International Journal of Science and Research (IJSR)
ISSN: 2319-7064
SJIF (2022): 7.942

Analysis and Characterization of Microorganisms from Heavy Metal Containing Wastewater

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Abstract: ***In overcrowded metropolitan cities emerging in nations like India, industries compete with one another for growth and advancement to meet people's needs. Toxic chemicals and heavy metals are not effectively removed from the effluents that are produced. This could harm living organisms, and plants when effluents are dumped directly or indirectly into water bodies. The current study aims to characterize and analyse the microorganisms present in the effluents collected from the Rajarajeshwari sewage outlet and Peenya industrial discharge in Bengaluru. Lead, Cadmium, and Chromium heavy metals were present in the collected samples. The microorganisms from the effluents were isolated and Bio-chemical tests were done for identification. The consortium of bacteria and Gram-negative Pseudomonas was found to be abundantly present. The enumeration of bacteria was observed by calculating CFU/ml (Colony Forming Unit/ml).***

Keywords: Effluents, microorganisms, heavy metals, *Pseudomonas*, CFU

1. Introduction

Water is a natural resource available in groundwater, rivers, lakes, and oceans. Human civilization is contaminating these water bodies with rapid urbanization which is leading to overconsumption and over-dependence on water like daily usage in manufacturing industries for the upstream and downstream processes. The release of effluents directly into the water bodies without treatment of the effluents and toxic chemicals has led to a rapid spike in the heavy metals in water. An increase in toxin levels has led to the death of aquatic bodies and made the water to be unfit for daily human needs, this contaminated water acts as a vector for transmission of diseases. This can be withheld by the detoxification process using techniques such as bioremediation [1]. Bioremediation is a technique that employs the use of living organisms like microbes and bacteria to decontaminate affected areas [2]. It is used in the removal of contaminants, pollutants, and toxins from soil, water, and other environments.The use of either naturally occurring or deliberately introduced microorganisms to consume and break down environmental pollutants, to clean a polluted site [3]. It has been introduced recently in most parts of the world for the betterment of our living habitats. Bioremediation is mostly used in the reduction of pollution in various polluted means, here we have used this technique the for reduction of Heavy Metal contamination in water bodies. Two major areas are selected -Rajarajeshwari Nagar of Bangalore South and Peenya Industrial area of Bangalore North for bioremediation.

2. Materials and Method

Materials

Raw materials: Samples from the Peenya industrial area and Rajarajeshwari Nagar, Bangalore.

Methods

Raw effluent samples were collected in 100ml bottles from Rajarajeshwari Nagar and Peenya industrial area. 4 samples in total were collected. The serial dilution approach was used to dilute the acquired samples.

Serial dilution is a technique used to gradually lower the sample's concentration by suspending it in a predetermined volume of liquid diluent until the desired concentration is reached. The 10^{-4} and 10^{-2} concentration diluted samples were inoculated into liquid Nutrient broth in conical flasks and then plated onto solid Nutrient agar plates. Conical flasks and inoculated plates were incubated for 48 hours. A visual characterization technique called colony morphology is used to identify unidentified microorganisms by looking at the physical traits of the colony. Colony forming unit/ml (CFU/ml) was used to calculate the number of colonies formed.[4]-[7]

3. Results and Discussion

The serially diluted samples were plated onto Nutrient agar plates at different dilutions. The plates were labelled as V and P, 'V' represents Vrishabhavati samples i.e., samples collected from Rajarajeshwari Nagar sewage, and 'P' represents from Peenya industrial area. The dilutions plated were 10^{-2} and 10^{-4}.

Some similar-looking colonies were identified and their morphological analysis was done.

Morphological identification was done and tabulated in Table 1.

Table 1: Colony characteristics of bacteria present in sewage sample

Source and colony number	Form	Elevation	Margin	Light transparency	Pigmentation	Gram reaction	**CFU/ml**
1.V[colony 1]	Round	Flat	Smooth	Opaque	White	Gram-negative rods	1.12×10^4
2.V[colony 2]	Round	Flat	Smooth	Opaque	White	Gram-negative small rods	
3.V[colony 3]	Round and raised	Flat	Smooth	Opaque	Yellow	Gram-negative rods	
4.P[colony 1]	Round and raised	Raised	Smooth	Opaque	White	Gram-negative small rods	7.28×10^3
5.P[colony 2]	Round with scalloped margin	Raised	Wavy	Opaque	White	Gram-positive rods	

From the above study of bacterial characterization, one can get to know the shape, color, light transparency, and Gram reaction. The majority of obtained bacteria in the above consortium of bacteria were Gram-negative rods.

All of the bacteria' from Table 1, were analyzed by biochemical tests shown in Table 2.

Table 2: Various biochemical tests conducted to identify the colonies.

Biochemical Tests							
Test / Sl.no	Indole	Methyl Red	Gelatine	Starch Utilization	VP	CATALASE	OXIDASE
1	-	+	+	-	-	+	-
2	-	-	+	-	-	+	+
3	-	-	+	-	-	+	+
4	+	+	+	+	+	-	-
5	-	+	+	-	-	-	+

'+'- Positive result, '-' - Negative result

In the next stage of biochemical tests, a fermentation test was carried out using glucose, lactose, and maltose.

From tables 2 and 3, it is evidently seen that plates 2 and 3 have *Pseudomonas* majorly present, in the consortium of bacteria.

Table 3: Sugar fermentation tests

	Glucose		Lactose		Sucrose	
Sl. no	Acid	Gas	Acid	Gas	Acid	Gas
1	+	-	+	-	+	-
2	-	-	-	-	-	-
3	-	-	-	-	-	-
4	+	-	+	-	+	-
5	+	-	-	-	+	-

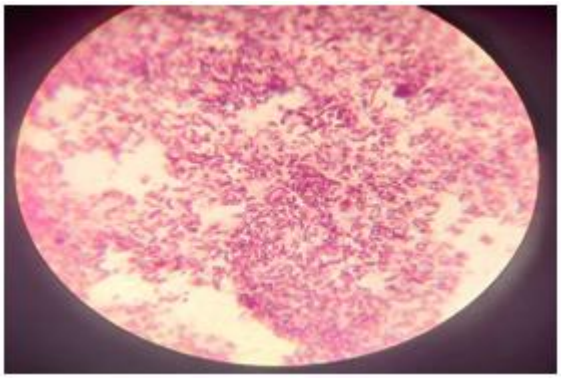

Figure1: Gram-negative *Pseudomonas*

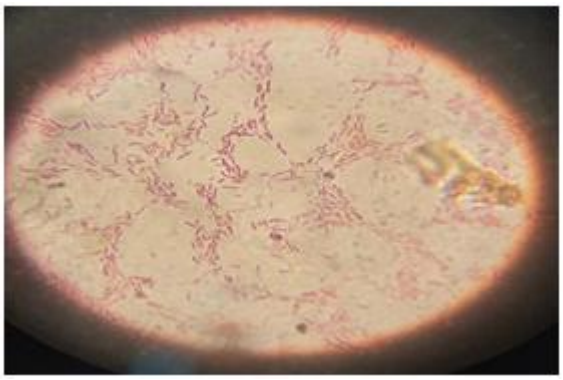

Figure 2: Gram staining of Consortium of Bacteria
Figure 1, 2: Gram staining of bacteria under 100x

4. Conclusion

The polluted water containing heavy metals and harmful toxins contains different types of bacteria. These bacteria survive depending on the contaminants present in the water, hence the bacteria can be used for reducing heavy metals and toxins.[8] The result showed that the majority of bacteria were Gram-negative *Pseudomonas sp*. and a consortium of bacteria from the polluted water sample, from the various biochemical tests and Gram staining [9],[10].

The number of bacteria present in RR Nagar sewage was more than in Peenya effluents. The Peenya effluents contain more industrial waste(heavy metals) whereas RR Nagar sewage contains predominantly domestic wastes.

5. Future Scope

The project work can be extended further by isolating the fungi present in the sewage water. The polluted water can be estimated for the presence of Heavy Metals and also the effects of these metals on the growth of microorganisms.

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Author Profile

Dr Madhumita Ghosh Dastidar has 30 years of teaching experience in the field of Microbiology. She is the former Head of the Department and Associate professor of the Microbiology Department, Vijaya College, Bengaluru. She has immense research experience and presented papers at various International and National conferences. She has published several research papers in peer-reviewed journals

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