Isolation, Screening and Biochemical Characterization of Effective Microorganism for Bioremediation from Surface Water in a Mula-Mutha River, Pune – India

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Abstract—*In the present study, an attempt has been made to isolate and identify bacterial pollution indicators and effective micro-organism. The dominant colonies were found such as coliforms, Staphylococcus species, and Bacillus species from surface water of Mula-Mutha River in Pune- India. The Microorganism was isolated during the monsoon season 2018-19; from surface water the outcomes revealed that there was the impact of anthropogenic activities on the bacterial quality of the surface water. The results confirmed changes inside the amount of the colonies and therefore the density of bacterial indicators of examining sites. The best sort of bacterial pollutants recorded via monsoon season. Many ecological factors affect the bacterial activity, also its impact on quantitative and qualitative of characteristic bacteria. Also, they may be used as a bio-indicator and as an Effective Microorganism for the removal of surface water pollutants in Mula- Mutha River. Total viable counts were found between 9×10^3 to 100×10^3 CFU/ 100 ml respectively.*

Keywords: *Bacteria, colony, Water; CFU/mL.*

Introduction

It is widely known that water is indispensable natural aid and maximum valuable gifts of nature without which no life survives on this earth. It's very essential for human life and a part of the ecosystem. Freshwater is the simplest aid which is significant for human existence, agriculture and also the industry. Without having clean water of getting good quality, the developments don't seem to be possible (Jadhav *et al*, 2013). The health and fitness of the human being are directly depending upon the quality of river water. River pollution is one amongst the main problems and concerns in front of the many countries on the planet. All the living and non-living factors affecting by the standard of water quality. It must be treated with unique mechanisms. Rivers has been considered as a lifeline of groups of people. These are vital freshwater structures of strategic importance across the planet, providing principal water assets for domestic, industrial, agriculture and recreational reasons at present, to safeguard of water assets, it is very critical to issue regarding achieving complete river water restoration and to bring back to its original nature. A rise in globalization and industrialization, we are facing the assorted challenges for providing smooth and secure water to the general public. The range of rivers is becoming polluted through the addition of pollutants created by the person while, our local bodies including municipalities are taking care of this or they're facing difficulties for the same (Sadguru *et al*, 2018).

Pollution of surface water by semisolid wastes is considerable in many countries. Most of the wastewater generated is released into river bodies without treatment or inadequately treated. (Jasim *et al*, 2012). Today water needs to end up to be the most exploited natural systems because man stored the planet. Biological pollution (human and livestock), still as physical and chemical pollutants (temperature, oxides, salts, and pesticides); have negatively affected the microbial community within the water and sediments (Jasim *et al*, 2012).

Pune became one amongst the winners of India's first smart town and has grabbed the interest of the many. The town is ever-developing with the fresh upcoming projects, studies proposals, and modifications that are made to remodel the metropolis into the next vicinity to measure in. At the identical time, it's growing into a poster enterprise centre. it's recognized jointly of the 9 cities international with citation "Hosts IT and automobile companies". The town has its historic cost and its miles located on the banks of river Mutha (Kadhum *et al*, 2017). There are three most vital claimants of water sources viz. Domestic, agriculture and industry. These claimants are utilizing the useful resource not only in terms of consumption however also in phrases of excessive water pollutants. The pollutants of the river are specifically because of these 3 claimants, Pune vicinity at which population density is excessive with industrial attention to its height level. This has caused pollute Mula- Mutha river to an irreversible extent. (PMC Report, 2018). Further, Pollutants enter into the groundwater float and reached too many kilometers far-flung from Pune and therefore the populace alongside Bhīma River at high risk. It's also found that the villages are either use the polluted water or grasp purified paying excessive value up to Rs. 0.50/- per litre. As towards Rs 0.01 / litre inside the urban

place as causing pollution. Therefore its miles need of the way to check water pollutants at supply and to purify it with rock bottom viable cost, during this context bioremediation are often the price-effective manner of removing pollutants from the water released in Mula-Mutha River. Sediment with complicated chemical constituents (natural and inorganic) and microbial biomass could be a critical issue of freshwater ecosystems. Sediment in freshwaters could be a derivative of diverse anthropogenic activities (Gao *et al*, 2018). Microbial variety within the freshwater water body could be practical assemblages of microorganism that include single cellular organisms affect key approaches in river nutrient cycles. The sudden deposition of organic and inorganic debris to sediments could be critical manner all told aquatic ecosystems. Approximately one-third of the terrestrial organic matter (OM) that Enters freshwater is sequestered in sediments. Prokaryotes are among the most essential participants to the transformation of complex organic compounds and mineral in freshwater sediments, therefore, analysis of Microbial aim freshwater sediments is of most significance for getting the next know-how of river ecosystems, the various culture-unbiased molecular approaches supported small-subunit 16S rRNA have also been used for studies of microbial ecology in freshwater sediments. However, records on microbial variety in freshwater sediments are limited, because of the very fact previous research have specially targeted on some precise functional agencies which include coliforms, nitrify, and denitrifies, sulphate reducers. During this study, we achieved comparative analyses of the bacterial range of freshwater sediment of a shallow eutrophic lake on the premise of 16S rRNA gene analysis and modified cultivation techniques that progressed the culturability of unknown species present inside the aquatic ecosystem. Freshwater microbial infection using various pollution derived from anthropogenic resources could be a problem throughout the worldwide; control of this contamination and recuperation of degraded habitats of freshwater can fee many thousands of dollars and sizable on-the-floor effort by way of water and land managers, Traditionally, indicator bacteria, which incorporates *E. coli* and coliform bacteria had been accustomed monitor potential contamination of polluted waters, Often, those bacteria aren't good enough to spot infection sources because of the very fact they'll originate from several warm-blooded animals and environmental sources (Khadum *et al*, 2017).

Materials and Method

A surface water pattern was gathered from exceptional sites of a Mula-Mutha river from Pune district Maharashtra. After collection, the samples were delivered to the laboratory and preserved at 4⁰ C in an exceedingly freeze by using the quality protocol (APHA, 1998) and used for further analysis and Bacterial characterization. Water samples had been serially diluted with sterile 0.9 % saline water and analyzed for diverse bacteria by using spread plate method and streak plate method. The general heterotrophic bacterial count becomes assayed using medium and Luria Bertani agar medium (Peptone 5 g/L,

NaCl 5 g/L, beef extract. Five g/L, yeast extract 1.five g/L, agar 15 g/L, pH 7.4). (Bergey *et al*, 2019). The bacteriological evaluation just like the number of bacterial colonies, style of general coliform, faecal coliform had been measured by well-known plate count (SPC), respectively. All the gathered water samples are analyzed within 24 hr. The numbers of bacterial colonies had been counted by using the usage of the colony counter. All estimations have distributed the usage of 5 replicates. The final coliforms and *E. coli* are determined simultaneously using selective Eosine- methylthionine chloride medium (EMB), Mix medium and Luria-Bertani Agar Medium. (Kora *et al*, 2017). Biochemical Characterization of Bacterial Indicators in surface water was analyzed per conventional strategies described in (APHA, 1998) and (Bergey *et al*, 2019).
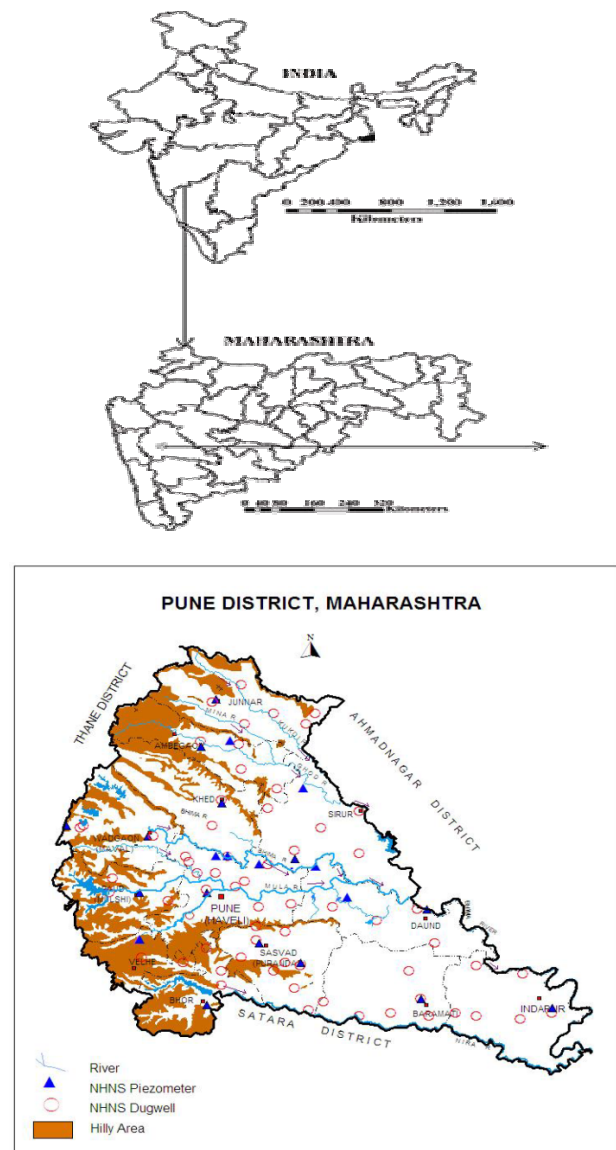

Figure 1: Distribution of sampling points across the Mula-Mutha River (Adopted from PMC, Report, 2018)

The bacterial colonies are isolated with the help of streaking them on fresh plates containing medium. To avoid repeated isolation of the identical bacterial isolates, the colonies showing similar cultural characteristics had been received through repeated streaking of diluted suspension prepared from remote bacterial colonies. The purified bacterial isolates were preserved on medium slants at 4˚C. All bacterial isolates had been subculture after 30 days (Bergey *et al*, 2019).

Identification

The promising bacterial isolates had been identified on the thought in their morphological, physiological and biochemical function features (Claus & Berkley, 1986).

Bacterial Consortium Preparation

Bacterial cultures were inoculated individually in pre-sterilized 100 ml of nutrient broth. The flask was kept in an exceedingly shaker at 120 rpm for 16 to 18 h at 30°C. The culture broth was centrifuged at 10000 rpm for 20 min. The cell suspension was prepared using sterile water and adjusted to 0.5 OD using UV-visible spectrophotometer 17. 5% of the above stock solution of Effective Microorganisms was added in Jaggery Solution (Jaggery-100g, Yeast extract-10g Distilled water- 1000ml pH-6.5. Dissolve all the ingredients in water and autoclaved at 121°C at 15 lbs.' for 15 min.). The inoculated Jaggery medium was incubated at 37°C temperature for five days. (Raghav and Shrivastav, 2014).

The efficiency of the bacterial consortium for the reduction of pollution load of Physico-chemical properties of Mula- Mutha River:

The efficiency of the bacterial consortium was evaluated for changes in physicochemical parameters of the wastewater samples after treatment by following the quality method of APHA (1998). For bacterial consortium treatment, 5% of Effective Microorganism solution containing bacterial consortium was inoculated into the water samples and incubated in an incubator. After incubation, the samples were analyzed for various physicochemical parameters. (Raghav and Shrivastav, 2014).

In present study for bio-remediation purpose, several indigenous bacterial strains were isolated from Mula-Mutha water and were tentatively identified by using various biochemical tests as *Bacillus species*, *Staphylococcus species*, *Escherichia-coli*, *Serratia species*etc. (Shrivastava *et al*, 2015) reported that the microbes employed in Effective microorganism technology are non-harmful, non- pathogenic, non-genetically engineered or modified and non-chemically synthesized. The bacterial consortium used has *Bacilli*, *Cellulomonas species, Lactobacillus species, Rhodobacter spheroids,* and *Rhodopseudomonas palustris*. microbes originated from their environment previously exposed to organic substances have greater degradation ability of related

waste within the biodegradation process. We got the identical *Bacillus* genus in our experiment.

Jin *et al*, 2018 showed that microbes have different abilities to degrade heavy metal, and therefore the degradation ability mainly depends on degradative plasmids and spores. Usually, *E. coli* K–12 adsorb the bulk of heavy metal ions, and therefore the adsorption capacity of *Pseudomonas* and *Bacillus* are strong species employed in bioremediation.
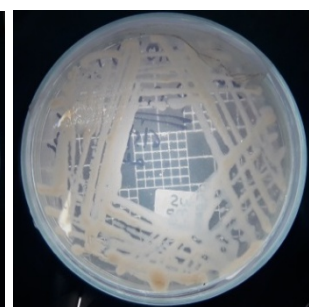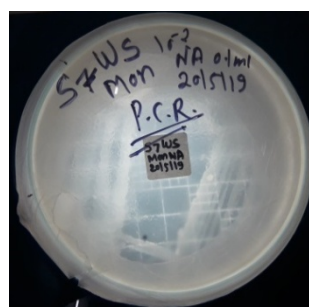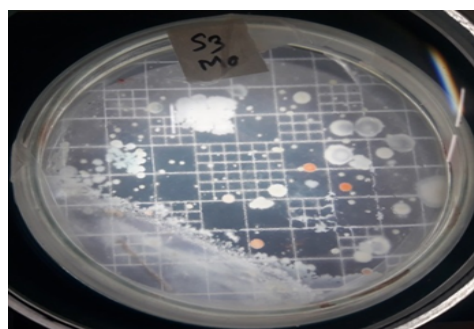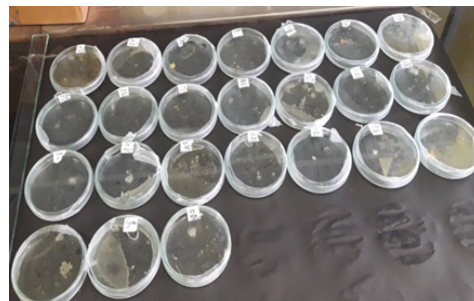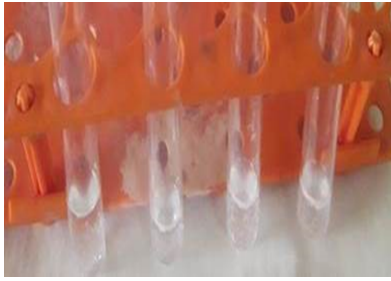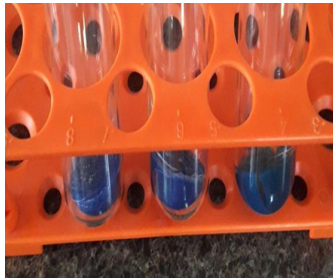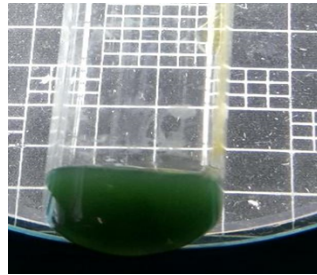
Results and Discussion

Fig. 2: Typical bacterial indicators in Monsoon river water

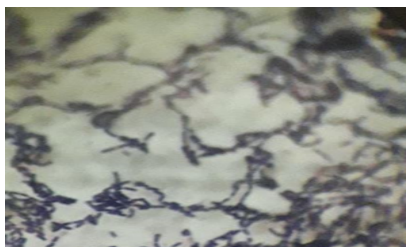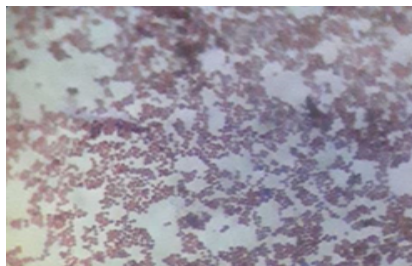**Catalase Positive Test**

Citrate Positive Test   **Citrate Negative Test**

Gram-Positive Test

Gram-Negative Test

Methyl Red –Voges Proskauer Test

Table 1: Colony Characteristics of isolated bacteria (Colony Forming Unit CFU/100mL)

S.R.No.	Name of Sampling Sites	Colony Color	Number of Colonies	Colony Shape	Cell Shape	Gram Test	CFU/100 ml
1	Sangam bridge	White, Whitish Yellow	50	Round	Purple rods	+ ve	50
2	Mundhwa Hadapsar	White	50	Round	Pink rods	- ve	50
3	Theur	Saffron, White	17	Round	Pink Dots	- Ve	17
4	Madanwadi (Bhigwan)	Whitish yellow	32	Round	Pink Dots	- ve	32
5	Dalaj No.3	Whitish Yellow	10	Round	Pink Dots	- ve	10
6	Ujjani Upstream	White	15	Round	Pink rods	- ve	15
7	Ujjani Downstream (Bhima River)	White	11	Round	Pink Dots	- ve	11
8	Bopodi	White	9	Round	Pink rods	- ve	9
9	Aundh	White	> 100	Round	Pink Dots	- ve	100

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Jin *et al*, 2018 showed that microbes have different abilities to degrade heavy metal, and the degradation ability mainly depends on degradative plasmids and spores. Usually, *Escherichia coli K–12* adsorb the majority of heavy metal ions and the adsorption capacity *of Pseudomonas* and *Bacillus* is a strong species used in bioremediation.

CONCLUSION

In the growing awareness of relationships between human health and pollution, it's essential to undertake regular monitoring and surveillance of important aquatic ecosystems. The results of this study indicate that Effective microorganism technology is helpful within the reduction of water impurities. The observation revealed that isolated bacterial consortium in water may indicate the pollution because we got faecal bacteria as a *Bacillus species, Staphylococcus species, Escherichia-coli, Serratia species*, which is additionally pathogens; it caused water-borne diseases like dysentery, Cholera etc. The uses of microorganisms are might be an eco-friendly solution for the reduction of pollution load from the natural water bodies.

Acknowledgement

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