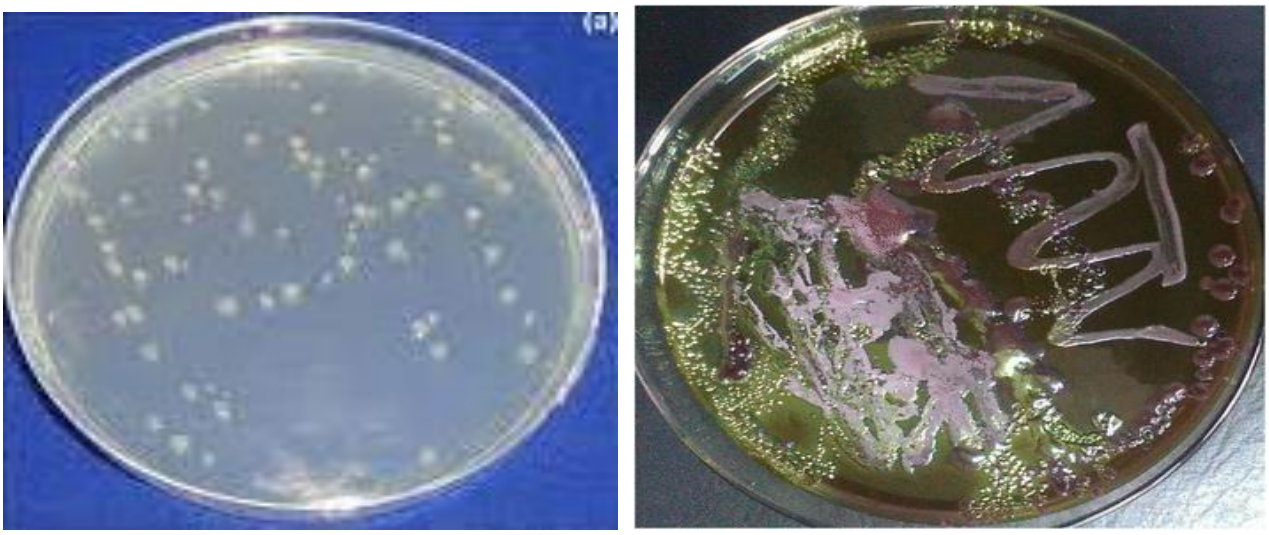


Figure 4. Isolation of Ecoli

The Membrane filter method was used to analyze coloiforms in water samples collected from three sampling sites were evaluated.

Machgora dam water total coliform, faecal coliform and faecal streptococcal coliform ranged between 26×10^4, 12×10^4 and 4×10^4 - 72×10^4, 53×10^4 and 22×10^4 cfu/100ml In pre monsoon and in post monsoon it ranged between 35×10^4, 21×10^4 and 5×10^4 - 112×10^4, 81×10^4, and 29×10^4 cfu/100ml during 2009 - 2011. Minimum value of total coliform, faecal coliform and faecal streptococcal coliform was observed 26×10^4, 12×10^4 and 4×10^4 and maximum value of total coliform, faecal coliform and faecal streptococcal coliform was observed 112×10^4, 81×10^4, and 29×10^4.

Total Bacterial Count

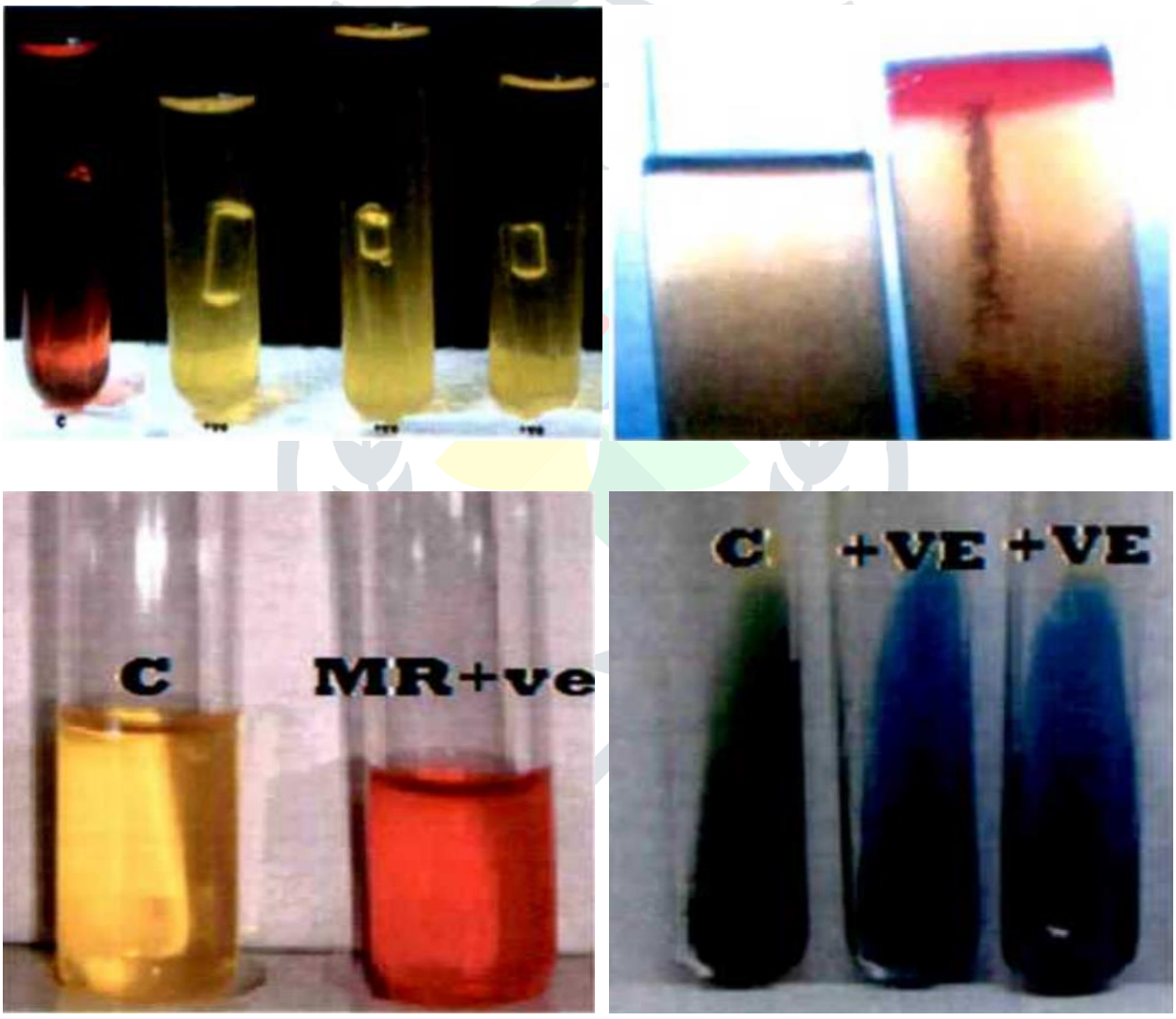
In Maximum value of Total Plate Count was found 19×10^4 cfu/ml for Machgora dam in post monsoon in year 2010 and minimum value of Total Plate Count was found 1.5×10^4 cfu/ml for Machgora dam water in post monsoon year 2009.

Biochemical analysis

Occurrence and seasonal variation of bacteria in water samples were detected on the basis of biochemical analysis. Five types of bacteria were isolated and identified from the various water samples collected from machagora dam water sources included in the study. These bacterial isolates are mainly gram-negative bacteria namely, *E. coli, E. aerogenes, Pseudomonas species* and *Salmonella species* and *Streptococcus species* is only gram positive bacteria reported in our study. 75% of gram negative and 25% gram positive bacteria were isolated from the machagora dam water samples.

The study points to possible fecal or surface runoff contamination of the drinking water sources in all districts of the state as indicated by microbiological indicators like total coliform and *E. coli* counts. The evaluated microbiological indicators were found to be well above the permissible limits of WHO for potable water. Though bacteria are naturally present in the environment like water bodies, soil, ponds and lakes, the level of coliform bacteria is generally considered as an indicator of the level of fecal contamination in water bodies and the associated health risk (Pal M, 2018). The presence of fecal coliforms above a permissible level is known to cause

ailments like nausea, cramps, diarrhea, vomiting, and headache. In rare occasions, high microbial contamination of the drinking water is associated with hemolytic uremic syndrome, which is a serious kidney disease and may cause lifelong complications (Pal M,2018). The detection of a high number of coliform bacteria in the water distribution network of Sikkim suggests fecal contamination of water, so other pathogenic bacteria like *Vibrio, Salmonella, Shigella,* and parasites like *Cryptosporidium, Giardia* may be also present. *V. cholera, Shigella,* and *Salmonella* cause diseases like cholera, bacillary dysentery and typhoid fever, respectively (von Reyn, 1994). Incidentally, several cases of acute diarrheal and enteric fever (typhoid) had been reported from Sikkim in the recent years, such as during 2014–2016 (Government of India *Water-Borne Diseases, 2017*). It prompts us to suggest possible contamination of the water distribution network of the state with the fecal material that needs to be immediately checked to improve the health index of the state residents. (Singh AK, 2019)



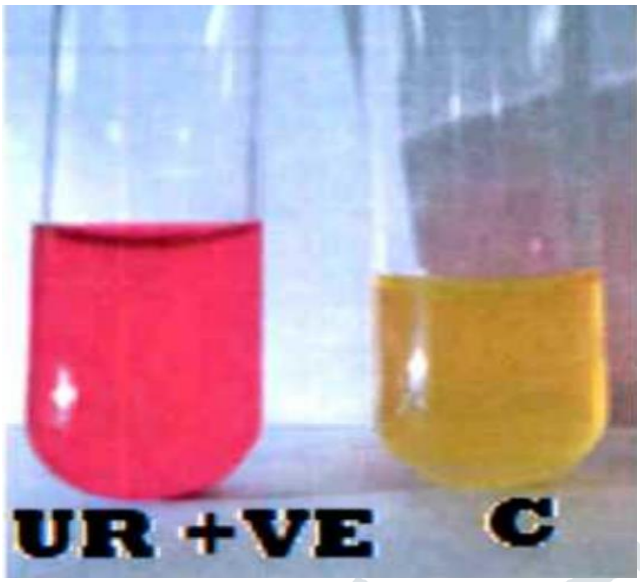


Figure 4. Biochemical Test

Conclusion

Seasonal changes observed in various microbial groups in all three sampling sites could be related to the influence of the physico-chemical properties of water. But human activities could also affect the microbial growth in pre and post monsoon season. Water of good quality and its availability is the basic importance to human physiology and man's continued existence. Before water from the source site included in the study should be checked routinely for its comply with certain physical, chemical and microbiological standards, which are designed to ensure that the water is safe to use for various purposes.

This work may help us to estimate the suitability of water of the studied area mainly for drinking & irrigation purpose. This study has found a treatment path for water pollutants and also adding a new and remarkable chapter to the knowledge of science and its role for the welfare of the mankind and society.

References

1. Jiang SC, Bischel HN, Goel R, Rosso D, Sherchan SP, Whiteson KL, Yan T, Solo-Gabriele HM. Integrating Virus Monitoring Strategies for Safe Non-Potable Water Reuse. Water. 2022; 14(8):1187. https://doi.org/10.3390/w14081187
2. Himshikha, Dobhal, S., Ayate, D., Lal, P. (2022). Influence of Anthropogenic Activities on the Biological Diversity of Forest Ecosystem. In: Rani, M., Chaudhary, B.S., Jamal, S., Kumar, P. (eds) Towards Sustainable Natural Resources. Springer, Cham. https://doi.org/10.1007/978-3-031-06443-2_12
3. Wassie, S.B. Natural resource degradation tendencies in Ethiopia: a review. Environ Syst Res 9, 33 (2020). https://doi.org/10.1186/s40068-020-00194-1
4. Sivakumar N, Sathishkumar R, Selvakumar G, Shyamkumar R, Arjunekumar K. Phyllospheric Microbiomes: Diversity, Ecological Significance, and Biotechnological Applications. Plant Microbiomes for Sustainable Agriculture. 2020 Mar 7;25:113–72. doi: 10.1007/978-3-030-38453-1_5. PMCID: PMC7123684.
5. Ashbolt NJ. Microbial Contamination of Drinking Water and Human Health from Community Water Systems. Curr Environ Health Rep. 2015 Mar;2(1):95-106. doi: 10.1007/s40572-014-0037-5. PMID: 25821716; PMCID: PMC4372141.

6. Coelho, L. M., Rezende, H. C. , Coelho, L. M. , de Sousa, P., Melo, D. F. , & Coelho, N. M. (2015). Bioremediation of Polluted Waters Using Microorganisms. In (Ed.), Advances in Bioremediation of Wastewater and Polluted Soil. IntechOpen. https://doi.org/10.5772/60770
7. Shanko, Kebede, Jelalu Kemal, and Dufera Kenea. "A Review on Confronting Zoonoses: The Role of Veterinarian and Physician." Journal of Veterinary Science & Technology (2015).
8. Pandey PK, Kass PH, Soupir ML, Biswas S, Singh VP. Contamination of water resources by pathogenic bacteria. AMB Express. 2014 Jun 28;4:51. doi: 10.1186/s13568-014-0051-x. PMID: 25006540; PMCID: PMC4077002.
9. Front. Sustain. Food Syst., 27 July 2018 Sec. Waste Management in Agroecosystems https://doi.org/10.3389/fsufs.2018.00042
10. Zahra Ghaemmaghamian, Reza Zarghami, Gavin Walker, Emmet O'Reilly, Ahmad Ziaee, Stabilizing vaccines via drying: Quality by design considerations, Advanced Drug Delivery Reviews, Volume 187, 2022,
11. Hassan Mahmoudi, Mohammad Reza Arabestani, Mohammad Yousef Alikhani, Iraj Sedighi, Hamed Farhadi Kohan, Mohammad Molavi GMS Hyg Infect Control. 2017; 12: Doc03. Published online 2017 Feb 2. doi: 10.3205/dgkh000288
12. Sanders ER. Aseptic laboratory techniques: plating methods. J Vis Exp. 2012 May 11;(63):e3064. doi: 10.3791/3064. PMID: 22617405; PMCID: PMC4846335.
13. American Public Health Association. 2020. Laboratory Procedures for the Examination of Seawater and Shellfish, 5th ed. APHA, Washington, DC.
14. Jain, A., Jain, R., Jain, S. (2020). To Perform Biochemical Identification of Microorganism: IMViC (Oxidative Fermentation and Carbohydrate Source Utilization) . In: Basic Techniques in Biochemistry, Microbiology and Molecular Biology. Springer Protocols Handbooks. Humana, New York, NY. https://doi.org/10.1007/978-1-4939-9861-6_37
15. Bhumbla U, Majumdar S, Jain S, Dalal AS. A study of isolation and identification of bacteria from lake water in and around Udaipur, Rajasthan. J Family Med Prim Care. 2020 Feb 28;9(2):751-754. doi: 10.4103/jfmpc.jfmpc_1032_19. PMID: 32318414; PMCID: PMC7113958.
16. Singh AK, Das S, Singh S, Pradhan N, Gajamer VR, Kumar S, Lepcha YD, Tiwari HK. Physicochemical Parameters and Alarming Coliform Count of the Potable Water of Eastern Himalayan State Sikkim: An Indication of Severe Fecal Contamination and Immediate Health Risk. Front Public Health. 2019 Jul 10;7:174. doi: 10.3389/fpubh.2019.00174. PMID: 31355173; PMCID: PMC6636254.
17. Pal M, Ayele Y, Hadush A, Panigrahi S, Jadhav VJ. Air and water borne diseases public health hazards due to unsafe drinking water. Air Water Borne Dis. (2018) 7:1–6. 10.4172/2167-7719.1000138 [CrossRef] [Google Scholar]
18. von Reyn CF, Marlow JN, Arbeit RD, Barber TW, Falkinham JO. Persistent colonisation of potable water as a source of Mycobacterium avium infection in AIDS. Lancet. (1994) 343:1137–41. 10.1016/S0140-6736(94)90239-9 [PubMed] [CrossRef] [Google Scholar]
19. Press Information Bureau Government of India Water-Borne Diseases New Delhi (2017). [Google Scholar]