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Microbial and Physico-Chemical Quality Assessment of Rivers of Kathmandu Valley

Santosh Poudel[1], Akash Paudyal[1], Bishnu Prasad Sharma[1], Kamana Sharma[1], Yubaraj Baral[1], Shailaja Adhikari[2], Manju Shree Shakya Hada[1]

[1]Department of Microbiology, Tri-Chandra Multiple Campus, Tribhuvan University, Kathmandu, Nepal

[2]Kathmandu Upatyaka Khanepani Ltd. (KUKL), Kathmandu, Nepal

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Abstract

Water quality refers to the chemical, physical, biological characteristics of water. It is a measure of condition of water relative to requirement of one or more biotic species and or to any human need or purpose. The main objective of the study is to detect the physio-chemical and microbiological parameters of water sample from the Bagmati river and its tributaries of Kathmandu valley along with antibiotic susceptibility. In physico-chemical parameters, turbidity, temperature, pH, Electrical conductivity, Dissolved Oxygen, Biological Oxygen Demand, Ammonia, Alkalinity, Hardness, Chloride, Phosphate, Iron, Nitrate, Total Dissolved solids, and color were analyzed. Iron and Turbidity was found to be above the World health organization and Nepal Standard guideline in all the samples (100%), while Ammonia was found to be above the WHO guideline in 10(90%) samples. Among 11 samples, 10(90%) showed a low Dissolved oxygen level. Most Probable Number method was followed for counting total load of coliform and fecal coliform. *Escherichia coli* was isolated from the sample and subjected to Antibiotic susceptibility. Coliform was detected in all the samples and *E. coli* was identified as highly resistant towards Gentamicin (81.8%) and sensitive towards Chloramphenicol (81.8%). High value of ammonia, turbidity and low value of Dissolved Oxygen in the lower belts of river was due to large inputs of wastewater and organic loads caused by anthropogenic activities. High value of Coliform in all the samples indicates bacterial contamination in river water. The comparative study for the water quality variables in the urban areas showed that the main rivers and its tributaries were equally polluted.

Keywords: *E. coli*, Bagmati, physico-chemical parameters, Antibiotic Susceptibility, Most Probable Number method

Corresponding author, email: Poudelsantosh550@gmail.com

Introduction

Water is the main constituent of Earth's hydrosphere and the fluids of all known living organisms. It is vital for all known forms of life [1]. Among the type of surface water, River is the most accessible and widely available source of water to human beings. River water has various uses like irrigation, drinking, and water hydropower, directly related to people. River water has an economic backbone in the upliftment of the country [2].

Bagmati is one, with religion and geographical importance. Various important religious shrines like Gokarneshwor, Pashupatinath, Guhyeshwari, Teku dovan, and others lie on the river's bank [3]. The Bagmati River is considered the source of Nepalese civilization and urbanization. In contrast to the various large snow-fed rivers of the Himalayas, it is a river originating from Baghdwar and Shivapuri hill at an altitude of 2650m. Bagmati River is the principal river of the Kathmandu valley river system. It drains the entire valley with its seven tributaries- Bishnumati River, Manohara River, Dhobi River, Nakkhu River, Balkhu River, Hanumante River, and Tukucha River [4].

The quality of the Bagmati River and its tributaries have been rapidly degrading. It contains large amounts of untreated sewage, and high levels of pollution of the river exist due primarily to the region's large population. Many residents of the Kathmandu valley empty personal garbage and waste into the river [5]. In Kathmandu, urbanization and industrialization contribute to deterioration in water quality with regional consequences for the aquatic system and health of downstream sub-basin user groups. Sewer lines for domestic and industrial wastewater have been connected to the river [6]. These activities are responsible for serious pollution and the production of foul odors near the riverside. River system water entering the core urban area is visibly black with filth stinks badly. In particular, the Hanumante River, Dhobi River, Tukucha River, and Bishnumati River are the most polluted [7]. A few kilometers of the uppermost section (High Mountain with a catchment area of 17 km^2) is only suitable for drinking water supply [8]. The remaining sections are not

used for potable purposes due to greater water quality deterioration [9].

Although many wastewater treatments plants have been constructed in the Kathmandu valley, only one of the Guheshwori wastewater treatment plant is currently functional. Various restoration project has been ongoing within Kathmandu valley focused on improving water quality and establishing minimum flow requirements [10].

Assessment of the Physico-chemical and microbiological parameters of the river water indicates the level of pollution and provides progression to understand its effect on the aquatic life as well as on the human population [11]. Water is the principal vehicle for the transmission of a wide range of communicable diseases. From the microbiological view, the presence of total coliform and fecal coliform shows then water quality. Coliform bacteria *E. coli*, which is a medically important bacteria causing many significant illnesses like Bacteremia, cholangitis, UTI, travelers' diarrhea, neonatal meningitis, pneumonia [12,13]. In addition, other bacteria like *Salmonella*, *Shigella*, *Pseudomonas*, *Proteus*, and *Vibrio* in drinking water are major causes of water-borne diseases [14].

Various antibiotics are used for the treatment of the diseases caused by *E. coli*. In recent years the number of antibiotic-resistant strains of *E. coli* is increasing by the haphazard use of antibiotics [15]. Antimicrobial resistance is a global public health concern contributing to increased morbidity and mortality, particularly in low-income countries. Studies on commensal bacteria are essential as they reflect the state of antimicrobial susceptibility patterns in populations. Overpopulation of Kathmandu valley and inappropriate use of antimicrobials signal significant rates of resistance among flora circulating within the community. Increased use of antibiotics in agriculture, domestic, livestock, and the hospital are likely to develop resistance by *E. coli* strains.

This study was carried out to analyze different Physico-chemical parameters and microbiological parameters of the Bagmati River and its tributaries.

Materials and Methods

Sampling site and sample

A total of 11 samples were tested in the study. Water samples were collected from four different Bagmati River sites (Sundarijal, Before Guheshwori treatment plant-Gaurighat, After Guheshwori treatment plant-Gaurighat and Chovar) and seven sites of its tributaries in Kathmandu valley (Bishnumati River-Teku dhovan, Balkhu River-Balkhu, Dhobi River-Baneshwor, Nakkhu River-Nakkhu, Tukuchu River-Tripureshwor, Mahohara River-Jadibuti, Hanumante River-Jadibuti). The sample was collected in a sterile bottle of 500 mL capacity for microbiological analysis and a sample bottle of 500 mL for physico-chemical analysis. The bottles were labeled with time, place, and date of sample collection and transported to the laboratory as soon as possible on the icebox. For Dissolved Oxygen (DO) and Biological Oxygen Demand (BOD), two BOD bottles each of 300 mL were taken at the site, processed at the lab. The study was conducted from November 2019 to January 2020 in Kathmandu Upatyaka Khanepani Limited (KUKL) laboratory, Mahankal Chaur, Kathmandu.

Sample Analysis

Analysis of Physico-chemical parameters

The standard method for examining water and wastewater [16] was followed to analyze the Physico-chemical parameters of water as Temperature (by mercury thermometer), pH (by digital pH meter), E.C. (by E.C. meter), Turbidity (by turbid-meter), Color and Ammonia (by colorimeter), Iron, Phosphate and Nitrate (by UV-visible spectrophotometer), Chloride, Alkalinity, and Hardness (by titration) and DO and BOD (by Winkler's method).

Analysis of microbiological parameters

Microbiological parameters analysis was carried out following standard methods [16]. Enumeration of total coliform count and fecal coliform were done by Most probable number (MPN) method.

Isolation of *E.coli* was done by enrichment in buffered peptone water and cultured in EMB (Eosin Methylene Blue)[17]. The colony was identified and confirmed by following respective biochemical characteristics[18] and subjected to antibiotic susceptibility using Kirby Bauer disk diffusion method following CLSI guidelines[19]. The antibiotics used were Ampicillin (10 µg), Gentamicin (10 µg), Cotrimoxazole (25 µg), Ciprofloxacin (5 µg), Ceftriaxone (5 µg) and (Chloramphenicol (30 µg).

Results

Physical parameters assessment

Temperature of sample ranges from 9^0C of Sundarijal (SL), Chobar (CHOB), and 16.5^0C of Hanumante (HAN) and Guheshwori Before treatment (GBT). Color was observed highest in Tukucha (TUK) i.e., 40HU and lowest in Sundarijal (SL) i.e., 2.5HU. Only the water sample from Sundarijal was clear while all other samples appeared turbid and hazy. SL sample was found to have lowest turbidity i.e., 2.7 NTU whereas Mahohara (MAN) sample

Table 1. Physical Parameters of water samples. (* Temperature during measurement)

Physical Parameter	Sundari jal (SL)	Guheswori Before Treatment (GBT)	Guheswori After Treatment (GAT)	Manohara River (MAN)	Hanumante River (HANU)	Tukucha River (TUK)	Dhobi River (DHOBI)	Nakkhu River (NAK)	Balkhu River (BAL)	Bishnu Mati (BM)	Chobar (CHOB)
Appearance	Clear	Turbid	Turbid	Turbid	Turbid	Turbid	Turbid	Turbid	Turbid	Turbid	Turbid
Temperature (°C)	9°	**19°**	16.5°	12°	16.5°	16°	13°	11°	13°	15°	9°
Color (HU)	**2.5**	25	30	35	25	**40**	20	35	25	15	25
Turbidity (NTU)	**2.7**	52.59	295.12	**759.32**	122.66	179.78	186.71	261.33	112.03	305.80	15.57
E.C.* (µS/cm)	**49.2 (9.1°)**	258.7 (13°)	167.0 (18.6°)	567 (13.0°)	1039 (16°)	1090 (14°)	820 (13.9°)	701 (14.5°)	**1109 (12.5°)**	986 (14°)	983 (11.6°)
TDS	**9**	89	57	143	**367**	371	300	162	354	349	268

Table 2. Chemical Parameters of water samples (* Temperature during measurement)

Physio Chemical Parameter	Sundari jal (SL)	Guheswori Before Treatment (GBT)	Guheswori After Treatment (GAT)	Manohara River (MAN)	Hanumante River (HANU)	Tukucha River (TUK)	Dhobi River (DHOBI)	Nakkhu River (NAK)	Balkhu River (BAL)	Bishnu Mati (BM)	Chobar (CHOB)
pH*	7.7 (9.1°)	7.41 (13°)	7.72 (18.6°)	**7.22 (13°)**	7.45 (16°)	7.3 (14°)	**8.65 (13.9°)**	8.29 (14.6°)	7.61 (13°)	7.27 (14°)	7.56 (11°)
DO (mg/L)	**12.4**	3.02	4.87	2.24	1.05	1.10	1.26	2.8	1.4	**0.77**	1.59
BOD (mg/L)	8.9	66	**48**	160	279	261	242	187	291	**354**	213
Ammonia (mg/L)	**0.2**	15	12	24	35	80	40	24	**90**	40	32.5
Alkalinity (mg/L)	**24**	108	88	88	264	288	248	160	**404**	296	294
Hardness (mg/L)	**12**	128	56	80	264	152	312	148	**344**	176	178
Chloride (mg/L)	**1.92**	26.88	19.2	65.28	103.68	**149.76**	69.12	46.08	111.36	80.64	88.46
Phosphate (mg/L)	**0.0**	0.4	0.2	1.0	2.0	**3.5**	2.5	0.5	2.0	2.5	2.5
Iron (mg/L)	**0.4**	1.6	6	**23.2**	8.8	4.4	6.4	3.2	6.4	15.2	4.8
Nitrate (mg/L)	**0.2**	0.9	2.1	3.7	**5.7**	**5.7**	4.8	1.0	4.6	5.3	2.1

had the highest i.e., 759.32. Only SL sample follows WHO and NDWQS guidelines (≤5 NTU). Electrical conductivity of samples varied from SL 49.2 µS/cm being lowest and BAL 1109µS/cm being highest. Out of 11 samples HANU, TUK, BAL donot follow WHO guidelines with value (1039, 1090, 1109) µS/cm respectively. Value of TDS was highest in TUK i.e. (371 mg/L) and lowest in SL (9 mg/L). All TDS value of samples lies within the guideline of WHO (1000 mg/L).

Chemical parameter assessment

pH of the sample Manohara was found to be lowest i.e., 7.22 and sample Dhobi(DHOBI) was highest i.e., 8.65 which doesn't follow WHO guidelines. Water in most of the river samples were slightly alkaline. Dissolved oxygen in the sample SL has highest i.e., 12.4 mg/L while Bishnumati has lowest i.e.,0.77 mg/L. In the study performed BOD of the sample Bishnumati has highest i.e., 354 mg/L and SL has lowest 8.9mg/L. Ammonia of the sample Sundarjal being lowest i.e., 0.2mg/L lies within the WHO guidelines while other sample don't follow WHO guidelines. Sample Balkhu has highest value i.e., 90 mg/L. Highest value of Total hardness was obtained from the sample Balkhu i.e., 344 mg/L and lowest value from Sundarijal i.e,.12 mg/L. All values were within the NDWQS guidelines (≤500 mg/L). Chloride value was highest in Tukucha i.e., 149.76mg/L and lowest in Sundarjal i.e., 1.92 mg/L. In the study performed phosphate value was highest in Tukucha i.e., 3.5 mg/L and lowest in Sundarijal i.e., 0.0 mg/L. All the values of iron don't follow the guidelines of WHO and NDWQS (≤0.3). Iron value was highest in sample

Manohara i.e., 23.2 and lowest in sample SL i.e., 0.4 mg/L. Hanumante and Tukucha sample have highest Nitrate value i.e., 5.7 mg/L while Sundarijal sample has lowest i.e., 0.2 mg/L.

Table 3. Total coliform count by MPN method

Sample	Number of tubes giving positive reactions			MPN index (cfu/100mL)
	1 of 50mL each	5 of 10mL each	5 of 1mL each	
Sundarijal	1	5	5	>180
Guheshwori Before Treatment	1	5	5	>180
Guheshwori Before Treatment	1	5	5	>180
Manohara	1	5	5	>180
Hanumante	1	5	5	>180
Tukucha	1	5	5	>180
Dhobi	1	5	5	>180
Nakkhu	1	5	5	>180
Balkhu	1	5	5	>180
Bishnumati	1	5	5	>180
Chobar	1	5	5	>180

Microbiological analysis

All 11 sample (100%) contain coliform above 180 per 100 mL.

Antibiotic Susceptibility

Table 4. Susceptibility patterns of *E. coli* isolates. R= Resistant, I= Intermediate, S= Sensitive

	Ampicillin 10 µg	Gentamicin 10 µg	Cotrimoxazole 25 µg	Ciprofloxacin 5 µg	Ceftriaxone 30 µg	Chloramphenicol 30 µg
Sundarijal	R	R	R	S	S	S
GBT	S	I	S	S	S	S
GAT	R	I	S	S	S	S
MAN	R	R	S	R	I	S
HANU	R	R	S	I	R	S
TUK	R	R	R	R	I	I
DHOBI	R	R	S	S	S	S
NAK	S	R	S	S	S	S
BAL	S	R	S	S	I	S
BM	R	R	R	R	I	S
CHOB	R	R	R	I	I	R

Table 4 showed that *E.coli* isolated from sites TUK, BM and CHOB were resistant against more than 50 percent of the antibiotics testes while isolates from sites GBT, GAT, DHOBI, NAK and BAL were susceptible to more than 50 percent of the antibiotics tested.

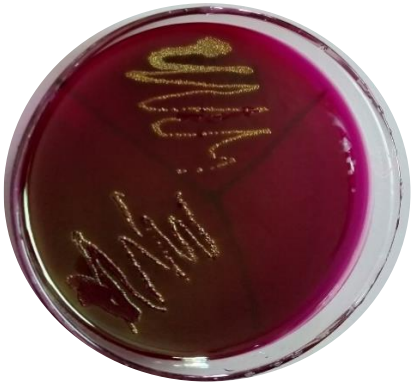

Figure 1. Green metallic sheen colony growth on M-Endo agar (*E. coli*).

From 11 isolates, 6 (54.4%) were found multi drug resistant (MDR) i.e., resistant to 3 or more class of antibiotics. Most of the MDR isolates (45.5%) were resistant to the Gentamicin in this study.

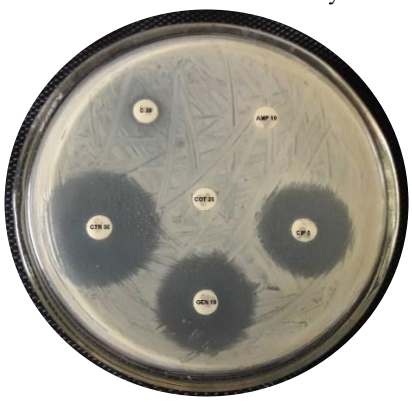

Figure 2. Antibiotic Susceptibility by disk diffusion method

Table 5. Antibiotic Susceptibility of *E. coli* (N=11)

	Sensitive	Intermediate	Resistance
Ampicillin (10 µg)	27.27% (3)	0	72.72% (8)
Gentamicin (10 µg)	0	18.18% (2)	81.82% (9)
Cotrimoxazole (25 µg)	63.6% (7)	0	36.4% (4)
Ciprofloxacin (5 µg)	54.55% (6)	18.2% (2)	27.27% (3)
Ceftriaxone (30 µg)	45.5% (5)	45.5% (5)	9.1% (1)
Chloramphenicol (30 µg)	81.9% (9)	9.1% (1)	9.1% (1)

Discussion

Turbidity in this study was recorded in range of 2.7NTU in Sundarijal to 759.32NTU in Manohara. The values of turbidity except Sundarijal were much higher compared

to the previous study conducted i.e. 14.2NTU before mixing of tributaries of Kathmandu valley[20]. Discharge of industrial effluents such as alums and chemicals might contribute to the high value of turbidity. Tributaries of Bagmati are major contributors to turbidness in Bagmati river.

The pH value was found within the range of WHO standards and NDWQS guidelines value except the sample of Dhobi river, which is found to be 8.65. It is higher than the value reported in the previous study be 8.20 [21]. High value of pH may be due to the increasing waste discharge and industrial effluent along with microbial decomposition of organic matters.

Temperature ranged from 9^0C to19^0C, while previous study conducted [4] during summer season recorded a maximum of 20^0 C. Since the study was conducted in winter explains the low temperature recorded.

Electrical conductivity of the samples Hanumante, Tukucha, and Balkhu were 1039 µS/cm, 1090µS/cm, and 1109µS/cm respectively that do not lie within the guidelines of WHO standard 0-1000 µS/cm. In the previous study [21], highest value was 889.59 µS/cm. Higher value indicates the presence of a higher amount of dissolved ions as well as plant nutrients in the water which might be due to the wash off of the fertilizer from agricultural lands.

Alkalinity refers to the capability of water to neutralize acid. In natural water, there are many salts of weak acids such as silicates, borate-causing alkalinity. In this study, highest alkalinity was of Balkhu 404mg/L. The alkalinity observed in this study is higher than the result of previous study 360 mg/L [20]. Samples were collected during winter season, the flow and level of water were low. Higher value of alkalinity might be due to increase concentration of natural soil and minerals.

The ammonia concentration for Sundarijal was only 0.2 mg/L which was limit for surface water recommended by WHO [22]. But the concentration of ammonia for other samples was a lot higher than the recommended value which might be due to more amount of municipal waste dumping in the river of Kathmandu valley. Highest ammonia concentration was from Balkhu sample, 90 mg/L, and Tukucha sample, 80 mg/L. Similar results reported by [20], also had a higher value of ammonia in Balkhu river indicating that it was more polluted than other rivers.

The highest value of chloride recorded was 149.76mg/L in Tukucha followed by Balkhu 111.36mg/L, indicating most contaminated about chloride content. Previous study [23] , presented a similar result being Teku and Sundarighat as most contaminated sites[23] considering Tukucha river and Balkhu river joins on Bagmati in Teku and Sundarighat respectively. High chloride concentration in river is toxic to aquatic life and also increases the potential corrosivity of water[24].

Nitrate was recorded highest in Tukucha and Hanumante i.e.5.7mg/L which is higher than the data previously recorded [23], 3.95mg/L. Both studies showed similarity in the increment of nitrate content from upstream to downstream[23]. Excess levels of nitrates can be considered to be a contaminant of river waters. Most sources of excess nitrates come from human activity. The source of excess nitrates can usually be traced to agricultural activities, human wastes, or industrial pollution. Rainwater can wash nitrates in the fertilizer into streams and rivers[25].

Highest phosphate value was 3.5 mg/L in Tukucha. Sundarijal sample phosphate value was 0.0 mg/L and that of Chobar 2.5 mg/L which are low compared to that of the data of similar research [23], 0.24 mg/L at Sundarijal and 12.3 mg/L at Chobar [26]. Significant increase was seen in the level of phosphate as the river enters an urban core area, which is from manmade sources such as septic systems, fertilizer runoff, and improperly treated wastewater.

At Sundarijal, BOD level was found to be within the guideline by BBWMSIP. However, the level of BOD increases with the increase in the organic waste in the river. BOD indicated the pollution of organic waste resulting low level of dissolved oxygen. Highest BOD was observed in Bishnumati. Previous study [26] showed a low level of BOD in Sundarijal but an increment in the level of BOD towards downstream.

Dissolved oxygen concentrations in the core urban areas were significantly lower than that of Sundarijal showing that the water is anoxic. As the river flows downstream, the dissolved oxygen gets more reduced. Lowest concentration was observed in Bishnumati of 0.77 mg/L. Bacterial decomposition of incorporated organic matter was most likely for low level of dissolved oxygen similar research [23,26] also observed low level of DO in Bagmati river.

In this study, coliform was present in the entire river water sample. The presence of a high amount of coliform might be due to the fact; samples were taken from the river before it was subjected to a treatment of disinfection. Previous studies on the river water from Kathmandu valley showed the presence of various viruses [27], Human Enteric Viruses, Protozoa, and Indicators of Pathogens[28] except in the sample of

Sundarijal. The water is contaminated by various anthropogenic activities as it flows downstream.

All 11 samples were positive for *E. coli*. Identification of *E. coli* during the study indicates the fecal contamination of human origin in Bagmati River and its tributaries. Although there was a low detection rate of pathogen in Sundarijal during a previous study[28] but the detection of *E. coli* from the Sundarijal suggest the contamination of water even upstream of the Bagmati river, which cannot be neglected.

Multiple drug-resistant (MDR) was 54.4% in this study. Other researchers have reported an increasing pattern of *E. coli* isolates against common antibiotics in Nepal[29], [30]. It is observed from the study that Gentamicin and Ampicillin were most resisted by 81.82% and 72.72% respectively by the *E. coli* isolates, similar results of high resistance against β-lactamase(Ampicillin)[31] and Aminoglycoside (Gentamicin) [30] were reported. Among the antibiotics, Chloramphenicol was more sensitive to the isolates. A high rate of effectiveness by the Chloramphenicol against MDR isolates of *E. coli* was reported in previous studies[31, 32].

Conclusion

The study showed the variation in water quality of the Bagmati River and its tributaries. The quality of water is worse in the urban core areas compared to that of upstream. The presence of fecal coliform in river water is the prime indication of a possible source of an outbreak for waterborne diseases and water is not suitable for drinking purposes without proper treatment. High resistance towards some antibiotics shows the threat to the exposure of antibiotics resistance bacterial strains by the population of Kathmandu valley. Immediate action is needed to prevent further deterioration of the river and amplify efforts to slow the emergence and spread of resistance.

Author's Contribution

The development of concept, preliminary work and laboratory analysis, was done by SP, AP, BPS, KS and YB under the guidance of MSSH and SA. All authors read and approved the final manuscript.

Competing Interests

The authors declare that they have no conflicts of interest.

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Ethical Approval and Consent

This study was carried out with the approval from the concerned authorities.

Data Availability

The data can be made available upon request.

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