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# Isolation and Identification of Bacteria from Hasdeo River Water in Champa Region and Level of Microbial Pollution

**Mrs. Rajshree Singh[1], Dr. Shweta Sao[2]**

[1]Assis. Professor Microbiology, Shri Agrasen Girls College, Korba
[2]H.O.D. Microbiology, Dr. C.V.Raman University, Kota, Bilaspur

**Abstract:** *River Hasdeo is life line for many district and villages that came across its bank. The water is used for various purposes like as drinking water, in agriculture etc. hence one of the greatest concerns for the water consumers with respect to the quality of drinking water is contamination with pathogenic microorganisms and various industrial effluents. Microbiological river water quality is obligatory for use-related aspects such as for drinking water production, irrigation or recreation according to technical standard. The present study was an attempt to understand the main cause of microbial contaminant of river Hasdeo in Champa region and microbial contaminant level different weather conditions in relation with physical parameter like pH, temperature, BOD, COD, DO.*

**Keywords**: *Water quality, indicator microorganisms, microbial pollution.*

## 1. INTRODUCTION

Environment is known to be the group of geographical setup of a region that includes both biotic and abiotic components of all the habitats i.e. aquatic, terrestrial. Water is one of the most important factor that play a crucial role in any change in environment by affecting both flora and fauna of the nearby places and its contamination in can create great harm to human beings as well as to the environment.

The quality of river water is greatly influenced by industrial, agriculture and plethora of human activities which affect its physic-chemical characteristics and microbial qualities [1]. Major industrial pollutant to river Hasdeo are coming from the NTPC, BALCO, CSEB thermal power and coal, bauxite mines wastes those were continuously discharged their waste in the Hasdeo river [2]. These effluents contain harmful toxic metal trace and other toxic elements. Apart from this sewer system, water discharge runoff from agriculture lands adds to pollution level. The continuous emanations of treated and untreated wastes from city drainage play a vital role in toxicating the river water quality of this region. Many studies showed the water pollution by microbial contaminant effect the growth of human pathogen. Source of entry of microbial pathogen into riveer includes rain water surface-runoffs, strom sewar spillages or overflow, discharge of untreated effluent came poor sanitation and poor treatment of waste water and catastrophic floods [4; 5]. and thus infecting and killing of populations of both human and animal [6] The impact of river pollution on human health depends mainly on the water uses, as well as the concentration of pathogens in the water [7].

Coliform bacteria, Escherichia coli and coliphages are normally used as indicators of water quality [8]. Two bacterial groups, coliforms and fecal streptococci, were used as indicators of possible sewage contamination [9] because they are commonly found in human and animal feces and are generally not harmful themselves and indicate the possible presence of pathogenic bacteria, viruses, and protozoans that are enteric [10]. Their presence in streams suggests the presence of pathogenic microorganisms [11], which makes *E.Coli* to be used as an indicator of fecal pollution for decades [12]. Detailed knowledge of microbial pollution in river water is crucial for watershed management activities to maintain safe waters for recreational and economic purposes [13]. The concentrations of heterotrophic bacteria correspond commonly with contamination by organic matter [14] that changes with the weather condition.

## 2. MATERIALS AND METHODS

### 2.1. Collection of Water Samples

Ten samples of water from the river Hasdeo were collected from Champa, during the period from June 2012 and September 2012. The samples were collected aseptically in sterile 500mL Durans

Schoot glass bottle using the standard procedure and methods prescribed by American Public Health Association [15].

### 2.2. Total Coliform count by Most Probable Number (MPN) Test

Most Probable Number (MPN) test was performed to assess the domestic pollution level in the selected area for total Coliform count. The technique involves three successive steps namely, presumptive test, confirmatory test and completed test. This method has direct application in quantification studies for media and for alternate microbiological methods. The number of broth tubes producing gas used to determine the statistical range of coliform. Confirmed and positive test are used to calculate the MPN.

### 2.3. Isolation by Membrane Filtration

Membrane filter techniques is considered to be most accepted techniques as a standard method for the microbiological examination of sewage water. Each sample was analyzed in triplicate.

### 2.4. Isolation of Bacterial Species from Water Samples

By using inoculation needle, the samples were streaked for the growth of isolated colonies on nutrient agar. Then the plates were incubated at 37°C for 24 hrs for bacteria. Once a bacterium has been obtained in pure culture, it has to identify. Identification of microorganisms is based on several criteria like morphology, staining reaction (gram staining), microorganism's shows diverse culture characters and the diversity also depends on the type of medium used for culturing. Growth parameter such as size, colour, texture, margin, elevation, consistency etc. is of value in identification of colonies. The characters morphology and staining reaction helps in preliminary identification.

### 2.5. Biochemical Characterization of the Isolates

The biochemical tests performed include Indole production test, Methyl red test, Vogues-Proskauer test, Citrate Utilization test, Oxidase production test, Catalase production test, Triple Sugar Iron Agar test, Carbohydrate Fermentation. These biochemical tests were performed as per standard Microbiological methods [16].

### 2.6. Determination of Water Quality Parameter

The water quality assessment parameters analyzed were; pH-measured using standard pH meter, BOD by dissolved oxygen loss method and chemical oxygen demand (COD) by potassium dichromate method.

## 3. RESULTS

The present study was undertaken to characterize the physicochemical parameters like pH, Temperature, BOD, COD, DO and comparative study of level of microbial contamination in water samples of Hasdeo River in the two different weather conditions i.e. June and September.

## 4. ENUMERATION OF BACTERIAL POPULATION

Data reflects the presence and percent concentrations of *E.coli, Klebsiella pneumonia, Salmonella typhi, Pseudomonasa aeruginosa, Shigella flexneri, Yersinia entrocolitica*. Generally, higher level of microbial pollution of *Klebsiella pneumonia, Streptococci sp., Staphylococcus aureus, Yersinia sp., Enterococcus faecalis* was observed in the month of June because of settled waster and optimum temperature for the growth of bacteria. Bacterial population of *Klebsiella pneumonia, Shigella sp., Salmonella sp*. were increased profoundly in the month of September, Low rainfall was associated with exponential increases in concentrations of indicator bacteria while the effect on turbidity attenuated with very heavy rainfall.

## 5. PHYSICAL PARAMETER

### 5.1. pH

pH which is a an important ecological factor that provides an important information about many types of geochemical equilibrium or solubility calculation that affect the flora and fauna of the aquatic system. Most of the aquatic organisms are adapted to an average pH and do not withstand abrupt changes. The pH values fluctuated between **6.8 to 7.3 in June and 7.1 to 7.5 in September (Table 2)**. The pH shows slightly alkaline trend with the change in season from June to September. Generally pH of water is influenced by geology of catchments area and buffering capacity of water.

### 5.2. Temperature

The temperature was recorded in between the range of 28.5 **to 30.5°C** during the period of June and changes to **27.5 to 28.5°C** in September **(Table 2)**. In the month of June higher value of water temperature observed in the due to the extreme hot weather in summer prevailed during the period of investigation. Whereas in September very less variation in temperature was recorded due to humidity and no change in environmental temperature.

### 5.3. Biological Oxygen Demand (BOD)

The BOD that indicate microbial pollution in water ranges from 4.4 to 5.4 mg/l in the month of June in the river water samples which was almost two fold higher than that of September i.e 2.1 to 2.5 mg/l. Heavy rain fall in the month of September was found to be reason for the reduction in the BOD for the month of September.

### 5.4. Chemical Oxygen Demand (COD)

Chemical oxygen demand represents the oxygen required for chemical oxidation of organic pollutant both degradable and non-biodegradable matter. The COD was recorded in June 25.6 as compared to 14.2 mg/l. the variation was because of rain fall in the month of September.

### 5.5. Dissolved Oxygen (DO)

Dissolved oxygen, an important parameter for the assessment of water quality and reflects the physical and biological processes prevailing in the water. Water saturated with oxygen should have taste and should have a solubility of **4.7mg/l at 30°C.** The DO of the Hasdeo river was observed **5.8 mg/l n June and 5.1mg/l in September,** in both the DO was lower than the permissible quantity, which are unfit for the aquatic animal.

**Table1.** *Water samples collected in June & September 2012*

| **Samples** | **Names of bacteria** | |
|---|---|---|
| | **June 2012** | **September 2012** |
| R-1 Pure sample | - | |
| R-2 | *E.coli, Klebsiella pneumonia, Salmonella typhi, Pseudomonasa aeruginosa, Shigella flexneri, Yersinia entrocolitica, Staphylococcus aureus* | *E.coli, Klebsiella pneumonia, Salmonella typhi, Pseudomonas aeruginosa , Shigella flexneri, Enterococcus faecalis* |
| R-3 | *E.coli, Klebsiella pneumonia, Pseudomonasa aeruginosa, Salmonella typhi, Shigella flexneri, Staphylococcus aureus* | *E.coli, Klebsiella pneumonia, Pseudomonasa aeruginosa, Shigella flexneri, Enterococcus faecalis* |
| R-4 | *E.coli, Klebsiella pneumonia, Salmonella typhi, Pseudomonasa aeruginosa,* | *E.coli, Salmonella typhi, Pseudomonasa aeruginosa* |
| R-5 | *E.coli, Klebsiella pneumonia, Salmonella typhi, Staphylococcus aureus* | *E.coli, Pseudomonasa aeruginosa* |

**Table2.** *Morphological Characteristics of bacteria isolates from water samples*

| S.No | Names of bacteria | Gram staining | Shapes | Arrangement | Sizes in (mm) |
|---|---|---|---|---|---|
| 1 | *E.coli* | -ve | rods | Single | 3mm |
| 2 | *Enterobacter faecalis* | -ve | rods | Single | 4mm |
| 3 | *Staphylococcus aureus* | +ve | cocci | Grapes like cluster | 1mm |
| 4 | *Pseudomonas aeruginosa* | +ve | cocci | Single/pair/short chain | 1mm |
| 5 | *Yersinia sp.* | -ve | rod | Single/chain/cluster | -. |
| 6 | *Shigella sp.* | -ve | rod | Single/pair/short chain | - |
| 7 | *Klebsiella pneumonia* | -ve | rod | Single/pair/short chain | 2mm |
| 8 | *Salmonella typhi* | -ve | rod | Single | 2mm |

**Table3.** *Cultural characteristics of bacteria isolates from water samples.*

| S.No. | Names of Isolated organisms | Form | Color | Margin | Elevation | Odour |
|---|---|---|---|---|---|---|
| 1. | *E. Coli* | Circular | White/metallic sheen | Entire | Raised | Fecal odour |
| 2. | *Enterobacter* | Irregular, | Lack of sheen | Entire | Convex | Fecal |

| | | | | | | |
|---|---|---|---|---|---|---|
| | *faecalis* | large | | | | odour |
| 3. | *Staphylococcus aureus* | Circular | Golden yellow | Entire | Convex | Unpleasant |
| 4. | *Pseudomonas aeruginosa* | Circular | Flour yellow | Undulate | Raised | Fruity |
| 5. | *Salmonella typhi* | Circular | Colourless | Entire | Raised | Unpleasant |
| 6 | *Yersinia sp.* | Circular | White | Entire | Raised | - |
| 7 | *Klebsiella pneumonia* | Irregular | Blue colour | - | Convex | Yeasty odour |
| 8 | *Shigella sp* | Irregular | Yellow colour | lobate | Convex | Fruity odour |

**Table4.** *Percent density of microorganisms in Champa region of Hasdeo river water*

| S.No | Name of organisms | Density of organism in % | |
|---|---|---|---|
| | | **June 2012** | **September 2012** |
| 1. | *E. Coli* | 32% | 31% |
| 2. | *Klebsiella pneumonia* | 14% | 11% |
| 3. | *Pseudomonas sp.* | 18% | 16% |
| 4. | *Shigella sp.* | 11% | 10% |
| 5. | *Salmonella sp.* | 10% | 9% |
| 6. | *Staphylococcus aureus* | 4% | 2% |
| 7. | *Yersinia sp.* | 5% | 4% |
| 8. | *Enterococcus faecalis* | 6% | 17% |

**Table5.** *Physical parameter of water sample from river Hasdeo collected in the month of June and September 2012.*

| Town | River | Year | Quality of parameters | | | | |
|---|---|---|---|---|---|---|---|
| | | **2012** | **pH** | **Dissolved Oxygen (mg/lit)** | **BOD (mg/lit)** | **COD (mg/lit)** | **Temperature** |
| Champa | Hasdeo | June | 6.8 to 7.3 | 6.2 to 7.5 | 4.4-5.6 | 25.6 | 30.5 to 28.5°C |
| | | Septe-mber | 7.1 to 7.5 | 9.2 to 10.8 | 2.1-2.5 | 15.2 | 28.5 to 27.5°C |

## 6. CONCLUSION

The study clearly indicates that river water is highly contaminated by bacteria those are present in all sort of environmental conditions that allows human involvement which are predominated by indicator organisms. Enumeration study on indicator microorganisms was found to be high in the month of June as relatively less in September due to rain fall. Also the rain fall results in low level of BOD, COD and increased level of DO that also influence the level of microorganisms in the river water. pH and the temperature of water samples were highly influenced by other physical factor like metal and industrial pollution level. The pH was found to be more in alkaline side in September where as in June it was towards slightly acidic. The bacteria seem to be responsible for the degradation of organic and inorganic compounds from where they derive their nutritional requirement hence the study strongly suggest to develop certain standard for the Hasdeo River water in Champa region for the health concern of the population residing in its bank, also the study on bacterial population those are used in remediation of metal pollutant deposited in river water from various industrial resources can help in removal of those metal from the river water.

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## REFERENCES

[1] Koshy, M., and Nayar, V.T., "Water quality aspects of river Pamba", Pollution Res. 18, 501–510 (1999)

[2] Vaishnav M.M. and Milan H., Effect of Paper Mills Effluents on ground and surface water bodies of some selected areas of Janjgir Champa, C.G., India. Indian J. Sci. Res. 4(2), 119-126 (2013)

[3] Rutgersson et al., 2011], Edwin Oakes Joran. The kinds of bacteria found in rivers, journals of hygiene, (1-27) (1900)

[4] Petersen T.M., Rifai H.S., Suarez M.P., Stein A.R., Bacteria loads from point and nonpoint sources in an urban watershed. J. Environ. Eng. 131 (10), 1414 – 1425 (2005)

[5] Donovan E., Unice K., Roberts J.D., Harris M. and Finley B., Risk of gastrointestinal disease associated with exposure to pathogens in the water of the lower Passaic River. Appl. Environ. Microbiol. 74, 994 – 1003 (2008).

[6] Abraham WR., Megacities as source for pathogenic bacteria in rivers and their fate downstream. Intl. J. Microbiol. 10, (1155): 13 (2010)

[7] Niyogi K., Shigellosis. J. Microbiol., 43 (2): 133-143 (2005).

[8] Anna E., Richard M. Vgl. Predicting fecal coliform bacteria levels in the Charles river, Massachusetts, J. of the American water resources asso. 1-15 (2005).

[9] Doron T A., Unacceptable level of sewage bacteria in river creek, Hudson – Catskill newspaper, 1-3 (2011)

[10] Lesley C.L., Extent of *E.coli* contamination of Cagayan De Oro River & factor causing contamination, Liceo J. of higher Edu. Res. CHED accredited Res. J. 1-20 (2008)

[11] Charles W., Hendricks, Enteric bacterial growth rates in river water, J. ASM, 24(2), 168-174 (1972)

[12] Edberg S.C., Rice E.W., Karlin R.J. and Allen M.J., *Escherichia coli*: the best biological drinking water indicator for public health protection. J. Appl. Microbiol. 88, 106S–116S (2000)

[13] Farnleitner A.H., Hocke L., Beiwl C., Kavka G.G., Zechmeister T., Kirschner A.K.T., and Mach, R.L., Rapid enzymatic detection of Escherichia coli contamination in polluted river water, Letters in Appl. Microbiol. 33, 246-250 (2001).

[14] Kohl W., Uber die Bedeutung bakteriologischer Untersuchungen für die Beurteilung von Fließgewässern, dargestellt am Beispiel der österreichischen Donau, Arch, Hydrobiol,/Suppl.44, (4), 392-461 (1975).

[15] APHA (American Public Health Association). American water works association and water pollution control federation. standard methods of examination of water and waste water. 19th edition, New York, U.S.A. (1991).

[16] Cappuccino J.G., Sherman N., Biochemical activities of microorganisms. In: Microbiology, A Laboratory Manual. The Benjamin / Cummings Publishing Co. California, USA. (1992)