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Comprehensive Study Of River Narmada At Dindori With Special Reference To Water Borne Pathogens.

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Abstract:- A comprehensive study was undertaken to analyze the physico-chemical and bacteriological characteristics of river Narmada at Dindori (M.P.), India from April to May, 2025, in order to assess its pollution status water sample were collected from five sampling stations and their physico-chemical and microbiological variables like p^H , temperature were determined as per BIS,2012 and APHA,1998. A total of 52 bacteria were isolated and tentatively identified. The virulence factors of these isolated bacteria quantitatively measured by plate assay method and their antibiotic resistance pattern was studied by standard disc diffusion method. However, physico- chemical variables did not indicate any pollution of river Narmada as they were well within limits but analysis of bacteriological variables indicate the high organic pollution of river Narmada. Analysis of virulence of environmental bacterial isolates revealed their potential to cause opportunistic infections these environmental isolates also exhibited the phenomena of multiple drug resistance against commonly used antibiotics from the present study it was inferred that Shankar ghat was the most polluted station of river Narmada and its water could be considered unsafe for drinking purpose due to high bacterial load. The physico-chemical and bacteriological quality of water needs to be carefully assessed for checking and solving the problem of pollution and such data would also be helpful in phylogenetic diversity study.

Key words:- Physico-chemical, River Narmada, Bacteriological, Virulence

Introduction:- Water is life on earth and it has no substitute. For most part of the earth and for much of its 4.6 billion year geological history most of the planetary water has been inexistence as liquid water. (Rajamani, 2005). Polluted water contains solids and dissolved organic compounds that impart an offensive odour and serve as an excellent medium for the growth and multiplication of microorganisms. (Aneja, 2003). Typical organisms found in different types of water belong to fungi, protozoa, algae, bacteria, actinomycetes and viruses. For example, the causative agents of dysentery (*Entamoeba histolytica*), typhoid fever (*Salmonella typhi*), hepatitis virus (**hepatitis A virus**). According to the world health organization 1980, at least 30,000 people die every day in developing countries of the world because of unsanitary water supplies. (Aneja, 2003). The greatest impact of water pollution is seen in drinking water and recreational water as large number of potentially pathogenic organisms has been encountered in recreational waters. (Joshi and Rajput, 1992 and Sharma and Rajput, 1996; 1998). Virulence provides quantitative measures of the pathogenicity or the likelihood of causing disease. In India the national standard bodies (NSBs) like the BIS (Bureau of Indian standards) have formulated certain drinking water standards. Which are being followed by different authorities. River Narmada is the fifth largest fresh water river of India. Its total length is 1312 km of which 1077 km flow through Madhya Pradesh. It is an important source of drinking water for Dindori.

The present investigation was carried out to study the physico-chemical analysis in the river Narmada at Dindori M.P., the water was sampled from April to May 2025 from Five sampling stations of river Narmada at Dindori (Upstream of kharighat, Damghat, Shanighat, Shankarghat, Hanumanghat). Four major waste stream discharge their wastes in River at Damghat and Shankarghat and these two sites is extensively used by people for bathing and washing of clothes daily, the catchment area of this site is thickly populated. The survey was carried out on five different sites at River Narmada according to which the people leaving and using the water for drinking and other purpose and suffered from all following various diseases like diarrhea, dysentery, were observed.

Water of Narmada at Dindori was analyzed with the following objectives.-

- Sampling:- Different sites of Narmada at Dindori were selected and periodically water samples were withdrawn for various analyses.
- Physico-chemical analysis:- To check for various physico-chemical parameters such as p^H , temperature etc. Which affect bacterial growth.
- Microbiological analysis:- To determine the level of contamination by checking the number and type of microorganisms found in the water from these sites.
- Isolation -: Bacteria would be isolated and cultured on selective media for tentative identification.
- Study of virulence:- Virulence factor of the isolated bacteria was qualitatively measured by plate assay method in order to assess the pathogenic potential.

Materials and methods: - Dindori is a district of Madhya Pradesh of central India. It was created on 25th May, 1998 with total 927 villages. The district is situated between the latitude 22.17N and 23.22N and longitude 80.35E and 80.58E. According to the 2011 census Dindori district has population of 704,218 roughly equal to the nation of Bhutan.

In the present study five location of River Narmada have been sampled for analysis of water quality and was assessed during the month of April and May 2025 .These were:- Upstream of kharighat, Damghat, Shanighat, Shankarghat and Hanumanghat.

Sampling procedure: - (APHA, 1998) All the five sites were sampled from April to May 2025. The water was analysed for physico-chemical and microbiological parameters.

Surface water from each site was collected in sterile BOD bottles for physico-chemical analysis and in separates a sterile BOD bottle for microbiological analysis. All the bottles were brought to laboratory under ice cold conditions in an ice box at 4⁰C temperature for further analysis and were processed immediately. All the physico-chemical and bacteriological parameters were studied following (APHA, 1998) and (BIS, 2012) standard methods. For all bacteriological studies, prepare media (Hi media) were used.

• PHYSICO-CHEMICAL ANALYSIS:-

Temperature – The air and water temperature were noted on the sampling spot, with the help of the thermometer and the respective readings were noted.

P^H - pH of water sample was measured with the help of digital tripar meter model 181E. For this, the instrument was calibrated with the known buffer solutions (4.0, 7.0 and 9.2) and then p^H readings were taken.

Total dissolved solids- To estimate the amount of total dissolved solids 100ml borosil beakers were washed and dried in a dessicator. Then, their initial weights were taken in the digital electronic balance contech model CA 123. Then 50 ml sample were poured in their respective beakers and kept on hot plate for evaporation. Thereafter, the beakers were cooled in the dessicator and were weighted again.

With the help of initial and final weights the amount of total dissolved solids was calculated in mg/lit.

$$\text{Total Dissolved Solids in mg/L} = \frac{\text{Final weight} - \text{initial weight}}{\text{Volume of sample in ml}} \times 10^6$$

Total Alkalinity – 50ml of sample taken in a 100 ml conical flask. If the p^H of the sample was over 8.3 then 2 to 3 drops of phenolphthalein indicator was added to it titrated with standard sulphuric acid solution till the pink colour observed by indicator just disappears (p^H 8.3). The volume of sulphuric acid used was recorded and 2 to 3 drops of methyl orange indicator was added in the same flask.

It was then titrated with standard acid light pink colour (p^H 3.7). The volume of sulphuric acid used was recorded.

$$\text{Total Alkalinity in mg/L} = \frac{(A+B) \times N \times 50,000}{V}$$

A = ml of standard sulphuric acid used to titrate to p^H 8.3

B = ml of standard sulphuric acid to titrate to p^H 3.7

N = Normality of H_2SO_4

V = Vol. in ml of sample taken in for test.

Total hardness – Estimated by titrimetric method (APHA- 1998) 50 ml of sample taken in a conical flask and 1.2 ml of ammonium buffer solution was added and properly mixed. Then 2-3 drops of Eriochrome black T was added to get a reddish colour resultant were red coloured solution was titrated with standard 0.01M EDTA solution to get a blue coloured end point.

$$\text{Total hardness as mg CaCO}_3/\text{L} = \frac{\text{ml of titrant} \times 10^3}{\text{ml of sample}}$$

Calcium hardness: - It was analysed by titrimetric method (APHA – 1998) 50ml of sample taken in a beaker and 2 ml of NaOH solution was added. After proper mixing a pinch of murexide indicator was added the pink colour solution obtained was titrated with standard 0.001M EDTA solution to get a violet colour end point.

$$\text{Ca hardness as mg CaCO}_3/\text{L} = \frac{\text{ml of titrant} \times 10^3}{\text{ml of sample}}$$

Magnesium hardness – (APHA- 1998) it can be calculated directly by using the following formula:

$$\text{Mg hardness as mg/L} = \text{Total hardness} - \text{Ca hardness}$$

• MICROBIOLOGICAL ANALYSIS OF WATER

Yeast and mould – 1ml of water sample was taken aseptically in sterilized Petri dishes and 20ml of yeast extract dextrose, chloramphenicol agar medium was passed and the inoculums was mixed by rotating the plates for the uniform distribution of sample. The plates were incubated at $28 \pm 5^\circ C$ for 4 to 7 days. When the colonies appeared yeast and mould colonies were counted by colony counter.

Detection of water borne pathogens – 1ml of water sample was taken aseptically in sterilized plates and 20ml of various solid enriched agar media (differential media) were poured and allowed to solidify, this were than incubated for 24 hours at $37^\circ C$.

Table-1: Colony characteristics of water borne pathogens on differential Agar media.

Medium	Colony characteristics	Possible organism
Eosine methylene Blue agar medium (EMB)	Blue- black with or without green metallic seen	<i>E.coli</i>
Glutamine starch phenol/red agar (GSP)	Shiny and pink coloured colonies. Circular yellow coloured colonies	<i>Pseudomonas sp.</i> <i>Aeromonas sp.</i>
Mannitol salt Agar medium	Circular yellow coloured colonies Surrounded with a yellow hallow	<i>Staphylococcus sp.</i>
Thiosulphate citrate bile salt sucrose agar medium (TCBS)	Yellow colonies with entire round margins.	<i>Vibrio sp.</i>
Xylose lysine deoxycholate agar medium (XLD)	Pin headed red coloured colonies Black centered pin headed colonies	<i>Shigella sp.</i> <i>Salmonella sp.</i>

- **Identification of isolated bacteria** – The various bacteria developing on the different solid enriched agar media were isolated and streaked on nutrient agar slants. These were then identified up to the species level by performing Gram staining and the following biochemical tests. (Aneja, 2003)

Indole test, Methyl red test, Voges-proskauer test, Citrate utilization test, Carbohydrate Fermentation test, Gelatinase test, Triple sugar iron agar test, Nitrate reductase test

Urease test, Amylase test, Motility test, Growth at 37°C and 20°C

Gram staining (Aneja, 2003) – A smear of bacterial sample was prepared on a slide and heat fixed. Then it was flooded with crystal violet for 1 min. and treated with Lugol's iodine for 30 seconds. Then slide was destained with alcohol for 30 seconds and counterstained with saffranin for 30 seconds. Bacterial cells appearing purple are Gram positive while those appearing pink are Gram negative.

- **Identification of bacteria** – Identification of the isolated bacteria up to species level was done on the basis of biochemical tests using Bergy's manual of systematic bacteriology (Kreig and Holt, 1994) and PIB (Probabilistic identification of bacteria) computer kit (Bryant, 1993).

After identification the pathogenic potential of the isolates was qualitatively ascertained by plate assay techniques –

- I. **Gelatinase activity** – (Jacob and Girstein, 1960) Screening of identified bacteria was done by plate assay method for their gelatinolytic and Gelatinase activity. Gelatin agar medium was point inoculated with the test bacterium and incubated for 24 hrs. at $37 \pm 2^\circ\text{C}$. After incubation the culture plates were flooded with mercuric chloride reagent and kept undisturbed for a few minutes up to formation of clear zone around the colony indicates a positive gelatin hydrolysis where as negative test is indicated by no zone formation. The enzyme activity in terms of zone size can be estimated by the following formula –

$$\text{Gelatinase activity} = (\text{Diameter of the zone} + \text{colony}) - (\text{Diameter of the colony})$$

- II. **Amylase activity**– The bacterial isolates were point inoculated on starch agar medium and incubated for 24 to 48 hrs. at $37 \pm 2^\circ\text{C}$. After incubation the colonies were flooded with iodine solution and the plates were left undisturbed for a few minutes. Formation of clearing zone around the colonies indicated a positive amylase test. A negative reaction is indicated by blue black colouration of the medium.

The enzyme activity in terms of zone size can be estimated by the following formula –

$$\text{Amylase activity} = (\text{Diameter of the zone} + \text{colony}) - (\text{Diameter of the colony})$$

- III. **Lipase activity** – Tributyrin agar medium was point inoculated with the test bacterium and incubated for 24 hrs. at $37 \pm 2^{\circ}\text{C}$. After incubation, a clear zone around the colony indicates a positive lipid hydrolysis whereas negative test is indicated by no zone formation

The enzyme activity in terms of zone size can be estimated by the following formula-

$$\text{Lipase activity} = (\text{Diameter of the zone} + \text{colony}) - (\text{Diameter of the colony})$$

- IV. **Hemolytic activity** – The hemolytic pattern of isolated bacteria was studied on blood agar medium (Aneja, 2003). The bacteria were point inoculated over the medium supplemented with defibrinated blood and were observed for the development of hemolytic zones after 24 hrs. incubation for $37 \pm 2^{\circ}\text{C}$.

Antibiotic sensitivity test – Muller- Hinton agar plates were seeded with the antibiotic disc (as per Bauer-Kirby, Disc diffusion method) using sterile cotton swabs for the antibiotic sensitivity. The plates were incubated in the test, Penicillin (P) $10\mu\text{g}$, Ampicillin (A) $10\mu\text{g}$, Amoxicillin (AMO) $30\mu\text{g}$, Cephalexin (CP) $30\mu\text{g}$, Ciprofloxacin (CI) $5\mu\text{g}$, Erythromycin (E) $30\mu\text{g}$, Gentamicin (G) $10\mu\text{g}$. clearing zones were observed around the antibiotic disc which gives the indication of the susceptibility of the microbes swabbed using the zone size interpretative chart the resistance and sensitivity was interpreted.

• RESULTS AND DISCUSSION. –

During the present investigation the water of river Narmada at Dindori was analysed for its physico-chemical and bacteriological parameters during the month April to May, 2025 and following results were obtained. The temperature of water body of river Narmada ranged from $23-25^{\circ}\text{C}$ and air temperature of $32-38^{\circ}\text{C}$ in April and May. Since temperature is influenced by the seasons, there are corresponding shifts in the microbial flora of water in water bodies.

Table-2: Temperature of Narmada water at Dindori during April to May 2025

S.NO.	Sampling sites	Temperature($^{\circ}\text{C}$)			
		April		May	
		Air Temp.	Water Temp.	Air Temp.	Water Temp.
1.	Upstream of kharighat	32	23	38	24
2.	Damghat	34	24	37	24
3.	Shanighat	33	24	38	25
4.	Shankarghat	34	24	38	24
5.	Hanumanghat	34	24	38	25

The p^{H} was found at 7.8 and 7.9 in upstream of kharighat in April and May and 8.3 at Hanuman ghat in the month of April. These were found to be within the desired range of 6.5 to 8.5 as per BIS, 2012.

Table-3: p^H of Narmada water at Dindori during April to May 2025

S.NO.	Sampling sites	p ^H of water sample(Units)	
		April	May
1.	Upstream of kharighat	7.8	7.9
2.	Damghat	8.1	8.2
3.	Shanighat	8.1	8.2
4.	Shankarghat	8.2	8.1
5.	Hanumanghat	8.3	8.2

According to BIS, 2012 the limit of total dissolved solids is at a maximum of 500 mg/L and it was observed that at all the sampling sites of Narmada water, the amount of total dissolved solids exceeded this limit except in the upstream region of kharighat. Since there is no human settlement in the surroundings of this area, while the water of Shankarghat is highly contaminated by human and animal activities, thus the value of total dissolved solids was maximum at 820 mg/L.

Table-4: Total Dissolved Solids of Narmada water at Dindori during April to May 2025

S.NO.	Sampling sites	Total dissolved solids mg/L of water sample	
		April	May
1.	Upstream of kharighat	540	560
2.	Damghat	700	700
3.	Shanighat	750	780
4.	Shankarghat	800	820
5.	Hanumanghat	650	650

According to BIS, 2012 the desirable range of total alkalinity is up to 200 mg/L and the values at all sampling sites was found to be within the permissible limit both during April and May.

Table-5: Total Alkalinity of Narmada water at Dindori during April to May 2025

S.NO.	Sampling sites	Total dissolved solids mg/L of water sample	
		April	May
1.	Upstream of kharighat	140	140
2.	Damghat	192	192
3.	Shanighat	200	200
4.	Shankarghat	192	200
5.	Hanumanghat	192	192

The total hardness of water of river Narmada at all the sampling sites ranged from 90 to 100 mg/L during April to May. It was found to be maximum (100mg/L) at Shanighat in both April and May and minimum (90 mg/L) at upstream of kharighat along with the Hanumanghat in the month of May. The calcium hardness in all the sampling sites ranged from 60 to 92 mg/L with maximum of 92 mg/L at Shanighat in the month of May. The magnesium hardness of water at all the sampling sites ranged from 6 to 30 mg/L. It was minimum (6 mg/L) in Damghat in the month of April and at Hanumanghat in the month of May while maximum magnesium hardness was recorded to be 30 mg/L in the upstream of kharighat in the month of May. The values of magnesium hardness at all the sampling sites were found to be within the permissible limit of 30 mg/L as per (BIS, 2012) with slightly high calcium content.

Table-6: Total hardness, Ca hardness & Mg hardness of Narmada water at**Dindori during April to May 2025**

S.NO.	Sampling sites	Total hardness, Ca hardness & Mg hardness mg/L of water sample					
		April			May		
		Total hardness	Ca hardness	Mg hardness	Total hardness	Ca hardness	Mg hardness
1.	Upstream of kharighat	92	64	28	90	60	30
2.	Damghat	92	86	06	92	84	08
3.	Shanighat	106	92	08	100	90	10
4.	Shankarghat	96	80	16	96	84	12
5.	Hanumanghat	96	84	12	90	84	06

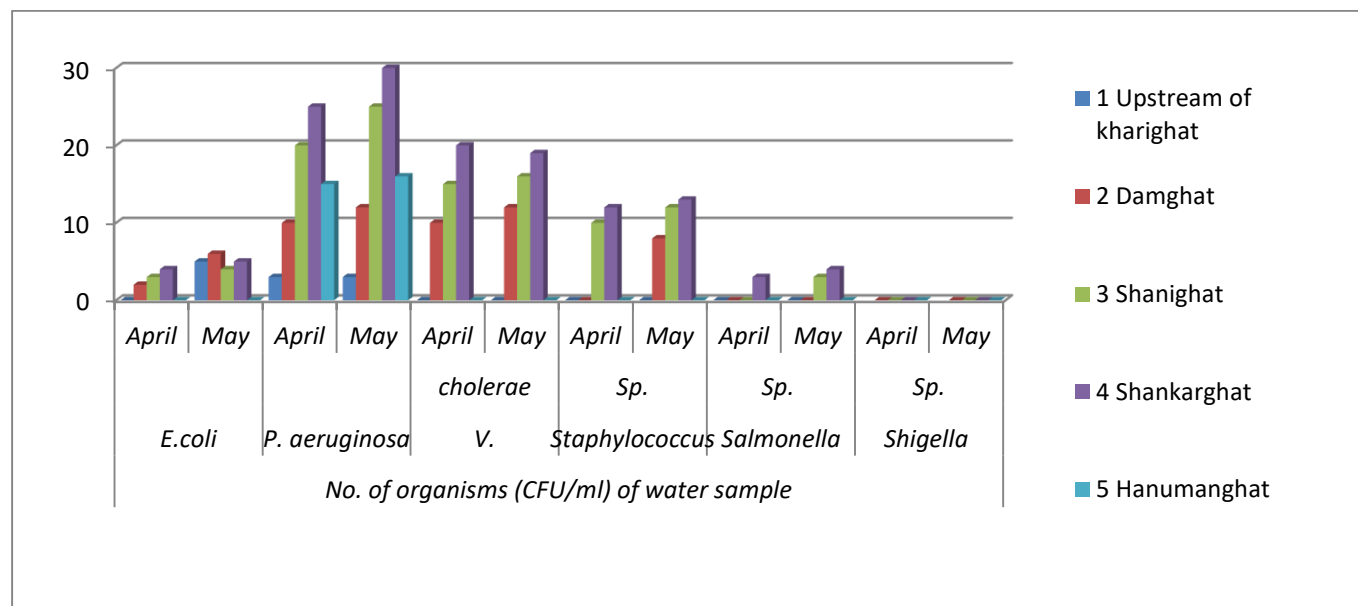
The disposal of sewage from increasingly urbanized population is causing problem in many parts of the world. Thus a careful microbiological assessment of the river water is required to ensure public safety. The yeast and mould count of water of river Narmada at Dindori during the month of April was nil. This was the least count among all the sites. The BIS, 2012 stated that the yeast and mould count of water used by humans should be nil so that there is a least chance of skin diseases. According to the present study Shankar ghat showed a high level of yeast and mould count because it receives solid organic wastes such as house hold wastes, flowers, coconut coir etc. It also reveals that the fact population using such water might suffer from superficial skin disease.

Table-7: Yeast and Mould count of Narmada water at Dindori during April to May 2025

S.NO.	Sampling sites	Yeast and Mould count CFU/ml of water sample	
		April	May
1.	Upstream of kharighat	00	04
2.	Damghat	13	14
3.	Shanighat	24	24
4.	Shankarghat	26	27
5.	Hanumanghat	20	19

On performing the bacteriological analysis for the detection of different water borne Micro-organisms at it was observed that *E.coli* was maximum at Damghat and Shankar ghat in the month of April whereas it was again at maximum levels at Damghat during the month of May. The most abundant bacterial spp. Encountered during the month of April and May were *Pseudomonas aeruginosa*, and *vibrio cholerae*. It can be concluded from these results that at all sampling stations, river water is being continuously mixed with polluted water that has resulted in such high CFU for these water borne pathogens. According to BIS, 2012 water should be totally free of these pathogens, thus these outcomes present an alarming situation as the river water from these stations is being used for various human recreational purpose. Moreover, such highly contaminated water can be a source of sporadic outbreaks of various water borne disease.

Fig-1:- Bacteriological analysis of Narmada water at Dindori during April to May 2025



The hemolytic activity was tested for different bacteria isolated from different stations of river Narmada at Dindori. It was observed that out of 42 isolates tested about 64.3% (27 isolates) showed α hemolysis and the remaining 35.7% (15 isolates) showed the γ hemolytic pattern. β hemolysis was not observed in any of the isolated bacteria. However, α hemolysis also is an indicator of pathogenesis as the bacteria are potent enough to cause opportunistic infections in several individuals. (Fig-2)

Antibiotic sensitivity assessment of isolated bacteria spp. was performed as all the tested isolates were resistant towards penicillin while the other antibiotics exhibited variable drug resistance pattern 45 isolates showed resistance to Erythromycin, Amoxicillin, Cephalexin and Ampicillin was resisted by nearly 75% of the isolates. Gentamicin was the most effective antibiotic. It can be said that environmental isolates showed multiple drug resistance pattern. (Fig-3)

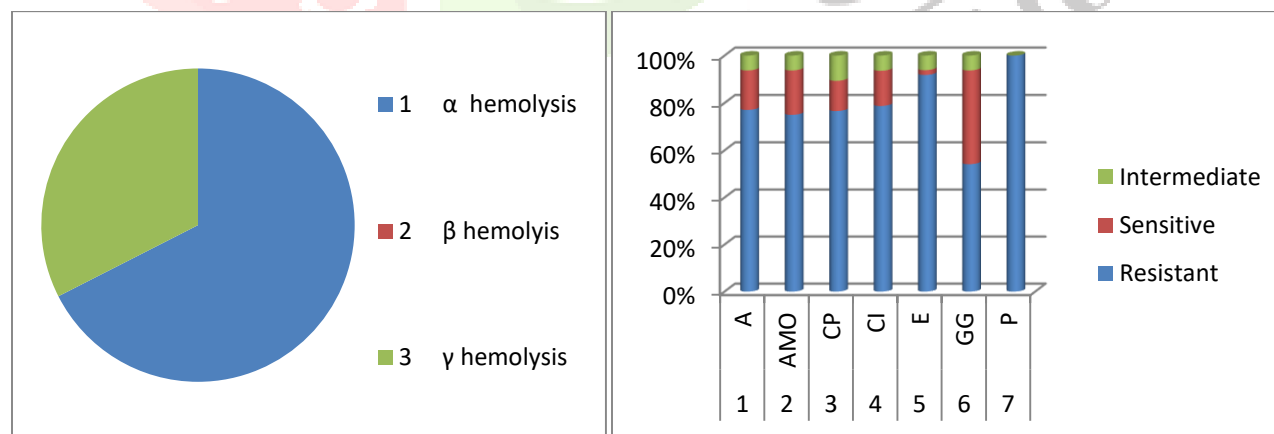


Fig. 2 Hemolytic activity was tested for

Different bacterial isolates.

Fig.3 Multiple drug resistance pattern of bacterial

Isolates. (A= Ampicillin, AMO=Amoxicillin

CP=Cephalexin, CI=Ciprofloxacin, E=Erythromycin

GG= Gentamicin, P= Penicillin.

• SUMMARY AND CONCLUSION:-

The present work focused on “Comprehensive study of river Narmada at Dindori with special reference to water borne pathogens”. Sampling for this five sites was conducted for two consecutive months, April and May 2025 and the water sample were analysed for selected physico-chemical and microbiological variables such as p^H , temperature, total dissolved solids, total hardness, Ca hardness, Mg hardness, total alkalinity and bacteriological analysis as per standard methods prescribed in APHA (1998) and BIS (2012).

Conclusively the present undertaking revealed that in the Dindori region, Shankarghat is most polluted station of river Narmada and its water can be considered unsafe for drinking purposes. The bacteria isolated from these stations poses pathogenic potential and also exhibited the phenomena of multiple drug resistance. Hence such poor water quality might give rise to water born outbreaks. It is proposed that an extensive planned and systematic survey of river Narmada should be conducted at different sites to analyze the physico-chemical and bacteriological quality of water which can further help in solving the problem of pollution and reduce the chances of human diseases.

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