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Assessment of Heavy Metals and Microbial Pollution in the Ganga River and its Source Streams at Panch Prayag, Uttarakhand, India

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Abstract: The Panch Pyayag Ganga River and its headstreams in Uttarakhand, India, hold significant cultural, ecological, and hydrological importance. However, increasing human activity, agricultural runoff, and industrial discharge threaten the river's health, potentially compromising its water quality. This study aims to assess the nutrient and microbial contamination levels in the Ganga River and its individual headstreams with a focus on identifying key pollution sources. Water samples were collected from multiple locations across the river system and analyzed for key parameters: biological oxygen demand, chemical oxygen demand, coliform bacteria, and other microbial indicators of contamination. The results revealed elevated levels of nutrients and microbial contaminants in specific stretches, correlating with nearby urban settlements, agricultural runoffs, and religious activities. The study highlights the need for comprehensive water quality management strategies that address the multi-faceted pollution sources affecting the river ecosystem. Understanding these contamination dynamics is essential for preserving the Ganga River's ecological integrity and ensuring safe water access for millions of residents and pilgrims in the region.

Keywords: *Sustainable Agriculture, Heavy Metals, Microbiological Analysis.*

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I. INTRODUCTION

Water is an essential part of the environment. It is crucial for human beings' health. All living beings depend in various ways on rivers as natural water sources for their daily requirements. Information concerning Ganga River water superiority is vital for the thoroughness of life. Water pollution-related health hazards have been one of the most significant challenges to mankind for centuries. Heavy metals and bacterial contamination in river water are the most critical occupational hazardous pollutants in the environment. Due to the indispensable physicochemical properties of non-biodegradable nature and these, levels are accumulating in the environment at very high alarming rates. Due to their significant toxicity, the Central Pollution Control Board decided the acceptable limit for all physicochemical, heavy metals, and microbiological parameters in river water in India. In addition, USEPA regulates and controls the heavy metal contamination in human blood. The Ganga River is considered the most holistic and sacred by Indians because of its important cultural, economic, and environmental values. It is a lifeline for millions of people who live along its path and provides water for roughly 550 people per square kilometer, or around 45 crore people (1). People take baths in the holy river and accomplish their rituals 2 (2). Several picnic areas for tourists, pilgrims, and spiritual facts have been recognized to accomplish various religious and entertaining activities (3). Thus, the Ganges is reflected as the most sacred river among

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different rivers in the country. River contamination through various anthropogenic activities is depriving the overall quality of river water, being affected due to sewage waste disposals directly in rivers agricultural pesticides flow, and the expansion of urban industrial belts along river banks. There is a direct relationship between river water quality and its impact on public health. River pollution has increased daily and is well documented in studies conducted globally in several developing countries (4, 5, and 6). Great attention to Heavy metals and Pathogenic Microbes are often a puzzle for Biota and ecosystem management. Changes in river water quality significantly depend on the energetic environmental conditions caused by the multifaceted interplay between terrestrial soil erosions and water domains (7). The Ganges allows sideways twenty-nine class I cities, twenty-three class II cities, and almost fifty settlements that discharge various forms of trash into this robust river ecosystem (8). Direct dumping of home garbage, industrial wastes, agricultural runoff, and human activities along the river bank results in accumulating these pollutants and contaminating river water. These wastes contain toxic substances that pose a health risk, such as salts of Arsenic, lead, cadmium, chromium, copper, and mercury, which interact with the aquatic environment and disrupt the river ecology (9, 10). Industries that contribute to the presence of heavy metals in river water include those that produce paint pigment, metals, varnishes, pulp and cotton textiles, paper, rubber, steel, sugar mills, and thermal power plants, galvanizing of iron products, mining. In addition, the haphazard use of pesticides and fertilizers containing heavy metals in agricultural fields has also contributed to polluting the river water (11). Several studies have determined the amount of metal pollution in rivers, markedly the Gomati (12) and Ganga (13) in India, Tigris River in Turkey (14), Krotova in Bangladesh (15), and Brisbane River in Australia (16). Pollutants found as heavy metal collects in the water column, sediment, and living things like plants and animals (17,18), which may have deleterious effects on human beings consuming them. Different indicators of microbes have been used universally to indicate contamination of river water through human wastes. Studies have shown that the physio-chemical and biological aspects of the water in the River of Ganga have changed and have beyond acceptable or necessary limits (19). The values of the Most Probable Number (3.5×108 & 4.6×108) and Standard Plate Count (5.4×105 & 6.8×106 SPC) in 100 ml of river water and ranged at different sampling sites as Har ki Pauri (HKP), 3 Pul Jatwara (PJ), Vishnu Ghat (VG), Daksh Mandir (DM). At the same time, these were relatively upper to the considered values of MPN (3.2×108 MPN100 ml -1) and SPC (4.5×105 SPC ml -1) in the assessment of control site Bhimgoda Barrage (BGB) (2). According to the Central Pollution Control Board (CPCB) criteria, the permissible BOD level of water is 2 mg/L or less, and the permissible DO level is 6 mg/L. According to the latest data of (CPCB) most of the Ganga River Water in the Uttar Pradesh, West Bengal stretch was found unfit for consumption and bathing. To the best of our knowledge, since there was no holistically approached evidenced-based documented data available in published literature for the Ganges River overall water quality and level of pollution index flown all along in hilly and plain regions of Uttarakhand state India, the present observational analytical study was planned and conducted. This work aimed to find effects on contamination levels and variations of physical, biochemical, and microbiological aspects of sacred river water flown in headstream at Panch Prayag river bank sites of the Ganges. The study evaluated physicochemical variables and eight metal analyses and identified microbiological indicators in water samples collected from the Ganges, specifically at five hilly and six plain regions of water sampling sites. British microbiologist Felix Twort and French-Canadian microbiologist Felix d'Hérelle discovered the bacteriophage in 1915 and 1917. These are viruses that infect and can kill bacteria (20). D'Hérelle and other scientists thoroughly examined bacteriophages' potential as medicinal agents and defined their nature (21, 22, 23, and 24). The history of bacteriophage therapy is extensive.

II. MATERIAL AND METHODS

Fig 1: Collection Sites from Google Earth

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Fig 2: Panch Prayag Collection Sites of Ganga River for Analysis Purposes

- **Ethical Clearance –** Ethical clearance was obtained from the institutional ethics committee on 8/03/2018 for this Research project.
- **IEC code: 42/IEC/Ph.D./2018.**

A. Reagents, Chemicals

All reagents and chemicals of standard quality were purchased from Gen Biotech Pvt. Limited and Sun Bio Pvt. Company.

B. Sampling Sites

- **Vishnuprayag (VP) -** Vishnu Prayag, located in Uttarakhand, India, is one of the four sacred Prayags (confluence points) of the Alaknanda River. It lies at an altitude of 1,372 meters, near the town of Joshimath in Chamoli district. Vishnu Prayag is the confluence of the Alaknanda and Dhauliganga rivers and holds religious significance in Hinduism, as it is believed to be a place where Lord Vishnu meditated. It is also an important pilgrimage site for devotees traveling to the Char Dham. The region is known for its scenic beauty, surrounded by majestic Himalayan peaks and lush landscapes.
- **Nandprayag (NP):** Nand Prayag, located in the Chamoli district of Uttarakhand, India, is one of the sacred Panch Prayags (five confluences) of the Alaknanda River. Situated at an altitude of 1,494 meters, it marks the confluence of the Alaknanda and Nandakini rivers. Nand Prayag holds religious significance in Hinduism, with its name derived from Nanda, the foster father of Lord Krishna. The site is an important pilgrimage destination for devotees traveling to the Char Dham and is known for its serene beauty, surrounded by the towering peaks of the Himalayan range.
- **Karanprayag (KP):** Karan Prayag, located in Uttarakhand's Chamoli district, is one of the five sacred Prayags (river confluences) of the Alaknanda River. Situated at an altitude of 813 meters, it marks the confluence of the Alaknanda and Pindar rivers. The site is named after the warrior Karna from the Mahabharata, who is believed to have worshipped here. Karan Prayag holds both religious and cultural significance, drawing pilgrims end route to the Char Dham. The area is also known for its picturesque landscapes, nestled in the lap of the Himalayas.
- **Rudraprayag (RP)**: Rudra Prayag, located in Uttarakhand, India, is one of the five sacred Prayags of the Alaknanda River. Situated at an altitude of 610 meters, it marks the confluence of the Alaknanda and Mandakini rivers. The town is named after Lord Shiva (Rudra), as it is believed that the deity meditated here. Rudra Prayag is a significant pilgrimage site, especially for devotees traveling to the Char Dham, and is known for its scenic beauty, surrounded by lush landscapes and majestic Himalayan peaks.
- **Devprayag (DP)**: Dev Prayag, located in Uttarakhand, India, is one of the five sacred Prayags of the Alaknanda River. Situated at an altitude of 472 meters, it marks the confluence of the Alaknanda and Bhagirathi rivers, forming the Ganga River. Dev Prayag holds immense religious significance, as it is believed to be the spot where the Ganga River took its holy form. The town is an important pilgrimage destination for Hindus, especially those visiting the Char Dham, and is renowned for its spiritual aura and stunning natural beauty. All sampling site details are given in table no.1.

C. River Water Sample Collection Procedure

According to the requirement of the test protocol water Samples were collected in three different new sterile plastic (polypropylene) containers (Tab.1).

Note- Water-resistant marker used for labeling on this slip, mention the date, time, location, and type of sample.

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Table 1: Specific Collection Container for the Test

Ist container(1litre) For Physico-chemical analysis	2nd container(1litre) Heavy metal analysis	3rd container (1litre) Microbiological analysis
The water sample was taken in a clean plastic bucket., which was then transferred to a labelled bottle and carried to the lab on ice before being kept in a deep freezer (-20^0 C) until analysis.	Each site's surface water samples (0–10 cm in depth) were collected manually using a sampler, placed in a sterile polyethylene container, and brought to the lab on ice. In addition, kept until the analysis process at -20^{0}C.	Using a sterilized glass bottle, water samples were taken from the River at a depth of 0.5 meters. 1. Remove the cap and cover of the sterile sample bottle aseptically. 2. Upstream, turn the bottle mouth. 3. Plume the neck downward until it is about 30 cm below the water's surface. 4. Carefully replace the cap and cover after allowing the neck to fill completely by tilting it slightly upward.

A. *Details of Analytical Methods Used in Physio-Chemical Analysis*

All physiochemical Analysis were done by the use of APHA guidelines. We measured pH using fresh samples and the HACH HQ40D portable meter, based on the electromotive force (emf) between a glass indicator electrode and a calomel reference electrode. For calibration, we prepared pH 4, 7, and 9.2 buffer solutions: pH 4 (10.12g potassium hydrogen phthalate in 1L distilled water), pH 7 (1.42g sodium hydrogen phosphate and 1.361g potassium dihydrogen phosphate in 1L distilled water), and pH 9.2 (3.81g borax in 1L distilled water, boiled and cooled). Electrodes were rinsed, dried, and calibrated with the buffer solutions before measuring the sample pH. Temperature is a key factor influencing the physical, chemical, and biological properties of water, including H_2O chemistry. It fluctuates constantly in rivers due to changing climatic conditions. Temperature was measured using a digital thermometer (Model: Pacer DTM 902). Conductivity was measured using a HACH HQ40D multi-parameter device. It reflects the water's ability to conduct electricity, which depends on the types and concentrations of ions present. The presence of ionized inorganic substances in water affects its conductivity, providing a quick estimate of dissolved mineral content.

Dissolved Oxygen (DO) is essential for the metabolic processes of all living organisms and is crucial for aerobic wastewater treatment, which relies on free oxygen. It is also necessary for the precipitation and suspension of inorganic elements in water. DO levels in tap water and wastewater are influenced by various physical, chemical, and biological factors. A portable HACH HQ40D multi-parameter device measures DO in water samples, which is vital for assessing aerobic biological treatment processes. The manual procedure for measuring DO involves collecting a sample in a BOD bottle, adding reagents ($MnSO_4$ and alkali-iodide-azide), and mixing to allow the precipitate to settle. After removing the supernatant, concentrated H_2SO_4 is added, and the sample is titrated with a standard $Na_2S_2O_3$ solution using starch as an indicator to determine the oxygen content. Total Dissolved Solids (TDS) refers to the residue left after evaporating and drying a water sample at 103-105°C. It includes both "Total Suspended Solids" (TSS) and TDS, which are crucial in assessing water quality and treatment processes, particularly in wastewater management. TDS analysis is vital for determining appropriate treatment methods and ensuring compliance with discharge regulations. The manual procedure involves placing a known volume of sample in a dish, drying it to constant weight at 103-105°C, then igniting it at 500-550°C to burn off organic matter. After cooling and weighing the dish at different stages, the concentration of solids is calculated as a percentage of the sample's weight. Biochemical Oxygen Demand (BOD) is a standardized laboratory test used to measure the oxygen required for the aerobic oxidation of biodegradable organic and some inorganic substances in water, wastewater, and contaminated waters under controlled conditions. This test helps assess pollution levels, a water body's ability to assimilate pollutants, and the effectiveness of wastewater treatment. The procedure involves incubating water samples at 20°C for five days and measuring the dissolved oxygen (DO) levels at the start and end of the incubation. The test quantifies both carbonaceous (organic) and nitrogenous (inorganic) oxygen demand. Using iodometric titration, the DO is measured in BOD bottles containing the sample and dilution water. The difference in DO levels before and after incubation determines the BOD value. The COD analysis is performed to determine how much organic pollution is present in wastewater. Here, for the estimation of COD, a secure reflex method is used. For the estimation of COD, take 20 mL of water sample in a flask of reflux unit. This added 10 ml potassium dichromate, a pinch of $AgSO4$ and $HgSO4$, and 30 mL of $H2SO4$. Refluxed the unit for 2 hours (temperature 60°C) and cooled the unit. It has added distilled water to dilute the contents to 150ml. Titrated against Ferrous Ammonium Sulphate (F.A.S.) with ferroin indicator; at the endpoint, blue-green color changes to reddish blue.

B. *Heavy Metals /Toxic Metals Analysis*

River Water samples from Panch Prayag (Ganga River headstreams) were analyzed for metal content at IIT Roorkee. This technique combines the high-temperature ionization of samples in an ICP (10,000°C plasma) with the mass spectrometer (MS) to detect and quantify metals. The ionized metals are sorted by their mass-to-charge ratio and then measured by an electron multiplier tube detector. ICP-MS is highly sensitive and precise, making it ideal for analysing metals in various samples, such as water, serum, and blood.

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C. Sample Preparation for Metals Analysis

- **Surface Water Sample -** 10 ml of pure HNO3 was used to digest 50 ml of water until the solution turned translucent at 80°C (APHA 1998). The solution will be diluted to 50 ml with double-distilled water after being filtered with what man no. 42 filter paper. All collected water samples were subjected to eight metal analysis Cadmium (Cd), Nickle (Ni) Mercury (Hg), Copper (Cu), Lead (Pb) , Zinc (Zn), Chromium (Cr), Arsenic (As), and using a standard protocol of Inductively Coupled Plasma-Mass Spectrometry (ICPMS) manufactured by Perkin Elmer. The instrument was calibrated with multi-elemental standards of metals before running water samples of each site. 45 ml Ganga water samples were digested with 3 ml of concentrated HNO3 and 2 ml H2O2 at ~ 80oC until the solution remains about 5 ml (APHA 2005). The solutions were filtered through what man filter paper no. 42 and dilute to 50 ml with double distilled water (APHA 23th ED-3111.C). The filter paper was rinsed with diluted nitric acid solution and used to remove the siliceous impurities from the digested solution. The concentration of nitric acid was so adjusted that it remained the same as that of blank and standard solutions. In ICP-MS, elements from water samples at high temperatures were ionized and directed further into MS. The ions were then directed to an electron multiplier tube detector after being sorted by the MS based on their mass/charge ratio. On the instrument's display unit, this detector then detected and quantified each ion from the processed water samples.

D. Methods used in Microbiological Analysis of Water samples

Most probable number test (MPN Test) – Included presumptive test, confirmation test, and complete test. In the MPN method by the use of a sterile pipette 10 ml, 1ml and 0.1ml of water samples from sterile containers were inoculated in a 50 ml test tube having 10 ml double strength Lauryl Tryptase (LT) broth medium and 5 ml single strength and 5 ml LT broth respectively. All tubes were incubated for 1 day at 37°C. After the growth of mixed culture, they were inoculated in the Broth culture tube for verification test viz. EC Broth, Brilliant Green Bile Broth (BGBB), and Tryptone water, Azide Dextrose for a complete test. Selenite F Broth and alkaline peptone water was used to a culture of some specific bacteria like Vibrio cholera and salmonellae *typhi* species, growth present was then subculture on Xylose Lysine Deoxy Cholate (XLD), Thiosulfate-Citrate-Bile Salt-Sucrose (TCBS) Agar culture plate respectively. Various morphological characteristics of improved isolates, colony morphology shape, color, arrangement, biochemical tests, and Gram staining were carried out for the identification of isolates. Antibiotic sensitivity was done to check out the cultural sensitivity of significant bacterial cultures. The water samples were also checked for Fungi using two methods, direct plate and dilution plate with the use of two types of growth media potato dextrose agar (PDA) and Sabouraud's dextrose agar (SDA) incubated for 7 days on 25°c.

III. RESULTS

A. Physicochemical Analysis

The water quality analysis across five sampling points (VP, NP, KP, RP, and DP) reveals that most parameters fall within the WHO (2017) guidelines, indicating generally good water quality. pH values for VP, NP, and DP are within the acceptable range of 6.5–9.2, but KP and RP show acidic pH levels (5.83 and 4.99, respectively), which may pose risks to aquatic life and soil health. Total Dissolved Solids (TDS) at all points are well below the 500 mg/L guideline, indicating low levels of dissolved solids. Conductivity values, ranging from 49.1 to 179.7 µS/cm, are within the recommended range of 200-800 µS/cm, though RP exhibits lower ion concentration, suggesting a more diluted sample. Biochemical Oxygen Demand (BOD) and Chemical Oxygen Demand (COD) values are all below the WHO thresholds of 5.0 mg/L and 10.0 mg/L, respectively, indicating low organic and chemical pollution. Dissolved oxygen (DO) levels, ranging from 6.5 to 9.4 mg/L, are also within the healthy range for aquatic ecosystems. Temperature values, ranging from 4°C to 10.2°C, are typical for cooler climates and do not present immediate risks as shown in Fig. 3. Overall, while most parameters are within safe limits, the low pH levels at KP and RP highlight potential concerns that need to be addressed to prevent adverse environmental impacts.

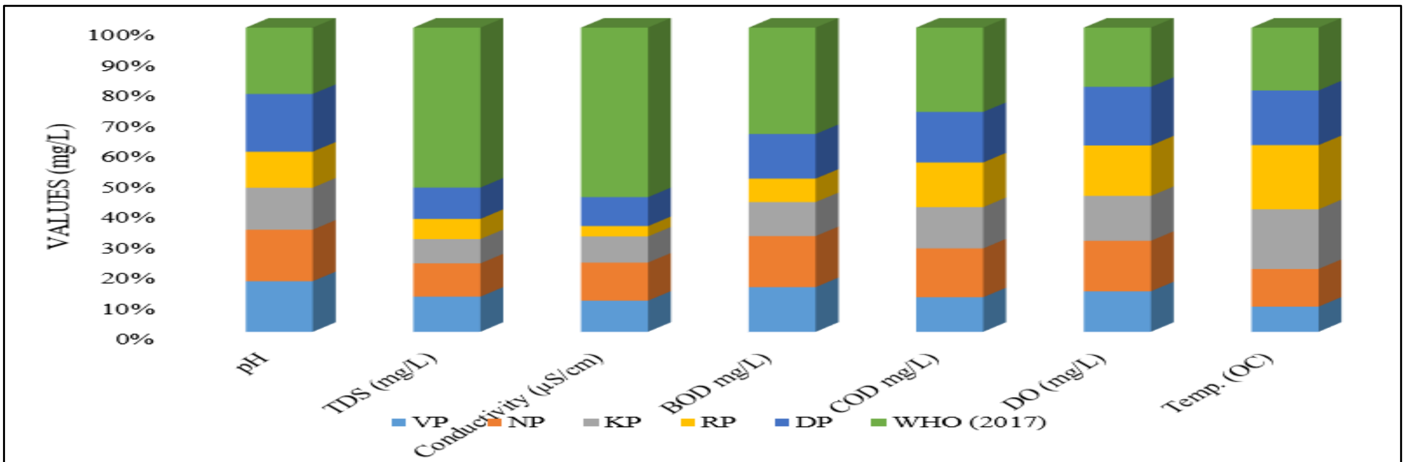

Fig 3: Physicochemical Analysis of Panch Prayag River Water Collected Samples

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B. Heavy Metals Analysis

The concentrations of various metals in different water samples (VP, NP, KP, RP, DP), along with the WHO (2017) maximum allowable concentrations for each metal in water. The metals analyzed include As (Arsenic), Pb (Lead), Hg (Mercury), Cd (Cadmium), Cr (Chromium), Cu (Copper), Zn (Zinc), and Ni (Nickel) From the data, it can be observed that for Arsenic (As), the concentrations across all samples range from 2.211 µg/L (VP) to 5.143 µg/L (KP), all of which are below the WHO threshold of 10 µg/L. For Lead (Pb), the concentrations range from 0.008 µg/L (NP) to 0.077 µg/L (DP), which are well below the WHO limit of 2 µg/L. Mercury (Hg) concentrations are notably higher, ranging from 2.106 µg/L (VP) to 6.32 µg/L (DP), all of which exceed the WHO limit of 2 µg/L. Cadmium (Cd) levels are generally low, ranging from 0.003 µg/L (RP) to 0.377 µg/L (VP), all well below the WHO threshold of 5 µg/L. Chromium (Cr) concentrations vary from 0.371 µg/L (NP) to 1.581 µg/L (DP), all remaining below the WHO limit of 100 µg/L. Copper (Cu) levels range from 0.309 µg/L (NP) to 1.157 µg/L (RP), which are far below the WHO limit of 1300 µg/L. Zinc (Zn) levels are higher in some samples, with values ranging from 1.637 µg/L (DP) to 8.622 µg/L (NP), all well below the WHO threshold of 5000 µg/L. Lastly, Nickel (Ni) concentrations range from 0.185 µg/L (DP) to 8.125 µg/L (NP), with all values being below the WHO limit of 25 µg/L. The concentrations of most metals (As, Pb, Cd, Cr, Cu, Zn, and Ni) in the water samples fall within the recommended limits set by WHO. However, Mercury (Hg) levels exceed the WHO limit in all samples, indicating potential contamination that requires attention as depicted in Fig 4.

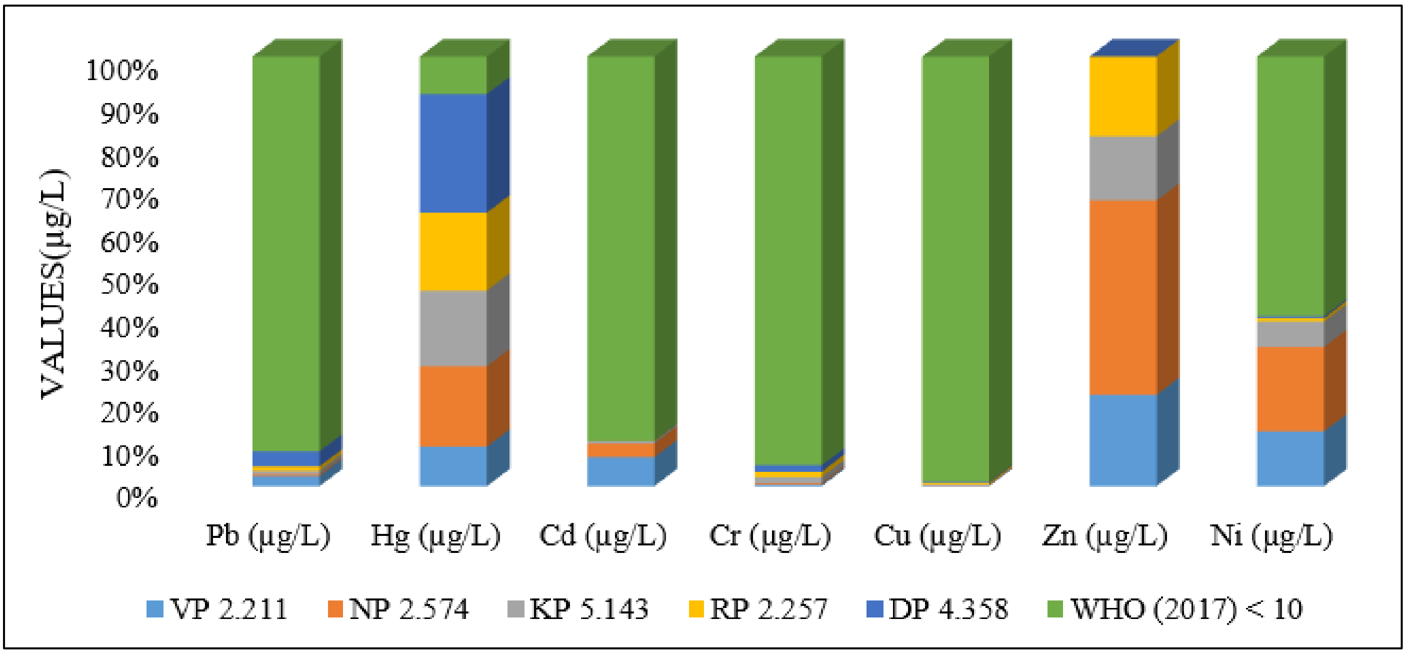

Fig 4: Bar Diagram of Heavy Metals Concentration in Collected River Water Samples of Panch Prayag.

C. Microbiological Analysis

All microbiological analysis was carried out at the Microbiology Department, AIIMS Rishikesh. , according to microbiological reports. The microbiological data on different water samples (VP, NP, KP, RP, DP) for various parameters: MPN (Most Probable Number), TPC (Total Plate Count), FC (Faecal Coliforms), E. coli count, FS (Faecal Streptococci), and the absence of pathogens such as *Salmonella*, *Shigella*, *Vibrio cholerae*, and fungi. For MPN, which measures the concentration of potentially harmful microorganisms, the values are 0 for VP and NP, 9 for KP, 14 for RP, and 75 for DP. This indicates a significant increase in microbial contamination from KP to DP. TPC, which represents the total number of microorganisms in the water, ranges from 0 for VP and NP to 3×10^2 (300) for KP, 2×10^3 (2000) for RP, and 4×10^4 (40,000) for DP, suggesting a considerable rise in microbial load as we move from VP to DP. Fecal coliforms (FC) and *E. coli* counts are 1×10^2(100) for KP,3×10^2 (300) for RP, and 2×10^3 (2000) for DP. all the samples, indicating no fecal contamination in these parameters. Similarly, fecal streptococci (FS) and the pathogens *Salmonella*, *Shigella*, *Vibrio cholerae*, and fungal growth show no growth in any of the samples, suggesting the absence of these specific harmful microorganisms. While the samples from VP and NP appear to have very low microbial contamination, with no growth of harmful pathogens, the samples from KP, RP, and especially DP show increasing levels of microbial contamination, with DP showing the highest MPN and TPC values. However, no fecal coliforms, E. coli, or specific pathogens such as *Salmonella* or *Vibrio cholerae* were detected in any of the samples, which is a positive sign in terms of public health risks associated with these particular microorganisms as shown in Fig 5.

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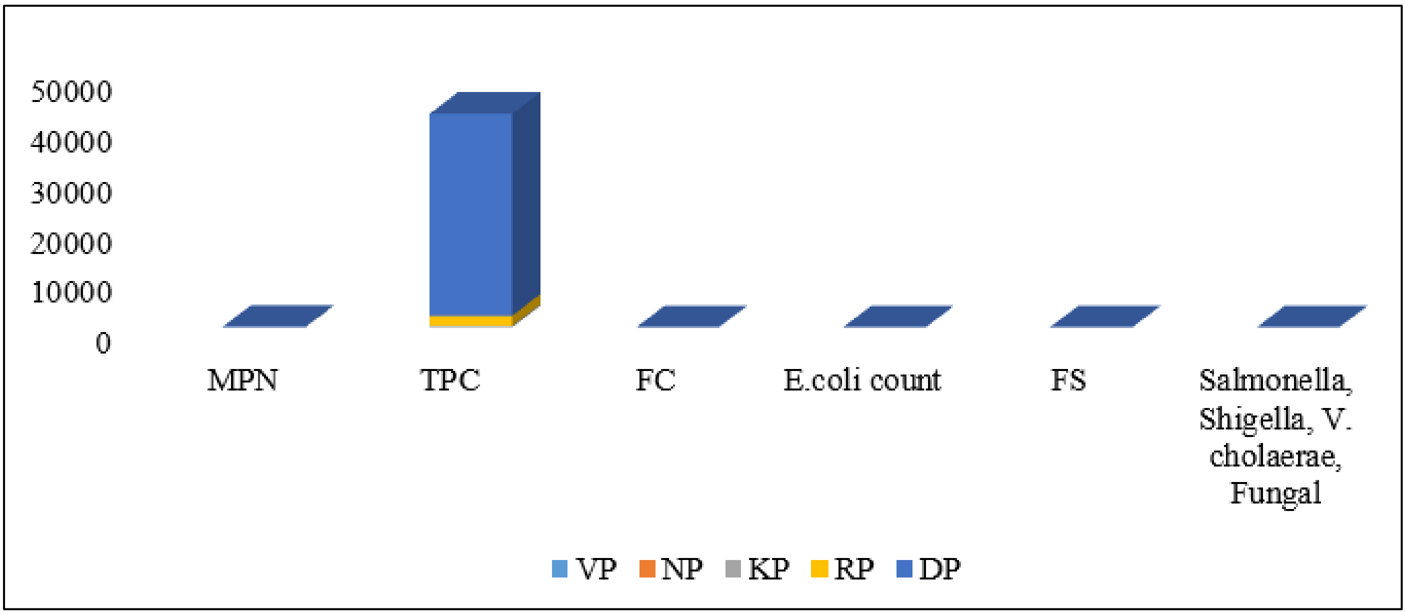

Fig 5: Microbiological Analysis in Ganga Water Collected Samples from Panch Pryag

D. Antibiotic Susceptibility Testing.

The antibiotic susceptibility pattern was determined against 18 commonly used antibiotics (Table 2) by the Kirby-Bauer disk diffusion methods. According to Clinical Laboratory Institute guidelines (CLSI-2018), Cefotaxime, Cefodoxime, Cefazidime, Aztreonam, Ceftriaxone, Amikacin, Azlocillin, Carbenicillin, Cefepime, Cefoperazone, Cefotaxime, Ciprofloxacin, Ceftazidime, Doripenem, Doxycycline, Gentamycin, Gatifloxacin, and Levofloxacin are the antibiotics used in this study. Antibiotic susceptibility testing was performed for all isolated bacteria. The maximum number of strains showed drug-sensitive patterns towards the tested antibiotics.

Table 2: Antibiotic Susceptibility Test using Kirby Bauer Disk Diffusion Methods

Name of Organism	**Klebsiella**	**Pseudomonas**	**Proteus**	**Citrobacter**	**E.coli**	**Acinetobacter**
Antibiotic drugs						
Cefotaxime	S	R	S	S	S	S
Cefodoxime	S	S	S	S	S	S
Cefazidime	S	S	S	S	S	S
Aztreonam	S	S	S	S	S	S
Ceftriaxone	S	S	S	S	S	S
Azlocillin	S	S	S	S		S
Carbenicillin	S	S	S	S	S	S
Cefepime	S	S	S	S	S	S
Cefoperazone	S	S	S	S	S	S
Cefotaxime	S	S	S	S	S	S
Ceftazidime	S	S	S	S	S	S
Doripenem	S	S	S	S	S	S
Doxycycline hydrochloride	S	S	S	S	S	S
Gentamycin	S	S	S	S	S	S
Gatifloxacin	S	S	S	S	S	S

*S= Sensitive *R=Resistant

The antibiotic sensitivity profiles of six bacterial organisms (*Klebsiella*, *Pseudomonas*, *Proteus*, *Citrobacter*, *E. coli*, and *Acinetobacter*) to various antibiotics. *Klebsiella*, *Proteus*, *Citrobacter*, and *E. coli* all exhibit sensitivity (S) to all antibiotics tested, indicating that these organisms are effectively controlled by the antibiotics in the list. *Pseudomonas*, however, is resistant (R) to Cefotaxime, although it remains sensitive (S) to all other antibiotics. *Acinetobacter* shows sensitivity (S) to nearly all antibiotics, with the exception of Azlocillin, for which no result is provided. Overall, the organisms display high levels of sensitivity to a broad spectrum of antibiotics, with *Pseudomonas* being the only exception, demonstrating resistance to Cefotaxime. This suggests that, for the most part, these antibiotics should be effective in treating infections caused by these bacteria, although resistance to certain drugs, like Cefotaxime, should be taken into consideration when choosing an appropriate treatment. Some of the representative figures of the result is shown below Fig. 6.

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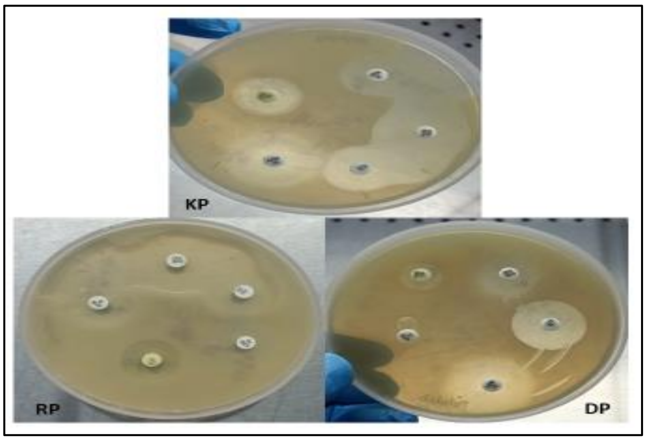

Fig 6: A Diagrammatical Presentation Showing Different Antibiotic-Resistant Zones of Inhibition Patterns

IV. DISCUSSION

➢ *Physico-Chemical Analysis:*

The quality of any water generally depends on its Physico-chemical characteristics. The Physico-chemical parameters of the river Ganga headstreams were studied in water samples at five selected sites at panchprayag for pH, TDS, Conductivity, BOD, COD, DO, and temperature. In the present study, water samples were obtained and analyzed from various locations (n=5) and we found pH under the acceptable limit of three collection sites but KP and RP had low pH values. In 2010, Kumar et al. showed that most of metabolic processes depend on pH, and aquatic organisms are impacted by pH. The ideal pH range for aquatic life is in between 6.8 and 8.2. The pH of an aquatic system is a crucial sign of the quality of the water and the level of pollution in the watershed areas (25). According to Effendi H et al., 2015, who observed that the pH levels might also be influenced by various human activities, such as washing, bathing, and using restrooms near water bodies (26). In 2009, Joshi reported that the pH of the Ganga River in Haridwar ranged from 7.06 to 8.35, making it mildly alkaline (27). Matta et al., 2014 analyzed two different sites, the Shivpuri site -1 with pH of 7.83, and Pashulok Barrage site-2 with pH of 7.91. They recorded that pH levels were not very variable (28). The total dissolved solids (TDS) in this study were determined to be between minimum (63mg/l) to maximum (110mg/l). The highest TDS reading (110 mg/l) was recorded in the Vishnu Prayag. Extreme TDS leads to a rise in water temperature, hinders photosynthesis, and decreases water clarity. The WHO guideline value for TDS has to be below 500 mg/L (29). Haritash AK et al., 2016 evaluated the water quality in Rishikesh and discovered that the TDS level was between 18.0 and 85.0 mg/l. His research has established the viability of drinking water from the Ganga River (30). In our study, the conductivity was estimated by the HACH multi-parameter analyzer on the spot during the collection of river water sampling. We have found a higher amount of conductivity in the Nandpryag (second collection site). We observed that the level of conductivity in Nandpyag areas is higher than in other Prayag. Electrical conductivity (EC) quantifies the concentration of ions in water, which significantly influences taste. The WHO acceptable limit was met by the mean value observed for all collected water samples. Electrical conductivity is a common way to gauge the total concentration of ionized water elements, and a higher conductivity indicates more water contamination (31).

In 2007, Misra and Tripathi observed that the average concentration of DO in Varanasi's River Ganga ranged from 1.8 to 5.9 mg/l (32). According to Verma (2013), the Ramganga River in India's Kumaun Himalaya had the highest DO level in the month of December at 10.72 mg/l, while the lowest DO content, 7.6 mg/l, was recorded in the month of July as a result of the higher temperatures in Post-Summer (33).

In our study, we have found the Biological Oxygen Demand in water samples within the acceptable range in all collected samples depicted in figure no.3. BOD measures how much oxygen is used by bacteria and other microbes to oxidize accessible water-borne materials (34, 35, and 36). In 2018, Kumar conducted a study of the Ganga river water during the Kanwar Mela 2017 at Haridwar. They observed and concluded that the human and ritual activities were more, and the level of BOD was found to be more concentrated (37). By utilizing a potent chemical oxidant, such as potassium dichromate under reflux circumstances, COD may calculate the quantity of oxygen necessary for the chemical oxidation of organic matter. Different seasons effect on their level. According to, Kumar P et al 2020, we reported high levels of chemical oxygen demand in river water samples from the post-monsoon, Post-winter, and Post-Summer seasons (15.1, 16.6, and 14.0, respectively) at the Shukartal site (38).In a previous study, Khanna et al., 2012 found that Post-winter had the lowest COD values (4.52±0.29 at S1 and 4.47±0.17), whereas monsoon had the highest COD values (5.74±0.37 at S1 and 5.46±0.48 at S2) (39).

Heavy metals in River Water and the Ganges River have been one of the chief receivers of industrial discharges in India. In this research study, we have analyzed eight heavy metals in Ganga river water from Panch Prayag collected samples from uttrakhand. According to our study, the As concentration was found highest at the karanprayag (KP) collection site which was 5.143.1µg/l, and lowest at 2.211µg/l at Vishnuprayag. Gupta et al., 2022 analysed Arsenic concentration was 32-153µg/L in River water collected samples and showed high levels of Arsenic in Human blood samples (66.6 to 76.9µg/L) at Kanpur city (40). In 2022, Smolikova V reported a significantly higher level of arsenic in Zenne River water in Belgium (41). Jolly et al., 2018 discovered As linked to hypertension and was found above acceptable level (10 µg/L) in water from the Shitalakhya River (42). In this study, the amount of lead in the devprayag region has been found to be in higher concentrations; 0.077µg/l. In a previous study Ahmed et al., 2010 analyzed the Buriganga River in Bangladesh and found lead in a range of 58.17 µg/l to 72.45 µg/l in the post-monsoon season (43). And Islam et al., 2015 also noted the seasonal change in Pb 138 concentration in the River Korotoa, which had a Pb level of 35.0 g/L in the Post-winter and 27.0 g/L in the Post-Summer (44). In 2018, Ahmed et al., reported significantly high levels of lead in blood, hair, and nails in automobile technicians and battery workers in certain districts of Pakistan

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(45).In this study we found mercury concentration above the acceptable limit of all collection sites. In 2007, Sinha et al., reported mercury pollution in the Ganga river in Varanasi. They analyzed mercury in the water sample and found high concentrations in soil and fish (46).

In 2019 Saha et al., reported the presence of bacteria in surface water proposes faecal pollution of the water as well as potential threats to human health. Typhoid, cholera, and diarrhoea are the most severe illnesses that could develop from bacterial pollution of the groundwater and surface water (47). According to CPCB, the Coliform contamination levels for drinking water are supposed to be zero per 100 ml, while levels for bathing are supposed to be 500 or less per 100 ml. In this present study, the Microbiological Analysis, the MPN count were found at 9, 14, and 75 at Karanprayag, Rudraprayag, and Devprayag respectively. In total plate count, we found (3×10^2), (2×10^3), and (4×10^4) at karanprayag, Rudraprayag, and devprayag respectively in collected water samples. In 2009, a similar study by Mishra et al., found a high level of faecal contamination in water due to more sewage and faecal matter through surface runoff in the Monsoon season (48). The E. coli load has been detected throughout the year and ranged between 2×10^2 CFU/ml and 1.4× 104 CFU/ml, according to a related study by Saha et al. in 2019 (47). In our study, we found a significant positive association between Physicochemical parameters and the overall bacterial count, suggesting that these variables affected bacterial growth. It is thought that the presence of these bacteria in water sources could have an adverse effect on consumers' health. In 2020, Adesakin TA found that Physicochemical factors and the total number of bacteria were found to be strongly positively correlated and suggesting that the parameters may have influenced bacterial growth (48). In Our study, various biochemical tests were performed to study the biochemical characterization of all isolates from the water samples. The high bacterial load of genera like klebsiella, Pseudomonas, Proteus, Escherichia, Citrobacter, Enterococci, and Acinetobacter were isolated from river water samples. The high abundance of bacterial isolates in a plain area water sample as seen in this study indicates the presence of high faecal contamination and health risk for human consumption due to the presence of the high pathogen in the water samples. According to WHO recommendations, there should be no faecal coliforms in drinking water and the reason for the gross contamination of waters by pathogens as observed in this study may be due to shallowness of this river water with various sewage drainage that allowed the easy entrance of particles from the surroundings. It may also be due to poor sanitary conditions around the areas. Water-borne bacterial pathogens in surface waters of Nairobi river and health implications to communities downstream Athi river recorded a high number of total coliform count, which exceeded the WHO permissible limit from water sources in river Ogun (49). The bacterial species identified from the water samples might be a result of farming activities practices occurring near the surface water by habitat of the community living 142 around this waterbody, which could result in open defecation along the farmland and there is a tendency that the runoffs from these farmlands may be washed into the River. Contamination of surface water may be due to human activities like bathing, farming, and washing, and human or/animal faces seepage run-offs enter the waterbodies and are capable of transmitting a large number of infectious diseases. The bacteria identified in this study can cause meningitis, pneumonia, and urinary tract infections in consumers. The coliforms are the primary bacterial indicator for faecal pollution in water and they are most abundant bacteria in water responsible for waterborne diseases such as typhoid, dysentery, and diarrhea and also been implicated in mortality across the world. A high abundance of bacteria such as klebsiella, Pseudomonas, Proteus, Escherichia, Citrobacter, and Acinetobacter were recorded in Ganga river water. A similar study by Pandey et al., 2014 on reservoir water reported the combination of sewage effluents, such as agricultural run-off and direct faecal contamination from natural fauna (50). As similar to our study, the high bacterial counts mean was recorded among the Post-Summer season samples could be due to runoff from the environment which can increase the microbial load especially coliforms in water. The finding is obtained by Esharegoma OS (51). Juma et al., 2016 reported high microbial counts during the rainy season compared to dry season could be attributed to increased nutrient levels occasioned by the concentration of water through evaporation during Post-Summer season (52). In the present study, we found that bacterial strains isolated from Shukratal sites with high serum arsenic levels are much more resistant to antibiotics than people with low serum arsenic levels are. In 2022, Kothari et al., sheds light on imminent threats to global health in which improper clinical, industrial, and other waste disposals increased antibiotic concentrations in the environment and increased human interference which can easily transform commensal and pathogenic bacteria found in environmental niches into life-threatening multidrug-resistant superbugs (53).

V. CONCLUSION

- The temperature values were found in maximum River Ganga at site Rudrapryag at 10.2 in the year June 2023 and minimum average value at site Vishuprayag Upstream at 4.0.
- The pH value observed for all Panchprayag was within acceptable limits. Except below, the range found karanprayag and Rudraprayag. The permissible limit was established by US Public Health Standards (USPHS, 1997) and the Bureau of Indian Standards (BIS, IS 10500:2012)
- The average Total dissolved solid was found to be highest in Vishuprayag at 110mg/dl. In addition, the minimum all-season average value at Rudraprayag is 63mg/dl. It was found that the level of TDS recorded for the considered rivers was found below the guideline set up by USPHS, which is 500 mg/l. and 600 by BIS.
- Electrical conductivity was recorded Maximum of 178.7µmhos/cm at Nandprayag. In addition, a minimum of 49.1 µmhos/cm at rudraprayag. It was noted that EC for the River has not exceeded at any study site the permissible limit of 300 µmhos/cm setup by USPHS and BIS.

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- The Absence of fecal coliform bacteria was indicated that no significant contamination has been found at all these panch Prayag collected samples.
- The study strongly advises concerned citizens and the Indian government to plan and implement reductionist approaches to the environmental management of natural water resource facilities by improving anthropogenic sources, managing natural environmental terrestrial soil erosion, and propagating proper Ganges water treatment facilities to improve river water quality for domestic use and have a positive impact.
- On public health.

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