Original Article

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Physicochemical and Bacteriological Analysis of Bagmati River in Kathmandu Valley

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ABSTRACT

Background: The Bagmati River, flowing through the heart of Kathmandu valley, has undergone considerable degradation in water quality that possesses a threat to the river ecosystem and public health. The study had been envisioned to assess certain physicochemical parameters i.e. pH, temperature, Total Dissolved Solids (TDS), chloride, Dissolved Oxygen (DO) and Biochemical Oxygen Demand (BOD), bacteriological analysis i.e. Total Plate Count (TPC), Total Coliform Count (TCC), isolation and identification of *Salmonella* spp., *Vibrio cholerae* and their Antibiotic Susceptibility Test (AST).

Methods: Six sites (B1-B6) were allocated along the Bagmati River from Baghdwar to Chobhar. Four samples were collected from each site and analyzed by standard procedures.

Result: Maximum average of pH, temperature, TDS, Chloride, DO, BOD was recorded to be 7.6, 29°C, 386ppm, 201mg/l, 9.68mg/l, 229.25mg/l respectively. Maximum and minimum average of TPC was 5.39×10^{6}cfu/ml and 7.09×10^{3}cfu/ml while that of TCC was 1.91×106cfu/ml and 5.38×102cfu/ml respectively. 45.83% (N= 11) samples exhibited growth of *Salmonella* spp.of which 12.5% (n=3) isolates were found to be *S*. Typhi, 8.33% (n=2) to be *S*. Paratyphi and rest 25% (n=6) to be other *S. enterica* serovar. All *Salmonella* spp. isolates were sensitive to Ofloxacin. 41.67% (N=10) samples showed growth for *Vibrio cholerae*. All isolates were sensitive to Chloramphenicol and resistant to Erythromycin.

Conclusion: This study reflected the current physicochemical and bacteriological status of Bagmati river and emphasizes the need to reduce pollution so as to prevent the transmission of bacterial pathogens.

Keywords: *Bagmati River, Physicochemical Parameters, Bacteriological Analysis, Salmonella spp.; Vibrio cholerae, AST*

Introduction

Water is the most vital resource for the existence of all life and ecosystems in Earth. Certain standards in terms of its physical, chemical and biological parameters determine its suitability for intended purposes. Water is considered polluted when these parameters shift from the acceptable range of quality standards [1].

The Bagmati is a perennial river that originates at Shivapurilekh, north of Kathmandu and drains into the Ganges. It forms a catchment area of 3710sq. km in Nepal which includes area in 8 districts and constitutes about 15% of the total area in Kathmandu valley [2,3]. Most of its water is contributed by run-off and the river exhibits wide seasonal variation in flow and water quality [4].

Various religious and economic activities like cremation, irrigation, hydropower generation, sand quarrying and industrial manufacturing occur in the river vicinity. The most severe problem at present is the mismanagement of sewers into the river [5]. These activities have led to water quality deterioration resulting serious public health and environmental challenges [6,7].

The study aims to assess the quality of river water in terms of certain physicochemical and bacteriological parameters. The findings of the study might be suggestive of the appropriateness of water for intended use and generate awareness about the probable chances of infection and other health problems associated with the use of river water.

MATERIALS AND METHODS

Sampling Sites and Sample Size: This study was conducted in the Microbiology laboratory of St. Xavier's College from January to June 2017. Six different sites along the river (B_1-B_6) were allocated. Bagmati River from Baghdwar to Sundarijal, Gokarna to Jorpati, Guheshwori to Pashupati, Tilganga to Tinkune, Thapathali to Teku and Balkhu to Chobhar were specified as sites B_1, B_2, B_3, B_4, B_5 and B_6 respectively. Four samples were collected from each site.

Sample Collection and Transportation: Samples were collected in sterilized bottles with their mouth directed against the water current. Physicochemical parameters were determined at the site itself. Dilutions were made to those samples that were highly polluted and turbid. Samples for bacteriological analysis were taken to laboratory within 4 hours in an ice box and processed immediately. For less feasible sites, samples were processed within 24 hours [1].

Physicochemical Analysis: Physicochemical parameters were assessed as per standard guidelines. pH was measured using pH meter. Temperature was recorded by mercury thermometer and Total Dissolved Solids (TDS) using a TDS meter. Chloride content was determined by Argentometric titration. The Dissolved Oxygen (DO) and respective Biochemical Oxygen Demand (BOD_5) was determined by Winkler's Iodometric method [1].

Total Plate Count (TPC) and Total Coliform Count (TCC)

The TPC and TCC were determined by Pour plate method. Samples were serially diluted. 1ml of the diluted sample was transferred to sterile plates and agar medium was added. Sample and medium were homogenized, solidified and incubated at 37ºC for 24 hours. Plate Count Agar (PCA) was used for TPC and Violet Red Bile Agar (VRBA) for TCC. The colony growth was enumerated and total organism per ml of the sample was determined [8].

Isolation and Identification of Salmonella species: 5 sample was inoculated in 45 ml Selenite F broth and incubated at 37ºC for 24 hours. This enriched sample was cultured on Xylose Lysine Deoxycholate (XLD) Agar and incubated overnight at 37ºC. Presumptive colonies obtained were sub cultured on Nutrient Broth (NB) and identified based on their colony characteristics, gram staining and biochemical properties [8].

Isolation and Identification of Vibrio cholerae: 1 ml sample: was transferred to 10 ml Alkaline Peptone Water (APW) and incubated for 4 hours at 37ºC. The enriched sample was cultured on Thiosulphate Citrate Bile Salt (TCBS) Agar and incubated overnight at 37ºC. Presumptive colonies were sub-cultured on NB. Gram staining, colony morphology and biochemical characteristics of the isolates were noted. Additionally, Cholera Red Test and String Test were also performed [8].

Antibiotic Susceptibility Test (AST): All isolates were tested for their antibiotic susceptibility by modified Kirby Bauer disc diffusion method. Standard inoculums (the density of the McFarland standard 0.5) of the isolates were swabbed onto the surface of a Mueller Hinton Agar plate and respective antibiotic discs were placed. After overnight incubation at 37ºC, the diameter of the zone of inhibition was measured and interpreted as Susceptible, Intermediate or Resistant [9].

Result

A total of 24 samples were collected from six different sites specified as B_1 -B_6. The samples were then tested for physicochemical and bacteriological characteristics; the results have been presented as the average of these parameters at respective sampling sites (Table 1).

The average value of pH was slightly alkaline (7-8) at all sites. TDS and the chloride content was observed to be in increasing trend downstream from site B_1 -B_6 whereby site B_2 showed more TDS and chloride concentration than the corresponding site B_3. DO value presented decreasing tendency towards the urban core of the valley corresponding to an increasing trend in BOD values.

The maximum average and minimum average of TPC recorded was 5.39×10^6 and 7.09×10^3 cfu/ml at sites B_6 and B_1 respectively. Similarly, the maximum and minimum average of TCC recorded was 1.91×10^6 cfu/ml and 5.38×10^2 cfu/ml at sites B_5 and B_1 respectively.

Of the 24 samples processed, 11(45.83%) samples showed growth positive for *Salmonella* spp. while the rest 13 (54.17%) samples were growth negative. Of the positive samples, 3(12.5%) were found to be *S.* Typhi, 2 (8.33) to be *S.* Paratyphi and the rest 6 (25%) to be other *S. enterica* serovar (Figure 1). All *Salmonella* isolates were sensitive to Ofloxacin. (Table 2)

10 (41.67%) samples were growth positive for *V. cholerae* and the rest 14 (58.33%) were growth negative (Figure 2). All *V. cholerae* isolates were resistant to Erythromycin and sensitive to Chloramphenicol while the susceptibility to other antibiotics varied. The AST of *V. cholerae* is presented in Table 3.

Discussion

This study was undertaken to assess certain physicochemical and bacteriological parameters of Bagmati river within the valley. Analysis based on these parameters revealed progressive degradation as the river passes through site B_1-B_6; sites B_5 and B_6 presented the maximum deteriorating condition. This can be correlated to other studies which presented that the water quality values, mostly towards urban centre of the valley, were beyond the limit of water quality standards for various uses [10,11].

The average pH was in the range 7.10 to 7.62. pH was nearly neutral in the headstream areas while alkaline towards the downstream. This could be because of higher bicarbonates production due to higher organic inputs and high residence time. Similar studies on the pH of the river reported comparable values where the pH ranged between 7.1 to 7.7 [12] and 7.06 to 7.62 [13] respectively.

The maximum and minimum average temperature recorded was 29°C and 14.25°C. This variation can be attributed to factors like season, time and sampling station. Sampling for site B_3 was done during winter so that the least temperature was recorded. Low temperature range at site B_1 might be due to altitude differences since sampling was done at and around the origin site which lie along the Shivapuri Hills.

The average range of TDS was 386 ppm to 6.75ppm. Higher values recorded at sites downstream to B_3 can be linked with variation in inputs of organic matter associated with population density and activities. Poudyal et al. also presented an increasing trend from headwaters to semi urban to urban section along the river; the average TDS recorded was 42.06 ppm, 130.10 ppm and 240.15 ppm respectively [14].

Average chloride concentration was in the range 123.54mg/l to 201mg/l at respective sites B_1 and B_6; the maximum was observed at regions stretching from Balkhu to Chobhar. Gautam et al [15] presented Teku and Sundarighat as the most contaminated sites with reference to chloride content; the concentration was about 17 times higher in urban stretch as compared to the headwaters.

Maximum and minimum average DO was 9.68mg/l at site B_4 and 0 mg/l at sites B_5 and B_6. The low level of DO was likely due to bacterial decomposition of incorporated organic matter resulting in oxygen depletion. The DO value was in the range 0-8 mg/l, the value being zero at Sundarighat, Teku and Balkhu [16]. A similar study by Bhatt et al [17] presented that the DO ranged between 8.4 mg/l to 2.1mg/l but not nil at any sites.

Average maximum and minimum BOD was 229.25mg/l and 1.12 mg/l. The lowest BOD at site B_1 can be correlated with lowest microbial count and minimal human intervention. Gautam et al. reported comparable values in the range 71 mg/l to 293 mg/l. However, Regmi and Mishra presented much higher content; maximum BOD of 409 mg/l at Teku followed by 384.3 mg/l at site after Bishnumati confluence.

The maximum and minimum average TPC was 5.39×10^6cfu/ml and 7.09×10^3 cfu/ml while that of TCC was1.91×10^6cfu/ml and 5.38×10^2cfu/ml respectively. Microbial analysis in a study at different sites along the river presented the average geometric mean of TPC being 2.8×10^{14}cfu/100ml and TCC being 3.1 10^{12} cfu/ 100ml [19].

11(45.83%) samples were growth positive for *Salmonella* spp. of which 3(12.5%) were found to be *S.* Typhi, 2 (8.33) to be *S.* Paratyphi and the rest 6 (25%) to be other *S. enterica* serovar (Figure 1). 15 strains of *Salmonella* were isolated in a similar bacteriological study by Aryal [19] of which 9 strains were *S.* Typhi (22.5%) and 6 were *S.* Paratyphi (15%).

All *Salmonella* isolates were sensitive to Ofloxacin, while sensitivity towards other antibiotics varied. Least sensitivity was observed with Ampicillin, i.e. 66.67%, 50%, and 66.67% isolates were resistant in case of *S.* Typhi, *S.* Paratyphi and other *S. enterica* serovar respectively. The best drug against these isolates was Ofloxacin since all strains were sensitive to it.

10 (41.67%) samples were growth positive for *Vibrio cholera*e. Cholera agents in water may be discharged by humans who are either infected or who are the carriers of the disease. *V. cholerae* was isolated in comparable range-43.5% of the samples along the Bagmati river network [20].

The AST of *V. cholerae* isolates showed varied patterns of sensitivity. None of the isolates were Multi Drug Resistant (MDR). However, two MDR strains of *V. cholerae,* i.e. resistant to Tetracycline, Chloramphenicol and Nalidixic Acid were isolated along the river sites [20]. The best drug against these isolates might be Chloramphenicol since all strains showed sensitivity to Chloramphenicol.

Table 1: Average range of Physicochemical parameters and Bacteriological count from each site.

Site	pH	Temperature (°C)	TDS (ppm)	Chloride (mg/l)	DO (mg/l)	BOD (mg/l)	TPC (cfu/ml)	TCC (cfu/ml)
B_1	7.1	16	6.75	2.01	9.68	1.12	7.09×10^3	5.38×10^2
B_2	7.25	27	114.5	30.17	3.95	5.53	1.2×10^5	2.79×10^4
B_3	7.21	14.25	91.5	14.91	4.31	5.12	2.26×10^5	3.75×10^4
B_4	7.85	22.75	160.25	30.53	3.94	76	5.45×10^5	6.25×10^4
B_5	7.1	24	353	86.26	0	190.25	3.56×10^6	1.91×10^6
B_6	7.62	29	386	123.54	0	229.25	5.39×10^6	1.05×10^6

(TDS-Total Dissolved Solids, DO- Dissolved Oxygen, BOD-Biochemical Oxygen Demand, TPC- Total Plate Count, TCC-Total Coliform Count)

Table 2: Antibiotic Susceptibility Test pattern (%) of *Salmonella* spp.

Susceptibility No. (%)	Antibiotics					
	AMP	C	CTR	CIP	NA	OF
***Salmonella* Typhi (n=3)**						
Sensitive	1(33.33)	1(33.33)	3(100)	3(100)	1(33.33)	3(100)
Intermediate		2(66.67)				
Resistant	2(66.67)				2(66.67)	
***Salmonella* Paratyphi (n=2)**						
Sensitive	1(50)	2(100)	2(100)	1(50)	2(100)	2(100)
Intermediate				1(50)		
Resistant	1(50)					
Other *Salmonella enterica* (n=6)						
Sensitive	2(33.33)	3(50)	5(83.33)	4(66.67)	1(16.67)	6(100)
Intermediate		2(33.33)	1(16.67)	2(33.33)	1(16.67)	
Resistant	4(66.67)	1(16.67)			4(66.66)	

(Amp=Ampicillin, C=Chloramphenicol, Ctr=Ceftriaxone, Cip=Ciprofloxacin, NA=Nalidixic Acid, OF=Ofloxacin)

Table 3: Antibiotic Susceptibility Test pattern (%) of *Vibrio cholerae*.

Antibiotic	Susceptibility No. (%)		
	S	I	R
Ampicillin		2(20)	8(80)
Chloramphenicol	10(100)		
Ceftriaxone	9(90)	1(10)	
Ciprofloxacin	8(80)	1(10)	1(10)
Erythromycin			10(100)
Tetracycline	3(30)	6(60)	1(10)

(S= Sensitive, I= Intermediate, R= Resistant)

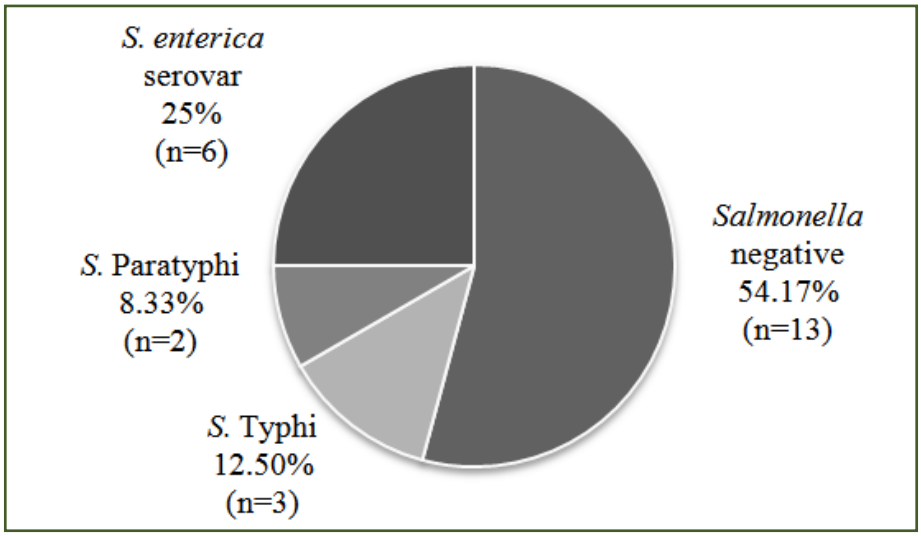

Fig. 1: Identification (%) of *Salmonella* spp. (N=11).

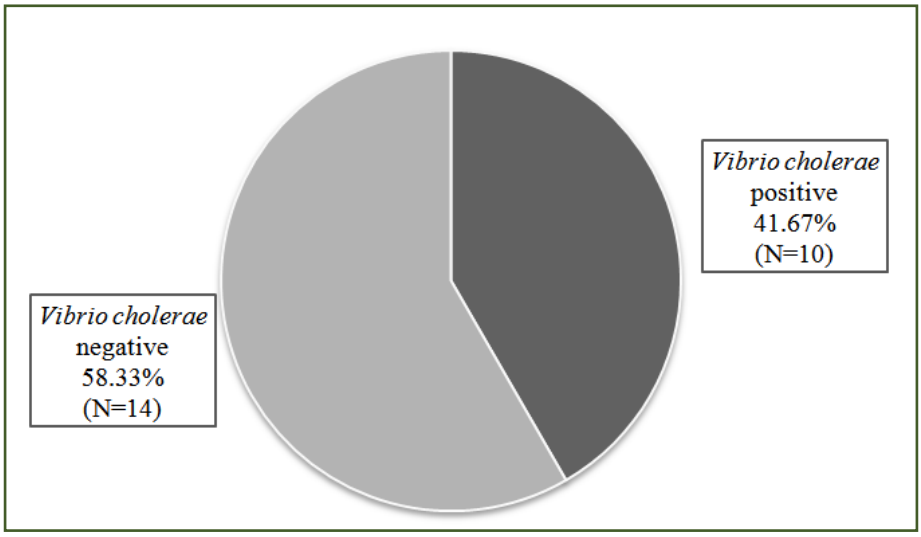

Fig. 2: Identification (%) of *Vibrio cholerae.*

Conclusion

The study has shown the spatial variation in water quality of Bagmati River within the valley. The water quality seemed to be deteriorating as the river passes from the headwater regions towards the valley core and mostly towards the end of the valley. The presence of medically important *Salmonella* spp. and *Vibrio cholerae* in the river network should not be ignored. This is the crucial indication for possible outbreak of *Salmonella* infections and/or Cholera any time in future and therefore demands proper sanitation, appropriate management and water quality improvement measures, supply or consumption of safe water and practice of personal hygiene.

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Reference

1. APHA. Standard Methods for the Examination of Water and Wastewater. 20th ed., Analysis of Water and Wastewater, American Public Health Association, Washington DC, USA: 2000.
2. DHM. Water Quality Summary (1992-2006). Department of Hydrology and Meteorology, Water quality and sediment section, MoEST, GoN: 2008.
3. NEP: KVWMP. Kathmandu Valley Wastewater Management Project. Ministry of Urban Development, Government of Nepal/ Asian Development Bank. 2013.
4. Dhital YP, TangQ, Shi J. Hydroclimatological Changes in the Bagmati River Basin, Nepal. Journal of Geographical Sciences. 2013; 23(4): 612-626.
5. Dahal A, Khanal M, Ale M. Bagmati River Festival: Conservation of Degrading River. Nepal River Conservation Trust, 2011; Georgia: Georgia Water Resources Conference.
6. Kannel P, Lee S, Kanel S, Khan S, Lee YS. Spatial-temporal variation and comparative assessment of water qualities of urban river system: A case study of river Bagmati (Nepal). Environmental Monitoring Assessment. 2007; 129:433-459.
7. Mehta KR, Kushwaha UKS. An Assessment of Aquatic Biodiversity of River Bagmati Nepal. Ecology and Evolutionary Biology. 2016; 1(2): 35-40.
8. Cappuccino JG, Sherman N. Microbiology: A Laboratory Manual.10th ed., Boston; Pearson Education: 2014.
9. Clinical and Laboratory Standards Institute. Performance Standards for Antimicrobial Susceptibility Testing. 26th ed., M100S Wayne, PA: 2016.
10. Mishra BK, Regmi RK, Masago Y, Fukushi K, Kumar P. Assessment of Bagmati River pollution in Kathmandu valley: Scenario-based modeling and analysis for sustainable urban development. Sustainablity of water and Ecology. 2017; 9:67-77.
11. GoN. Water Quality Measurement and Hoarding Board Installation. DHM, Ministry of Population and Environment, 2016.
12. Regmi RK Mishra BK. Current water Quality Status of Rivers in the Kathmandu Valley. Water and Urban Initiative Working Paper Series Number 06, March 2016, United Nations University Institute for the Advanced study of sustainability.
13. KAPRIMO. Kathmandu Participatory River Monitoring- A model for South Asia, 2007.

14. Poudyal P, Kang S, Sharma CM, Tripathee L, Sillanpaa M. Variations of the Physicochemical Parameters and Metal levels and their risk assessment in urbanized Bagmati River, Kathmandu, Nepal. Journal of Chemistry. 2016; 12(2): 240-246.
15. Gautam R, Shrestha JK, Shrestha GKC. Assessment of River Water Intrusion at the Periphery of Bagmati River in Kathmandu Valley. Nepal Journal of Science and Technology. 2013; 14(1): 137-146.
16. NEP: BRBIP. Environmental Assessment of Nepal: Emerging Issues and Challenges. Asian Development Bank/ ICIMOD, 2016.
17. Bhatt MP, McDowell WH, Gardner KH, Hartmann J. Chemistry of the heavily urbanized Bagmati River system in Kathmandu Valley, Nepal: Export of organic matter, nutrients, major ions, silica and metals. Environmental Earth Sciences. 2013; 71(2).
18. Regmi RK, Mishra BK, Luo P, Toyozumi-Kojima A, Fukushi K, Takemoto K.. Preliminary Trend analysis of DO and BOD records in Kathmandu, Nepal: Towards Improving Urban Water Environment in Developing Asian Countries. International Symposium on Southeast Asian Environment, 2014; 11: 371-376.
19. Aryal M. Detection of Salmonella species from surface water of Kathmandu valley. European Journal of Biomedical and Pharmaceutical Sciences, 2016; 3(4): 596-600.
20. Rai KR, Rai SK, Bhatt DR, Kurokuwa M, Ono K, Thapa MD. Study of Medically important Vibrios in the sewage of Kathmandu valley, Nepal. Nepal Medical College Journal. 2012; 14(3): 212-215.

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