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## Physiochemical and microbial analysis of Pre-Monsoon Ganga water from Saran district

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**Abstract-** The human activities in and around the river including the one from religious point of view have greatly affected the quality of river water. The consistent irresponsible activities have now reached to an extent where if proper measures are not taken the water pollution can never be reverted and the world would face a major biodiversity loss from aquatic ecosystem. This loss will directly and indirectly affect every living organism on the planet. The present study was performed to evaluate the Physio-chemical and microbial parameter of river water collected from three different locations. The present work shows the analysis result of before monsoon study. The parameters for quality check comprised of pH, alkalinity, electrical conductivity, total solids, hardness, alkalinity and acidity, phosphorous and sulphate content, BOD, COD, DO and elemental analysis (As, F, Na, K, Ca). The outcome of the present work evaluates that the river water is polluted mainly from the human activity and domestic waste disposal from nearby settlements. The high level of hardness ( $164.1 \pm 0.047$ ,  $196.2 \pm 0.047$ ,  $240.3 \pm 0.163$  mg/L) and total solids ( $190.3 \pm 0.125$ ,  $375.2 \pm 0.850$ ,  $440.5 \pm 0.082$  mg/L) represents that the water is being contaminated with domestic discharge that are leading to accumulation of calcium, phosphate and sulphate. The lower value of dissolved oxygen ( $8.5 \pm 0.021$ ,  $8.9 \pm 0.082$ ,  $8.3 \pm 0.050$  ml/L) and higher value for Coliform count ( $3 \times 10^5$  to  $18 \times 10^5$ ) indicated a higher microbial load in the water. The water quality can only be improved by taking proper measures regarding the waste disposal in and around the river.

**Key words:** River water, Physio-chemical, Microbial analysis, Water quality

### INTRODUCTION

Water, the most abundant, widely circulated and essential source from nature plays the crucial role in existence of life on earth. The biological and Physio-chemical characteristics of water justify it to be a healthy ecosystem for the aquatic life. The pollution in surface and ground water are a result of increased rate of industrialization, agricultural and various other human activities.<sup>1</sup> The urbanization coupled with the anthropogenic

activities has brought the ecological pressure on the aquatic environment that directly or indirectly affects the human health. The aquatic ecosystem is a reflection of environmental degradation from the different anthropogenic activities.<sup>2</sup>

The reason behind major water crisis globally is the water pollution caused by the uncontrolled and frequent dispose of industrial and domestic waste in the fresh water rivers. This practice has led to the reduction in available resources for us as well as other members of the ecosystem. The shortage of fresh water is faced by both developed

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and developing countries like India, China and African countries. Globally, 2.1 billion people are deprived of accessibility to clean water and about 4.5 billion have no access to adequate sanitation.<sup>3</sup> A recent UN report indicates that by 2025, two-thirds of the population of the world could face water stress. The scarcity of water could be in the form of physical scarcity, where water availability is limited and demands are not met, or it could be in the form of economic scarcity, where although water is available, there are no means or the infrastructure to provide required quality and quantity of water.<sup>4</sup>

A well-known fact is that many low-income nations have no industrial and environment standards and where there it is available the mitigation facilities and instrumentations are inefficient. Lack of a reliable and efficient monitoring system for the industrial emission and lack of compliance with standard protocol are the main reasons behind such scenario.<sup>5</sup> Non-restricted disposal of several tones of effluents into the lagoon, rivers and streams had become a treat to the aquatic environment. A study reported that disposal of untreated waste compost result in compromise in quality of the receiving water bodies.<sup>6</sup> The released wastewater gets distributed in the soil, destroying some micro-fauna, hence hindering the biodiversity of microbial organisms in the soil as well.

The urgent need arises for the treatment of wastewater prior to discharge as it is essential to prevent pollution. Analysis of the water from water bodies that are under industrial dispose influence or frequently under use by people for various purposes either traditional or domestic is required. Such analysis will give an insight about the level of contamination and pollution acquired by the water body. Accordingly, the measures can be designed and applied for the treatment of such water and also the prevention of further pollution. The analysis of water on Physio-chemical and microbial parameter would give insight about various parameters that directly or indirectly depict the extent of pollution in the respective water. The present study carries the similar aim where the Physio-chemical and microbial analysis of river water samples from different locations were performed to deduce their extent of pollution.

## **MATERIALS & METHODS**

### **Sample collection**

For the present study river water samples were collected from three areas that are Doriaganj, Dighwara

and Pahleza ghat of the Saran district, Bihar, India. The water samples were collected in sterile bottles and immediately brought to laboratory for analysis. The samples were stored at room temperature away from sunlight in the laboratory.

### **Physiochemical and Microbial Analysis**

For the collected water samples (3), physiochemical analysis on various parameters which include, the determination total solids, hardness, electrical conductivity, pH, alkalinity, acidity, BOD, COD, DO, temperature, quantification of phosphate, sulphate, chlorine, arsenic, fluoride, magnesium, calcium, potassium and sodium was conducted. All the analysis was performed under standard conditions.

#### **Total Solids, pH and Electrical conductivity**

The total solid in water sample was determined by gravimetric method, where the water sample placed in the pre-weighed crucible is evaporated completely. The change in weight of crucible is used to estimate the total solids content per liter using the formula mentioned below.<sup>7</sup> A digital pH meter (Mettler Toledo Five Easy Plus) was used to measure the pH value of samples. Whereas the electrical conductivity was determined by a digital conductivity meter (AMPEREUS 3 in 1) and the reading for EC value was recorded in  $\mu\text{S}/\text{cm}$ .

$$\text{Total Solids} \left( \frac{\text{mg}}{\text{L}} \right) = \frac{(A - B) \text{ in mg} * 1000}{\text{ml of sample}}$$

A= weight of crucible after drying; B= blank weight of crucible

#### **Total Hardness, Acidity and Alkalinity**

The Titrimetric approach was followed for the determination of total hardness, alkalinity and acidity in the water samples. Total hardness was determined using Eriochrome Black T as an indicator and 0.01M EDTA solution as titrant where titration was carried from wine red to blue color formed at the endpoint.<sup>8,9</sup> Total alkalinity was determined using phenolphthalein indicator and mixed indicator (100mg Bromocresol green and 20mg Methyl red). Titrant for the same was 0.02N Sulphuric acid,<sup>10,11</sup> whereas for Total acidity 0.02N sodium hydroxide was used as titrant with methyl orange and phenolphthalein acting as the indicators.<sup>12</sup>

$$\text{Total Hardness} \left( \frac{\text{mg}}{\text{L}} \right) = \frac{\text{Titre value of EDTA (ml)} * 1000}{\text{ml of sample}}$$

$$\text{Total Alkalinity} \left( \frac{\text{mg}}{\text{L}} \right) = \frac{\text{Titre value of Sulphuric acid (ml)} * N * 50 * 1000}{\text{ml of sample}}$$

$$\text{Total Acidity} \left( \frac{\text{mg}}{\text{L}} \right) = \frac{\text{Titre value} * \frac{N \text{ of sodium hydroxide (ml)}}{50} * 1000}{\text{ml of sample}}$$

### Determination of BOD, DO and COD

The analysis for Biochemical oxygen demand, Chemical oxygen demand and Dissolved oxygen was performed immediately after sample collection. For all three analyses Titrimetric approach was used. For the determination of BOD, the change in level of dissolved oxygen at day 0 and day 5 was estimated via titration approach using Alkali-Azide reagent, Starch indicator and Sodium thiosulfate as titrant.<sup>1</sup> The dissolved oxygen level was estimated as ml/L using the Winkler's methodology.<sup>7,13</sup> For chemical oxygen demand the water sample was digested with oxidizing agents i.e., sulphuric acid and potassium dichromate. The digestion step follows the titrimetric estimation using Ferroin indicator and 0.1M Ferrous Ammonium sulphate as titrant.<sup>7,12</sup> For each analysis a blank using the distilled water also analyzed simultaneously. The formula used in estimating the final values were as follows:

$$\text{COD} \frac{\text{mg}}{\text{L}} = \frac{(B - A) * 8000 * \text{Molarity of FAS}}{\text{Volume of sample}}$$

B= Titre value for Blank; A= Titre value for sample;

FAS= Ferrous ammonium sulphate

$$\text{BOD} \frac{\text{mg}}{\text{L}} = \frac{\text{Titre value of sample at } (D_0 - D_5) - \text{Blank correction} * \text{dilution factor}}{\text{Volume of sample}}$$

R, R std. and R blank = volume of thiosulfate used to titre sample, standard and blank respectively.

N IO3= Normality of standard KI solution (0.01N); V IO3= volume of standard (10ml)

E= 5598 ml O<sub>2</sub>/ equivalent; V bottle = volume of sample bottle (ml) - 200ml

DO rgt = oxygen added in reagents (0.0017ml O<sub>2</sub>/L); V rgt = volume of reagents (2ml)

### Quantitative estimation of Phosphate & Sulphate

The quantitative estimation of phosphate and sulphate in water sample was done by Spectrophotometric approach, where the estimation was done from comparison with standard curve. For sulphate the water sample was treated with a buffer solution (30 g magnesium chloride, 5 g sodium acetate, 0.111 g sodium sulfate, and 20ml acetic acid (99%) make up to 1000ml) followed by barium chloride crystals followed by optical density measurement at 420nm. Sodium sulphate (149.13±0.012mg/L, 154.64±0.021mg/L, 168.22±0.012mg/L) was used to

form the standard curve.<sup>12,14</sup> Whereas for quantification of phosphate, water sample (10ml) was treated with ammonium molybdate (2.5ml) and hydrazine sulphate (1ml). After incubation for 30 minutes at 60°C, the optical density was measured at 860nm. The solution of potassium dihydrogen phosphate (3.66±0.012mg/L, 3.06±0.017mg/L, 4.57±0.016 mg/L) was used to form standard curve.<sup>15,16</sup> The concentration of phosphate and sulphate was estimated in mg/L using the given formula:

$$\text{Sulphate or Phosphate} \left( \frac{\text{mg}}{\text{L}} \right) = \frac{\text{mg of phosphate or sulphate} * 1000}{\text{ml of sample}}$$

### Elemental Analysis (Ca, Mg, As, Na, K, F & Cl)

For the elemental analysis the quantitative determination of Calcium, Magnesium, Sodium, Potassium, and Arsenic was done by Atomic Absorption Spectroscopy following the standard protocols from Indian Standard 3025 - Methods of Sampling and Test (physical and chemical) for Water and Wastewater. For the determination of Ca, Mg, As, Na, and K the protocols from IS3025 (P-40), IS3025 (P-46), IS3025 (P-37) and IS3025 (P-45) respectively were followed. Estimation of fluoride content in sample was done by the process from Indian Standard 3025-Part 60. The electrochemical technique method is directly suitable for measuring fluoride concentrations from 0.012±0.001mg/l to 1.2±0.047mg/l. When a fluoride ion-selective electrodes comes into contact with an aqueous solution containing fluoride ions. A potential difference develops between the measuring electrode and the reference electrode. The value of this potential difference is proportional to the logarithm of the value of the fluoride ion activity in accordance with the Nemst equation. Atomic Absorption Spectrophotometer analytical instrument is based on the principle of atomic absorption spectroscopy. It is particularly effective at detecting the presence of metal ions in drinking water samples. An element is transformed into its atomic vapor when a sample solution is aspirated into a flame. Element atoms can be found in flame. Furthermore, while most atoms stay in their ground states, others are thermally activated by flame. The radiation of a particular wavelength emitted by the source, such as a hollow cathode lamp made of that particular metal, is then absorbed by the ground state atoms. Now, the source's or lamp's radiation's wavelength is comparable to that of the radiation absorbed by the atoms in the flame. Estimation of elements like Ca, Mg, As, Na, and K in water samples were done using this technique.<sup>17</sup>

The chloride content in water sample was determined by titrimetric approach by Mohr's Methodology using silver nitrate (0.01N) as titrant and potassium chromate (5%) as the indicator for color change from yellowish-green to reddish-brown endpoint.<sup>7</sup> The chloride content was estimated using the below given formula:

$$\text{Total Chloride } \left(\frac{\text{mg}}{\text{L}}\right) = \frac{\text{Titre value (ml)} \times \text{Nof Silver nitrate} \times 35.45 \times 1000}{\text{ml of sample}}$$

### Microbial Analysis

For the microbial analysis three parameters were studied for all three water samples, which include total plate count (TPC), yeast and mold count (YMC) and total Coliform count (TCC). For this Plate count agar media, Sabouraud dextrose agar media and MacConkey agar media was used for TPC, YMC and TCC respectively. The culture media was prepared with standard composition and the analysis was carried out with pour plate and spread plate methodology.

## RESULT & DISCUSSION

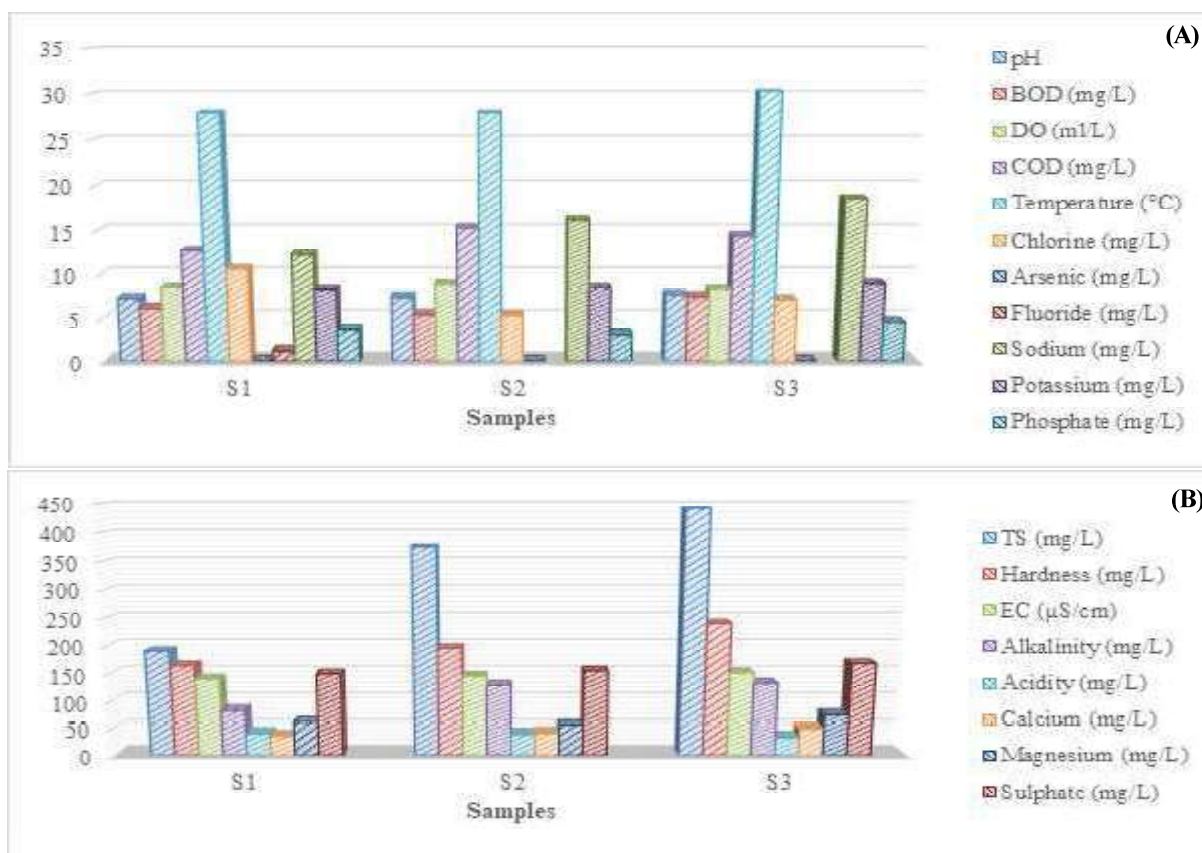
For the present study river water samples were collected from three areas namely Doriaganj, Dighwara and Pahleza ghat of the Saran district, Bihar, India and they were coded as S1, S2 and S3 respectively. The results for physiochemical analysis and microbial analysis are presented in the Table 1 and Table 2 respectively. The result shows that water sample S3 has the highest values for pH, EC, TS, Hardness and Alkalinity which are all the interrelated parameters. The sample S2 showed a lesser pollution level than other two samples by showing a higher DO (8.9±0.082mg/L) and lower BOD value (5.4±0.082mg/L). Water sample 3 also showed the high concentration than other two samples for sodium, potassium, calcium, phosphates and Sulphate as tabulated in Table 1.

**Table 1- Showing the results for Physiochemical analysis of water samples under several parameters**

S.No.	Parameters	Water sample	Mean ± STDV
1	TS (mg/L)	S1	190.3 ± 0.125
2		S2	375.2±0.850
3		S3	440.5±0.082
4	Hardness (mg/L)	S1	164.1±0.047
5		S2	196.2±0.047
6		S3	240.3±0.163
7	EC (µS/cm)	S1	140±0.082
8		S2	145.2±0.047
9		S3	151.5±0.082

S.No.	Parameters	Water sample	Mean ± STDV
10.	pH	S1	7.2±0.005
11.		S2	7.34±0.008
12.		S3	7.6±0.005
13.	Alkalinity (mg/L)	S1	82.4±0.163
14.		S2	128.4±0.047
15.		S3	132.4±0.094
16.	Acidity (mg/L)	S1	40.1±0.047
17.		S2	38.6±0.082
18.		S3	32.7±0.082
19.	BOD (mg/L)	S1	6.1±0.082
20.		S2	5.4±0.082
21.		S3	7.4±0.163
22.	DO (ml/L)	S1	8.5±0.021
23.		S2	8.9±0.082
24.		S3	8.3±0.050
25.	COD (mg/L)	S1	12.7±0.047
26.		S2	15.3±0.082
27.		S3	14.4±0.047
28.	Temperature (°C)	S1	28±0.000
29.		S2	28±0.000
30.		S3	30.3±0.471
31.	Chlorine (mg/L)	S1	10.64±0.017
32.		S2	5.33±0.012
33.		S3	7.08±0.012
34.	Arsenic (mg/L)	S1	0.012±0.001
35.		S2	0.012±0.000
36.		S3	0.012±0.001
37.	Fluoride (mg/L)	S1	0.8±0.082
38.		S2	1.0±0.047
39.		S3	1.2±0.047
40.	Sodium (mg/L)	S1	12.23±0.008
41.		S2	16.15±0.009
42.		S3	18.55±0.012
43.	Potassium (mg/L)	S1	8.22±0.012
44.		S2	8.43±0.005
45.		S3	8.97±0.031
46.	Calcium (mg/L)	S1	35.24±0.024
47.		S2	40.65±0.012
48.		S3	48.14±0.029
49.	Magnesium (mg/L)	S1	61.34±0.029
50.		S2	55.43±0.012
51.		S3	75.15±0.029
52.	Sulphate (mg/L)	S1	149.13±0.012
53.		S2	154.64±0.021
54.		S3	168.22±0.012
55.	Phosphate (mg/L)	S1	3.66±0.012
56.		S2	3.06±0.017
57.		S3	4.57±0.016

\*All the experiments were conducted in triplicates and the recorded value are expressed as mean ± STDV (standard deviation)



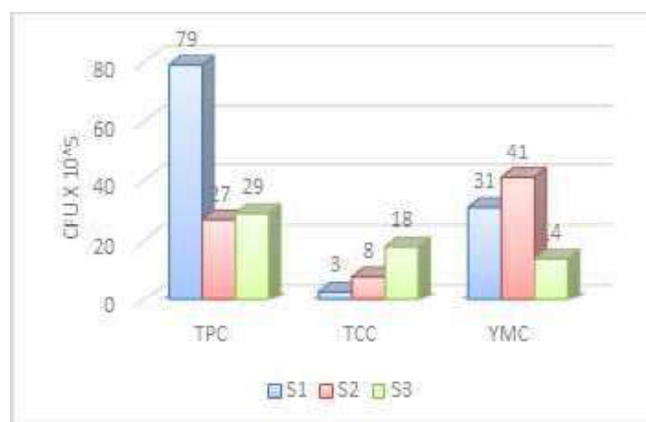
**Graph 1:** The Graph A and B depicts a comparative representation for the outcomes of physiochemical analysis for water samples, (A) shows pH, BOD, DO, COD, Chlorine, Arsenic, Fluoride, sodium, potassium, phosphate. (B) shows total solids, hardness, electrical conductivity, calcium, magnesium, sulphate, alkalinity, acidity

For the microbial analysis, highest load for Coliform count was in sample S3 ( $18 \times 10^5$  CFU/ml) followed by sample S2 ( $8 \times 10^5$  CFU/ml) then sample S1 at last with least count of  $3 \times 10^5$  CFU/ml. Whereas the total plate count with highest CFU/ml was for sample S1 and lowest for sample S2. The result for the same is given table 2 below.

**Table 2- Showing the result record for Microbial Analysis of water samples**

Total Plate Count			
Sl. No	Sample code	Count	CFU/ml
1	S1	79	$79 \times 10^5$
2	S2	27	$27 \times 10^5$
3	S3	29	$29 \times 10^5$
Total Coliform Count			
Sl. No	Sample code	Count	CFU/ml
1	S1	3	$3 \times 10^5$
2	S2	8	$8 \times 10^5$
3	S3	18	$18 \times 10^5$
Yeast and Mold Count			
Sl. No	Sample code	Yeast Count	CFU/ml
1	S1	31	$31 \times 10^5$
2	S2	41	$41 \times 10^5$
3	S3	14	$14 \times 10^5$

\*CFU= Colony Forming Units



**Graph 2:** A comparative representation for the outcomes of microbial analysis for water samples S1, S2 and S3, three parameters were studied for all three water samples, which include total plate count (TPC), yeast and mold count (YMC) and total Coliform count (TCC).

The quality of water is not a static condition of a system and hence cannot be defined by the measure of single parameter only. The water quality of India's major rivers did not improve significantly during the lockdown imposed to curtail the spread of the novel coronavirus disease (COVID-19) pandemic, according to Central Pollution Control Board (CPCB) report. The quality of water in a certain water body is affected by a range of chemical, physical and biological components. Such parameters give an insight about the level of water pollution and also enable a direct tracking of source of pollution.<sup>18</sup> The reduced rate of photosynthetic activities reduces the assimilation of carbon dioxide and bicarbonates which are ultimately responsible for change in pH.<sup>19</sup> The temperature of the samples was within the range of 28-30°C. The pH value of water samples ranged between 7.2±0.005, 7.34±0.008 and 7.6±0.005 and is under the acceptable range.<sup>18,20</sup> A sufficient supply of dissolved oxygen is must for the survival of aquatic life<sup>21</sup> and the river water samples under present study showed an approximately optimum value of dissolved oxygen that ranges from (8.5±0.021, 8.9±0.082, 8.3±0.050 ml/L). S2 has the highest DO than other two samples i.e., 8.9±0.082

The measure of the substance with acid neutralizing ability is denoted by the Alkalinity value.<sup>22</sup> The nature of materials being discharged is solely responsible for the alkalinity status of water which was highest in S3 (132.4±0.094mg/L) and lowest in S1 (82.4±0.163mg/L), and this indicates the discharge of some alkali either from the industrial or the domestic source nearby the site of sample S3 (Pahleza ghat, Bihar). On the other hand, acidity of S3 was found to be lowest i.e., 32.7±0.082mg/L. Due to the dominance of calcium and magnesium salts the water becomes hard and unfit for drinking purpose. The hardness results from surge in eutrophication of river leading to greater pollution level.<sup>23</sup> The hardness value for all the samples was found to be quite high that is in the range of (164.1±0.047, 196.2±0.047, 240.3±0.163mg/L). Highest value was measured in S3. Chlorine is highly soluble in water and is very toxic to aquatic life and its accumulation is indication for burgeoning anthropogenic pressure.<sup>24</sup> In present study the concentration of chlorine was not very high and ranged between 5.33±0.012mg/L and 10.64±0.017mg/L.

The presence of higher level of solids in water increases the density of water and thus, reduces the

solubility of gas and diminishes the water for drinking or irrigation purpose. The present study reports a higher value for Total solids in the two samples (S2 and S3) ranging from 375.2±0.850 to 440.5±0.082mg/L while the sample S1 showed a TS value of 190.3 ± 0.125mg/L, that is lowest amongst all the samples. To deduce the number of organic compounds, present in water, the biochemical oxygen demand is estimated. For oxidation of more organic matter more oxygen is required by the microbes.<sup>25</sup> BOD was estimated to be the highest in S3 i.e., 7.4±0.163ml/L.

Due to pollution brought on by human activity, the concentration of metals in the environment, particularly in water resources, increases to dangerous levels. Industrial wastewater, agricultural drainage water, sewage water, and oil pollution are the main human activities that cause water contamination. Chlorine concentration was found to be highest in S1 sample i.e., 10.64±0.017mg/L. F, Na, K, Ca, Mg, Mn, S was found to be highest in S3 water sample. Phosphate concentration was highest in S1 i.e., 3.66±0.012mg/L. Not only the Physio-chemical parameter but also the biological parameter (Microbial load) affects the quality of water. Total plate count was measured to be more in S1, and least in S2. Water is taken fit for drinking when the Coliform load is around 4 Coliform/100ml. The Coliform count gives the direct insight about the deleterious effect of pollution caused through the human settlements near the respective water bodies.<sup>26</sup> In present study all water sample showed the high population of Coliform which ranged from 3 ×10<sup>5</sup> to 18 ×10<sup>5</sup>. In which, S3 has the highest TCC i.e., 18×10<sup>5</sup>. This high Coliform content indicated that these water bodies are being contaminated with human excreta coming from the nearby settlements or through the open defecation near the water bodies. Often fungal contamination in the samples can be traced to specific sources such as standing water, dead plant material, contamination from outside sources, or improper drying and curing. Yeast and mold count was measured that showed the highest count in S2 (41×10<sup>5</sup> CFU/ml) and lowest count in S3 (14×10<sup>5</sup> CFU/ml).

Traditional water purification techniques include winnowing sieve filtration (common in Mali), cloth filtration (common in villages in India, Mali, and the southern part of Niger), clay vessel filtration (common in Egypt), plant material filtration (common in Tamil Nadu and Kerala, India), and the Jempeng stone filter method (used in Bali, Indonesia). Solar Water Disinfection

(SODIS), LifeStraw, Nanofilter, Ceramic water filter, Bio-sand filter, and Kanchan Arsenic filter are examples of contemporary water treatment techniques. In contrast, current techniques of water treatment use updated research technologies in the removal of pathogens and hazardous substances to make drinking water more potable and safer. Traditional methods of water treatment use rudimentary methods that have shown efficient in relation to filtering. Adeniyi.<sup>27</sup>

## CONCLUSION

The present study concludes that the quality of water from all three sites Doriaganj, Dighwara and Pahleza ghat of the Saran district, Bihar, India, is not at all fit for drinking. The high total solids and hardness value and high population of Coliform in these water samples make it non-potable. This condition of the water is mainly due to the domestic waste discharge from the nearby settlements and also due to open defecation nearby these water bodies. The low level of BOD and DO indicates the higher microbial load in them. Such condition can only be improved by checking the type of discharge in the river and also controlling and improving the waste disposal and human activities nearby the river. It requires a proper authorized measure to overcome this issue and improve the quality of this river water. For treating heavy metals pollution, many laboratory and field procedures have been suggested, including electro-osmosis, ion exchange, electro-kinetic sludge activation, and phytoextraction. Different techniques, including flotation, membrane filtration, aeration, precipitation, coagulation-flocculation, ion exchange, and electrochemical treatment, are traditionally used to remove water pollutants. It has been determined that methods for the management of aquatic resources should be taken into account within the dynamics of the ecosystem conditions so that their exploitation for human needs continues to be sustainable. The protection of human health and the well-being of aquatic ecosystems is a fundamental concern throughout the world.

## CONFLICT OF INTEREST

There is no conflict with other interest in the manuscript content

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