Seasonal Fluctuation of Water Quality and Ecogenomic Phylogeny of Novel Potentional Microbial Pollution Indicators of Veshaw River-Western Himalaya

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Abstract

Almost a billion people, especially in developing countries, need safe water. Several studies in India show that most drinking water sources have high coliform counts. Standard quality control is needed. This study was conducted in rural parts of South Kashmir in the Western Himalaya from February to January, 2020–2022. Water samples were taken from the river from upstream to downstream using standard water sampling techniques. The purpose of this study was to investigate the detection and molecular identification of coliforms in drinking water and the chemical water quality associated with coliform-contaminated drinking water, which summarises river water pollution. The results showed significant variation in water quality measures, indicating increased pollution levels downstream. In all seasons, the highest coliform count was recorded at Sangam (downstream) of the river. In the summer season at Sangam it was recorded (72.2600 cfu 106/l) and no coliform count was found at Kongwaton (upper stream) during the winter season near the source of the Veshaw river. In all seasons, the highest values of water quality parameters (pH 6.847, EC 71.620 dS/m, BOD 1.120 mg/l, and COD 24.637 mg/l) were recorded at Sangam (downstream) of the river during the summer season. In the winter season at Kongwaton, the lowest values of water quality parameters were pH 8.947, EC 253.680 dS/m, BOD 4.963 mg/l, and COD 51.440 mg/l. The existence of coliforms in water indicates current faecal contamination and determines the presence of disease-causing pathogens. The goal of this work is to examine the level of total coliform contamination, coliform species identification, and chemical water quality properties of drinking water and its associated factors, which implies the pollution level in the river. Total DNA was collected and sequenced for 16S rDNA and metagenomics. Universal primers were used to amplify the bacterial 16S rRNA. Using BLAST, the amplified 16S rRNA gene sequence was matched to the NCBI database. A metagenomic study found 27 species that had different relative abundances. These species include *E. coli, Escherichia fergusonii, Escherichia albertii, Klebsiella grimontii, and Shigella dysenteriae.* This study is thought to be the first to discriminate against *E. fergusonii, Escherichia albertii, Klebsiella grimontii*, and *Shigella dysenteriae* from *E. coli* and to report on *E. fergusonii* and E. albertii, *Klebsiella grimontii,* and *Shigella dysenteriae* in the river Veshaw water sources in Kulgam, Western Himalaya.

Introduction

For irrigation, agricultural, electrical, and recreational purposes, rivers are essential (Karunanidhi et al., 2021). Liang et al., 2021). Huang et al., 2019. Toxic pollution, such as industrial sewage, agricultural runoff, and livestock discharge, as well as aquaculture effluent, is the primary cause of blackening and odour formation in water bodies (Cao et al., 2020; Zhang et al., 2020; Zhang et al., 2021). Disfiguring the city's appearance and endangering human health are some of the negative effects of the contaminated river systems (Wang et al. 2021; Zhu et al.2022). Remediation solutions and long-term river management hinge on an understanding of the influence of various anthropogenic disturbances on water quality (Zhao et al., 2020). Water quality evaluation is vital for determining the origins, spread, and potential health effects of water-borne contaminants (Samanta et al.2016; Wu et al., 2021; Ghosh et al., 2022; Bera et al. 2022). Various water quality evaluation approaches, including statistical techniques, modelling analysis,

and the water quality index (WQI), have been used in recent decades to classify and evaluate riverine water (El Najjar et al., 2019; Liu et al., 2021). Safe water and hygienic methods of excreta disposal are universal needs and basic human rights, but many people around the globe do not have access to these necessities. Many developing countries' public health is in danger due to poor water quality and the possibility of contracting diseases transmitted by contaminated water (Ntajal et al.2022; Rusca et al.2022; Singh et al.2022).

To put that into perspective, some 1.1 billion people live without clean water since they live in the world's most impoverished countries. An estimated 1.6 million people die each year from curable diarrheal illnesses, the majority of whom are children under the age of five. (World Health Organization, 2008). Anthropogenic activities are degrading the world's water resources, reducing their accessibility for domestic, drinking, irrigation, fishing, outdoor enjoyment, and industrial purposes (Shit, 2021 ; Zhang et al. 2021; Bera et al.2022). Water quality is the most critical aspect in protecting the environment and public health (Das et al. 2020; Chakraborty et al 2021a; Raimi et al. 2021; Hossain et al.2022), which means improving water quality is essential for economic and health sector management. Ecosystems of rivers and streams are vulnerable to a wide range of natural and anthropogenic influences (such as variations in precipitation, erosion, and weathering processes) (Whitehead et al. 2009; Khatri et al. 2015; Issaka et al. 2017;Bano et al. 2018; Khan et al. 2021; Giri et al. 2021; RamyaPriya et al. 2022). Due to its role as a main stream for household, industrial, and agricultural waste and runoff, a stream's sensitivity to pollution is well-founded (Wu et al., 2019; Gnanachandrasamy et al., 2020;Rather et al., 2022a). More and more pressure is being put on river waters around the world due to growing populations and asymmetry in urban development; wastewater discharge from domestic areas; inefficient management in agriculture; and the removal of gravel from rivers and streams to channel water for generating electricity (Ustaoglu & Tepe, 2019; Ustaoglu et al. 2021; Bano et al. 2022). Concerns have also been raised about the urban streams that run through these neighbourhoods being used as dumping grounds by both homes and businesses (Bhat et al., 2011; Phiri et al., 2005; Enguito et al., 2013; Sharma et al., 2020; Mihai et al., 2020; Datta et al., 2022). The worsening of water quality as a result of river system disruption is a major worldwide environmental challenge (Ustaoglu et al., 2020; Chakraborty et al. 2021b). We live in a society that requires us to protect our natural resources (streams, rivers, lakes, etc.) and to monitor the causes of their degradation on a regular basis. If the deterioration of surface water in streams continues unchecked, it might cause a serious hazard to public health, economic stability, and social progress. For effective water management, there is a requirement to analyse water quality changes over time and reduce river pollution by identifying and eradicating its sources (Buniaet al. 2016; Hajigholizadeh & Melesse, 2017; Jatoi et al. 2021; Han et al. 2021; Patra et . 2022; Balla et al. 2022; Sun et al. 2022).

Concerns about the water resources of the Himalayas, both in terms of quality and quantity, have been extensively noted as being essential for economic growth, human well-being, and biological preservation (Khanday et al., 2021; Hoque et al., 2022). The current study focused on characterization and monitoring of drinking water quality parameters and coliform bacteria as indicators of faecal contamination with the hope of improving access to clean drinking water. The information gathered is then put to use in an effort to determine any connections between the contamination, the population, and the elevation. The Vishav

River, a renowned tributary of the Jhelum River in the Kashmir Himalaya, can serve as a baseline for future research (Hussain et al., 2021; Khanday et al., 2021). The huge population constantly depends on this particular river in order to receive food, fish, irrigation, washing, and drinking water. As a result of anthropogenic and natural influences on the stream's health, the economic improvement of the community as a whole and the health of those who live in the places where the river originates and empties are closely tied to the fineness of its water quality. Seasonal variation of microbes, water quality attributes, and molecular identification as a means of determining water pollution in the Veshaw River in the Western Himalayas were evaluated and many statistical methods were used to accumulate anthropogenic pressures and urbanisation in the river. As a result, the area's economy and public health could benefit from more frequent inspections of this important aquatic resource. Because of the resemblance between *E. coli and E. fergusonii*, 16S rRNA identification is critical. A number of factors have led researchers to depend on 16S rRNA gene sequences as the most prevalent housekeeping genetic marker used to explore the phylogeny and taxonomy of bacteria. A number of factors contribute to its widespread use, including the fact that it is present in nearly all bacteria. According to informatics, the 16S rRNA gene is strong enough and is present in multigene families or operons (1,500 bp) (Patel, 2001; Suardana et al.2014; Fadhil et al.2022). Therefore, this study aimed to identify coliform pollution-indicating species using 16S rRNA from water sources (Layton et al. 2006; Kawser et al. 2022).

To enhance the quality of drinking water in the Veshaw River in the Western Himalayas, this study investigated seasonal change in microorganisms, monitoring drinking water quality attributes, and molecular identification of coliform bacterial species. Many statistical methods were used of accumulated anthropogenic and urbanization stress on the river. A number of factors have led researchers to depend on 16S rRNA gene sequences as the most prevalent housekeeping genetic marker used to explore the phylogeny and taxonomy of bacteria for characterizing novel species from rive water Veshaw. Therefore, this study also aimed to identify novel new Coliform pollution indicating species using Metagenomic 16S rRNA from water sources in Veshaw River.

Material And Methods

2.1: Overview of the research area

In the Kashmir Himalaya, the Vishaw River is a prominent continuous tributary that flows left to right into the Jhelum River. This river is known as a backbone for the community deoends on it. This glacier-fed river, which goes by the name "Teri," can be found close to the base of Kousarnag, which is situated at an elevation of approximately 3840 metres above sea level (masl) on the northern side of the 'Pir Panjal Range in the Kashmir Himalayas'. This stream is located between ‘33° 39' to 33° 65' N latitude and 74° 35' to 75o 11' E longitudes’. For example, (Rather et al. 2022b, c) and The Vishav basin has a surface area of 1062.48 square kilometres and a length of 75 kilometres in the main channel (Hamid et al., 2016;). The study region has a moderate climate, with cold, wet winters and warm, dry summers (Romshoo et al., 2018; Guerri et al.2022). The Vishaw River has an increased food peak, which causes it to dump massive flooding into the Jhelum near Sangam down stream (Romshoo et al., 2018). There are numerous human

settlements along and downstream of this stream's route. After meeting the famous Jhelum River in the Kashmir Himalayas, this stream discharged a significant amount of water. Figure 1 shows the locations of the six survey locations that were chosen to represent the upstream, midstream, and downstream reaches of the Vishaw river, where substantial seasonal sampling was conducted.

2.2: Sampling procedure and analysis of water quality chemical biological parameters

Three different locations were used to collect water samples for testing the quality of the ricer waters viz., Site-I and II (Kongwaton and Aharbal an upstream site), Site-III and IV (Nihama and Kulgam a midstream site) and Site V and VI (Khudwani and Sangam, a completely downstream site) mentioned in Figure 1. The locations were chosen depending on the impact of human activity or we can say visual analysis of pollution source like agriculture inputs, horticulture inputs, social geography, commercial and population pressure along banks of river Veshaw s, from these sites. Every two months, during the months of February 2020 and January 2022, a seasonal sampling was carried out and the samples were stored in sterilised bottles. In order to analyse the chemical features of the water samples, one was immediately refrigerated at -4 °C and transferred to the lab; the other, which had been filtered, was stored at - 20 degrees Celsius to identify the microbial species for the Metgenomics sequencing process. In addition to sorting data by season, the results were analysed to identify seasonal fluctuations.

2.3 pH

Acidic substances have a pH below 7, while basic substances have a pH between 7 and 14. The pH of a solution is said to be neutral if the concentrations of H+ and OH– ions are equal. Glass electrodes (e.m.f.) and calomel reference electrodes (reference electrodes) are used to monitor hydrogen ion activity in a solution. pH was measured at room temperature (25 ºC) (AWWA, 1964).The pH meter instructions given by manufacturer have been strictly followed. Then the important aspect to use the pH meter is to calibrate it with suitable buffers has been performed with buffer 4, 7 and 9.2. In a clean dry 100 ml beaker, water sample has been placed in it and the electrode has been put into the sample and the instrument was allowed to stabilize and then the reading has been noted. pH was measured by Systronics pH meter (Model 306) (Rather et al. 2022c).

2.4: Electrical conductivity

Electrolytes in a solution break up into their ions and make the solution conduct electricity. The higher the conductivity of water, the less it resists the flow of electricity. This indicates that there is a greater quantity of salt that has dissolved in the water. The mhos unit was used to measure the electrical conductivity, which is the reciprocal of ohms, or siemens. Electrical conductivity was measured by Systronics conductivity meter (Model 361). The results were expressed in µS/cm. The conductivity was measured at room temperature. A metre and a conductivity probe are used to test conductivity. Electrodes in the water sample probe were connected by a voltage. The conductivity every centimeter is calculated from the voltage drop produced by water resistance. Ohm's law was used to calculate conductivity, which is the inverse of resistance. Measurement data is converted to micro mhos per centimeter and shown on the

screen for user convenience (Jones and Bradshaw, 1933). Conductivity meter has been calibrated before analyzing the samples. After calibration, electrode has been fully sterilized with deionized water and properly wiped with a tissue paper. Water sample (200 ml) was put into a beaker and electrode has been dipped into the sample solution and left undisturbed for a steady reading. Reading in millisiemens on the display has been recorded (Rather et al. 2022c).

2.5: Biochemical oxygen demand

According to Winkler's approach, the BOD (biochemical oxygen demand) was calculated (1988). The procedure involves filling the simple BOD bottle to the capacity and incubating it for five days at the specified temperature. The BOD is calculated by subtracting the initial DO from the final DO, which is measured at the beginning and end of incubation. After dilution, all oxygen uptake is included in the BOD measurement because the initial DO is calculated so quickly. Winkler's modified Iodometric technique was used to identify the initial dissolved oxygen level in the BOD bottle. An additional BOD bottle containing water from the same sampling location was then incubated for 5 days at a temperature of 20 1°C in the BOD incubator. The sample's final concentration of dissolved oxygen was assessed after five days of incubation.BOD was calculated as follows:- [(DOi - DOf) DF;BOD5 = Five-day biochemical oxygen demand; DOi = Initial dissolved oxygen; DOf = Final dissolved oxygen after five days of incubation;DF = Dilution factor]

2.6: Chemical oxygen demand

Chemical oxygen demand (COD) was estimated by using open reflux titrimetric method (APHA, 2005). A sample pre-treated with strong acid solution and known surplus of potassium dichromate ($K_2Cr_2O_7$) is allowed to reflex in a reflux apparatus. It is determined by titration with ferrous ammonium sulphate that the concentration of $K2Cr_2O7$ absorbed over the course of digestion and oxidised organic matter is estimated in terms of excess oxygen equivalents. To 500 mL refluxing flask containing 50 mL of water sample, 1 g $HgSO_4$, and few glass beads were added. Then contents were slowly added with 5 mL sulphuric acid reagent and carefully mixed to dissolve $HgSO_4$. To avoid the loss of volatile ingredients, the flask then allowed to cool while being constantly mixed. 0.004 M $K2Cr_2O_7$ was added to the flask after cooling and re-mixing. 70 mL of sulphuric acid reagent was introduced to the refluxing flask through the open end of the condenser and the contents were refluxed for two hours while being constantly stirred and mixed. Distilled water was added to the digested mixture to dilute it nearly twice its volume. It was chilled and titrated against 0.025 M ferrous ammonium sulphate solution using 2 to 3 drops of ferroin indicator and the end point was taken as a dramatic change in colour from blue-green to reddish brown. Blank was run in similar fashion for all sample tests. COD was calculated by using following formula:

$$\text{COD mg/L} = \frac{(A-B)\times M\times 8000}{\text{mL of sample}}$$

A =mL of FAS used for blank; B =mL of FAS used for sample; M = molarity of FAS (0.025 M)

2.7: Coliform Detection in river water:

It is commonly recognized that the coliform population is composed of the genera *Escherichia, Klebsiella, Enterobacter*, and *Citrobacter* (Clark and Pagel, 1977). Counting these microbes in the water's microbial ecosystem is a common way to check the cleanliness of water/pollution load. It's been questioned if the total coliform count is an accurate indicator of bacterial water pollution because the detection methods for coliforms can be influenced by other factors (Geldreich et al., 1978: Hutchison et al., 1943; Rather et al. 2022d).

2.8: Coliform enumeration

The total Coliform species (*E. coli*) were isolated from the river water by serial dilution plate method Seeley and Vandemark (1981) using modified ECD Coliform Agar medium containing Agar 15 g/L, potassium dihydrogen phosphate 5 g/L, bile salt 1.5 g/L, tryptone 20 g/L, disodium hydrogen phosphate 5 g/L, sodium chloride 5 g/L and yeast extract 5 g/L with final Ph 7.2±0.2 at 25^{o}C. After performing successive dilutions of the water samples all the way up to 106, 0.1 ml of the diluted soil suspension was plated on ECD Coliform Agar media plates. The results were examined. After three to four days of incubation at a temperature of 28.2 degrees Celsius in a biological oxygen demand (BOD) incubator, the plates were read. Using a colony counter, we determined the total number of colonies that had developed after 3–4 days of incubation (Rather et al 2022d; Rather et al 2022e).

2.9: Sampling procedure and analysis of water for Metgenomics molecular identification

The samples were sent to the Triyat Scientific Co. (Biotechnology company in Nagpur Mahrastra India. for molecular identification of Coliform pollution indicating bacteria

2.10: DNA extraction and 16S metagenomic analysis

In two different experiments, we isolated genomic DNA from water samples from each site as follows: A 0.2 lm filter was used to filter a litre of water (Merck Millipore, Darm- stadt, Germany). Eppendorf tubes with 2 ml of filter paper were then used to cut the paper into smaller pieces. The MagMax Microbiome Ultra Nucleic Acid Isolation Kit (Applied Biosystems, USA) was used to extract DNA from the filter paper, following the manufacturer's instructions. Using Intelli-Mixer'sTM (ELMI, Riga, Latvia), 800 mL of the lysis buffer was poured to the filter paper and mixed for 30 minutes at room temperature before centrifugation at 14000 x g for 2 minutes. Binding beads solution made with kit chemicals was used to purify DNA from supernatant, followed by washing steps with washing buffer and 80% alcohol. Using the elution buffer, we heated the binding beads for 5 minutes at 75°C before pelleting them against the magnet rack. The clear solution we obtained was used to extract the DNA. Qubit 4.0. fluorometer and Qubit dsDNA HS Assay kit were used to determine the DNA concentration (Thermo Fisher Scientific, Waltham, MA, USA). 16S rDNA sequencing and metagenomic analysis were performed on 40 ng of DNA (Shin et al. 2017; Padder et al.2021;Padder et al 2022)

2.11: PCR amplification

A PCR amplification of the V34 region of the 16S rRNA gene was performed using a two-step procedure (Matar et al.1999) The first thing that was done to amplify the sequence that was particular to the locus, which was x460 base pairs long. The universal primers V13F (5' AGAGTTTGATGMTGGCTCAG3') and V13R (5' TTACCGCGGCMGCSGGCAC3') were used to do this, as described by Klindworth et al (2013). The following adaptors and indices were used for Illumina sequencing during the second step:-

a) DNA QC

Before PCR amplification, the DNA extracted from the samples was submitted to Nano Drop and GEL Check. In order to identify the DNA's quality, the Nano Drop measurements at a value of 1.8 to 2 are used (Esfandani et al. 2019)

b) PCR Ampliqon QC

Purification and quality control (GEL Check and Nanodrop QC) are performed on the amplified 16s PCR Product. In order to determine the DNA's quality, the Nano Drop measurements at a value of 1.8 to 2 are used (Sarkar et al.2022)

c) Overview of Sequencing Protocol

An additional eight cycles of PCR using Illumina barcoded adapters were done to prepare the sequencing libraries after the amplicons from each sample were purified using Ampure beads to eliminate unused primers. Ampure beads were used to purify the libraries and the Qubit dsDNA High Sensitivity assay kit was used to quantify the libraries. With either the Illumina Miseq 2x300PE or ITS sequencing kit, we sequenced our data (Sanschagrin et al. 2014; Richardson et al. 2015)

d) NGS sequencing

We used an Illumina MiSeq system, which is capable of high throughput profiling at low cost and supports reads of up to 2x300 bp. Used the Titary assembler version 13.2 (Janin et al. 2020; Gaio et al.2021)

Statistical Procedures

The mean values of chemical parameters and Coliform were presented and analysed using descriptive analysis. Using the four distinct seasons of the year, we were able to categorise the information into 4 seasons: spring, summer, autumn, and winter (December, January, and February). Correlation matrix through XL-stat after two-way ANOVA SPSS statistic version 23 was used to collect the data. ANOVA was used to examine the differences between all of the data points (ANOVA).The other analyses like Heatmap, Phylogeny tree, etc were done using Microbiomeanalyst (online tool:

https://www.microbiomeanalyst.ca/). For Metabolic pathway visualization another online tool name Metageneassist is used (http://www.metagenassist.ca/).

Results And Discussion

The river water samples were taken at six different locations along the river Veshaw, study sites include Kongwattan and Aharbal (upstream), Nihama and Kulgam (middle stream), Khudwani and Sangam (down stream during the all season from February to January 2020-2022 in order to do a quantitative assessment of the Total Coliform (TC) load in the River Veshaw flowing the TC values in the river water sample ranged from 0.00 - 72.600 (CFU 10^{x6}) during all the four seasons. The faecal contamination of drinking water was the primary emphasis of this research. *E. coli* was used as an indirect way to discover pollution by faecal matter, which is associated with severe hazards to one's health (WHO 2008; Pantha et al.2021; Kongprajug et al. 2021). The bacterial research's findings revealed highest Coliform count was recorded at Sangam (72.600 CFU 10^{x6}) followed by Khudwani (47.567 CFU 10^{x6}) down stream of the river during summer and in spring season highest Coliform count was also found at Sangam (61.133 CFU 10^{x6}) and Khudwani (39.567 CFU 10^{x6}) [table1 and figure2] showed significant *E. coli* and coliform contamination in down stream of the river Veshaw . Related research has been done by Sharma and his colleagues (2010) and Bhat and his colleagues (2015), as well as Dimri and Bhat et al. (2021) who focused on surface water (rivers and lakes) in the SNP. It was determined that E. coli and coliform bacteria were present in every sample of surface water examined, with contamination rising dramatically as altitude decreased. This is in line with the findings of other researchers who found that contamination levels in the Ganges River decreased as one descended from the higher elevations of the Gangotri Glacier (Uttarakhand, India) to the lower altitudes of the Ganges River (Americo-Pinheiro et al.2021; Selvam et al.2022). The findings of Baghel et al. (2005), Sharma et al. (2010), and Bowes et al. (2020) imply that the rise in bacterial contamination in their study regions' lower altitudes was caused by an increase in anthropogenic and socio-cultural activity. Total coliform and E. coli were also found to be at their highest in the summer months, which researchers linked to an rise in the number of pilgrims and hikers in the area. A preliminary study by Nicholson et al. (2016) supports the concept that water pollution is highest at lower altitudes and may be linked to the tourist and/or resident populations there. Our findings support those of Baghel et al. 2005; Sharma et al. 2010;Nicholson et al. 2016) relived the correlation between bacterial counts and additional measures of water quality with altitude in the river from upstream to down stream. Bacterial contamination has a direct correlation with water quality (Figure 7).This indicates the pollution level in the river increase with decrease in altitude and there is positive correlation between coliform and the chemical parameters of the river water (figure 7). Reports of large influences on Nakdong River water quality by Cho and Um, 2005, were found in the upstream to midstream section of the Nakdong River. The river water quality may be harmed by excessive pollution amounts from discharges, according to a number of researches (Jung et al. 2016; Oh et al. 2017). Polluted tributaries to the Nakdong River have been linked to an increase in coliform bacteria, according to Lee et al. (2016). The water quality of the mid- and downstream part of the Geumho River, where the Nongong station is located, may be harmed by secondary tributaries that reach the river from severely contaminated sources

(Yu et al.2015; Na et al.2016; Elassass et al.2022). While faecal coliform levels were low in the upstream stations, they steadily rose as the study moved downriver (Table 2 and Figure 1). Its hilly upstream region is home to the Veshaw River, which is also home to pollution sources such as agricultural land and animal sheds as well as industries like as agriculture and tourism. The river's midstream and downstream regions are also home to important tributary inflows. High coliform concentrations at downstream sites could be the result of such a complicated interaction between point and non-point pollution sourcesThroughout the months of spring and summer, both total and faecal coliforms had significant population densities and broad distribution ranges (Figure 2 and Table S1). Similarly, the frequency status of total coliform bacterial species like absent, present and rare, common, dominant and abundant are mentioned in Table 1 This may have been a contributing cause due to the impact of rainfall, non-point pollution sources, and rising water temperatures over the summer. According to the findings of this study, coliform bacteria populations in the Nakdong River were reported to be high throughout the summer and fall due to rainfall in the middle to downstream part of the river. This finding is similar with the findings of the previous study (Ramteke et al.1992; Baek et al. 2014; Seo et al.2019; Bisimwa et al. 2022).

Table 1: Seasonal variation in the presence of Coliform populations at selected sites of River Veshaw

Season / Site	Up stream		Middle stream		Down stream	
	Kongwaton	Aharbal	Nihama	Kulgam	Khudwani	Sangam
Spring	+	++	++	+++	++++	++++
Summer	+	+	++	+++	++++	++++
Autumn	+	+	++	++	+++	++++
Winter	-	+	+	++	++	+++

*Frequency status: (-, 0%) Absent; (+, < 20%) Present and Rare; (++, >20-50%) Common; (+++, >50%) Dominant; (++++, 100%) Abundant

From upstream to downstream of the river Veshaw there were important differences (p 0.05) in the following parameters: TC, pH, EC, BOB, and COD. Water from Sangam downstream had the highest pH, EC, BOB, and COD readings during the summer (Figure 3-6). In order to survive, aquatic fauna required a pH of 7.4, although photosynthesis and respiration are the primary metabolic pathways that influence pH in aquatic habitats (Hamid et al., 2020). As shown in Figure 3 and Table S2, there was a significant difference (p 0.05) in the alkaline pH of the Vishav stream across the sampling sites, with the highest pH (8.947) discovered in summer at Sangam (downstream) and the lowest pH (6.847) found in winter at Kongwattan (upstream). While testing the pH of the Doodhganga stream in the Kashmir valley, the same kind of supportive evidence were found (Dar et al., 2020). As the temperature rises, the pH likewise rises, which can be linked to a change in the process of photosynthesis, which is also affected by the rise in pH (mean value of 7.90.3). The high concentration of buffering chemicals (calcium and magnesium carbonates) in the riverside area and downstream can be blamed for the study's overall alkaline pH.

(Kang et al., 2001). Analyses of the pH of the Doodhganga stream in the Kashmir Valley produced comparable supporting evidence (Husaini, 2020). Also, the pH indicated a significant difference between seasons (p0.05), and the higheer value (mean value of 7.90.3) during summer and autumn can be linked to the rising temperature, which also governs the photosynthetic activity. Carbonates of calcium and magnesium, which contribute to the high alkaline pH, are found in the riverside area downstream due to a significant quantity of human activity (Kilonzo et al.2014;Indu et al., 2015; Adesakin et al. 2020; Chakraborty et al. 2022).

Temperature variations have a significant impact on electrical conductivity (EC), which can rise by 2 to 3 percent for every 1 degree Celsius increase (Yilmaz & Koc, 2014). Water's electrical conductivity (Rana et al. 2016) is directly related to the amount of total dissolved solid in the water (sodium, potassium, calcium, carbonate, bicarbonate, chloride, and sulphate). While it has long been believed that water flowing through granite bedrock has less of an EC than water flowing through clay soils, this theory has recently been challenged (Gupta & Paul, 2013). They argue that this is because granite is composed of inert material, which does not conduct electricity when washed away by water (Gupta & Paul, 2013). (Bhateria & Jain, 2016; Denise et al. 2022;). During the present study, average mean EC varied significantly, from (71.620 -253.680dS/m), highest EC (253.680dS/m) was recorded during summer at Sangam (down stream) of the river and lowest EC (71.620 dS/m) was recorded at Kongwattan (up stream) during winter season showed in (figure 4 & Table S3) all of which may be the result of agricultural runoff and/or enhanced bank erosion during food preparation in the downstream regions, a rise in total dissolved solids, and an increase in temperature, The Doodhganga stream in the Kashmir Himalaya has also shown a close similarity in the spatial variance of EC (Husaini et al., 2020;Dar et al. 2020). An increase in water conductivity due to temperature fluctuations can be attributed to the winter low EC (mean of 80.622.9 s cm-1) and the summer high (mean of 176.876.4 s cm-1) reported during the seasons of winter and summer, respectively. Also in Kashmir Himalaya's Jhelum basin, summer and winter seasons show the same trend (Indu et al.2015; Khanday et al., 2021).

Water from Sangam (downstream) had the highest BOD (4.963 mg/l) during summer seasion and the lowest BOD (1.120 mg/l) was recordrd during winter at Kongwattan (upstream) figure 5 & Table S4. Organic wastes must be oxidised aerobically in order for bacteria to meet their biochemical oxygen demand (BOD) (Masters and Ela, 2014). The domestic sewage and wastewater from industries are laden with a variety of biodegradable organic pollutants. When this waste is discharged into the water body, it is readily decomposed by the microorganisms into the simpler organic and inorganic compounds. This decomposition process consumes a lot of dissolved oxygen, thereby decreasing the DO level in the water body upto a critical minimum level (hypoxia) which ultimately disrupts the biota and causes the death of fish (Zaghloul et al. 2019; Mudau, 2021). Therefore, BOD is considered as an important tool in the determination of the extent of dissolved oxygen required for the stabilization of organic waste from domestic and industrial settings. High BOD value reveals the higher organic pollution load in the stream (Lkr et al. 2020; Lkr et al. 2022; Krishan et al. 2022). In the presence of ample oxygen, the end product of the microbial decomposition of the organic waste is non-objectionable substances like carbon dioxide, sulphate, phosphate and nitrate. Whereas, under anaerobic conditions, potentially objectionable and

odorous substances such as hydrogen sulphide, ammonia and methane are generated (Watson and Juttner , 2017). We observed a significant increase ($p < 0.05$) in the mean BOD values of River Veshaw stream from least polluted upstream sites to more polluted downstream sites during all the seasons. For each of the four seasons studied, BOD exhibited significant differences (p 0.05). The highest mean BOD was observed during the summer season when DO was less which was followed by summer and the least mean BOD was observed during the winter when the oxygen level was fairly high as compared to spring. The BOD values in the present study showed a positive correlation with the pH and TC. It is pertinent to mention here that the mean BOD values of the Veshaw stream were most of the time < 2 mgL^{-1} in the upper reaches revealing oligosaprobic nature (unpolluted to slightly polluted) of the stream (Taylor et al. 2007; Fernandez et al. 2018; Yorulmaz et al. 2021). The mean BOD in the middle and lower reaches were however > 2 mgL^{-1} and < 4 mgL^{-1} during spring and autumn seasons which shows the *β*-mesosaprobic nature (moderately polluted) of the Veshaw River downstream in these seasons. In the summer season, the mean values of BOD were > 4 mgL^{-1} revealing *β*-*α*-mesoosaprobic nature (critical levels of pollution) in the lower reaches of the stream (Taylor *et al.*, 2007; Fernandez et al. 2018; Yorulmaz et al. 2021). The highest mean BOD during the summer season is related to increased biological activities at higher temperatures and low dissolved oxygen available in this season. The minimum BOD during the winter season is related to the ample availability of oxygen due to high phytoplanktonic photosynthesis in less discharge and slow flow. A decrease in water temperature also increases the solubility of dissolved oxygen in water resulting in low BOD. Our findings are in agreement with Venkatesharaju et al. 2010; Huang et al. 2022; Zhang et al. 2022; George et al.2022) who also reported the high DO and less BOD as an outcome of plentiful phytoplanktonic growth in lean water flow resulting in high photosynthesis. The gradual increase in BOD concentration from upstream to downstream indicates that the BOD is most significantly contributed by the discharge of sewage, agricultural runoff and organic wastes from the catchment area into the stream (Indu et al. 2015;Hamil et al. 2018; Lkr et al. 2020) reported a similar trend of BOD from upstream to a downstream gradient of the Gravatai river. They observed the increased BOD with the increase in the organic contamination gradient. Further, they found high BOD values and severe eutrophication in the spring and summer and related it to the prevalence of the high temperature in these seasons.

The average mean COD varied significantly, from (24.637 to 51.440 mg/l) maximum COD 51.440 mg/l was recorded at Sangam downstream during summer season and the minimum COD 24.637 mg/l was found at Kongwattan upstream during winter stream figure 6 & Table S5. In order to convert all physiologically accessible and inert organic matter into carbon dioxide and water, a metric known as the chemical oxygen demand (COD) must be used (Kaur and Kaur, 2015; Azer et al. 2022; Cai et al. 2022).Some fraction of the organic matter such as cellulose, phenols, benzene resists biodegradation and others such as pesticides and industrial pollutants are toxic to microorganisms and thus not degraded easily. COD is a measurable quantity in which whole organic matter (biodegradable plus inert) gets oxidized chemically and independently without relying on the type of substance or microorganism present. Therefore, COD gives quicker and higher estimates of organic waste and its values are always higher than BOD (Watson and Juttner , 2017). COD test is frequently employed in combination with the

BOD test for the quantification of the total of non-biodegradable organic material in a water body (Elsheikh et al. 2012; Choi et al. 2022). In the Veshaw stream, like BOD, mean COD values also exhibited significant ($p < 0.05$) downstream increase with the highest values observed at Sangam Downstreamin all the seasons. The COD values increased with the increase in temperature and its highest mean value was recorded during the summer season. The higher value of COD downstream is attributed to the increasing anthropogenic activities such as discharge of the agricultural waste, domestic waste and untreated municipal sewage into the stream and is an indication of high pollution from various organic wastes. Our results are in agreement with (Indu et al. 2015; Hamil et al. 2018) who reported a similar spatial variation in the COD as a result of anthropogenic activities in and around Ghrib Dam, Algeria. The high COD in summer may be related to increased water temperature coupled with low discharge resulting in the high rate

Figure 7 depicts the results of an investigation into the relationship between coliform bacteria and various aspects of water quality. A positive correlation has been found between pH, EC, BOD, and COD, as well as between faecal coliforms and total coliforms. This is the degree of association among the following water quality factors: In this order, TC, pH, BOD, and COD. It's shown here in Figure 6. Positive relationships were found between BOD, pH, EC, and COD, as well as moderate correlations between pH and COD. In addition to coliform bacteria, total coliforms had high correlation values of more than 0.5. Rainfall's impact on coliform concentration was discussed by Seo et al.2019. There is a significant association between rainfall and coliform bacteria abundance in rivers due to increased suspended particles, agricultural wastes, and phosphorus that has a strong potential to adsorb. Point pollution sources located along the Veshaw River had a favourable impact on the link between COD and BOD and coliform bacteria in terms of organic matter. A prior investigation of the Nakdong River and its major tributaries proved successful that resembled those of the present study, therefore these findings appeared to be reliable (Hong et al. 2015;Jung et al. 2016 ;Seo et al.2019). A rise in nitrite nitrogen was shown to be inversely proportional to coliform concentration in Beck and Sohn (2006), as well. The correlations between pH and coliforms in the river's mid-and downstream stations could be explained by a combination of the previously mentioned factors. Other research have identified favourable correlations between organic matter and coliform bacteria, but this was not the case with BOD (Beck and Sohn 2006;Kim et al. 2006; Mamun et al. 2022). According to Cho and Song, 2008, in a river system, coliform bacteria dropped significantly after an average of three days. Rather than upstream stations, it was thought that such natural die-offs (Van der et al.2000; Im and Mostaghimi,2004; Saraswat et al.2022) were responsible for the negative connection. In the Nakdong River's main stem, Lee et al. 2016 found a weak negative connection between BOD and coliform bacteria. Eutrophication and the consequent spread of Chl-a, an indication of algal blooms, could result from the influx of contaminants and slow flow velocity downstream. Increased production of Chl-a as self-replicating organic matter may limit the growth of coliform bacteria. Ahmad et al. (2014) and Seo et al. (2019) looked on the removal of coliform bacteria by a freshwater algae species (see references). It's possible that rainfall or the discharges of nearby streams contribute to the EC's positive association with coliform bacteria. There are numerous tributaries flowing into the Veshaw River's middle and lower regions. Kim et al. 2007; Mamun et al. 2022)

found negative associations between pollutants and precipitation, as well as between contaminants and discharges. Electrolyte loss and the EC drop that follows could be the result of pollutant dilution. Correlations between the presence of coliform bacteria and EC were shown to be negative (Yun et al. 2017).

Outcomes of Regression Analysis Total coliform level for all locations was impacted by BOD, COD, EC and pH. Total coliforms showed proportionate correlations with pH, EC, BOD and COD. The relative influence of the factors ranks as follows: precipitation pH >EC>BOD >COD As for faecal coliforms, EC was included along with the abovementioned five water quality parameters, and these factors accounted up to the concentration of faecal coliforms (figure 7 and 8). The regression statistics of the physicochemical data set of the Vishav stream is summarised in Figure8. The connection with water quality variables was equal to that of the total coliforms. EC exhibited similar proportionate association with faecal coliforms figure 7 and 8. The relative influence of the components evaluated as follows: pH >BOD >COD>EC. In general, organic matter had a dominant impact on total coliforms, whereas pH and EC and BOD have this effect on faecal coliforms. BOD was the most influencing factor for the proportion of both types of coliforms. The regression findings using all station data indicated the significant water quality characteristics at each location. At each site, the water quality parameters affecting the level of coliforms were BOD, COD, EC and pH (figure 8). However, the water quality parameters could be defined at the up and midstream locations and the mid- and downstream locations beneath Vehaw River, depending on dominating patterns. (Hong et al. 2015;Jung et al. 2016;Seo et al. 2019) similarly spatially categorised the Nakdong River as the up- and midstream and mid- and downstream portions at a non-weir station between Dasa (Gangjeong-Goryeong weir) and Nongong (Dalseong weir) locations using cluster analysis.

A strong tool in environmental studies, Metgenomics analysis can be used to determine the microbial diversity at a specific area and may be useful for understanding how microbial-communities communicate and interact (Yoo, et al. 2017; Chen et al. 2022). Table 2 and figure 9 provided a summary of the sequence results. In National Center for Biotechnology Information (NCBI) gene bank result showed that total of 27 accession numbers were developed from NCBI gene bank. The Coliform bacterial species identified with accession numbers include *Escherichia coli* strain Rather Veshaw61 (accession number ON197775), *Escherichia coli* strain Rather Veshaw68 (accession number ON227007), *Escherichia coli* strain Rather Veshaw69 accession number (ON227008), *Escherichia coli* strain Rather Veshaw70 accession number (ON227009), *Escherichia coli* strain Rather Veshaw71 accession number (ON227010), *Escherichia coli* strain Rather Veshaw72 (accession number ON227011), *Escherichia coli* strain Rather Veshaw73(accession number ON227012), *Escherichia coli* strain Rather Veshaw74 (accession number ON227013), *Escherichia coli* strain Rather Veshaw75 accession number (ON227014), *Escherichia coli* strain Rather Veshaw76 accession number (ON227015), *Escherichia coli* strain Rather Veshaw77 accession number (ON227016), *Escherichia coli* strain Rather Veshaw78 accession number (ON227017), *Escherichia coli* strain Rather Veshaw79 accession number (ON227018), *Escherichia coli* strain Rather Veshaw80 accession number (ON227019), *Escherichia fergusonii* strain Rather Veshaw1 accession number (ON614213), *Escherichia fergusonii* strain Rather Veshaw2 accession

number (ON622781), *Escherichia fergusonii* strain Rather Veshaw3 accession number (ON622786), *Escherichia fergusonii* strain Rather Veshaw4 accession number (ON622784), *Escherichia fergusonii* strain Rather Veshaw5 accession number (ON622780), *Escherichia fergusonii* strain Rather Veshaw6 accession number (ON622785), *Escherichia fergusonii* strain Rather Veshaw7 accession number (ON622783), *Escherichia fergusonii* strain Rather Veshaw8 accession number (ON622782), *Escherichia albertii* strain Rather Veshaw9 accession number (ON622787), *Klebsiella grimontii* strain Rather Veshaw10 accession number (ON631203), *Klebsiella grimontii* strain Rather Veshaw11 accession number (ON631204), *Klebsiella grimontii* strain Rather Veshaw12 accession number (ON631205) and *Shigella dysenteriae* strain Rather Veshaw13 accession number (ON631212) mentioned in (Table 2 & Fiure S1).

Figure 9 showed the results of a Multiple Sequence Analysis (MSA) using MEGA 11 to determine the evolutionary relationships among the 27 Coliform genomes that have so far been identified for phylogenetic analysis. Biological connections were established using the MSA. *Escherichia coli* species found in both cluster 1 and 2 with the highest bootstrap values in clusters 1 and 2 of the three clusters studied. Cluster 3 contains *Escherichia fergusonii* , *Escherichia alberti* , *Klebsiella grimontiand, Shigella dysenteriae* species that have a close relationship with each other (Figure 2). These total 27 coliform species of bacteria are all genetically connected to each other, as depicted in Figure 9 by their phylogenetic tree. Phenotypic traits have been traditionally used to identify bacteria, but genotypic characteristics, which are more recently discovered, are more accurate and dependable. The ribosomal RNA sequence based analysis is an implicit and special way for understanding microbial diversity within and across a group and also for identifying novel strains of micro-organism (Magray *et al*., 2011; Matsuo *et al*., 2021). The 16S rRNA gene, which has highly conserved and hyper variable sections and is used to identify novel strains of bacteria, is present in all bacterial species. There has been an increase in use of genetic techniques that use comparisons of bacteria's 16SrRNA gene sequences to known bacteria in databases. Many studies have relied on the 16S rRNA gene sequence for inferring evolutionary relationships among bacteria (Sujatha *et al*., 2012; Dueholm *et al*., 2022). As a result of our analysis, we were able to identify the species with certainty. This revealed that all the 27 coliform species were of different genus. While 14 bacterial species were identified as *E. coli*, eight were identified as *E. fergusonii* as new emerging water polluting indictors. Besides this three species of *Klebsiella grimontii,* one species of each *Escherichia albertii* and species of *Shigella dysenteriae* were identified in the water as pollution indictors and new reported bacterial species in Veshaw River of Kashmir Himalaya figure 2. Another investigation by Wragg *et al*., in 2009 and Ori *et al*., in 2019 found that *E. fergusonii* and *Escherichia albertii* could not be distinguished from *E. coli*. It has been shown that both the morphological and genotypic characteristics of *E. fergusonii* and *Escherichia albertii* Coliform bacterial species are similar to those found in the pathogenic strains of *E. coli*, as well as the evidence that these strains may be opportunistic pathogens. Using molecular tools, researchers from Walk *et al*., 2009, Olowe *et al*., 2017, and Lin *et al*., 2022 were able to distinguish and identify closely related bacterial species belonging to the same genus that had previously been considered cryptic lineages of the genus *Escherichia.*

Pseudomonadota the most common phylum in the six areas examined, with varying levels of diversity. Because of its great ability to perform biogeochemical activities in high-nutrient environments, the microbial community. Pseudomonadota, commonly known as coliforms, is well-adapted to high-nutrient environments where it may proliferate quickly (Krishna et al., 2020; Senevirathna et al. 2022). Similar research has been performed by (Alotaibi et al. 2022). (Nunez Salazar et al., 2020) that Proteobacteria was found to be the most abundant phylum in all of their research , with the maximum abundance in the area where human intervention was discovered, according to a recent metagenomic study. Proteobacteria belonging to the Alpha/B eta/Gamma-proteobacteria family like high pH conditions. . Another study found that the presence of such a phylum was significantly higher in areas with low salinity (Rath et al. 2019). There may be valid reasons for the higher abundance of Pseudomonadota, such as decreased conductivity and elevated pH (Brad et al. 2022). According to a study by Siles and Margesin (2016), alpine forest soils with high microbial diversity had lower EC values than soils with low microbial diversity. However, this phylum was shown to be the primary taxon in the water that had been contaminated by humans (Yadav and Sharma, 2019; Dubey et al., 2019). Also, in Veshaw River water samples, Pseudomonadota was the dominant phylum; however, its decreased abundance in upstream may be linked to lower anthropogenic contamination/lower pollution levels, as was found to be the case with the continuous discharge of human waste, agricultural inputs, and so on.

Table 2: Molecular identification of pollution indicating bacteria from water samples in Veshaw River based on their 16s rDNA sequences deposited in NCBI, USA.

S.No.	Strain	NCBI gene bank accession number	Gene size amplified (bp)
01	*Escherichia coli* strain Rather Veshaw61	ON197775	300
02	*Escherichia coli* strain Rather Veshaw68	ON227007	301
03	*Escherichia coli* strain Rather Veshaw69	ON227008	317
04	*Escherichia coli* strain Rather Veshaw70	ON227009	300
05	*Escherichia* coli strain Rather Veshaw71	ON227010	312
06	*Escherichia coli* strain Rather Veshaw72	ON227011	304
07	*Escherichia coli* strain Rather Veshaw73	ON227012	316
08	*Escherichia coli* strain Rather Veshaw74	ON227013	308
09	*Escherichia coli* strain Rather Veshaw75	ON227014	309
10	*Escherichia coli* strain Rather Veshaw76	ON227015	302
11	*Escherichia coli* strain Rather Veshaw77	ON227016	303
12	*Escherichia coli* strain Rather Veshaw78	ON227017	300
13	*Escherichia coli* strain Rather Veshaw79	ON227018	318
14	*Escherichia coli* strain Rather Veshaw80	ON227019	318
15	*Escherichia fergusonii* strain Rather Veshaw1	ON614213	772
16	*Escherichia fergusonii* strain Rather Veshaw2	ON622781	773
17	*Escherichia fergusonii* strain Rather Veshaw3	ON622786	773
18	*Escherichia fergusonii* strain Rather Veshaw4	ON622784	780
19	*Escherichia fergusonii* strain Rather	ON622780	792

	Veshaw5		
20	*Escherichia fergusonii* strain Rather Veshaw6	ON622785	867
21	*Escherichia fergusonii* strain Rather Veshaw7	ON622783	764
22	*Escherichia fergusonii* strain Rather Veshaw8	ON622782	764
23	*Escherichia albertii* strain Rather Veshaw9	ON622787	1502
24	*Klebsiella grimontii* strain Rather Veshaw10	ON631203	301
25	*Klebsiella grimontii* strain Rather Veshaw11	ON631204	298
26	*Klebsiella grimontii* strain Rather Veshaw12	ON631205	300
27	*Shigella dysenteriae* strain Rather Veshaw13	ON631212	301

Conclusion

This study assessed the dynamics of the Vishaw physicochemical properties using multivariate statistical approaches to monitor its ecological status. The water quality metrics demonstrated indirectly affected, most notably in nutrient content and microbial contamination (also known as pollution load), both of which risen downstream in comparison to midstream and upstream locations as a result of both point and non-point waste sources as well as anthropogenic stress. The quality of the water in the Veshaw stream is determined by its temperature, pH, electrical conductivity, biological oxygen demand, and chemical oxygen demand. Veshaw rivers total Coliform and physicochemical analyses was predominantly affected by both organic and inorganic contaminants resulting from anthropogenic factors, especially downstream. Proper supervision will be needed to protect and manage this biological asset, despite the stream's favourable water quality criteria for freshwater and fish life. The latest study will help policymakers assess the alterations that will occur in the water quality in the future consequences on this River ecology. This awareness will reveal disturbing variables that could affect this stream's ecological stability.

- Water quality analysis shows oligsaprobic (least pollution load) nature in upstream while as β-mesosaprobic (moderate pollution load) to α-β-mesosaprobic nature (critical pollution load) of water in the middle and downstream reaches.
- Spatial and seasonal variation in the water quality parameters is regulated by constant inputs of pollutants from the natural and anthropogenic sources in this stream.

- Coliform (*E.coli*) and new emerging species like *Escherichia fergusonii*, *Escherichia albertii*, *Klebsiella grimontii* and *Shigella dysenteriae* distribution pattern be used to monitor the pollution load of the River. Increase in Coliform load in downstream is the indicator of increased availability of nutrients.

Bacterial species can be utilised to monitor the stream's heavy metal, inorganic, and organic pollution. This research explains microbial activity and interaction in a certain environment. Microbiologists are concerned about pathogens with new genetic resources. This metagenomics inquiry will address knowledge gaps in the microbial profile structure of the influent and effluent environment, as well as projected species interactions based on the environment itself. This national-scale investigation successfully revealed influent and effluent water profiles of microbial composition, ARGs, and ARG–species relationships. All influent and effluent wastewater samples have comparable taxonomic composition profiles, indicating an efficient filtration and digesting mechanism. The research met health and environmental protection requirements, Thus, municipal WWTP sanitations have successfully prevented health hazards, most notably those related to water-borne bacterial infections, as well as environmental challenges related to pathogen-contaminated effluent and influent water.

Declarations

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Authorship contribution statement

Rauoof Ahmad Rather provided the conceptualization and Methodology, Visualization and Investigation by N.M. Mubarak, Shoukat Ara; Software Shahid Ahmad Padder, Validation and framed the manuscript by Rauoof Ahmad Rather, Shahid Ahmad Padder, N.M. Mubarak, S. Ara, S.A. Mir, F. A. Lone, Tawseef Rehman Baba, Sanjeev Sharma; Rauoof Ahmad Rather, N.M. Mubarak, Shahid Ahmad Padder, performed the analysis and formatting; Rauoof Ahmad Rather, S. Ara, Tawseef R Baba, Shahid Ahmad Padder helped data collection data analysis; Sanjeev Sharma, N.M. Mubarak helped in the initial draft of the manuscript text and revised and commented on the manuscript critically.

Consent to Participate

Not applicable

Consent to Publish

Not applicable

Availability of data and materials

Not applicable.

Competing Interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Figures

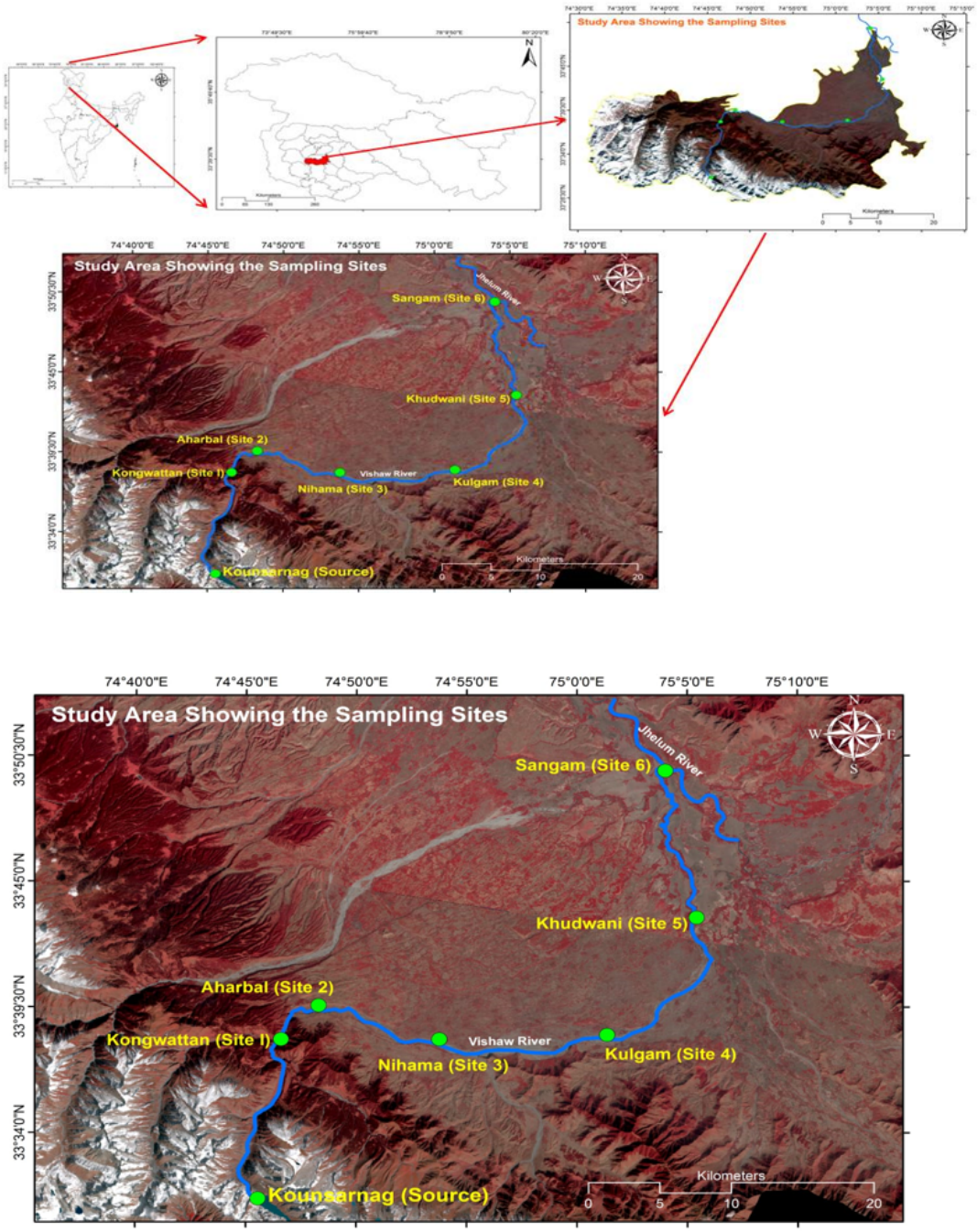


Figure 1

A map showing the area under study and the locations of the sampling sites

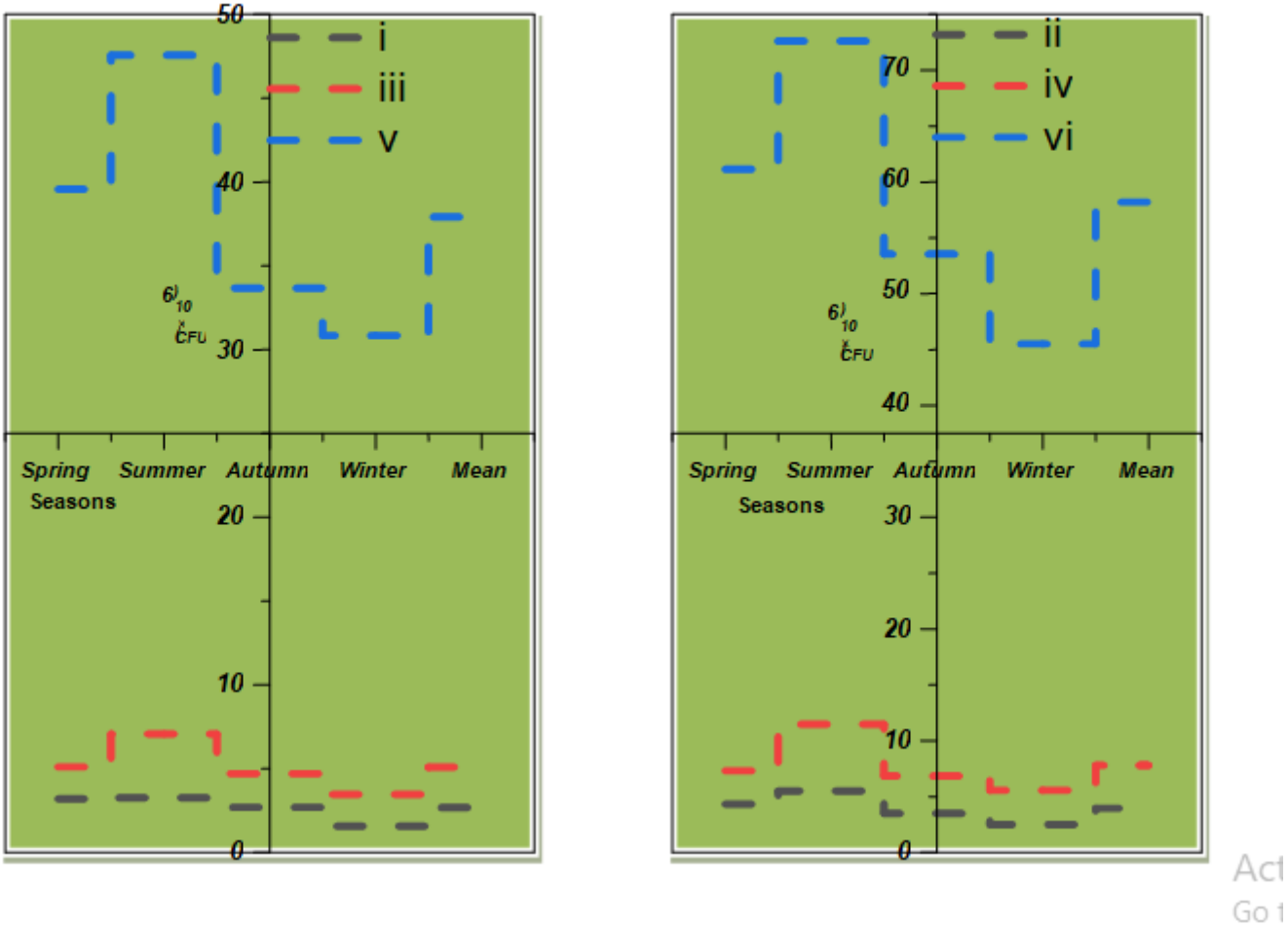


Figure 2

Figure depicting the comparative time-series seasonal variation analysis of Total Coliform (TC) in the water of the River Veshaw at six different sites (I:Kongwattan;II:Aharbal;III:Nihama;IV:Kulgam;V:Khudwani;VI:Sangam) collected during all the seasons from Spring to Winter. Each symbol represents the mean of triplicate values

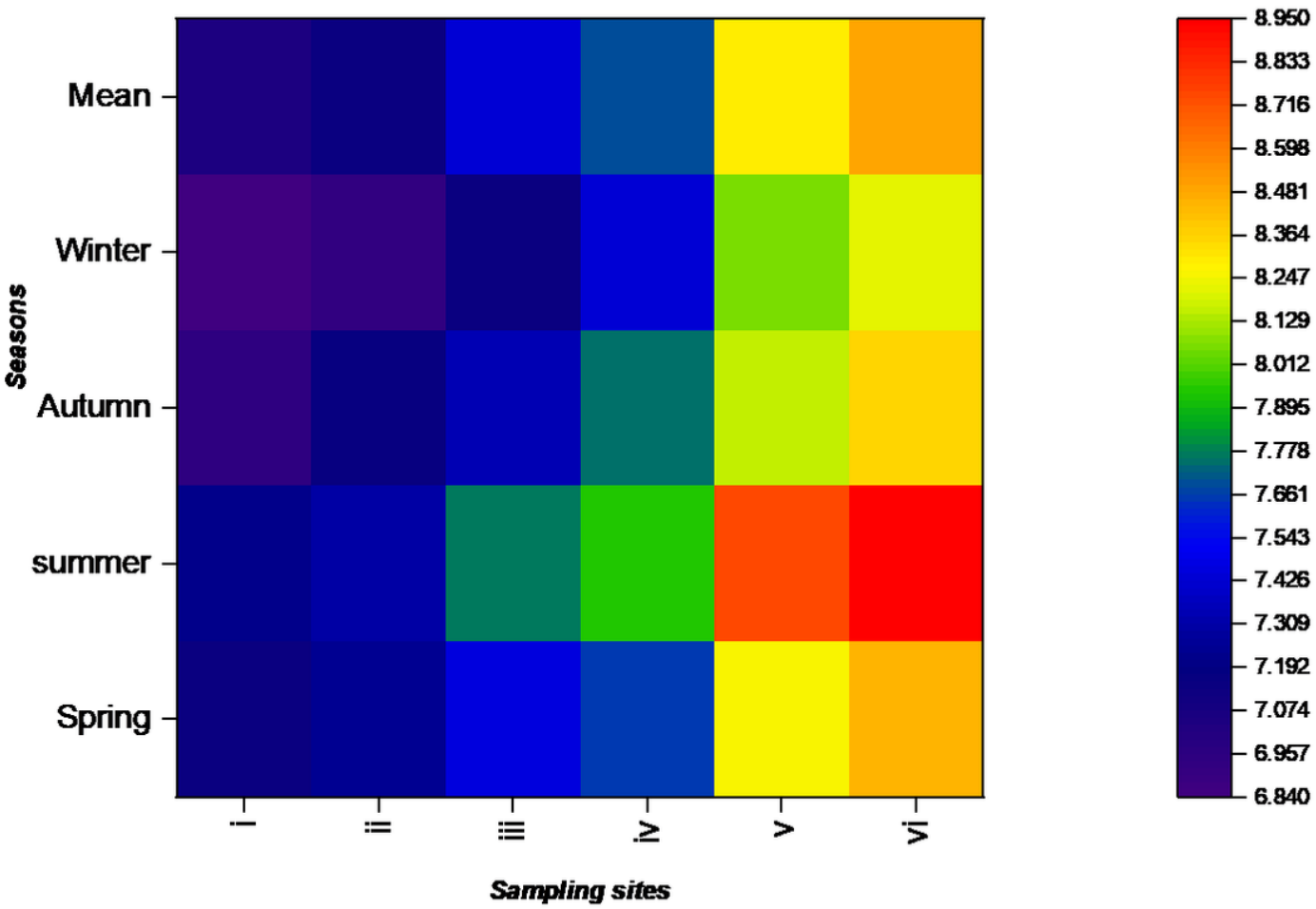


Figure 3

Figure depicting the comparative time-series seasonal variation analysis of (pH)in the water of the River Veshaw at six different sites
whichinclude(I:Kongwattan;II:Aharbal;III:Nihama;IV:Kulgam;V:Khudwani;VI:Sangam) collected during all the seasons from Spring to Winter. Each symbol represents the mean of triplicate values

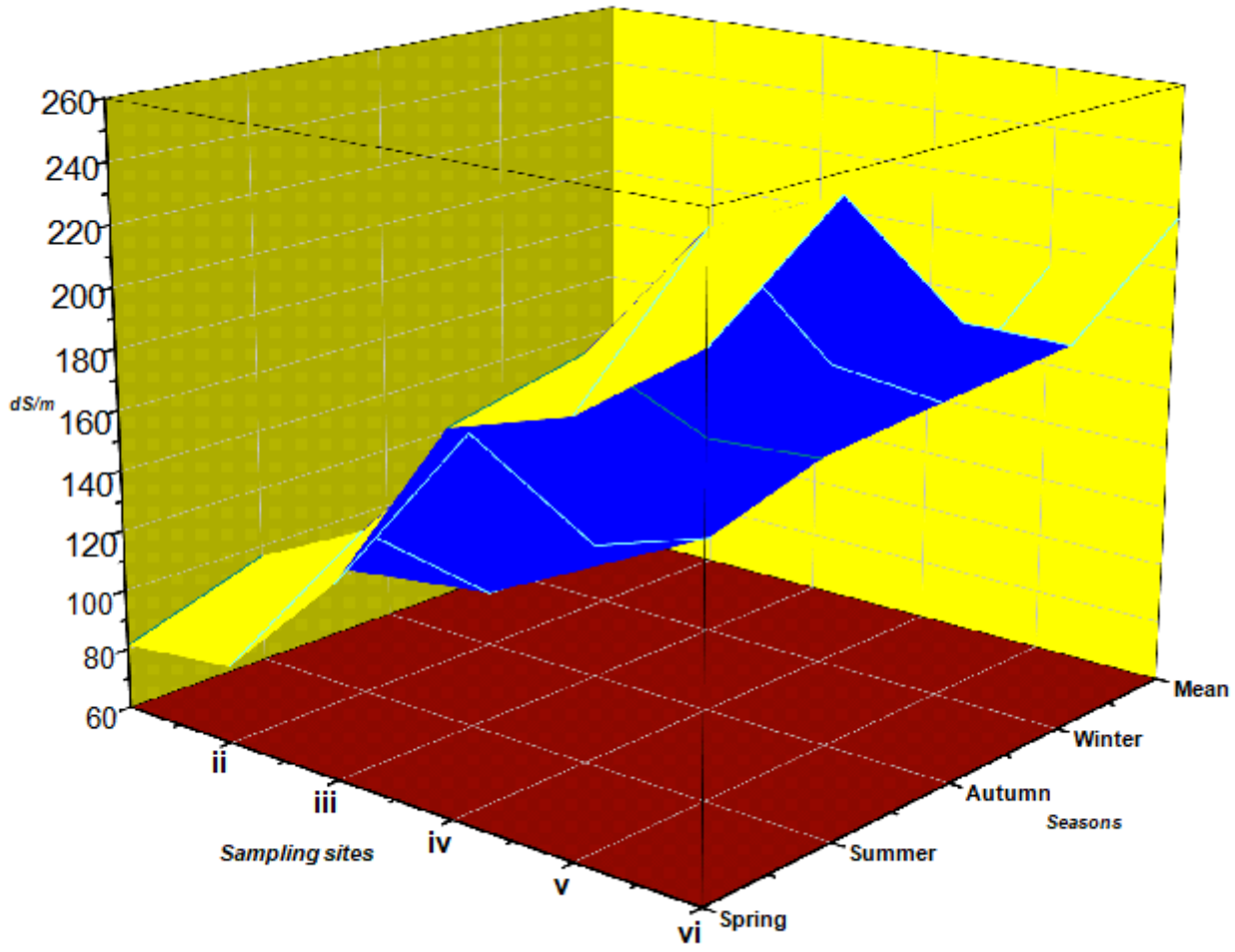


Figure 4

Figure depicting the comparative time-series seasonal variation analysis of (EC)in the water of the River Veshaw at six different sites (I:Kongwattan;II:Aharbal;III:Nihama;IV:Kulgam;V:Khudwani;VI:Sangam) collected during all the seasons from Spring to Winter. Each symbol represents the mean of triplicate values

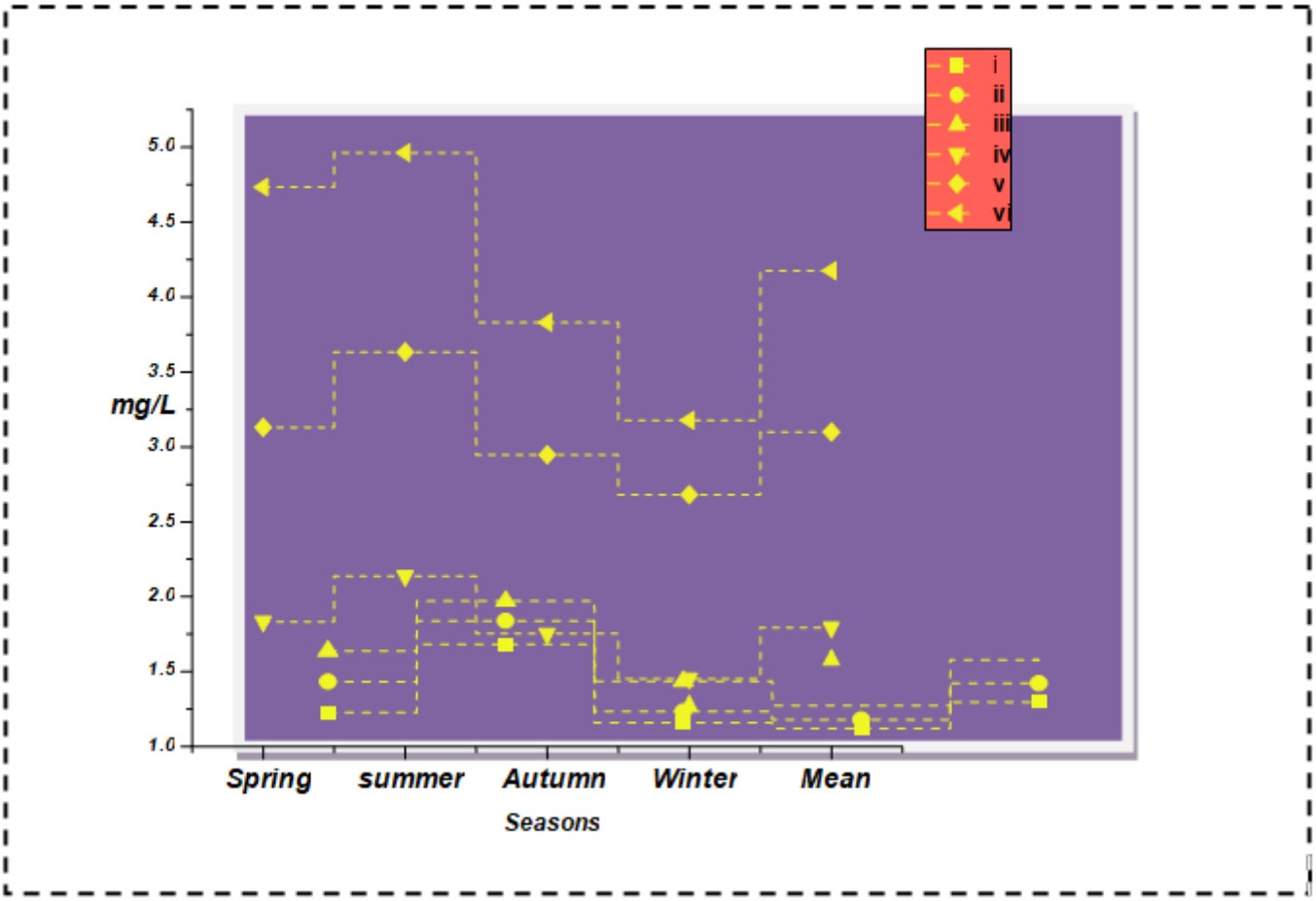


Figure 5

Figure depicting the comparative time-series seasonal variation analysis of (BOD)in the water of the River Veshaw at six different sites (I:Kongwattan;II:Aharbal;III:Nihama;IV:Kulgam;V:Khudwani;VI:Sangam) collected

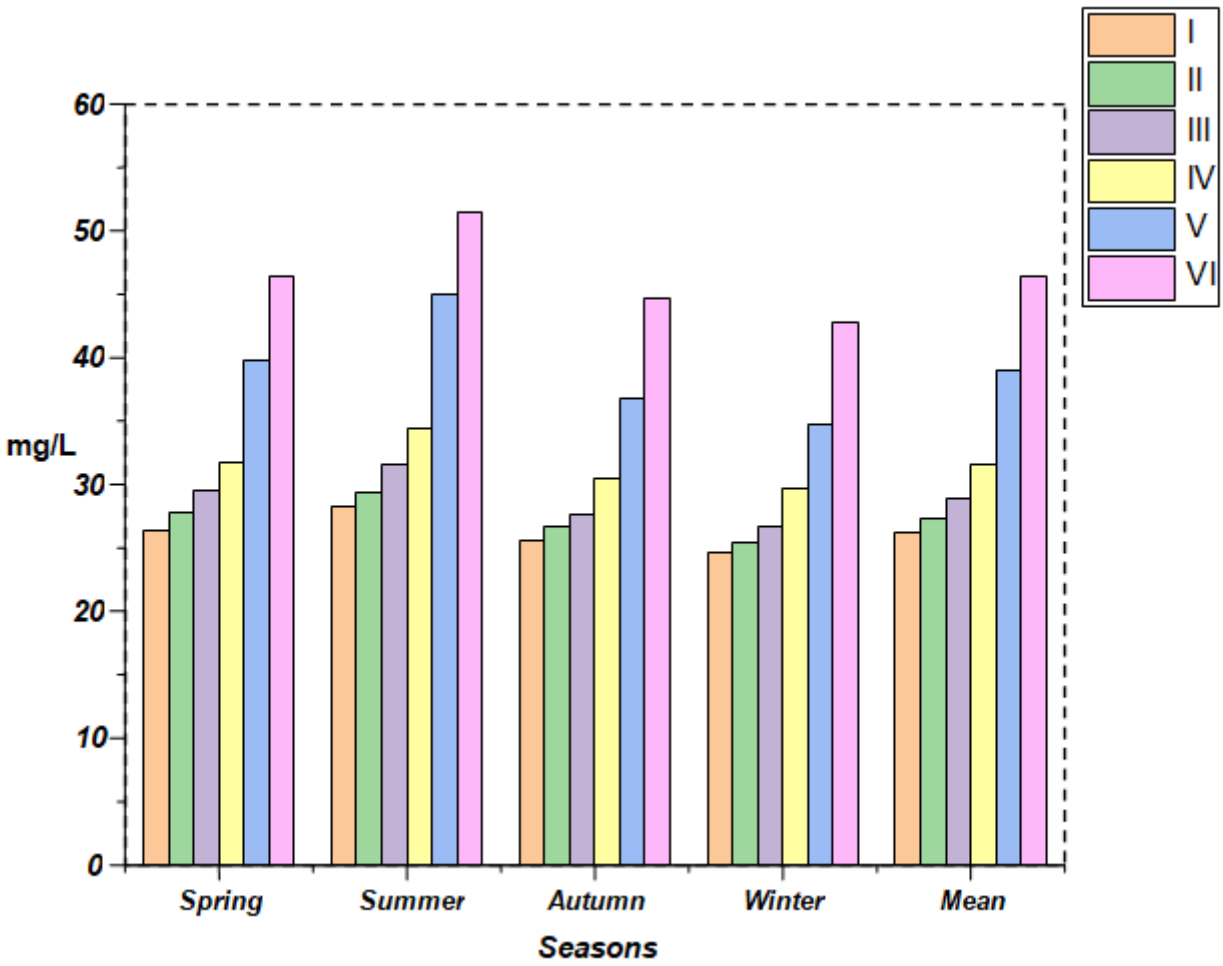


Figure 6

Figure depicting the comparative time-series seasonal variation analysis of (COD)in the water of the River Veshaw at six different sites (I:Kongwattan;II:Aharbal;III:Nihama;IV:Kulgam;V:Khudwani;VI:Sangam) collected during all the seasons from Spring to Winter. Each symbol represents the mean of triplicate values

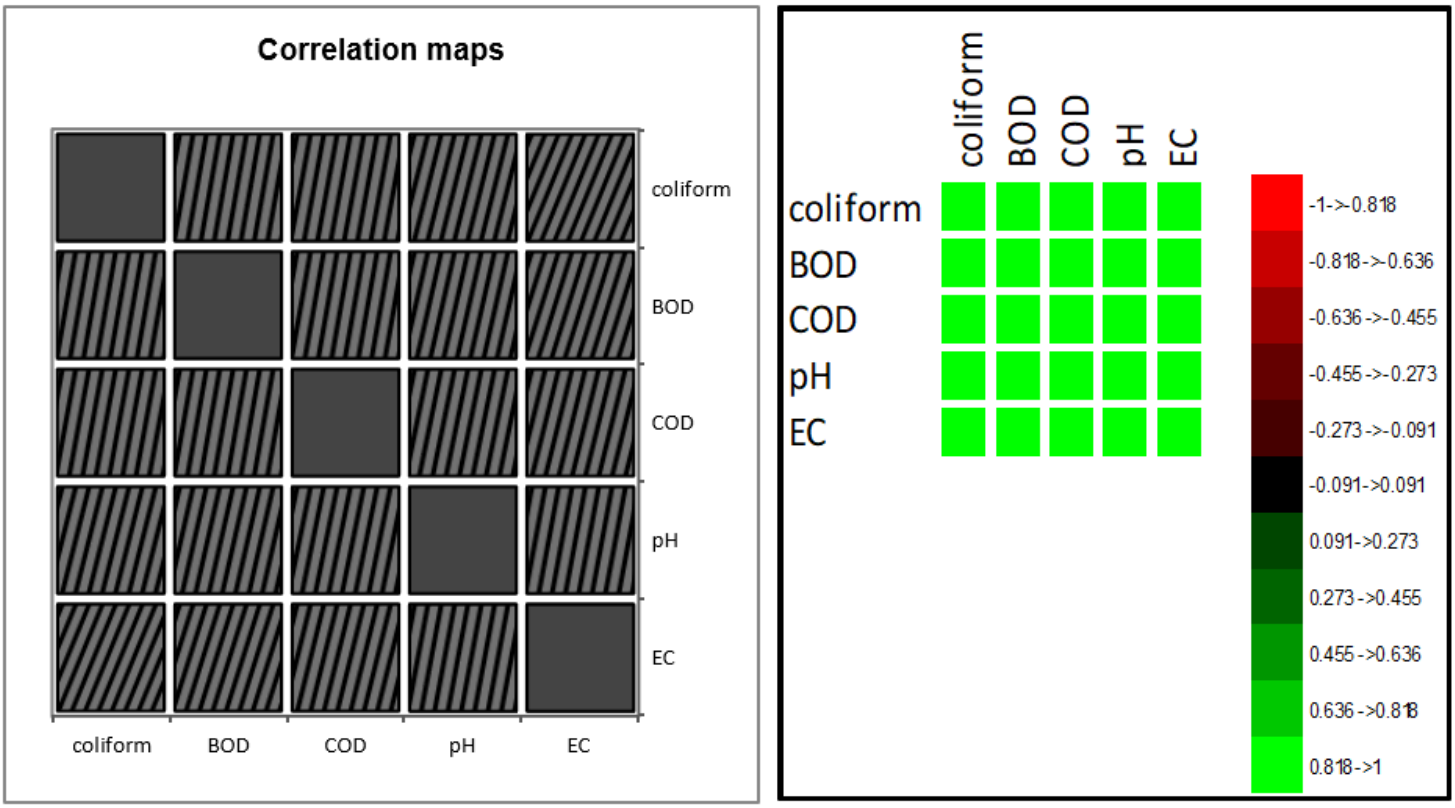
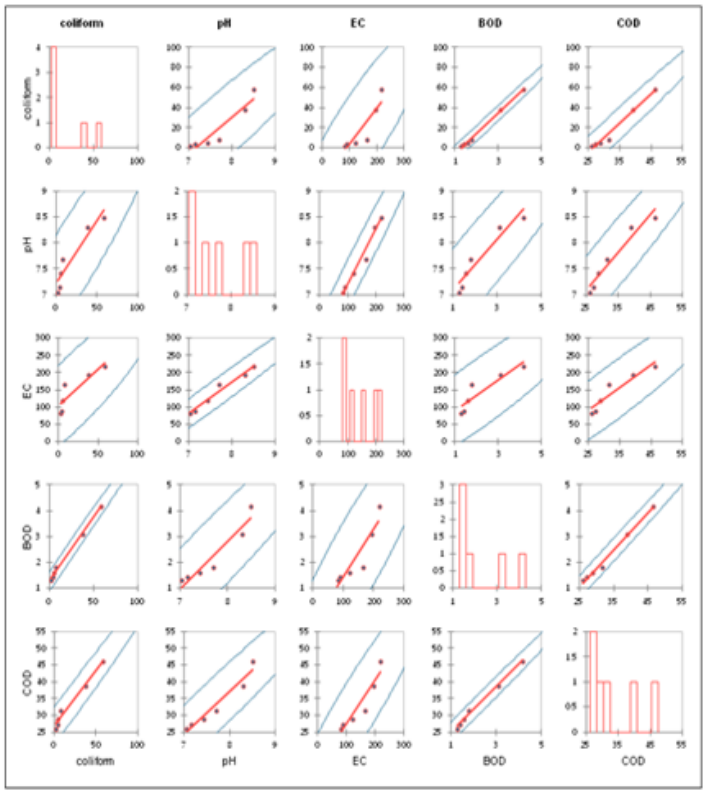


Figure 7

Correlation Coefficient (r) matrix of various water quality parameters studied from Veshaw River of Kashmir Himalaya



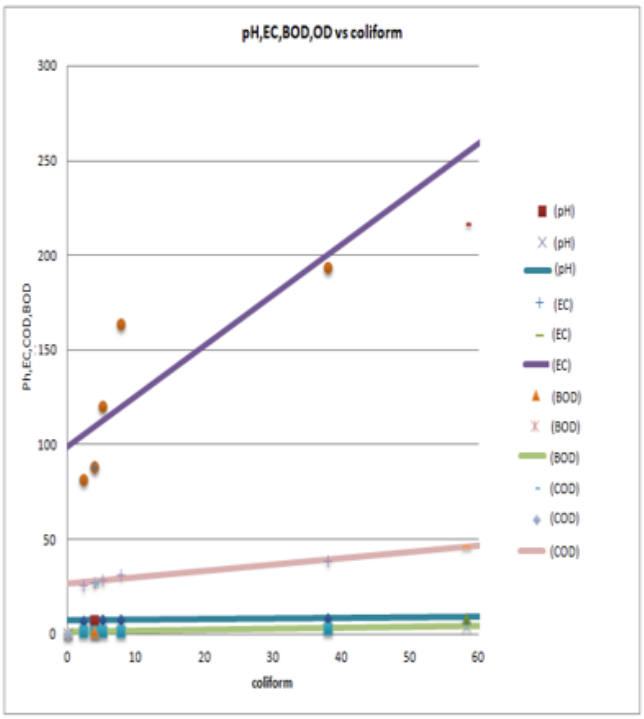


Figure 8

Scatter plots: Linear regression model indicating the relationship among the various physicochemical parameters of the Veshaw stream. Normal P-P plots of the regression standardized residual between the observed (x-axis) and estimated (y-axis) cumulative probabilities for the total (left) and fecal (right) coliforms at each weir station.

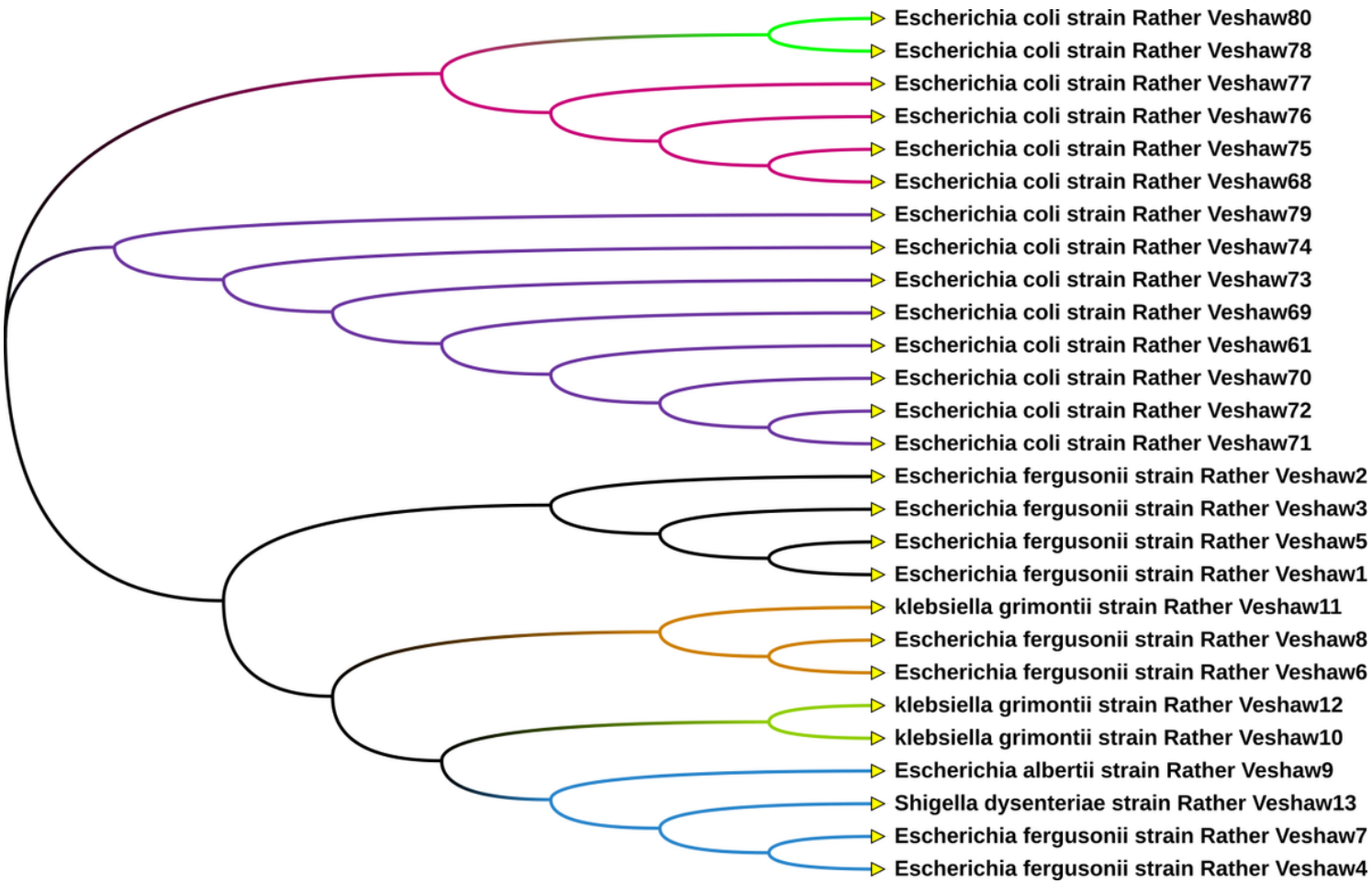


Figure 9

Phylogenetic tree analysis of the 16S rRNA gene sequence of 27 identified pollution indicator bacterial species derived from the water of the river Veshaw. Figure also depicting the total of four clusters which include 27 bacterial species.**Cluster1:**[*Escherichia coli* strain Rather Veshaw80, *Escherichia coli* strain Rather Veshaw78, *Escherichia coli* strain Rather Veshaw77, *Escherichia coli* strain Rather Veshaw76, *Escherichia coli* strain Rather Veshaw75, *Escherichia coli* strain Rather Veshaw68];**Cluster2:***Escherichia coli*strain Rather Veshaw79, *Escherichia coli*strain Rather Veshaw74, *Escherichia coli* strain Rather Veshaw73, *Escherichia coli* strain Rather Veshaw69, *Escherichia coli*strain Rather Veshaw61, *Escherichia coli* strain Rather Veshaw70, *Escherichia coli* strain Rather Veshaw72, *Escherichia coli* strain Rather Veshaw71];**Clustr3** [*Escherichia fergusonii* strain Rather Veshaw2, *Escherichia fergusonii*strain Rather Veshaw3, *Escherichia fergusonii* strain Rather Veshaw5, *Escherichia fergusonii* strain Rather Veshaw1];**Cluster4:**[*Escherichia fergusonii* strain Rather Veshaw4, *Escherichia fergusonii*strain Rather Veshaw6, *Escherichia fergusonii* strain Rather Veshaw7, *Escherichia fergusonii* strain Rather Veshaw8, *Escherichia albertii* strain Rather Veshaw9, *Klebsiella grimontii* strain Rather Veshaw10, *Klebsiella grimontii* strain Rather Veshaw11, *Klebsiella grimontii*strain Rather Veshaw12 and *Shigella dysenteriae* strain Rather Veshaw13]

Supplementary Files

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