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MICROBES USED AS A TOOL FOR BIOREMEDIATION OF HEAVY METALS FROM WASTEWATER

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Abstract: The drastic increase in population rate, in a developing country like India has led to rapid industrialization and has been a major contributor in polluting the environment. The pollutants released into the surroundings contain hazardous chemicals, domestic products, organic and inorganic substances, heavy metals, and other toxic elements. These are let out into water bodies which cause adverse effects on living organisms and humans. The wastewater containing toxic effluents can seep over time into the ground causing deterioration in groundwater quality. The industries that induce the pollutants into surface and groundwater sources may not strictly regulate pollutants to safe limits. If the industrial effluents are not treated properly before letting them out into water bodies, they can cause serious damage to mankind. The present study aims at analyzing the microbial characterization of wastewater from the Peenya industrial area and Rajarajeshwari Nagar of Bengaluru. These samples also to analyse the types of heavy metals such as nickel, lead, cadmium, chromium, mercury etc present in waste water by Atomic Absorption Spectroscopy. The isolated microorganisms were incubated with waste water sample to monitor the utilization of heavy metals. After quantification it was observed that microorganisms present in the collected samples were Gram-negative *Pseudomonas* and a Consortium of bacteria. Both these microbes were efficiently utilized heavy metals Cadmium, Chromium and Lead present in waste effluents.

Keywords: **Pollutants, Heavy Metals, *Pseudomonas*, Consortium of bacteria, Atomic Absorption Spectroscopy**

I. INTRODUCTION

Water is inorganic, transparent, tasteless, odorless, nearly a colorless chemical substance forming the hydrosphere layer of the earth. It plays a vital role in the lives of living organisms being an essential nutrient in metabolism, hydration, carrying nutrients to the cell, and much more. Due to more and more production of industrial products to meet human needs, water pollution is now turning into a crisis which is leading to changes in the environment[1]. Huge quantities of toxic effluents released into the water bodies play a major role in causing pollution. Industries such as leather, tanning, chemical, pesticide, oil and refineries, dyes, and pharmaceutical industries use a variety of heavy metals like cadmium, manganese, arsenic, lead, copper etc., a variety of chemicals containing sulfides, carbonates, nitrates, other organic and inorganic compounds are used and these are released as effluents into the water bodies, improper and lenient wastewater treatments are done by the industries as it is expensive and excessive amounts of energy is needed [2]. Water is used as irrigation in growing fruits, vegetables, and crops not only that the air we breathe surrounded by these heavy metals all these accumulations lead to increased toxicity to the lives of aquatic and other living organisms, thus declining the quality of water.

The correct definition for heavy metals varies among various authors. These are sometimes defined as metals with high density with a high atomic number or mass number with the features of metalloids both physical and chemical characteristics with essential and non-essential features involved in characterizing the heavy metal. The consumable limits of heavy metals like cadmium, zinc, lead, arsenic, and others range from 0.2mg/kg to 99.4 mg/kg as recommended by WHO. The common heavy metals density range is around 5g/cm3, and beyond this level leads to toxicity and chronic diseases like neurotoxicity, carcinogenic, infertility, liver and kidney damage, and other health hazards. These toxins accumulate in the water and are unable to be detected through the naked eye, by taste, or odor it can only be analysed by chemical estimations [3]. Thus, the 1-1.3 % of the water that is available for consumption if left for further toxification due to lack of management and control may lead to scarcity of water in the future generation.

Our research project aims to reduce the heavy metal concentration in water using wastewater as a source. Rajarajeshwari Nagar sewage outlet and Peenya industrial area wastewater were collected and processed for results. Filled toxic wastes and effluents, by incorporating the method of bioremediation a technique used in science where naturally available microbes, and plants are used in reducing the effluent in soil, and water concentration. This method is found to be effective, efficient, quick, eco-friendly,

inexpensive, and durable, with no side effects. 100ml of samples were collected containing heavy metals, Cadmium, chromium, and lead. Further processes were done using an Atomic Absorption Spectrometer, it is a special type of Spectrometer instrument that is widely used in the analytical technique of qualitative analysis that is the concentrations of heavy metals are determined and further analysed.
This estimation was carried out in the Ganga Enviro tech lab at Nelamangala, Bengaluru.

II. MATERIAL AND METHOD

Methods:

2.1 Collection And Analysis of The Sample

The samples collected are based on domestic sewage and industrial outlets, which were acquired from Rajarajeshwari Nagar and Peenya industrial area of Bangalore. The maximum microbial flora present in the samples was found to be Consortium of bacteria and Gram-negative *Pseudomonas*. These microorganisms were further subculture onto nutrient agar plates. The isolated *Pseudomonas* and Consortium of bacteria were separately grown and incubated in collected waste water for up to 9 days to assess the reduction of heavy metal concentrations in waste water.

2.2 Estimation of Heavy Metals by Atomic Absorption Spectroscopy

The inoculated samples were processed to check the presence of heavy metals using an Atomic Absorption Spectrometer. Atomic absorption spectroscopy is a technique used to measure the metal ions in various concentrations present in different materials, using Beer-Lambert law. As an analytical technique, it uses electromagnetic radiation from a light source of the respective metal, a cathode lamp. The metals are first converted into free ions by evaporation, which is absorbed by the cathode lamp and emits a different wavelength of radiation. This difference in wavelength gives a picture of the concentration of a specific element present in the given sample. Instrumentation of atomic absorption spectroscopy includes atomization, a detector, and the recorder connected to a monitor.This technique requires a standard known parameter to estimate the unknown sample [4]. In this technique, air was used for oxidation, acetylene, and nitrous oxide as fuel were used to create the flame. Lead and cadmium were oxidized in low flame, whereas chromium required high flame comparatively. 100ml of freshly collected sewage samples (uninoculated samples) were filtered using WHATMAN filter paper 44. The filtered samples were then reduced to 10ml by heating them on a hot plate at 90°C. To the reduced samples, 5ml of 60% nitric acid was added to acidify the sample which was then made up to 100ml by adding distilled water. Further, the samples were boiled at 90°C for 5 minutes, after which the samples were estimated to check the concentration of heavy metals- Chromium, Cadmium, and Lead along with 4 and 5 ppm standards for each. The same method was used for the estimate of heavy metals after an incubation period of 2 weeks of the inoculated samples [5].The reduction percentages of heavy metals- Cadmium, Chromium, and Lead for each sample were analyzed to conclude the role of microorganisms in reducing the heavy metal concentrations[6, 8].

2.3 The optical density of inoculated samples

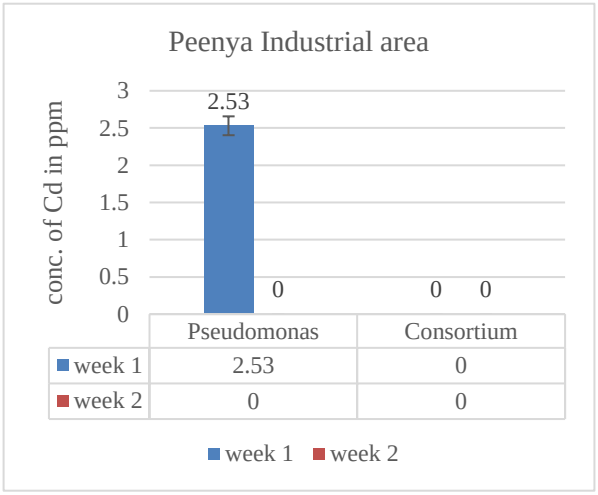
The microbial growth was constantly observed by using optical density as a parameter. The increase in turbidity of the inoculum indicated the growth of microorganisms. A colorimeter is a light emitting diode (LED) – based instrument commonly used for the measurement of transmittance or reflectance of solutions, transparent or opaque samples. Wherein a light ray passes through the test solution and reads the optical density of the solution. The microorganisms (Pseudomonas and consortium of bacteria) which were isolated from the sewage samples themselves were introduced into saline media as the primary inoculum for the reduction of heavy metals [7].1ml of each primary inoculum was separately inoculated into the saline media of sewage samples containing Heavy Metals from both the places (Rajarajeshwari Nagar and Peenya industrial area) and were incubated for a period of 2 weeks. The growth of microorganisms was constantly observed by monitoring Optical Density using a colorimeter [9].

III. RESULT AND DISCUSSION:

Table: 3.1 Initial Concentration of Heavy Metals at Week 0

Chromium	Cadmium		Lead
Peenya	Rajarajeshwari Nagar	Peenya	Rajarajeshwari Nagar
1.01 ppm	2.76ppm	2.77ppm	2.97ppm

Fig 1: Estimation of Cadmium concentration of Peenya Industrial waste and Rajarajeshwari Nagar sewage sample.



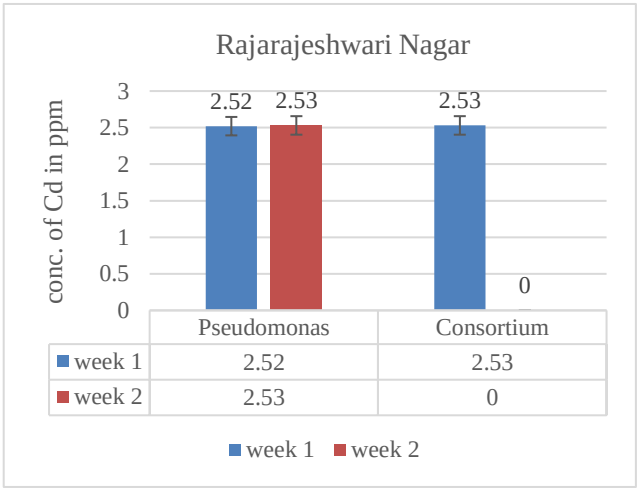


Fig 2: Estimation of Chromium Concentration of Peenya Industrial wastewater.

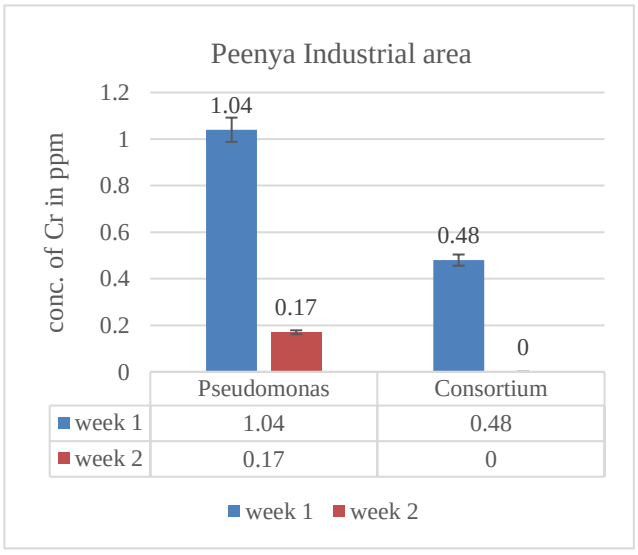


Fig 3: Estimation of Lead concentration of Rajarajeshwari Nagar water sample

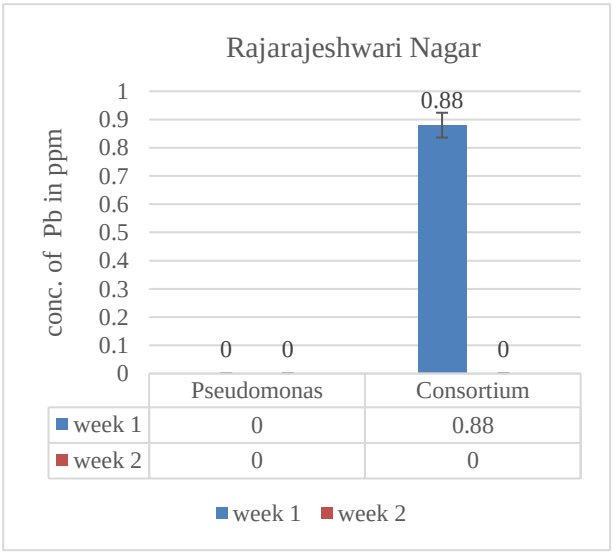


Fig 4.1: Percentage Reduction of Heavy Metals by Consortium of Bacteria

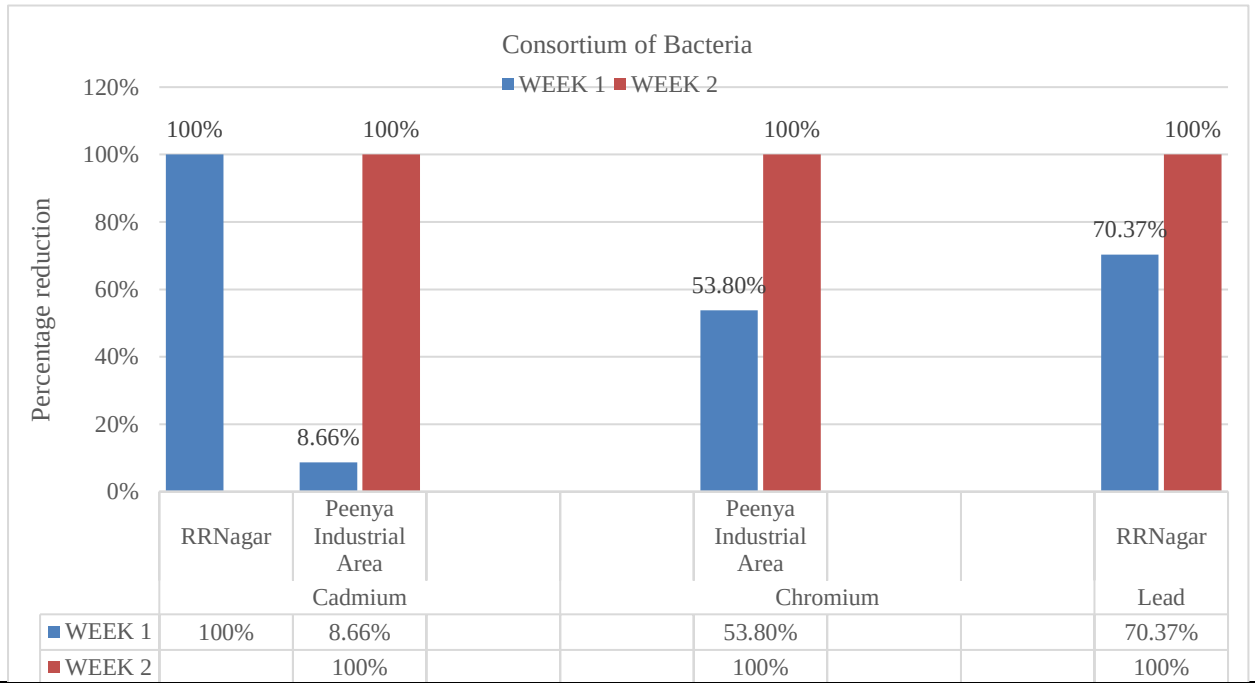
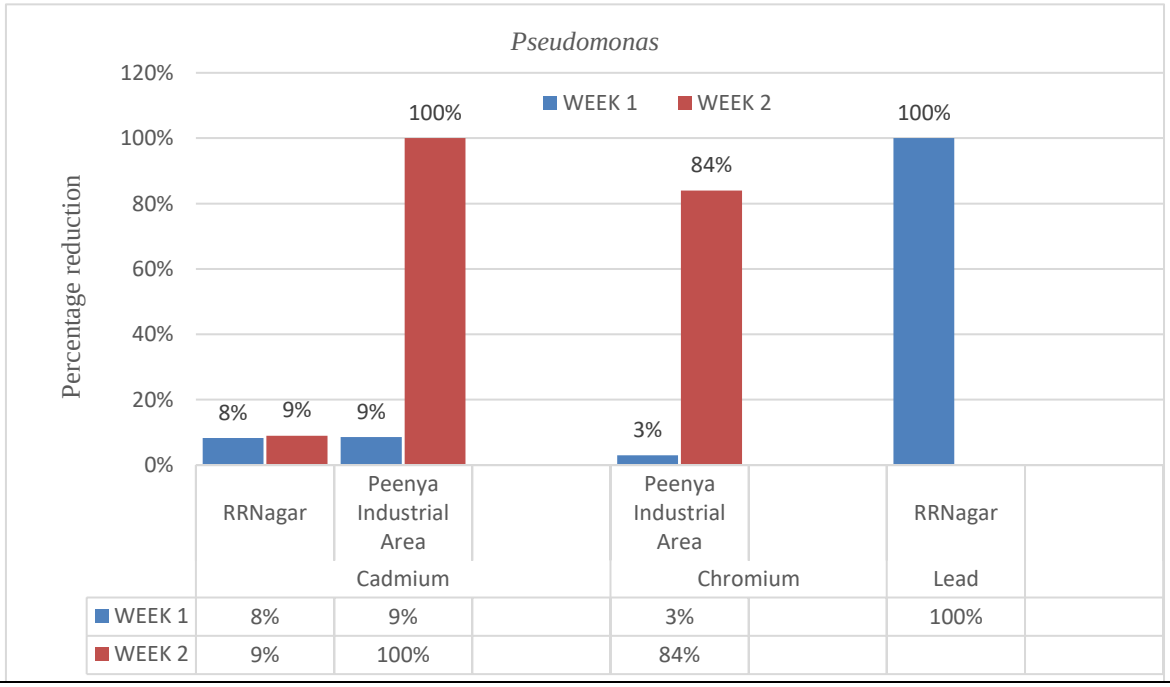


Fig 4.2: Percentage Reduction of Heavy Metals by *Pseudomonas*



Both *Pseudomonas* and Consortium of bacteria reduced Cadmium concentration in Peenya Industrial area and Rajarajeshwari nagar (Fig 1) effectively. From initial concentration of 2.76ppm *Pseudomonas* could utilize the cadmium and 100% reduction was observed by 2nd week in Peenya waste sample whereas *Pseudomonas* couldn't degrade the cadmium completely when incubated in Rajarajeshwari nagar sample. The consortium of bacteria on the other hand reduced the cadmium fully in Rajarajeshwari nagar sample.

The initial concentration of Chromium (1.01ppm) in Peenya industrial waste in week 1 was raised to 1.04 by *Pseudomonas*, whereas it was reduced to 0.48 by consortium of bacteria. By the end of week 2 *Pseudomonas* reduced the concentration of chromium to 0.17 but consortium of bacteria reduced the concentration completely (Fig 2).

The initial concentration of Lead was found to be 2.97ppm in Rajarajeshwari nagar and by week 1 *Pseudomonas* reduced the lead concentration to 0. In consortium of bacteria could reduce the Lead in week 1 by 0.88 followed by complete degradation by week 2(Fig 3)[10, 11].

The percentage reduction of heavy metals is calculated by the formula:

(Initial Concentration –Final Concentration) / Initial Concentration

Pseudomonas reduces cadmium to 8% in week 1 and 9% in week 2 in Rajarajeshwari nagar and reduces to 9% in week 1 and no reduction in Peenya industrial waste.

Pseudomonas reduces chromium to 3% in week 1 and 84% in week 2 in Peenya industrial waste. *Pseudomonas* does not lead in week 1 in Rajarajeshwari nagar sewage water and 70.17% in week 1 and no reduction in week 2 Peenya industrial waste water.

Consortium of bacteria reduces Cadmium to 2.00% in week 1 in Peenya industrial waste water and reduces chromium to 53.80% in Peenya industrial waste water in week 1 and reduces lead to 70.37% in week 1 in Rajarajeshwari nagar sewage water and there is no reduction seen in week 2 in Rajarajeshwari nagar sewage water.

Table: 2. OPTICAL DENSITY

		Pseudomonas		Consortium of bacteria	
	Date	Peenya	R R Nagar	Peenya	R R Nagar
Week-1 and 2	Day-1	0.13	0.03	0.13	0.03
	Day-3	0.10	0.01	0.10	0.02
	Day-5	0.10	0.05	0.11	0.05
	Day-7	0.10	0.04	0.10	0.07
	Day-9	0.09	0.04	0.09	0.08
	Day-12	0.07	0.05	0.10	0.08
	Day-15	0.06	0.06	0.10	0.07

The growth of *Pseudomonas* and consortium of bacteria was monitored by measuring Optical Density over a period of two weeks. The results of our present study show the ability of *Pseudomonas* and Consortium of bacteria to degrade heavy metals cadmium, chromium and lead obtained from waste water and industrial effluent samples in different concentrations [12].

It was observed that after 2nd week the growth of *Pseudomonas* was reduced by 50% in Peenya Industrial waste whereas in Rajarajeshwari nagar showed increased in growth of *Pseudomonas.*The prominent heavy metals were found to be Cadmium and Lead in RR nagar whereas Peenya Industrial area contained Cadmium and Lead. Pseudomonas efficiently reduced Cadmium and partially lead. The consortium of Bactria again increased in RR naga waste whereas marginal drop of Optical density aws observed in Peenya Industrial area. Her the prominent heavy metal was found to be Cadmium and Chromium.

The isolated bacteria that is *Pseudomonas* and Consortium of bacteria was inoculated to understand the original environment of microbes in the collected waste water. This recreates the microbial in-vivo environment in the laboratory [13].

Pseudomonas proves to be a novel bacterium with ability to degrade heavy metals over a period of time [14]. This bacterium very efficiently reduced the Cadmium, Chromium and Lead (100%). The consortium of bacteria reduced 100% Cadmium and Lead but not Chromium. A microbial consortium or microbial community, is two or more microbial groups living in harmony.

Heavy metal pollution has become a major threat to the environment as it causes certain health hazards like respiratory cancer, anaemia, skin irritation etc. the water contaminated with heavy metals like cadmium, chromium and lead cannot be used for domestic purposes and agriculture [16]. Chromium (Cr) and cadmium (Cd) are capable of inducing oxidative damage and denaturation of microorganisms as well as weakening the bioremediation capacity of microbes. Cadmium (Cd) and lead (Pb) pose deleterious effect on microbes, damage cell membranes, and destroy the structure of DNA[17].

The use of biological systems bioremediation is more efficient, cost effective and environmental friendly compared to traditional treatment of heavy metal containing effluents [18].

Accumulation of such heavy metals can also cause bio magnification, harming the marine life forms. Our study was based on bioremediation of heavy metals chromium, cadmium and lead polluted waste water. Results evidently showed the ability of *Pseudomonas* and Consortium of bacteria to degrade the heavy metals present in the waste water [19].

The microbes have evolved to maintain stable state of living even in adverse conditions and their ability to degrade heavy metals in toxic environments and can be used in larger scale or even before the effluents, wastes are let out into water bodies, which can be a potent way to reduce heavy metal from entering into the water bodies or agricultural fields as toxic pollutants [20, 21, 22].

IV. CONCLUSION

In this study Pseudomonas showed as a very efficient bacteria to degrade the heavy metals in waste water. The consortium of bacteria also could degrade the Lead and Cadmium more than Chromium. These microorganisms also sustained in waster after degradation which can be utilized in future for large scale degradation process.

V. ACKNOWLEDGMENT

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VI. CONFLICTS OF INTEREST

The authors declare no conflict of interest regarding publication of the paper.

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