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Seasonal Microbial variations in stream-waters from Sikkim

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ABSTRACT

Availability of clean and safe water is a critical indicator of overall health as well as developmental status. Because of growing population and developing economy safe water availability has come under severe constraints. This is true for, Sikkim, AN east Himalayan State of Sikkim, despite being rich in fresh water. Water quality remains an intensely researched area because of its criticality as also because of its varying impact across time and geography. Microbial contamination in Sikkim is yet to be studied specifically, despite a few existing studies. Historically, the state has experienced a large number of deaths of particularly infants and young children during the monsoon season due to diarrhea, typhoid or other water borne diseases. This study had been undertaken to identify pathogenic micro-organisms in the water streams of the state, particularly near human-habitation. The study concludes that 13 species of microbial species abound the streams of water in Sikkim and which are being used regularly by human inhabitants. Escherichia coli and klebsiella spp. are found most frequently. The study also reports seasonal variations with monsoon season showing highest microbial contamination and the premonsoon the least. Escherichia coli, klebsiella spp, Citrobacter spp and Enterobacter are prevalent during monsoon season and during post-monsoon phase Salmonella spp, Salmonella spp, Proteus spp and Providencia are more common. There is a strong correlation across seasonal variations and thus it gives prospects to forecast monsoon counts on the basis of premonsoon counts and fow which a regression model has been developed.

Key words : *Microbial variations, Stream-waters from Sikkim*

Introduction

Water is a unique and vital substance that we have and which plays a critical role in meeting day-to-day requirements of human existence. Across the world, necessity of fresh water will continue to increase considerably over the coming decades to meet the needs of rapid increased populations, growing economics, way of consumption and living style (Shrivastava *et al*., 2021). Around 780 million populations do not get the fresh and safe water and around 2.5 billion people do not getting proper sanitation, results around 6 to 8 million people die every year due to waterborne diseases and disaster Rahmanian *et al*. (2015). One of the most critical factor defining human healthcare and development matrix is availability of clean and safe drinking water (Anonymous, 2017; Gift *et al*., 2020). Availability of safe water has come under tremendous constrains because of exploding population, anthropogenic activities and trends towards increased urbanization (Fronczyk 2016; KC *et al.*, 2020). This is particularly concerning for the developing countries such as India (Anonymous 2018; Paul *et al.*, 2020). In India this crisis has deepened because of presence of antibiotic resistant bacteria in drinking water (Casanova *et al.*, 2016; Singh *et al*., 2016; Ram *et al.* 2020) and Blasco *et al.* (2008). Many microbial communities survive in water and given favorable conditions these grow exponentially and can create a severe health issues (Ikonen *et al.,* 2017). Human and animal fecal contents are primarily responsible for the microbial contamination of fresh water in India (Khurana *et al*., 2008). Diarrhea, caused by microbial contamination, is one of the leading causes of death and morbidity in under-five children (Srivastava *et al*., 2021). As disease and deaths due to microbial contamination of water continues to increase continuous research across geography and time remains relevant and critical.

Sikkim is a small state in the eastern Himalaya, India. It borders Tibet in north and northeast, Bhutan in the east, Nepal in the west and west Bengal in the south, Sikkim is also known for its biodiversity (Subba *et al.,* 2021). Total area of Sikkim is 7,096 sq. km with total population 6.11 Lakhs.Out of total population 25.15% people live in urban areas whereas 74.85 % people live in the rural areas (Subba *et al.,* 2021). Rural habitations of Sikkim primarily depend upon spring water (also called as dhara) and it is one of the foremost source to meet the basic needs in daily life such as drinking and other domestic purposes. Generally it is considered that spring is natural and freshwater for drinking and other domestic purposes but steadily increase the contamination of water through various sources such as improper discharge of human and animal excreta in nearby water sources, leaching of drainage, runoff water, soil erosion and landslide which is common in Sikkim due to geological condition and intensity of heavy rainfall. Therefore for the good health of community and prevention of numerous waterborne diseases, consumption of water is free from bacterial contaminants. Due to heavy rainfall it may increase the pollutants load and reaches to the water bodies.

This paper explores the presence of pathogenic microbial content of water springs in the state of Sikkim, specially focusing on the seasonal variations.

Methodology: The study is conducted in rural areas of East Sikkim, India from August 2015 - July 2017. A total of 54 spring water samples, nine sample from each area during monsoon, post-monsoon and pre- monsoon. A liter of water sample for analysis of physical parameters are collected in sterile air tight container from the selected sources. Each sample bottle is labelled with full details of the selected source of spring water at the time of sample collection. The analysis is performed within 24 hours of sample collection. Pathogenic microbial contents have been identified. .

Result and Discussion

Phase-1 results

Findings of Phase-1 has been provided in Table 1. From the table it is evident that the most common microorganisms that pollute stream-water in Sikkim are *Escherichia coli* and *Klebsiella* spp. Least common ones are *Providencia* and *Morganellamorganii*. Singh *et al.* (2019) in their study also found Escherichia coli to be highly prevalent. In terms of season monsoon emerges as the time of highest number of micro-or-

Table 1. Phase-1

Organisms	Monsoon Phase-I	Postmonsoon Phase-I	Premonsoon Phase-**I**	Total
Escherichia coli	32	27	20	79
klebsiellaspp	31	24	24	79
Citrobacterspp	12	4	2	18
Shigellaspp	3	6	3	12
Salmonella spp	6	9	5	20
Proteus spp	6	7	1	14
Pseudomonas spp	5	5	3	13
Acenitoacter	3	1	5	9
Enterobacter	4	2	2	8
Providencia	1	4	1	6
Morganellamorganii	2	2	1	5
Serratia	2	1	5	8
Aeromonashydrophilia	1	1	7	9
Total	108	93	79	280

ganisms and winters (post monsoon) as having relatively lower number of water-borne micro-organisms. An interesting observation is that whereas *Escherichia coli, Klebsiella* spp, *Citrobacter* spp and Enterobacter are found in higher numbers during monsoon, during post-monsoon phase *Salmonella* spp, *Salmonella* spp, Proteus spp and Providencia are more common during post-monsoon season. Further, *Serratia* and *Aeromonas hydrophilia* are more common during summer season (pre-monsoon). Monsoon being the time of highest water-borne microorganisms is confirmed by the traditional beliefs of Sikkim, with Lepchas, the earliest inhabitants of Sikkim calling the months of Blung (June-July) and Numkum (July-August) as dreaded months when most children die of diarrhea and corresponding dehydration.

Phase-2 results

Table 2 provides the details of second phase and which has been undertaken in. From this table it can be seen that details are very similar to the ones that were noted for the first phase. *Escherichia coli* and *Klebsiella* spp remain most prevalent water-borne micro-organisms. Also again we find that intensity of presence of microorganisms is highest during monsoon, followed by post monsoon and least during pre monsoon season.

Correlation across seasons

One question that we explore is that is the prevalence of various microorganisms across seasons correlated. To find answer to this we measured correlation coefficient across the three seasons. The results are provided in the Table 3. Pearson Correlation coefficients across Monsoon and Pre-monsoon is 0.966;

Table 2. Phase-2

Organisms	Monsoon Phase II	Postmonsoon Phase II	Premonsoon Phase-II	Total
Escherichia coli	31	28	24	83
klebsiellaspp	31	27	26	84
Citrobacterspp	11	5	4	20
Shigellaspp	6	8	4	18
Salmonella spp	6	6	4	16
Proteus spp	4	6	2	12
Pseudomonas spp	9	6	3	18
Acenitoacter	3	2	1	6
Enterobacter	5	4	2	11
Providencia	2	3	2	7
Morganellamorganii	2	1	0	3
Serratia	2	2	4	8
Aeromonashydrophilia	1	2	3	6
Total	113	101	78	292

Table 3. Correlation across seasons

		Correlations		
		Monsoon	Pre-monsoon	Post-monsoon
Monsoon	Pearson Correlation	1	.966	.935
	Sig. (2-tailed)		0	0
Premon-soon			1	0.942
	Sig. (2-tailed)			0

Table 4. Model Summary

Model	R	R Square	Adjusted R Square	F-value	p-value
1	.935	.874	.869	167	0

a. Predictors: (Constant), Pre-monsoon

between Monsoon and Post-monsoon is 0.935 and between Pre-monsoon and post-monsoon is 0.942. A very strong correlation therefore exists across seasons and which is confirmed by significance values that are below 0.001. Such a strong relation implies that relative percentage of such micro-organism remains more or less steady.

Regression Model

Such a strong correlation calls for developing a regression model for predicting the prevalence of microorganisms during monsoon season on the basis of their counts in pre-monsoon season. To explore this a regression analysis has been undertaken with monsoon-count being the dependent variable and pre-monsoon count as the independent variable. Before developing a regression equation, goodness of forthcoming model needs to be assessed. These are given in Table 4. From the table we find that value of R^2 is 0.874 and which is very high and implies that proposed model will explain 87.4 % of the variations. The fitness of model is denoted by high F-value and which is 167. Fitness of model is confirmed by corresponding p-value which is almost zero.

Having confirmed the fitness the next step is to develop regression model. Table 5 provides the details of regression model.

From the analysis it transpires that value of constant is 1.24 and that of independent variable (pre-

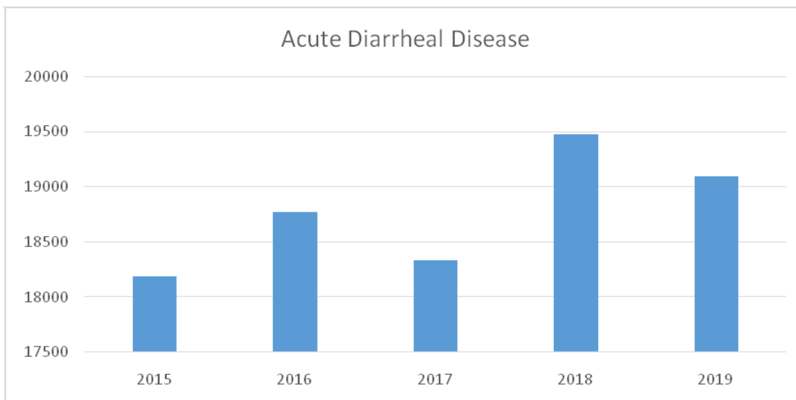

Chart 1. Acute Diarrheal Disease Trends

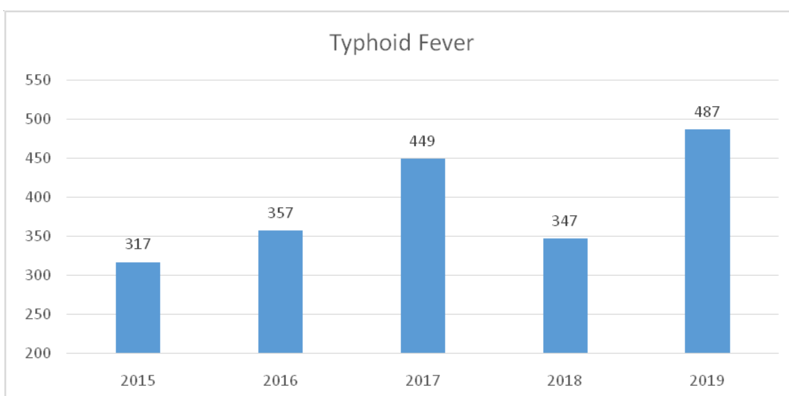

Chart 1. Typhoid Fever Trends

Table 5. Coefficients

Model		Unstandardized Coefficients		t-value	p-value
		B	Std. Error		
1	(Constant)	1.24	.926	1.34	.193
	Pre-monsoon	1.228	.095	12.927	0

a. Dependent Variable: Monsoon count

monsoon count) is 1.228. Corresponding t-values are 1.34 (which is on the lower side) and 12.927 and which implies strong significance. The regression equation, therefore, can be written as:

Monsoon count of micro-organisms = 1.24 + 1.228*pre-monsoon count.

e. Correlation across Phase: From the result of Phase-1 and phase-2 there appeared to be a strong correlation. To test this Pearson correlation coefficient across two phases is also calculated and is found to be 0.975 – again depicting a strong correlation. This needs to be corroborated using studies much longer across time. This conclusion may or may not be generalizeable.

f. Trends in Prevalence of water borne diseases: We studies the prevalence of diseases caused by the pathogenic micro-organisms in the state of Sikkim. Chart-1 depicts the recorded cases of Acute Diarrheal from the study area from 2015 to 2019. There is a distinct upward trend in the prevalence of the disease perhaps as a consequence of rising population and increased anthropogenic activities This calls for a greater action on part of civil authorities to run sensitization campaigns and use effective preventive and curative measures. Chart 2 provides details of the trends of typhoid fever as recorded in the state for the same period. Again an increasing trend is perceptible calling the healthcare workers to initiate strong measures to control such a trend.

Conclusion

Safe drinking water is recognized as a fundamental need in modern societies. Sikkim still has a large number of people depending on water streams for water supply and which has been suspected of far from being clean and safe supply of water. Historically, the state has experienced a large number of deaths of particularly infants and young children during the monsoon season due to diarrhea, typhoid or other water borne diseases. This study had been undertaken to identify pathogenic micro-organisms in the water streams of the state, particularly near human-habitation. The study concludes that 13 species of microbial species abound the streams of water in Sikkim and which are being used regularly by human inhabitants. *Escherichia coli* and *Klebsiella* spp. are found most frequently. The study also reports seasonal variations with monsoon season showing highest microbial contamination and the premonsoon the least. *Escherichia coli*, *Klebsiella* spp, *Citrobacter* spp and *Enterobacter* are prevalent during monsoon season and during post-monsoon phase *Salmonella* spp, *Salmonella* spp, *Proteus* spp and *Providencia* are more common. There is a strong correlation across seasonal variations and thus it gives prospects to forecast monsoon counts on the basis of premonsoon counts. As monsoons are dreaded for death of infants due to polluted water, this could provide ample warning for the healthcare providers and civil authorities to take preventive measures. Towards the end the researchers identified the trends in prevalence of Acute Diarrheal disease and typhoid in Sikkim, through hospital records. It is evident that numbers are increasing and which a cause of concern is particularly in the context of climate change. A warmer climate could cause waterborne diseases to become more frequent, including cholera and diarrhoeal diseases (Hales *et al.,* 2003).

REFERENCES

Anonymous World Health Organisation. *Water Safety and Quality*. Geneva 2018.

Anonymous World Health Organization. *Guidelines for Drinking-Water Quality.* Geneva (2017).

Asheesh Shrivastava*, Shalini A Tandon, Rakesh Kumar 2015. Water Quality Management Plan for Patalganga River for Drinking Purpose and Human Health Safety. *International Journal of Scientific Research in Environmental Sciences.* 3(2) : 0071-0087.

Blasco, M.D., Esteve, C. and Alcaide, E. 2008. Multiresistant waterborne pathogens isolated from water reservoirs and cooling systems. *J Appl. Microbiol.* 105 : 469–75. doi: 10.1111/j.1365-2672.2008.03765.x

Casanova, L.M. and Sobsey, M.D. Antibiotic-resistant enteric bacteria in environmentalwaters. *Water.* 2016. 8:1–7. doi: 10.3390/w8120561

Fronczyk, J., Radziemska, M., Dynowski, P., Mazur, Z. and Bazyd³o, M. 2016. Quality of water in the road drainage systems in the Warsaw agglomeration, Poland. *Water.* 8 : 1–12. doi: 10.3390/w8100429

Geldreich, EE. 1989. Drinking water microbiology-new directions toward water quality enhancement. *Int J Food Microbiol.* 9 : 295–312. doi: 10.1016/0168-1605(89)90098-6

Gift, R. A., Raimi, M., Owobi, O. E., Oluwakemi, R. M., Anu, B. and Funmilayo, A. A. 2020. Nigerians crying for availability of electricity and water: a key driver to life coping measures for deepening stay at home inclusion to slow covid-19 spread. *Gift RA, Olalekan RM, Owobi OE, Oluwakemi RM, Anu B, Funmilayo AA (2020). Nigerians crying for availability* *of electricity and water: a key driver to life coping measures for deepening stay at home inclusion to slow covid-19 spread. Open Access Journal of Science*, 4(3), 69-80.

Hales, S., Edwards, S.J. and Kovats, R.S. 2003. Impacts on health of climate extremes. In: McMichael AJ, Campbell-Lendrum DH, Corvalan CF, Ebi KL, Githeko A, Scheraga JD, Woodward A, editors. *Climate change and human health: Risks and responses.* Geneva, Switzerland: World Health Organization. pp. 79–102.

Ikonen, J.M., Hokajärvi, A.M., Heikkinen, J., Pitkänen, T., Kolehmainen, M. and Pursiainen, A. 2017. Drinking water quality in distribution systems of surface and ground waterworks in Finland. *J Water Secur.* 3 : 1–10. doi: 10.15544/jws.2017.004

Khurana, I. and Romit, S. 2008. *Drinking Water Quality in Rural India : Issues and Approaches.* New Delhi: Water Aid.

Koç, C., Bayazýt, Y. and Bakýþ, R. 2020. A study on assessing the urban growth, population, and water resources of Bodrum Peninsula, Turkey. *Environmental Monitoring and Assessment*. 192(10) : 1-12.

Rahmanian, N., Siti Hajar Bt Ali, M. Homayoonfard, N.J. Ali, Rehan, M., Sadef, Y. and Nizami, A.S. 2015. *Journal of Chemistry.*, Article ID 716125, 10 pages.

Onesmus Nzung'a Sila, 2019. Physico-chemical and bacteriological quality of water sources in rural settings, a case study of Kenya, Africa. *Journal of Scientific African.* 2 (2019)e00018.

Paul, S. K., Kumar, R., Pal, R. and Ghosh, A. 2020. Safe drinking water and toilet facility in public places in India: What we need to do!. *Journal of Family Medicine and Primary Care*. 9(6) : 2593.

Ram, B. and Kumar, M. 2020. Correlation appraisal of antibiotic resistance with fecal, metal and microplastic contamination in a tropical Indian river, lakes and sewage. *NPJ Clean Water*. 3(1) : 1-12.

Singh, A.K., Das, S., Singh, S., Gajamer, V.R., Pradhan, N., Lepcha, Y.D. 2018. Prevalence of antibiotic resistance in commensal *Escherichia coli* among the children in rural hill communities of Northeast India. *PLoS ONE*. 13 : e0199179. doi: 10.1371/journal.pone.0199179

Singh, A. K., Das, S., Singh, S., Pradhan, N., Gajamer, V. R., Kumar, S. and Tiwari, H. K. 2019. 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. *Frontiers in Public Health*. 7 : 174.

Srivastava, P., Mishra, C. P., and Nath, G. 2021. Everything is not on track in management of diarrhoea in under-five children: Evidence from rural area of India. *Journal of Family Medicine and Primary Care*. 10(4) : 1582.

Subba, A., Pandey, M., Jha, S., Jha, A. and Dolma, K. G. 2021. Sustainable Supply of Spring Water as a Social Challenge: A study of Seasonal Variations at Sikkim. *Ilkogretim Online*. 20(5).

UN-Water, An increasing demand, factsans figures,UN-Water, coordinated by UNESCO in collaboration with UNECE and UNDESA,2013,http://www.unwater.org/water-cooperation-2013/ en/.