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Evaluation of Water Quality by Physicochemical Parameters, Heavy Metal and Use of Metal Resistant Property of Bacteria for Bioremediation of Heavy Metals

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Abstract: Pollution of water is one of the major concerns for whole world. Heavy metals, toxic waste and various effluents from industrialization and anthropogenic sources causes pollution of river water. These pollutants have adverse effects on health of human and other living beings in terrestrial and aquatic environment and also affect the food chain. The present study was an attempt to evaluate the water quality of river Hasdeo at Sarvamangla Nagar, Korba (Chhattisgarh). Pollution level was measured by estimating the water quality parameters [physicochemical i.e. temperature, pH, DO, BOD, COD and Level of heavy metal pollutants]. Pollution of heavy metals were observed in following order [Fe>Pb>Cd>Zn]. In addition, the bacteriological analyses involved most abundant bacterial species tolerant to heavy metal pollution and isolation identification of these heavy metal resistant bacteria from the river water of Hasdeo. The bacteriological analyses showed the population density [in %] of most abundant metal resistant bacterial species were Pseudomonas sp. [38%], Bacillus sp. [27%], E. Coli [20%] and Enterobacter sp. [15%]., The results of the present investigation revealed that, the river Hasdeo water at Sarvamangla Nagar was highly contaminated with heavy metals and require suitable action for the removal of pollutant by using the these metal tolerant bacteria as bio-remediating heavy metal pollutant which are very harmful to human health and environment.

Key words: Heavy metal remediation · Fecal contaminant · Indicator microorganisms · Metal resistance microorganisms

INTRODUCTION

Environment is defined as combination of all external physical factors that affect and influence the growth, development and survival of an organism. It includes both biotic and abiotic factors. Water pollution is a major global problem that requires proper evaluation of level and source of pollution. Water pollution involves the release of toxic substances, pathogenic microorganisms, etc. that settled at the bottom and their bio-accumulations will interfere with the balance of ecosystems. The quality of river water is greatly influenced by industrial, agriculture and plethora of human activities which affect its physicochemical characteristics and microbial diversity present in the river water [1].

River Hasdeo is the tributary river of Mahanadi in Chhattisgarh that flows from Bilaspur, Champa, Korba, Korea districts. Korba Known as power hub of Chhattisgarh with almost 11 main power plants projects plus number of small power plant units running and various other industries in the surrounding and holds 5th position in the list of critically polluted industrial areas/clusters in descending order with CEPI index 83 as per Chhattisgarh Environment Conservation Board. Most of the pollutants were coming from this power plant those are Karnataka power plant Ltd, NTPC, BALCO, CSEB, SECL etc., there are many fly ash and other small factories that discharge their effluents in to the river [2]. These effluents contain harmful toxic metal traces and other toxic elements. Apart from industrial pollutant sewer system, water discharge and 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 [3], poor sanitation and poor treatment of waste water results in accumulation of heavy metals and introduction of

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pathogenic microorganisms and thus infecting and killing of populations of both human and animal [4]. Water sources contaminated with faecal matter causes number of diseases, like typhoid, cholera and dysentery.

"Heavy metals" term refers to any metallic element with relatively high density greater than 4 g/cm^3, or 5 times or more, greater than water [5- 7] and toxic or poisonous even at low concentration [8]. As these heavy metals are toxic and non degradable, they pose a serious threat entire ecosystem [9] as they persist for long time and hence causing various diseases to human and animal as well those are using this contaminated water [10,11]. There is significant effect of heavy metal on aquatic animal present in river water [9,12] Heavy metal toxicity can result in damaged or reduced mental and central nervous function, lower energy levels and damage to blood composition, lungs, kidneys, liver and other vital organs. Long-term exposure may result in slowly progressing physical, muscular and neurological degenerative processes that mimic Alzheimer's disease, Parkinson's disease, muscular dystrophy and multiple sclerosis. Allergies are not uncommon and repeated long-term contact with some metals or their compounds may even cause cancer [13].

Microbial contamination of water due to pathogenic organisms is another most important problem for the water quality management and another important factor for water pollution for Hasdeo river water. These pathogenic microorganisms comes from the faeces of human and warm blooded animals which brought to the river water through various ways like the release of effluent from waste water and house drainage, surface runoff [14]. Contamination of river water due to pathogenic micro-organisms is considered as an indicator risk of pathogen-induced illnesses in humans [15]. In recent years there has been many studies carried out for physic-chemical and bacteriological analysis at different places viz. Nagpur district, Rajasthan [16], Akola [17], Hyderabad [18], Aurangabad [19], Beed district [20], Dehradun [21].

There has been good number of studies carried out for the estimation of pollution level in River water of Hasdeo in Korba region that mostly involves the physicochemical parameters like DO, BOD, COD, pH, temperatures [22, 23] and metal analysis for few heavy metals [22], In our Study we have estimated the lead [Pb], cadmium [Cd], zinc [Zn] and iron [Fe] in river water of Hasdeo. Also there is no data available for the microbial contamination and no one has worked on remediation of heavy metal by these metal resistant bacteria of river Hasdeo, in my study I have analyzed the level of physicochemical parameter like BOD, COD, DO, pH and temperatures at in Sarvamangla nagar of Korba, along with estimation of heavy metal concentration like Fe, Cd, Pb, Zn and also measured the bacterial species resistant to heavy metal, there are good number of studies that shows bacterial resistance towards these heavy metals *Bacillus sp.* [*Bacillus Cereus, Bacillus spaericus and Bacillus subtilis*] were observed to shows tolerance towards many heavy metals [24]. The metal resistance was observed in *Proteus vulgaris, Pseudomonas aeruginosa, Acinetobacter radioresistens* [25]. Heavy metal absorbing bacterias were also isolated from the industry effluent of the river Nagavali and identified as *Staphylococcus aureus Staphylococcus epidermidis* and *Staphylococcus saprophyticus* [26]. This study also aims to focus on the bacterial inherent properties [degrade environmental toxin and metal binding capacities] for resistant towards heavy metals can be used as bioremediation of river water from heavy metals with the help of new biotechnological approach. Further improvements in capabilities of bacteria to degrade environmental toxins and bind metals arose through the use of genetically engineered microorganisms [GEM].

MATERIALS AND METHODS

Collection of Water Samples: Water samples from the river Hasdeo were collected from Korba region near Sarvamangla nagar in three replicates, during the month of July 2014. The samples were collected aseptically in sterile 500mL Durans Schoot glass bottle using the standard procedure and methods prescribed by American Public Health Association [27].

Isolation and Identification of Metal Resistant Bacteria: Isolation of heavy metal resistant bacteria was done by enrichment culture technique, using metal salts supplement in nutrient agar medium. Serially diluted water samples were inoculated into the heavy metal enriched agar plate and incubated at 35 °C for 48hr, morphologically district colonies were picked up and then pure culture was prepared by repeated streaking and was preserved in NA slants under refrigerated [4°C] conditions for biochemical and morphological characterization. The biochemical tests were performed as per standard Microbiological methods [28].

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, margine, elevation, consistency etc. is of value in identification of colonies. The characters morphology and staining reaction helps in preliminary identification.

Determination of Water Quality Parameter: The water quality assessment parameters analyzed were; temperature, pH [measured using standard pH meter], BOD by dissolved oxygen loss method and chemical oxygen demand [COD] by potassium dichromate method.

Analysis of Heavy Metal Concentration: Contents of the heavy metals in water samples were analyzed by AAS [Thermo Scientific, USA] following the method of American Public Health Association [APHA, 27]. The water samples were digested with 20 mL aqua-regia [3:1, HCl/HNO_3] in a beaker on a hot plate, then 5.0 mL hydrogen peroxide was added to the sample to complete the digestion and the resulting mixture was heated again to near dryness in a fume cupboard and filtered by Whatman no. 42 filter paper and the volume was made up to 50 mL by double distilled water.

RESULTS AND DISCUSSION

The present study was undertaken to characterize the physic-chemical parameters like pH, temperature, BOD, COD, DO and level of water pollution both pollution due to heavy metal and isolation of bacterial species resistant to heavy metal in water samples of Hasdeo river in the month of July 2014.

Isolation and Identification of Metal Resistant Bacteria and Evaluation Metal Tolerance: Sample analysis from the river water of Hasdeo Data reflects the presence of *E.coli* [20%], *Pseudomonasa aeruginosa* [38%] and *Enterococcus faecalis* [15%] and *Baccilus subtillis* [27%] in the river water of Hasdeo and these are showing resistant to the heavy metal pollution.

Physical Parameter

Temperature and pH: pH and temperature are 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 optimum pH and temperature and do not withstand abrupt changes. The pH values fluctuated between 7.3 to 7.9, generally pH of water is influenced by geology of catchments area and buffering capacity of water. The temperature was recorded in between the range of 20.5 to 21.4°C.

Biological oxygen demand [BOD], Chemical Oxygen demand [COD] and Dissolved Oxygen [DO]: The BOD that indicates microbial pollution in water ranges from 2.9 to 3.8 mg/l. Whereas Chemical oxygen demand represents the oxygen required for chemical oxidation of organic pollutant both degradable and non-biodegradable matter. The COD was recorded in as 4.1 to 5.6 mg/L. 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 4.7 to 5.5mg/l at 30°C which was lower than the permissible quantity, which are unfit for the aquatic ecosystems. Dissolved oxygen [DO] is essential to maintain higher forms of biological COD are used as a measure of the oxygen equivalent of the organic content.

Assessment of Heavy Metal: Results of Heavy metals analysis was as follows:

Iron [Fe]: The concentration of iron in the river water of Hasdeo, in Korba ranges from 11.80 mg/L, which was very much high. According to the Bureau of Indian Standards [BIS, 29] the desirable limit were 0.3mg/L and 1mg/L as the maximum permissible limit for iron in drinking water [BIS, 29]. There is various health complication related with high concentrations may promote bacterial activities causing objectionable odours.

Lead [Pb]: The lead was most abundantly [0.09 mg/L] found in river water of Hasdeo. As per the recommendation by the Bureau of Indian Standards the desirable limit of Lead was 0.050 mg/L for drinking water [BIS, 29]. Beyond this limit, the water becomes toxic. The major source of lead contamination is the combustion of fossil fuel.

Table 1: 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	*Pseudomonas aeruginosa*	+ve	Cocci	Single/pair/short chain	1mm
4	*Bacllilus subtilis*	+ve	Rod	Chain/single	4-10mm long

Table 2: Cultural characteristics of bacteria isolates from water samples collected in Sarvmangla nagar

S.No	Isolated organisms	Form	Color	Margin	elevation	Odour
1.	*E. Coli*	Circular	White/metallic sheen	Entire	Raised	Fecal odour
2.	*Enterobacter faecalis*	Irregular, large	Lack of sheen	Entire	Convex	Fecal odour
3.	*Pseudomonas aeruginosa*	Circular	Flour yellow	Undulate	Raised	Fruity
4.	*Bacllilus subtilis*	Irregular	Cream color	Undulated	Convex	Foot odour

Table 3: Percent density of microorganisms in Sarvmangla nagar of Hasdeo river water. [Data are mean of three biological replicates]

S.No	Name of organisms	Density of organism in %
1.	*E. Coli*	20%
2.	*Pseudomonas aeruginosa*	38%
3.	*Enterococcus faecalis*	15%
4.	*Bacllilus subtilis*	27%

Table 4: Physical parameter and heavy metal analysis data of water sample from river Hasdeo collected in the month of July 2014. [Data are mean of four replicates]

Parameter	Observation
pH	7.3 – 7.9
Temperature	20.5°C – 22.4°C
BOD	2.9 – 3.8 mg/L
COD	4.1 – 5.6 mg/L
DO	4.7 – 5.5 mg/L
Pb	0.003 mg/L
Cd	0.09 mg/L
Fe	11.8 mg/L
Zn	0.088 mg/L

Cadmium [Cd]: Cadmium is a highly toxic heavy metal and present at concentration of 0.003 mg/L in river waster of Hasdeo. The desirable limit for drinking water [BIS, 29] was 0.001 mg/L as per the Bureau of Indian standard. Beyond this limit, the water becomes toxic. The drinking water with Cadmium concentration more than 0.0010 mg/L causes bronchitis, emphysema, anaemia, renal stone and causative agent for cancer. Sources of cadmium pollution including industrial emissions and the application of fertilizer and sewage sludge to farm land.

Zinc (Zn): The concentration of zinc was found most abundantly in river water of Hasdeo in the range from 0.088 mg/L. the desirable limit for Zn prescribed was 5mg/L according to the Bureau of Indian Standards and 15mg/L as the permissible limit for drinking water [BIS, 29].

CONCLUSION

Rapid industrialization and various other anthropogenic activities add to the level of pollution to the river Hasdeo in Korba. The river water was found to be polluted with heavy metal and microbial pollutant. There has been great impact on physicochemical parameter like DO, BOD, COD that affects the every component of ecosystems. Level of heavy metal pollution is very critical as it posses' significant health hazard to any form of life and causing global problem and warrant ultimate remediation measurement. Our study shows that the quality of Hasdeo river water at Korba region is not for the drinking, bathing and agricultural use. Also the bacteria present in the river water were found to be resistant to heavy metals present that open as door that they have potential to bioremediation of metal pollution to the river water so that river water can be used for human necessities.

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