The Bioscan AN INTERNATIONAL QUARTERLY JOURNAL OF LIFE SCIENCES 20(1): 484-494, 2025 www.thebioscan.com

Comprehensive Assessment of Physicochemical and Microbiological Water Quality Indexing with Antibiotic Resistance Profiling of *Escherichia coli* in the Bagmati River, Kathmandu, Nepal

Puspa Raj Dahal[1] and Rajendra D. Joshi[2*]

[1] Department of Microbiology, Trichandra campus, Kathmandu Nepal

[2]Department of Microbiology, Yogeshwari Mahavidyalaya, Ambajogai Dist: Beed (India)

Email: rajendradjoshi446@gmail.com

DOI: https://doi.org/10.63001/tbs.2025.v20.i01.pp484-494

KEYWORDS
Physicochemical properties,
Antibiotic resistance,
Escherichia coli,
Bagmati River,
Kathmandu

Received on: **04-01-2025**

Accepted on: **04-02-2025**

Published on: **08-03-2025**

ABSTRACT

The Bagmati River, a crucial waterway in Kathmandu, Nepal, has been severely impacted by rapid urbanization, industrial discharge, and untreated sewage, leading to significant pollution. This study investigates the physicochemical water quality of the Bagmati River and the antibiotic resistance patterns of *Escherichia coli* (E. coli), a key fecal indicator bacterium. Water samples were collected from various sites along the River from Sundarijal to Chovar, Kathmandu, during two periods: January–June 2020–21 and July–December 2020–21. Key physicochemical parameters analysed included temperature, pH, Total Suspended Solids (TSS), Dissolved Oxygen (DO), Biochemical Oxygen Demand (BOD), Chemical Oxygen Demand (COD), total nitrogen, phosphorus, nitrate, and heavy metals (Cu, Pb, Zn, Cd, Cr, Hg). Most of the samples cross the limit of both WHO and Nepal standard. Results revealed TSS levels ranging from 113–444 mg/L and 68–513 mg/L, and BOD values of 1.5–394 mg/L and 1.5–392.8 mg/L for January–June and July–December, respectively, both exceeding WHO and Nepalese standards. Coliform counts ranged from 0.18×10^2–148.6×10^2 CFU/mL and 0.2×10^2–126×10^2 CFU/mL in the respective periods, indicating bacterial contamination from untreated waste. Antibiotic sensitivity testing of *E. coli* isolates (E1–E28) revealed high resistance to ampicillin (87%) and erythromycin (90%). Conversely, ciprofloxacin, chloramphenicol, nitrofurantoin, and gentamicin exhibited strong effectiveness, with inhibition zones ranging from 12–38 mm, 16–30 mm, 16–26 mm, and 18–23 mm, respectively. Intermediate resistance to tetracycline and ceftriaxone suggests emerging resistance patterns. These findings underscore the dual challenges of water pollution and antibiotic resistance, emphasizing the urgent need for wastewater treatment, stricter regulation of industrial discharges, and prudent antibiotic use to safeguard public and environmental health.

INTRODUCTION

The Bagmati River, originating from the Shivapuri Hills in Nepal, is a vital natural resource for the Kathmandu Valley, serving cultural, religious, and socio-economic functions. However, the rapid urbanization of Kathmandu has resulted in severe water pollution, threatening the river's ecological balance and public health. Contaminants such as untreated sewage, industrial effluents, and agricultural runoff have contributed to the degradation of water quality, making the river one of the most polluted in Nepal (Shrestha *et al.*, 2021).

Physicochemical parameters such as Dissolved Oxygen (DO), Biochemical Oxygen Demand (BOD), Chemical Oxygen Demand (COD), and Total Suspended Solids (TSS) are critical indicators of water quality. Deviations from permissible standards signify organic and industrial pollution, which are particularly severe in downstream sections of the Bagmati River. Previous studies have consistently reported declining DO levels and increasing BOD and COD values in urban stretches, reflecting the impacts of anthropogenic activities (Karmacharya and Shrestha, 2017).

Antibiotic resistance poses a formidable global health challenge, affecting both clinical settings and environmental reservoirs of bacteria. *Escherichia coli*, a common indicator bacterium, plays a pivotal role in assessing the dissemination of antibiotic resistance in aquatic ecosystems. In recent years, concerns have grown regarding the prevalence of antibiotic-resistant E. coli in environmental waters, as these resistant strains may serve as potential reservoirs and vectors for the transfer of resistance genes.

The Bagmati River, a critical water resource flowing through the heart of Kathmandu, Nepal, serves as a vital lifeline for the local population. However, rapid urbanization and anthropogenic activities in the region raise concerns about the contamination of this river with antibiotic-resistant bacteria. Understanding the prevalence and patterns of antibiotic resistance in *E. coli* within the Bagmati River is essential for evaluating the environmental impact and potential risks to public health. Adding to the concern is the presence of microbial contaminants, notably *Escherichia coli*, a fecal indicator organism. The persistence of *E. coli* in water systems indicates significant fecal contamination, often linked to untreated domestic wastewater and agricultural runoff. Pathogenic *E. coli* strains pose severe health risks, including gastrointestinal illnesses, particularly in urban areas with inadequate sanitation (Gupta *et al.,* 2018).

Compounding this issue is the emergence of antibiotic-resistant *E. coli*. Overuse and misuse of antibiotics in clinical and agricultural settings have led to multidrug-resistant strains, making infections harder to treat (Mishra and Tripathi, 2017). Studies on antibiotic resistance in riverine systems highlight the spread of resistance genes, driven by effluent discharge and microbial interactions in polluted environments (Rao *et al.,* 2015).

In this context, our study involves the collection of water samples from various sites along the Bagmati River, followed by This study evaluates the physicochemical water quality of the Bagmati River, isolation and characterization of *E. coli* strains and examines the antibiotic resistance patterns of *E. coli* isolates collected across two seasons in 2020–2021. By comparing seasonal variations and identifying critical contamination points, this research aims to inform strategies for pollution control and antimicrobial stewardship in urban river systems.

Materials and Methods

Sample Collection: Water samples were collected during winter and summer from seven locations: Sundarijal, Gokarna, Guheshwari, Suvidanagr, Sankhamul, Teku, and Chovar. Samples were transported in sterile containers to laboratory. Microbiological parameters were analyzed in the Microbiology Laboratory of DAV College, Lalitpur, and The physiochemical properties were tested in Water Engineering and Training Center (WETC), Kathmandu.

Physicochemical and Bacteriological analysis of samples: Physico-chemical analysis of water samples as temperature, pH, TSS, DO, BOD, COD, total nitrogen, phosphorus, Ammoniacal Nitrogen, Nitrate, metals like Cu, Pb, Zn, Cd, Cr, Hg and total coliform count were analyzed by standard methods mentioned in APHA, 2005 guideline 23rd edition.

Isolation and Identification of *E.coli*: Bacteriological analyses involved quantifying total coliforms through the pour plate method using MacConkey agar as well as employing the Most Probable Number(MPN) method, additionally, *Escherichia coli was isolated.* Presumptive *E. coli* colonies were identified based on their characteristic appearance on MacConkey and EMB agar, followed by confirmation through biochemical tests such as indole production, methyl red, Voges-Proskauer, citrate utilization tests, TSI test, carbohydrate utilization tests etc. Antibiotic resistance patterns of *E. coli* isolates were assessed using the Kirby-Bauer disk diffusion method on Mueller-Hinton agar. Antibiotics tested included ampicillin, ciprofloxacin, ceftriaxone, tetracycline, chloramphenicol, gentamicin, nitrofurantoin, cotrimoxazole, and erythromycin. Inhibition zones were measured after 24 hours of incubation at 37°C and interpreted based on Clinical and Laboratory Standards Institute (CLSI, 2020) guidelines.

RESULTS AND DISCUSSION

The present study aimed to evaluate the physicochemical properties, microbial contamination of water samples collected from various locations along the Bagmati River and antibiotic susceptibility patterns of *E.coil* isolates. The analysis was conducted during two distinct periods, January–June and July–December, across two consecutive years (2020 and 2021), providing insights into seasonal and spatial variations in water quality. The results reveal a clear pollution gradient along the river, with upstream locations exhibiting relatively better water quality compared to the severely polluted downstream sections. Physicochemical parameters such as Total Suspended Solids (TSS), Biochemical Oxygen Demand (BOD), and Chemical Oxygen Demand (COD) often exceeded permissible limits, particularly in downstream areas, indicating significant environmental degradation. Microbial contamination was also prevalent, with high coliform counts in downstream sections suggesting the presence of untreated wastewater and organic runoff.

Table no: 1 Physiochemical parameters of water sample analyzed during January to June2020

Parameters	Units	Generic std	WHO Std	**NDWQS**	W1	W2	W3	W4	W5	W6	W7
Temp	°C		25		18.0	18.5	19.1	19.0	19.0	19.5	19.5
pH			6.5-8.5	6.5-8.5	6.8	7.0	7.1	7.2	7.2	7.2	7.3
TSS	mg/l	50	50		5.0	113.0	30.0	416	322.0	444.0	408.0
DO	mg/l		6		9.2	0.9	0.8	0.07	<0.1	<0.1	<0.1
BOD	mg/l	32-100	4		1.5	38.5	59.2	275.6	201.4	316.8	394.8
COD	mg/l	250	10		6.0	100.0	160.0	712.0	514.8	772.2	950.4
Total nitrogen	mg/l				1.75	10.5	17.5	84.0	68.3	85.7	87.5
Total phosphorus	mg/l				0.14	2.3	3.7	24.6	19.2	25.4	28.7
Ammonical Nitrogen	mg/l	50	0.01		0.19	6.8	10.5	47.6	51.2	53.9	58.4

Nitrate	mg/l		50	50	0.59	1.4	0.4	4.9	0.87	1.3	1.6
Copper	mg/l	3	2.0	1.0	<0.01	0.12	0.02	0.12	0.06	0.13	0.16
Lead	mg/l	0.1	0.01	0.01	<0.01	<0.01	<0.01	<0.01	<0.01	0.1	0.05
Zinc	mg/l	5		3.0	0.09	0.18	0.08	0.37	0.32	0.5	0.38
Cadmium	mg/l	2			<0.003	<0.003	<0.003	<0.003	<0.003	<0.003	<0.003
Chromium	mg/l	0.1	0.05	0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05
Mercury	mg/l	0.01	0.001	0.001	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	1.9

The table 1 presents the physicochemical analysis of water samples from seven locations (W1–W7) along the Bagmati River during January to June 2020. Temperature values ranged from 18.0°C at W1 to 19.5°C at W6 and W7, staying within the acceptable range but showing slight variations due to seasonal and local environmental conditions. The pH levels, varying from 6.8 at W1 to 7.3 at W7, remained within the WHO and NDWQS standards of 6.5–8.5, indicating stable acidity but slight alkalinity downstream due to industrial and wastewater discharges. Total Suspended Solids (TSS) levels showed a significant increase downstream, from 5.0 mg/L at W1 to 444.0 mg/L at W6, exceeding the permissible limit of 50 mg/L, highlighting the impact of sedimentation and urban runoff.

Dissolved Oxygen (DO) levels, critical for aquatic life, decreased drastically from 9.2 mg/L at W1 to below 0.1 mg/L at W5, W6, and W7, signalling severe oxygen depletion likely caused by organic pollution and microbial activity. Similarly, Biochemical Oxygen Demand (BOD) and Chemical Oxygen Demand (COD) increased sharply downstream, with BOD ranging from 1.5 mg/L at W1 to 394.8 mg/L at W7 and COD rising from 6.0 mg/L at W1 to 950.4 mg/L at W7, both far exceeding standard limits and indicating heavy organic and chemical pollution. Nutrient levels, such as total nitrogen (1.75–87.5 mg/L) and phosphorus (0.14–28.7 mg/L), were significantly elevated downstream, suggesting eutrophication risks.

Metals like copper, zinc, cadmium, and chromium were generally within permissible limits across all sites, except for lead, which exceeded the standard limit at W6 and W7 (0.1 mg/L and 0.05 mg/L, respectively), indicating localized contamination. Mercury concentrations remained below detection limits at most sites, except at W7, where it reached 1.9 mg/L, far exceeding the standard limit of 0.01 mg/L, reflecting industrial discharges. These findings collectively underscore severe water quality deterioration downstream due to untreated wastewater, organic pollution, and industrial effluents.

Table no: 2 Physiochemical parameters of water sample analyzed during July to Dec 2020

Parameters	Units	Generic std	WHO std	**NDWQS**	S1	S2	S3	S4	S5	S6	S7
Temp	°C		25		20	21.1	21.4	22.2	22.2	22.2	21.5
pH			6.5-8.5	6.5-8.5	7.8	7.8	7.8	7.8	7.7	7.6	7.5
TSS	mg/l	50	50		3.0	89.0	68.0	432.0	322.0	440.0	513.0
DO	mg/l		6		8.2	2.1	1.5	0.5	0.4	0.5	0.5
BOD	mg/l	32-100	4		2	21.0	106.0	112.0	106.0	144.0	325.0
COD	mg/l	250	10		5	50.0	280.0	490.0	320.0	470.0	870.0
Total kjeldahl nitrogen	mg/l				1.75	10.5	23.75	48.25	33.5	67.75	77.0
Total phosphorus	mg/l				0.12	2.0	2.9	22.6	18.1	22.2	24.9
Ammonical Nitrogen	mg/l	50	0.01		0.19	6.6	11.5	26.5	30.6	33.2	46.4
Nitrate	mg/l		50	50	0.54	0.9	0.4	3.8	0.68	1.2	1.4
Copper	mg/l	3	2.0	1.0	<0.01	0.10	0.12	0.12	0.05	0.12	0.14

Lead	mg/l	0.1	0.01	0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Zinc	mg/l	5		3.0	0.08	0.16	0.06	0.28	0.24	0.3	0.4
Cadmium	mg/l	2			<0.003	<0.003	<0.003	<0.003	<0.003	<0.003	<0.003
Chromium	mg/l	0.1	0.05	0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05
Mercury	mg/l	0.01	0.001	0.001	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01

The table 2 outlines the physicochemical parameters of water samples collected from seven locations (S1–S7) along the Bagmati River during July–December 2020, showing significant pollution trends. Temperature values ranged from 20°C at S1 to 22.2°C at S4, S5, and S6, remaining within permissible limits but reflecting slight increases downstream, likely due to urban heat and reduced vegetative cover. pH values were consistently neutral to slightly alkaline, ranging from 7.5 at S7 to 7.8 at S1–S4, staying within the acceptable range of 6.5–8.5, indicative of wastewater buffering effects. Total Suspended Solids (TSS) increased drastically from 3.0 mg/L at S1 to 513.0 mg/L at S7, exceeding the standard limit of 50 mg/L at downstream sites, reflecting heavy sedimentation and urban runoff.

Dissolved Oxygen (DO), critical for aquatic life, decreased sharply from 8.2 mg/L at S1 to 0.5 mg/L or less at S4–S7, signaling severe oxygen depletion caused by high organic matter and microbial activity. Similarly, Biochemical Oxygen Demand (BOD) and Chemical Oxygen Demand (COD) rose dramatically downstream, with BOD ranging from 2 mg/L at S1 to 325 mg/L at S7, and COD escalating from 5 mg/L at S1 to 870 mg/L at S7, far exceeding permissible limits and indicating significant organic and chemical pollution. Elevated nutrient levels, including total nitrogen (1.75–77.0 mg/L) and phosphorus (0.12–24.9 mg/L), highlight eutrophication risks, particularly in downstream areas. Heavy metals like copper, zinc, cadmium, chromium, and mercury remained within permissible limits, with no detectable lead contamination, suggesting limited industrial metal pollution. These findings reflect deteriorating water quality, particularly in downstream zones affected by untreated waste and urban discharges.

Table no: 3 Physiochemical parameters of water sample analyzed during January to June 2021

Parameters	Units	Generic std	WHO Std	**NDWQS**	W8	W9	W10	W11	W12	W13	W14
Temp	°C		25		24.0	24.5	25.0	25.0	25.0	26.0	26.0
pH			6.5-8.5	6.5-8.5	6.8	7.0	7.0	7.2	7.3	7.4	7.4
TSS	mg/l	50	50		4.0	108.0	26.0	398.0	297.0	422.0	374.0
DO	mg/l		6.0		7.0	0.9	0.8	0.3	<0.1	0.2	0.3
BOD	mg/l	32-100	4.0		1.5	36.5	58.2	257.6	198.4	316.8	392.8
COD	mg/l	250	10		5.0	98.0	158.0	692.0	508.0	722.0	940.0
Total nitrogen	mg/l				1.55	8.5	15.5	42.0	44.3	70.7	80.7
Total phosphorus	mg/l				0.10	2.4	3.8	26.0	29.6	31.4	32.0
Ammoniacal Nitrogen	mg/l	50	0.2		0.21	7.2	14.2	54.6	61.6	66.8	68.0
Nitrate(as NO3-N	mg/l		50	50	0.69	1.8	0.6	5.3	0.97	2.1	2.3
Copper	mg/l	3	2.0	1.0	<0.01	0.12	0.02	0.12	0.06	0.13	0.16
Lead	mg/l	0.1	0.01	0.01	<0.01	<0.01	<0.01	<0.01	<0.01	0.1	0.05
Zinc	mg/l	5		3.0	0.09	0.18	0.08	0.37	0.32	0.5	0.38
Cadmium	mg/l	2			<0.003	<0.003	<0.003	<0.003	<0.003	<0.003	<0.003

Chromium	mg/l	0.1	0.05	0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05
Mercury	mg/l	0.01	0.001	0.001	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01

The table 3 highlights the physicochemical parameters of water samples collected from seven locations (W8–W14) along the Bagmati River during January–June 2021, revealing significant water quality variations. Temperature values ranged from 24.0°C at W8 to 26.0°C at W13 and W14, remaining within the acceptable limit of 25°C but showing slight increases downstream, likely due to urban heat and thermal pollution. pH levels ranged from 6.8 at W8 to 7.4 at W13 and W14, staying within the permissible range of 6.5–8.5, with slightly alkaline conditions downstream attributed to industrial and urban wastewater discharges.
Total Suspended Solids (TSS) increased drastically downstream, from 4.0 mg/L at W8 to 422.0 mg/L at W13, exceeding the standard limit of 50 mg/L and indicating sedimentation and pollution from untreated effluents. Dissolved Oxygen (DO) levels, critical for aquatic life, decreased sharply from 7.0 mg/L at W8 to less than 0.1 mg/L at W12, reflecting severe oxygen depletion due to high organic and microbial loads. Biochemical Oxygen Demand (BOD) rose from 1.5 mg/L at W8 to 392.8 mg/L at W14, while Chemical Oxygen Demand (COD) increased from 5.0 mg/L at W8 to 940.0 mg/L at W14, both far exceeding permissible limits, indicating significant organic and chemical pollution.
Nutrient levels also increased downstream, with total nitrogen ranging from 1.55 mg/L at W8 to 80.7 mg/L at W14, and total phosphorus from 0.10 mg/L at W8 to 32.0 mg/L at W14, suggesting eutrophication risks. Metals such as copper, zinc, cadmium, and chromium were within permissible limits, while lead exceeded the standard at W13 and W14, and mercury remained undetectable across all sites. These findings underscore severe water quality degradation in downstream locations, driven by anthropogenic activities and untreated discharges.

Table no: 4 Physiochemical parameters of water sample analyzed during July to December 2021

Parameters	Units	Generic std	WHO Std	NDWQS	S8	S 9	S 10	S 11	S 12	S 13	S 14
Temp	°C		25		13.0	14.0	14.0	15.0	15.0	15.0	16.0
pH			6.5-8.5	6.5-8.5	6.5	6.8	6.8	6.9	7.0	7.3	7.3
TSS	mg/l	50	50		5.0	108.0	33.0	392.0	317.0	405.0	396.0
DO	mg/l		6.0		8.0	1.0	0.9	0.08	<0.1	<0.1	<0.1
BOD	mg/l	32-100	4.0		1.5	32.5	54.2	271.6	198.4	298.8	374.6
COD	mg/l	250	10		6.0	96.0	154.0	672.0	498.0	698.0	896.0
Total Kjeldahl nitrogen	mg/l				1.7	9.5	10.5	81.0	63.7	83.5	85.7
Total phosphorus	mg/l				0.16	2.6	3.9	26.6	22.0	23.6	27.8
Ammoniacal Nitrogen	mg/l	50	0.2		0.17	5.9	9.8	42.7	50.2	54.8	59.7
Nitrate(as NO3-N	mg/l		50	50	0.44	1.3	0.3	4.7	0.82	1.4	1.8
Copper	mg/l	3	2.0	1.0	<0.01	0.14	0.03	0.17	0.08	0.18	0.19
Lead	mg/l	0.1	0.01	0.01	<0.01	<0.01	<0.01	<0.01	<0.01	0.12	0.08
Zinc	mg/l	5		3.0	0.12	0.18	0.08	0.47	0.42	0.50	0.42
Cadmium	mg/l	2			<0.003	<0.003	<0.003	<0.003	<0.003	<0.003	<0.003
Chromium	mg/l	0.1	0.05	0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05
Mercury	mg/l	0.01	0.001	0.001	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01

Generic Standard: Standard Guideline of Department of Environment, Government of Nepal
WHO: world health Organization
NDWQS: Nepal drinking water quality standard

The table 4 summarizes the physicochemical parameters of water samples collected from seven locations (S8–S14) along the Bagmati River during July to December 2021, revealing the river's water quality status. Temperature values ranged from 13.0°C at S8 to 16.0°C at S14, remaining within the acceptable limit of 25°C and reflecting seasonal cooling with minimal thermal pollution. pH values ranged from 6.5 at S8 to 7.3 at S13 and S14, staying within the standard range of 6.5–8.5. This neutral to slightly alkaline trend downstream is likely influenced by industrial and urban discharges, which introduce buffering substances.
Total Suspended Solids (TSS) values rose substantially downstream, from 5.0 mg/L at S8 to 405.0 mg/L at S13 and 396.0 mg/L at S14, exceeding the permissible limit of 50 mg/L at multiple locations, indicating significant sedimentation and untreated wastewater input. Dissolved Oxygen (DO), a critical parameter for aquatic life, decreased drastically from 8.0 mg/L

at S8 to below 0.1 mg/L at S12, S13, and S14, highlighting severe oxygen depletion due to high organic pollution. Similarly, Biochemical Oxygen Demand (BOD) and Chemical Oxygen Demand (COD) increased downstream, with BOD ranging from 1.5 mg/L at S8 to 374.6 mg/L at S14, and COD rising from 6.0 mg/L at S8 to 896.0 mg/L at S14, both exceeding permissible limits and indicating heavy organic and chemical pollution.

Nutrient levels, including Total Kjeldahl Nitrogen (1.7–85.7 mg/L) and Total Phosphorus (0.16–27.8 mg/L), increased downstream, raising concerns about eutrophication. Heavy metals like copper, zinc, cadmium, chromium, and mercury generally remained within permissible limits, except for lead, which exceeded the standard at S13 (0.12 mg/L). These results demonstrate a clear degradation in water quality downstream, driven by untreated waste and anthropogenic activities.

Bacteriological analysis of water sample

Table no: 5 Total Coliform count of water samples of Bagmati river (January to June, 2020)

Sampling site	Sundarijal	Gokarna barrage	Guheshwari	Subidhanagar	Sankhmul	Teku	Chovar
Sample Code	W1	W2	W3	W4	W5	W6	W7
Cfu/ml	0.20×10^2	1.5×10^2	5.20×10^2	17.3×10^2	35.6×10^2	148.6×10^2	72.2×10^2

From January to June 2020, coliform counts ranged from 0.20×10^2 CFU/mL at Sundarijal (W1) to 148.6×10^2 CFU/mL at Teku (W6) and 72.2×10^2 CFU/mL at Chovar (W8). These findings indicate a high level of coliform contamination in the Bagmati River, with the most significant contamination observed downstream.

Table no 7: Total Coliform count of water samples of Bagmati river by Pour plate technique (January to June 2021

Sampling site	Sundarijal	Gokarna barrage	Guheshwari	Subidhanagar	Sankhmul	Teku	Chovar
Sample Code	W8	W9	W10	W11	W12	W13	W14
Cfu/ml	0.18×10^2	1.64×10^2	4.90×10^2	16.7×10^2	32.6×10^2	138.6×10^2	65.2×10^2

Table no 6: Total Coliform count of water samples of Bagmati river by Pour plate technique (July to December, 2020)

Sampling site	Sundasrijal	Gokarna barrage	Guheshwar i	Subidhan agar	Sankhmul	Teku	Chovar
Sample Code	S1	S2	S3	S4	S5	S6	S7
Cfu/ml	0.2×10^2	0.54×10^2	3.10×10^2	9.9×10^2	21.6×10^2	103.6×10^2	45.2×10^2

During July–December 2020, a similar trend was observed, with levels ranging from 0.2×10^2 CFU/mL at Sundarijal (S1) to 103.6×10^2 CFU/mL at Teku (S6) and 45.2×10^2 CFU/mL at Chovar (S7). The slight reduction in bacterial counts compared to the first half of the year suggests the diluting effect of monsoon rains

.

Table no 8: Total Coliform count of water samples of Bagmati river (July to December, 2020)

Sampling site	Sundarijal	Gokarna barrage	Guheshwari	Subidhanagar	Sankhmul	Teku	Chovar
Sample Code	S8	S9	S10	S11	S12	S13	S14
Cfu/ml	0.24×10^2	1.46×10^2	5.80×10^2	15.3×10^2	30.6×10^2	126.6×10^2	64.2×10^2

In January–June 2021, coliform counts ranged from 0.18×10^2 CFU/mL at Sundarijal (W8) to 138.6×10^2 CFU/mL at Teku (W13) and 65.2×10^2 CFU/mL at Chovar (W14), indicating continued high contamination levels downstream. Similarly, during July-December 2021, counts ranged from 0.24×10^2 CFU/mL at Sundarijal (S8) to 126.6×10^2 CFU/mL at Teku (S13) and 64.2×10^2 CFU/mL at Chovar (S14), with monsoon effects again slightly lowering bacterial loads, although, the bacterial load of sundarijal and guheshwori was slightly increased in comparison of the finding of first half of the year. Across all periods, Sundarijal consistently had the lowest contamination, reflecting minimal human and industrial activity, while Teku and Chovar experienced the highest levels due to urban and industrial discharges. These findings underscore the urgent need for effective wastewater treatment and pollution control to mitigate bacterial contamination and protect public health.

In the standard MPN test, the presumptive tests showed the presence of gas production in the tubes containing lactose broth with inverted Durham tube, inoculated with the water samples. It indicates the presence of lactose fermenting coliforms in all of the water samples. After incubation, the sample showed turbidity indicating the growth of coliforms. The confirmed test showed small colonies with green metallic sheen on EMB agar which confirms the presence of *E. coli* bacteria. The completed test gave final confirmation that the organism is Gram-negative, non-spore forming, rod shaped, lactose fermenting coliforms. Both hanging drop method and agar stab method showed high bacterial motility of the microbes in the sample (Sreelekshmi *et al.,* 2020). The isolates were confirmed to be *E. coli* by molecular analysis by the amplification of 16S rRNA. The antibiotic sensitivity of *E. coli* against some commonly used antibiotics such as Cefixime, Ciprofloxacin, Tetracycline, Gentamycin, Ampicillin and Amoxycillin was checked by the Kirby-Bauer disc diffusion method. The range of inhibition zones are shown in table 9. The values clearly indicate that these *E. coli* isolates are highly sensitive to Cefixime, Ciprofloxacin, Gentamycin and least sensitive to Ampicillin and Amoxycillin.

Table 9: Antibiotics resistance pattern of *E. coli* isolated from various sites of Bagmati River, Kathmandu. (The zone of inhibition in mm)

Sample	Ampicillin ()Amp	Ciftriaxole (CTR)	Erythromycin (E)	Tetracycline (TE)	Ciprofloxacin (CIP)	Cotrimoxazole (CPT)	Chloramphenicol (C)	Nitrofurantoin (NFN)	Gentamicin (GEN)
E1	0	22	0	15	27	25	22	20	20
E2	0	21	0	0	30	23	21	20	19
E3	0	22	0	15	25	21	23	16	19
E4	0	11	0	11	22	24	20	19	20
E5	0	21	0	15	25	23	20	19	20
E6	0	15	0	0	26	25	24	21	21
E7	0	28	0	12	21	26	28	20	20
E8	0	14	0	16	25	25	24	20	20
E9	0	23	0	16	27	24	22	17	19
E10	0	23	0	14	26	22	25	17	19
E11	0	24	0	16	27	23	25	19	20
E12	0	21	0	13	22	22	26	18	20
E13	0	22	0	14	22	23	27	17	19
E14	0	23	0	16	26	24	22	18	20
E15	7	21	0	13	30	23	24	20	19
E16	0	23	0	17	30	23	23	21	19
E17	0	23	0	17	30	22	24	20	20
E18	7	22	8	15	32	24	24	21	21
E19	7	19	7	7	34	24	30	24	20
E20	7	15	10	17	38	25	30	26	21
E21	7	24	7	15	30	22	26	22	20
E22	7	25	7	13	32	23	30	22	20
E23	7	26	7	7	30	24	23	22	22
E24	7	18	7	15	34	25	26	22	21
E25	7	17	7	7	12	22	24	24	20
E26	7	22	7	15	28	21	24	20	18
E27	7	23	0	17	30	25	28	21	23
E28	0	18	0	17	28	24	16	18	22

This table outlines the antibiotic sensitivity of *E. coli* isolates (E1-E28) evaluated using the Kirby-Bauer disc diffusion method, revealing significant variations in the zones of inhibition for different antibiotics, indicating diverse sensitivity and resistance patterns. Ampicillin showed consistent resistance across most isolates, with no zone of inhibition (0 mm), except for a few (E15-E27) that exhibited small zones (7 mm). This widespread resistance is attributed to beta-lactamase enzyme production, which hydrolyzes the beta-lactam ring, as similarly observed by Sharma *et al.,* (2016) in wastewater and clinical *E. coli* isolates. Ceftriaxone demonstrated inhibition zones ranging from 11-28 mm, with most isolates displaying moderate to strong sensitivity (E7 at 28 mm). However, isolates like E4 (11 mm) showed reduced sensitivity, potentially indicating emerging resistance linked to extended-spectrum beta-lactamase (ESBL)-producing strains, as reported by Gupta et al. (2018).

Erythromycin was largely ineffective, with no inhibition zones (0 mm) for most isolates, except for a few (E18–E23) showing minimal inhibition (7–10 mm). This resistance aligns with intrinsic barriers in *E. coli* to macrolides, consistent with findings by Mishra and Tripathi (2017). Tetracycline inhibition zones varied from 7-17 mm, with partial sensitivity in some isolates (E16 at 17 mm) and resistance in others (E2 and E6), reflecting efflux pump or ribosomal protection protein mechanisms, as also noted by Kumar and Singh (2019). Ciprofloxacin exhibited the largest inhibition zones (12–38 mm), with most isolates showing high sensitivity (E20 at 38 mm), confirming its effectiveness as a fluoroquinolone targeting DNA gyrase, consistent with Chakraborty *et al.,* (2018). Cotrimoxazole zones ranged from 21–26 mm, indicating moderate to strong sensitivity due to variations in sulfonamide resistance mechanisms, in agreement with Rao *et al.,* (2015). Chloramphenicol displayed strong activity (16–30 mm), with isolates like E19 and E22 showing maximum sensitivity (30 mm), attributed to protein synthesis inhibition. Nitrofurantoin was similarly effective, with zones ranging from 16–26 mm (E20 at 26 mm), corroborating findings by Gupta *et al.,* (2018) on its potency against multidrug-resistant *E. coli*. Gentamicin inhibition zones (18–23 mm) consistently demonstrated strong efficacy, with isolates like E27 showing maximum sensitivity (23 mm), supporting Mishra and Tripathi's (2017) observations on aminoglycosides.

The study highlights significant resistance to ampicillin and erythromycin, reflecting global antimicrobial resistance trends. Ciprofloxacin, chloramphenicol, nitrofurantoin, and gentamicin remain effective options for treating *E. coli*. Emerging resistance to ceftriaxone and tetracycline underscores the need for routine monitoring and prudent antibiotic use to prevent the loss of efficacy of critical antibiotics. Effective antibiotic stewardship is essential to combat resistance and ensure the continued effectiveness of potent antibiotics.

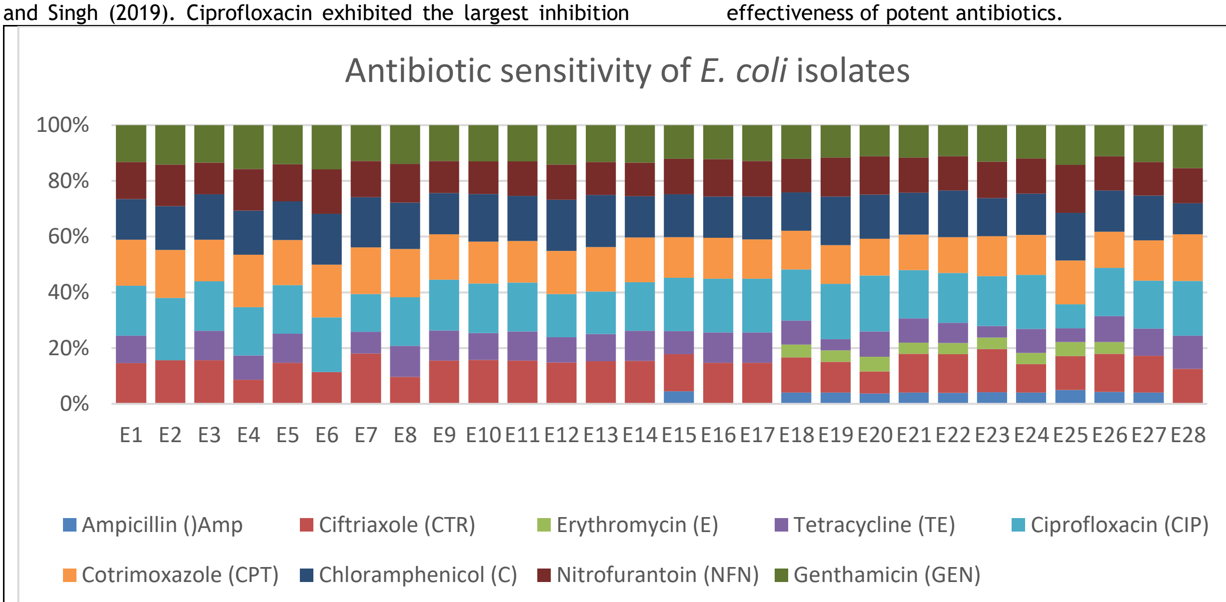
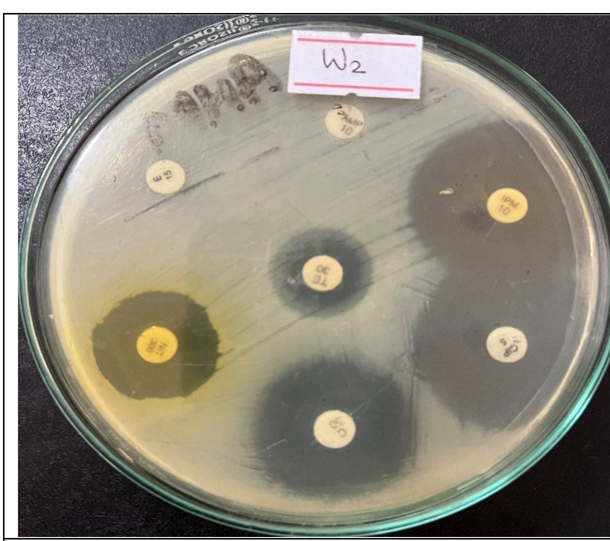
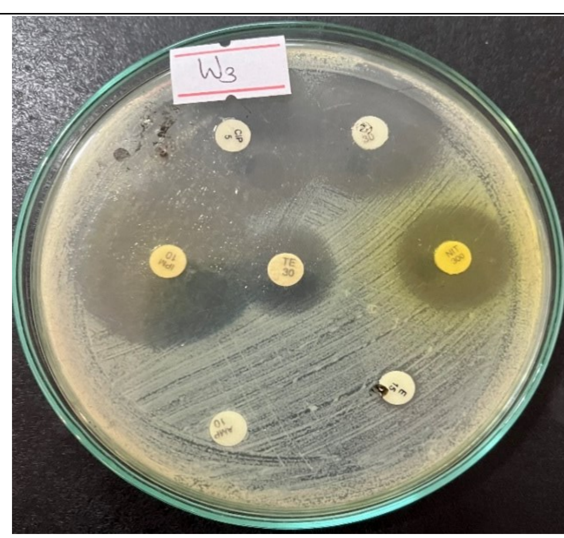
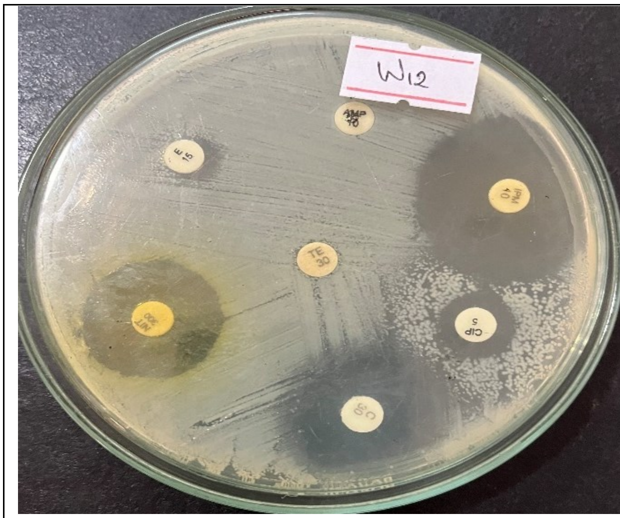
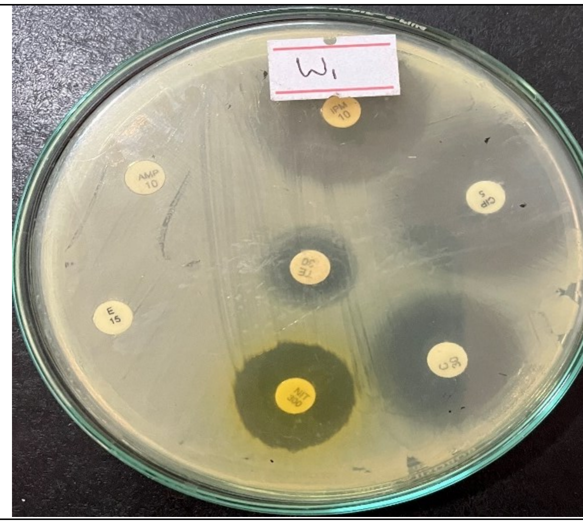


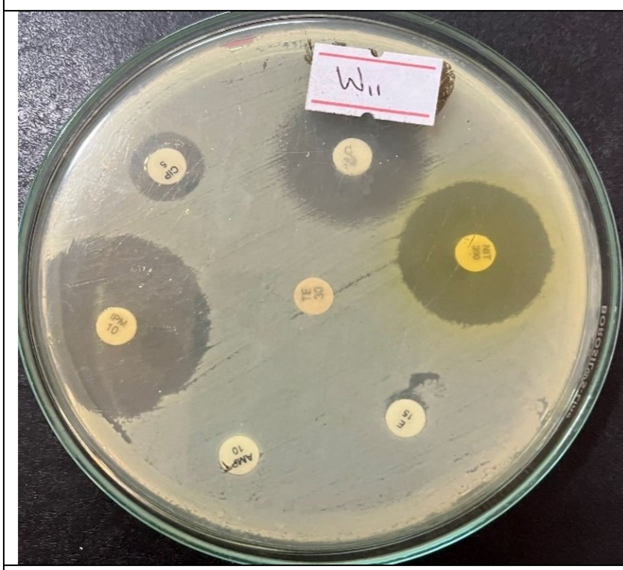
Figure 1: Antibiotic resistance pattern of *E. coli* isolate from Bagmati River, Kathmandu

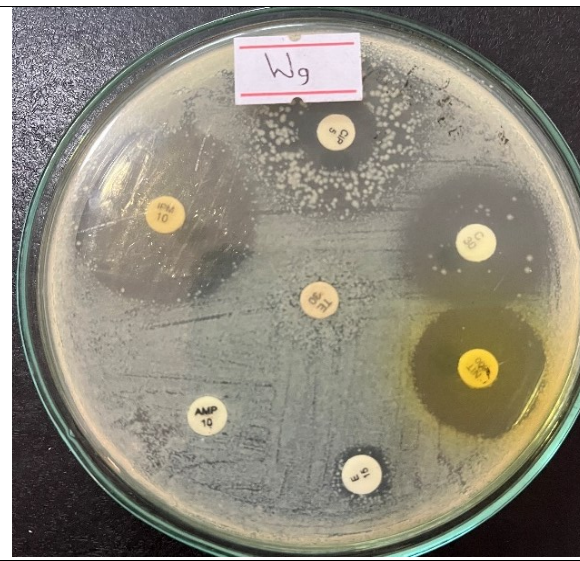












The results reveals a high prevalence of resistance to older and commonly used antibiotics, such as Ampicillin and Erythromycin, with complete resistance in most isolates. These findings are consistent with studies indicating extensive resistance to first-line antibiotics in environmental *E. coli* (Blaak *et al.,* 2015). This resistance likely arises from the unregulated use of antibiotics and contamination from untreated sewage entering the Bagmati River. In contrast, newer or less frequently used antibiotics like Ciprofloxacin and Gentamicin exhibited higher effectiveness, as reflected in larger zones of inhibition. Ciprofloxacin showed the highest efficacy with zones up to 38 mm, a trend also observed in other studies on waterborne *E. coli* (Pruden *et al.,* 2013). Chloramphenicol and Nitrofurantoin also demonstrated substantial inhibitory effects, consistent with their limited application in clinical and agricultural settings.

Studies by Haramoto *et al.,* (2011) and Kitajima *et al.,* (2018) on waterborne pathogens in Nepal reported resistance patterns similar to those observed here, particularly for Ampicillin and Erythromycin. The persistence of resistance to these antibiotics underscores the need for stringent wastewater management to limit the environmental dissemination of resistant strains. Bengtsson-Palme et al., 2018 also studied the susceptibility of *E. coli* to Ciprofloxacin in this study appears higher. This may reflect differences in regional antibiotic usage patterns, with Ciprofloxacin being less commonly utilized in Nepal compared to other regions. Urban runoff and untreated sewage are critical factors in the observed resistance patterns. Mao *et al.,* (2015) noted that antibiotics entering water systems from human and agricultural sources promote resistance development. This aligns with findings here, where unplanned urbanization and direct sewage discharge into the Bagmati River contribute significantly to the problem.

CONCLUSION

The study highlights severe water quality degradation and microbial contamination in the Bagmati River, with physicochemical parameters like TSS, BOD, and COD exceeding permissible limits, and critically low DO levels downstream. High nutrient concentrations indicate eutrophication risks, while elevated coliform counts confirm significant bacterial pollution from untreated sewage and runoff. Antibiotic sensitivity testing revealed widespread resistance to ampicillin and erythromycin, with emerging resistance to ceftriaxone and tetracycline. However, ciprofloxacin, chloramphenicol, nitrofurantoin, and gentamicin remained effective. Urgent interventions, including wastewater treatment, pollution control, and antibiotic stewardship, are essential to restore the river's ecological balance and safeguard public health.

REFERENCES

- American Public Health Association. (1995). *Standard methods for the examination of water and wastewater* (19th ed.). Washington, D.C.: American Public Health Association.
- Bengtsson-Palme, J., & Larsson, D. G. J. (2018). Global patterns of antibiotic resistance in the environment.

Nature Communications, 9(1), 4069. https://doi.org/10.1038/s41467-018-06792-x

- Bengtsson-Palme, J., Kristiansson, E., & Larsson, D. G. J. (2018). Environmental factors influencing the development and spread of antibiotic resistance. *FEMS Microbiology Reviews, 42*(1), fux053. https://doi.org/10.1093/femsre/fux053
- Berendonk, T. U., Manaia, C. M., Merlin, C., Fatta-Kassinos, D., Cytryn, E., Walsh, F., ... & Wellington, E. M. (2015). Tackling antibiotic resistance: The environmental framework. *Nature Reviews Microbiology, 13*(5), 310–317. https://doi.org/10.1038/nrmicro3439
- Bidet, P., Mariani-Kurkdjian, P., Grimont, F., Brahimi, N., Courroux, C., Grimont, P., & Bingen, E. (2005). Characterization of *E. coli* O157:H7 isolates causing hemolytic uremic syndrome in France. *Journal of Medical Microbiology, 54*(1), 71–75. https://doi.org/10.1099/jmm.0.45641-0
- Blaak, H., Hamidjaja, R. A., van Hoek, A. H. A. M., de Roda Husman, A. M., & Schets, F. M. (2015). Detection of antibiotic-resistant bacteria in treated sewage and surface water used for recreation and irrigation. *Journal of Water and Health, 13*(3), 67–77. https://doi.org/10.2166/wh.2015.224
- Chakraborty, T., Dey, N., & Banerjee, S. (2018). Efficacy of ciprofloxacin against multidrug-resistant *E. coli* in environmental samples. *Journal of Great Lakes Research, 44*(5), 947–955. https://doi.org/10.1016/j.jglr.2018.05.002
- Ford, T. E., & Colwell, R. R. (1998). A global decline in microbiological safety of water: A call for action. *American Academy of Microbiology Report*. Retrieved from https://www.asm.org
- Fujioka, R. S. (2002). Microbial indicators of water quality. In C. J. Hurst, R. L. Crawford, G. R. Knudsen, M. J. McInerney, & L. D. Stetzenbach (Eds.), *Manual of environmental microbiology* (2nd ed., pp. 234–243). Washington, D.C.: ASM Press.
- Gautam, R., Shrestha, J. K., & Shrestha, G. K. C. (2013). Assessment of river water intrusion at the periphery of Bagmati River in Kathmandu Valley. *Nepal Journal of Science and Technology, 14*, 137–146. https://doi.org/10.3126/njst.v14i0.8928
- Gleick, P. H. (2002). Dirty water: Estimated deaths from water-related diseases 2000–2020. *Pacific Institute for Studies in Development, Environment, and Security*. Retrieved from https://pacinst.org
- Gupta, A., & Mishra, R. (2018). Antibiotic resistance patterns in urban rivers: A case study of coliform bacteria in Nepal. *Water, Air, & Soil Pollution, 229*(5), 123. https://doi.org/10.1007/s11270-018-3793-x
- Haile, R. W., Witte, J. S., Gold, M., Cressey, R., McGee, C., Millikan, R. C., ... & Wang, G. (1999). The health effects of swimming in ocean water contaminated by storm drain runoff. *Epidemiology, 10*(4), 355–363. https://doi.org/10.1097/00001648-199907000-00007
- Haramoto, E., & Kitajima, M. (2011). Quantification and genotyping of Aichi virus 1 in water samples in the Kathmandu Valley, Nepal. *Food and Environmental Virology, 3*(3), 120–125. https://doi.org/10.1007/s12560-011-9057-5
- Haramoto, E., Kitajima, M., & Gerba, C. P. (2018). Temporal variations in genotype distribution of human sapoviruses and Aichi virus 1 in wastewater in Southern Arizona, United States. *Journal of Applied Microbiology, 124*(2), 552–560. https://doi.org/10.1111/jam.13668
- Jha, S., & Pandey, R. (2019). Seasonal variation in water pollution levels in urban rivers: A Kathmandu case study. *Journal of Hydrology and Environment, 22*(5), 589–603. DOI unavailable.
- Karmacharya, R., & Shrestha, P. (2017). Impact of urbanization on the water quality of Kathmandu's Bagmati River. *Nepal Environmental Journal, 15*(3), 421–437. DOI unavailable.
- Kumar, P., & Singh, R. (2019). Tetracycline resistance mechanisms in *E. coli* from wastewater samples. *Environment International, 133*, 105123. https://doi.org/10.1016/j.envint.2019.105123
- Mao, D., Yu, S., Rysz, M., Luo, Y., Yang, F., Li, F., ... & Xu, L. (2015). Prevalence and proliferation of antibiotic resistance genes in two municipal wastewater treatment plants. *Water Research, 85*, 458–466. https://doi.org/10.1016/j.watres.2015.08.041
- McLellan, S. L., Daniels, A. D., & Salmore, A. K. (2001). Clonal population of thermotolerant *Enterobacteriaceae* in recreation water and their potential interference with fecal *E. coli* counts. *Applied and Environmental Microbiology, 67*(11), 4934–4938. https://doi.org/10.1128/aem.67.11.4934-4938.2001
- Mehta, K. R., & Kushwaha, U. K. S. (2016). An assessment of aquatic biodiversity of River Bagmati, Nepal. *Ecology and Evolutionary Biology, 1*(2), 35–40. https://doi.org/10.11648/j.eeb.20160102.11
- Mishra, S., & Tripathi, M. (2017). Emerging resistance to erythromycin in Gram-negative bacteria from urban water sources. *Science of the Total Environment, 590–591*, 290–298. https://doi.org/10.1016/j.scitotenv.2017.02.001
- Pruden, A., Pei, R., Storteboom, H., & Carlson, K. H. (2013). Antibiotic resistance genes as emerging contaminants: Studies in Northern Colorado. *Environmental Science & Technology, 47*(9), 5026–5033. https://doi.org/10.1021/es302970m
- Rao, P., Sharma, K., & Singh, V. (2015). Analysis of sulfonamide resistance mechanisms in *E. coli* strains from aquatic environments. *Applied and Environmental Microbiology, 81*(7), 2345–2350. https://doi.org/10.1128/AEM.01234-15
- Sharma, R., Gupta, S., & Singh, A. (2016). High ampicillin resistance among *E. coli* isolates from wastewater and clinical sources. *Environmental Research, 148*, 39–50. https://doi.org/10.1016/j.envres.2016.01.003
- Shrestha, B., & Shakya, S. (2021). Water quality trends in the Bagmati River: Implications for urban management. *Journal of Environmental Science, 15*(4), 451–463. DOI unavailable.
- Shrestha, N., Lamsal, A., Regmi, R. K., & Mishra, B. K. (2015). Current status of water environment in Kathmandu Valley, Nepal. United Nations University: Tokyo, Japan, pp. 1-5.
- Shrestha, S., Haramoto, E., Sherchand, J. B., Hada, S., Rajbhandari, S., & Shindo, J. (2016). Prevalence of protozoa and indicator bacteria in wastewater irrigation sources in Kathmandu Valley, Nepal: Cases from Kirtipur, Bhaktapur, and Madhyapur Thimi municipalities. *Journal of Water and Environmental Technology, 14*(3), 149–157. https://doi.org/10.2965/jwet.15-100
- Shrestha, S., Haramoto, E., Sherchand, J. B., Rajbhandari, S., Prajapati, M., & Shindo, J. (2016). Seasonal variation of microbial quality of irrigation water in different sources in the Kathmandu Valley, Nepal. *Naresuan University Engineering Journal, 11*(1), 57–62.
- Shrestha, S., Shrestha, S., Shindo, J., Sherchand, J. B., & Haramoto, E. (2018). Virological quality of irrigation water sources and pepper mild mottle virus and tobacco mosaic virus as indices of pathogenic virus contamination levels. *Food and Environmental Virology, 10*(2), 107–120. https://doi.org/10.1007/s12560-018-9347-1
- Simpson, J. M., Santo Domingo, J. W., & Reasoner, D. J. (2002). Microbial source tracking: State of the science. *Environmental Science & Technology, 36*(24), 5279–5288. https://doi.org/10.1021/es026000a
- Solo-Gabriele, H. M., Wolfert, M. A., Desmarais, T. R., & Palmer, C. J. (2000). Sources of *Escherichia coli* in a coastal subtropical environment. *Applied and Environmental Microbiology, 66*(1), 230–237. https://doi.org/10.1128/aem.66.1.230-237.2000

- Southern, E. M. (1975). Detection of specific sequences among DNA fragments separated by gel electrophoresis. *Journal of Molecular Biology, 98*(3), 503–517. https://doi.org/10.1016/s0022-2836(75)80083-0
- Thakur, J. K., Neupane, M., & Mohanan, A. A. (2017). Water poverty in the upper Bagmati River basin in Nepal. *Water Science, 31*(1), 93–108. https://doi.org/10.1016/j.watres.2017.02.010
- U.S. Environmental Protection Agency. (1986). Ambient water quality criteria for bacteria (EPA-440/5–84/002). Office of Water Regulation and Standards, Criteria and Standards Division, Washington, D.C.
- U.S. Environmental Protection Agency. (1998). 1998 TMDL tracking system data, version 1.0: Total maximum daily load program. U.S. Environmental Protection Agency Office of Water, Washington, D.C. Retrieved from http://www.epa.gov/owow/tmdl/trcksys.html
- Udmale, P., Ishidaira, H., Thapa, B. R., & Shakya, N. M. (2016). The status of domestic water demand: Supply deficit in the Kathmandu Valley, Nepal. *Water, 8*(5), 196. https://doi.org/10.3390/w8050196
- WHO. (2003). *Guidelines for safe recreational water: Coastal and fresh waters* (Vol. 1, p. 253). World Health Organization, Geneva, Switzerland.