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Bioremediation of Sewage Waste from Sewage Treatment Plants in Kashmir

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Abstract: ***Background: Bioremediation is a process to detoxify environmental pollutants using living organisms. The main objectives of our study were to evaluate effectiveness & sustainability of bioremediation in treating sewage waste in Kashmir.***
Methods: Bacteria were isolated from various sewage treatment plants & then cultured into colonies. Spectrophotometry was used to detect the absorbance of heavy metals by the micro-organisms.
Results: Spectrophotometry detected absorbance of metals by bacteria, with some colonies having higher absorbance than others.
Conclusion: Bioremediation is an effective and useful technique for sewage treatment.
Key words: Bioremediation, Sewage treatment, Spectrophotometry, Absorbance, Heavy Metal

I. INTRODUCTION

The human population and industrialisation are increasing incessantly and together with rising anthropogenic activities, and unregulated agricultural activities are putting a pressure on our decreasing natural resources. Thus, a global rise in environmental pollution has been seen in the past few decades that has caused the accumulation of hazardous wastes.[1]
Bioremediation is a process that utilizes living organisms, primarily microorganisms, green plants, and their enzymes, to detoxify the hazardous components of environmental waste into less toxic forms.[2] It has been used to decrease the concentration and toxicity of several chemical pollutants such as pesticides, poly-aromatic hydrocarbons, halogenated petroleum hydrocarbons, nitroaromatic compounds, industrial solvents and metals.[3]
Contaminant compounds are transformed by living organisms through reactions that take place as a part of their metabolic processes.[4] Bioremediation has been used effectively in large- and small-scale applications; Alaska oil-spill cleanup is one of the good examples.[5]
Bioremediation is based on promoting the growth of specific micro-flora or microbial consortia which are native to the contaminated sites that are able to perform wanted activities. [6] Establishment of these microbial consortia can be done in numerous ways such as by promoting growth by adding nutrients or terminal electron acceptor or by controlling moisture and temperature conditions [7, 6, 8]. In this technology, microorganisms use the contaminants as energy or nutrient sources [7, 6, 9]

II. AIMS AND OBJECTIVES

1) To study the effectiveness of bioremediation in treating sewage waste from sewage treatment plants in Kashmir.
2) To evaluate the potential of bioremediation as a sustainable method for treating sewage waste from sewage treatment plants in Kashmir.

III. MATERIALS AND METHODS

This study was a prospective interventional study, conducted over a period of 3 months from April 2023 to June 2023 in Srinagar, capital city of Jammu & Kashmir, India.

A. *Sampling*

Sampling was conducted at three sewage treatment plants (STPs) chosen by simple random sampling in the Srinagar, Jammu and Kashmir on April 27, 2023. The STPs chosen were located at Brari-Nambal New, Habak, and Hazratbal, situated at geographical coordinates 34°05′12.88″N 74°48′50″E, 34°8'30"N 74°50'30"E and 34°08′06″N 74°50′29″E respectively. The effluent from these STPs is discharged into Dal Lake, River Jhelum, and Brari-Nambal Lagoon. Fresh sewage samples were collected in 250-mL polyethylene bottles that had been washed with distilled water. The samples were transported to the laboratory on the same day.

1) Sample Collection Number: 01 at STP Brari-Nambal New

The pH of the wastewater at Brari-Nambal New was alkaline, ranging from 7 to 7.2. The temperature was also within the standard range, from 4.1°C to 21.1°C. The Biochemical Oxygen Demand (BOD) and Chemical Oxygen Demand (COD) levels were slightly higher than the standard range, at 185 to 201 mg/L and 334 to 503 mg/L, respectively. The Dissolved Oxygen (DO) level was lower than the standard range, at 0.4 to 3.2 mg/L.

Figure 1: Sewage Treatment Plant Brari Nambal

2) Sample Collection Number: 02 at STP Habak

The pH of the wastewater at Habak site was slightly acidic, ranging from 6.8 to 7.1. The temperature was also within the standard range, from 6.3°C to 21.9°C. The BOD and COD levels were higher than the standard range, at 118 to 210 mg/L and 258 to 739 mg/L, respectively. The DO level was very low, at 0.2 to 0.4 mg/L.

Figure 2: Sewage Treatment Plant Habak

3) Sample Collection Number: 03 at STP Hazratbal

The pH of the wastewater at Hazratbal site was alkaline, ranging from 7 to 7.4. The temperature was also within the standard range, from 6.3°C to 22°C. The odor was pungent, indicating that the wastewater contained high levels of organic matter. The BOD level was higher than the standard range, at 112 to 280 mg/L. The COD level was also higher than the standard range, at 287 to 714 mg/L. The DO level was low, at 0.2 to 0.8 mg/L.

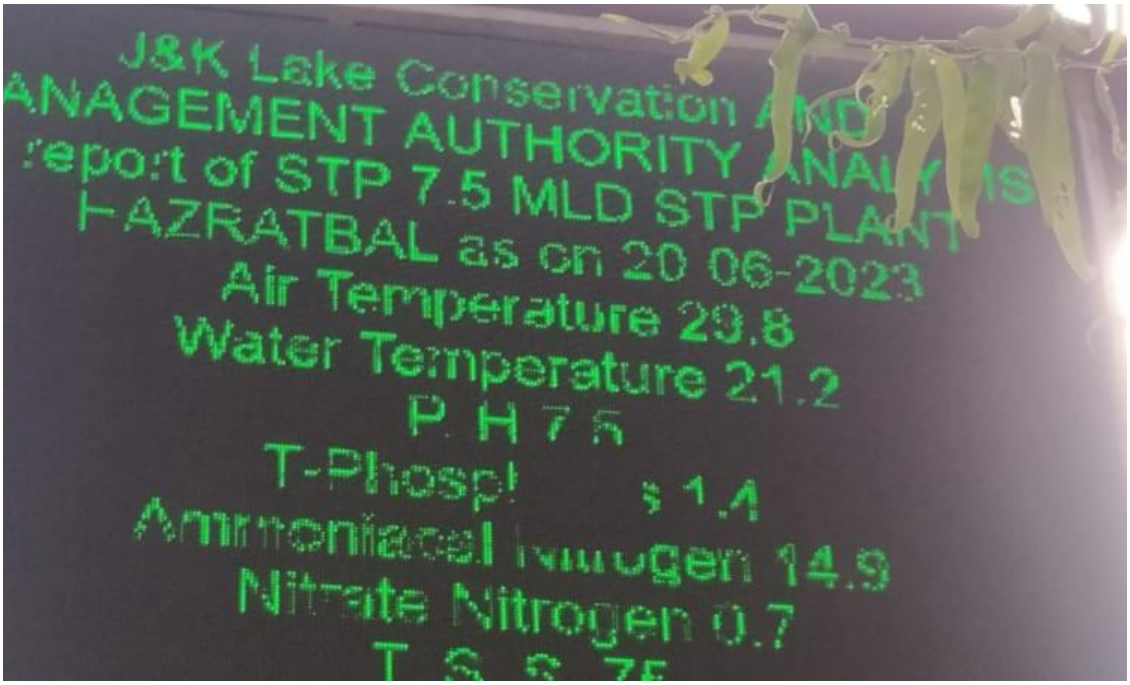


Figure 3: Sewage Treatment Plant Hazratbal

B. Media Preparation

Before carrying out any laboratory work autoclaving was done at 121 degree for 15 minutes, to eliminate all microorganisms. 28g of nutrient agar powder was dissolved in 1L of distilled water to prepare nutrient agar. This solution was poured into sterile petri-dishes, taking precautions to prevent contamination by maintaining a clean working environment. The poured agar was then left at room temperature for solidification.

Figure 4: Autoclave

Figure 5: Nutrient Agar

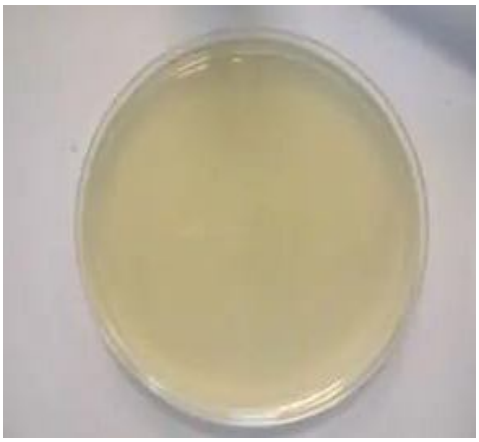


Figure 6: Solidified Nutrient Agar

C. Serial Dilution

Several test tubes with 9 mL of dilution liquid were prepared. These were used as dilution blanks. The first test tube received 1 mL of the undiluted sample. From there, 1 mL of the mixture in the first tube was transferred to the second tube, and this step is repeated for subsequent lines in the series. Each transfer results in a dilution factor of 10, and this process was continued until the desired dilution factor, usually reaching 10^{-4} & 10^{-5}, was achieved.


Figure 7: Serial Dilution

D. Inoculation

Using a sterile loop or pipette, we transfer 1 mL of the diluted sample onto the labelled solidified agar. The culture media was incubated at 20°C for 24-48 hours. Colonies of different morphology were observed on the media.

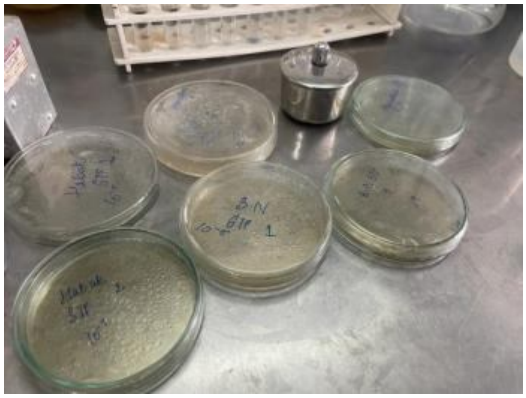


Figure 8: Inoculation

Figure 9: Incubator

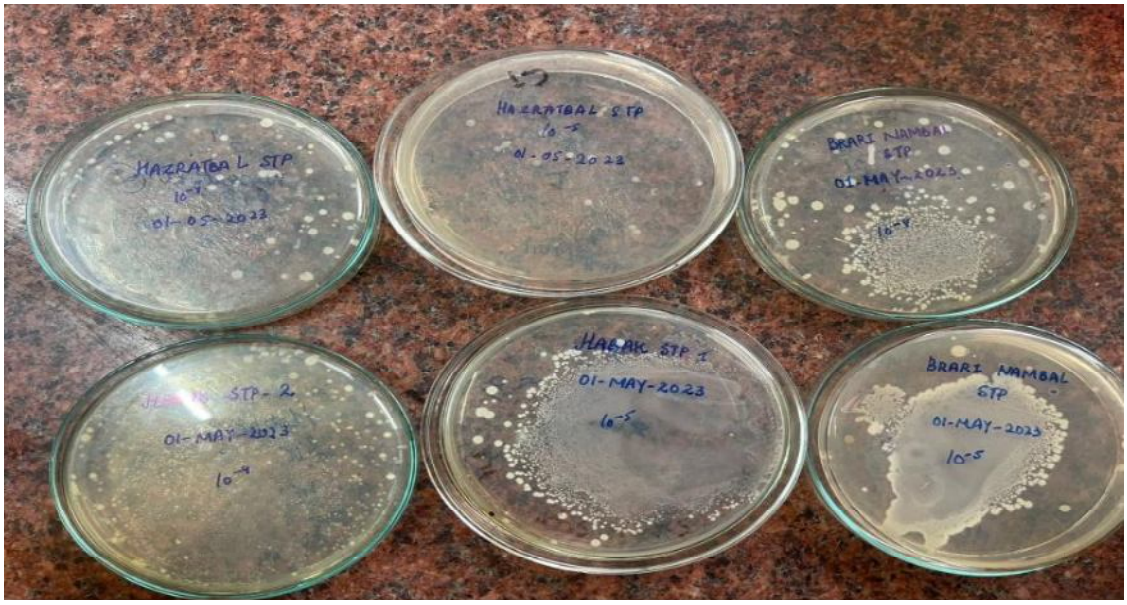


Figure 10: Mother Culture

E. Sub Culturing

Table 1: Constituents of media used:

Ingredients	Weight
Nutrient Agar	2.8g
Distilled Water	100ml
Cupric Sulphate (added in petri dish of all samples)	0.001g
Ferrous Sulphate (added in petri dish of all samples)	0.001g

The media after preparation was poured into a petri dish and placed in the incubator for 10 to 15 minutes at temperature 20 degree. The bacterial cultures obtained from the mother culture were well spread on the surface of a solid growth medium with the help of inoculating loop. The inoculating loop was sterilized by heating before collecting a small portion of the isolated colony from mother culture. This specimen was then streaked in a continuous zigzag pattern across the surface of a solid growth medium so that each colony arose from a single cell, allowing it to multiply independently and form visible individual clones**.**

Figure 11: Sterilization of Inoculating loop

Figure 12: Subculturing

The results were interpreted after incubating the plate for around 24 hours. Isolated colonies at the ends of the streaks were observed, and their morphologies noted for further analysis.

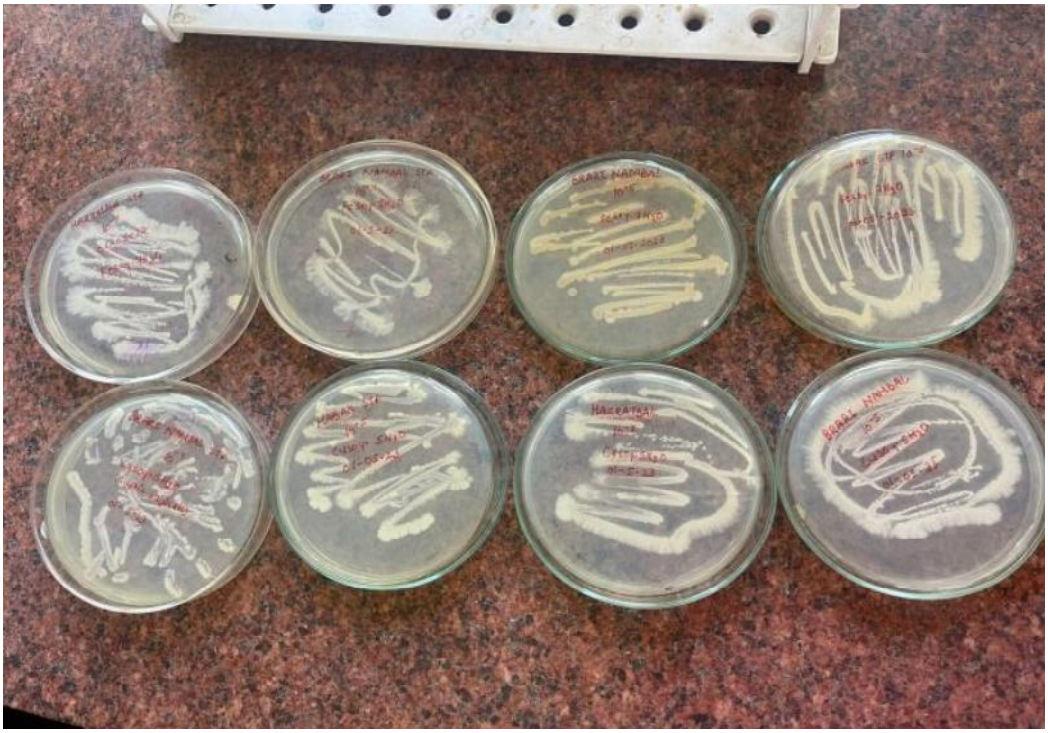


Figure 13: Pure Culture

F. Pure Culture Formation

Stock solution was created for pure culture formation using Iron (Fe) and Copper (Cu), specific chemical formulas are employed: Fe_2SO_4 for the Iron stock and $CuSO_4$ for the Copper stock. The volume of the sample (V_1) required to mix with a given book of media (V_2) and the normality of both the model (N_1) and media (N_2) was calculated using the equation $N_1V_1 = N_2V_2$. Assuming N_1 is 1000 and V_2 is 100 ml, the calculation yields $V_1 = 25$ ml. Therefore, 25 ml of each stock solution was used to prepare the media. To prepare the media, 7g of agar was combined with 25 ml of the Fe and Cu stock solutions. Distilled water was added to reach a final volume of 250 ml. The resulting mixture was then autoclaved to ensure sterilization.

Next, vaccination was performed using a previous subculture, and various colonies were streaked onto the media for further isolation and identification of pure cultures.

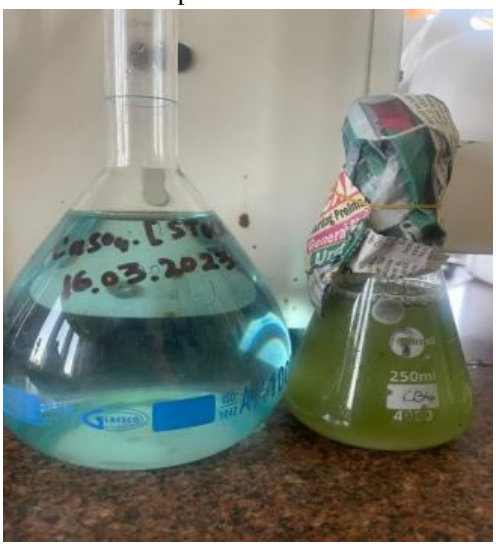


Figure 14: Stock Solution for Copper

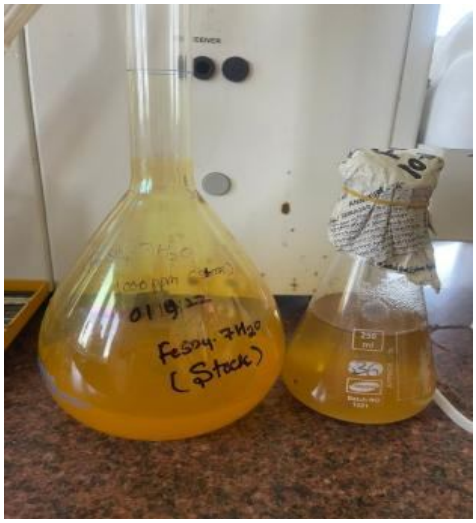


Figure 15: Stock Solution for Iron

Figure 16: Broth of Copper

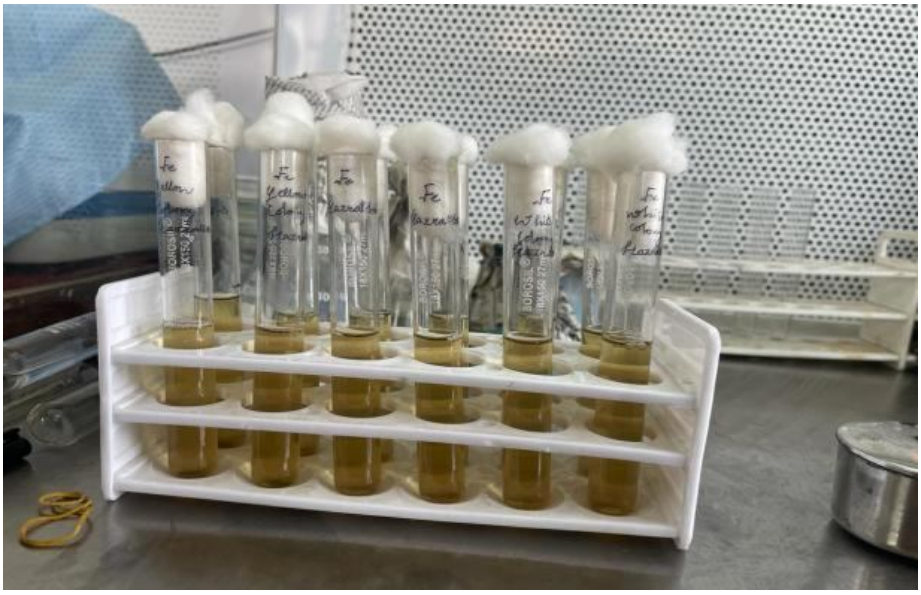


Figure 17: Broth of Iron

G. Gram Stainining & Microscopic Examination

Crystal violet stain was added over the fixed culture. After 10 to 60 seconds, the stain was poured off, and the excess stain was rinsed with distilled Water. The goal was to wash off the stain without losing the fixed culture. Iodine solution was used to cover the smear for 10 to 60 seconds. The iodine solution was poured off, and the slide was rinsed with running water. Excess water from the surface was shaken off. A few drops of decolourizer are added to the slide. The slide was rinsed with water for 5 seconds. The smear was counterstained with basic safranin solution for 40 to 60 seconds.

The safranin solution was washed off with water. The slide was air dried after shaking off excess water. All areas of the slide require an initial examination under microscope. Areas that had only one cell thick were examined. Thick areas in slides often give variable and incorrect results & hence weren't.

Figure 18: Chemicals used in Gram Straining

Figure 19: Sterilization of glass Slab

H. Spectroscopy

Double beam UV-VIS MODEL LT-2900 spectrophotometer was used. The spectrophotometer was set up with a double beam configuration. The wavelength was set to a wavelength that is known to be absorbed by the metal of interest. The absorbance of a blank solution that does not contain the metal was measured. The sample was placed in the sample cuvette and the absorbance was measured at the same wavelength that was used to measure the absorbance of the blank solution. The measurements were repeated to ensure that the results are accurate. The measurements were also be repeated at different wavelengths to confirm that the metal is absorbing light at the wavelengths of interest.

Figure 20: Double Beam UV -VS Spectrophotometer

IV. RESULTS AND DISCUSSION

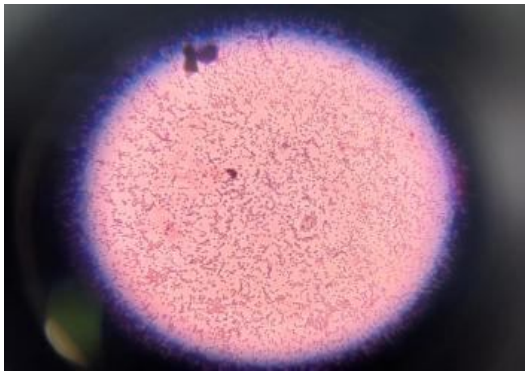


Figure 21: Microscopic view of Sample of Hazratbal (Iron)

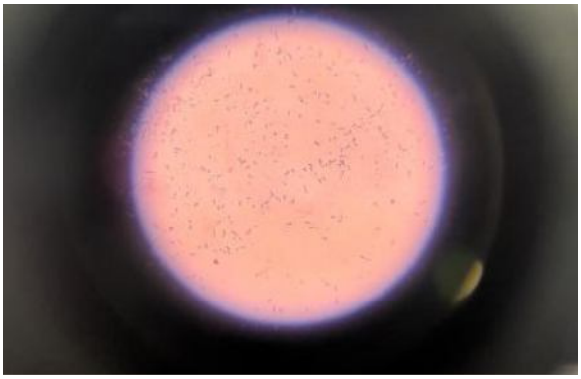


Figure 22: Microscopic view of Sample of Hazratbal (Copper)

Table 2

STP	Color of Colonies	Shape of Colonies	Elevation of Colonies	Metal present	Type of bacteria
Brari Nambal	Off-white Bluish Yellow	Circular	Raised	Fe	Gram negative bacteria
				Cu	Gram positive bacteria
Habak	Off-white Bluish Yellow	Circular	Raised	Fe	Gram positive bacteria
				Cu	Gram negative bacteria
Hazratbal	Off-white Yellow	Circular	Flat Raised	Fe	Gram positive bacteria
				Cu	Gram positive bacteria

Figure 23: Samples placed in cuvette for spectroscopy

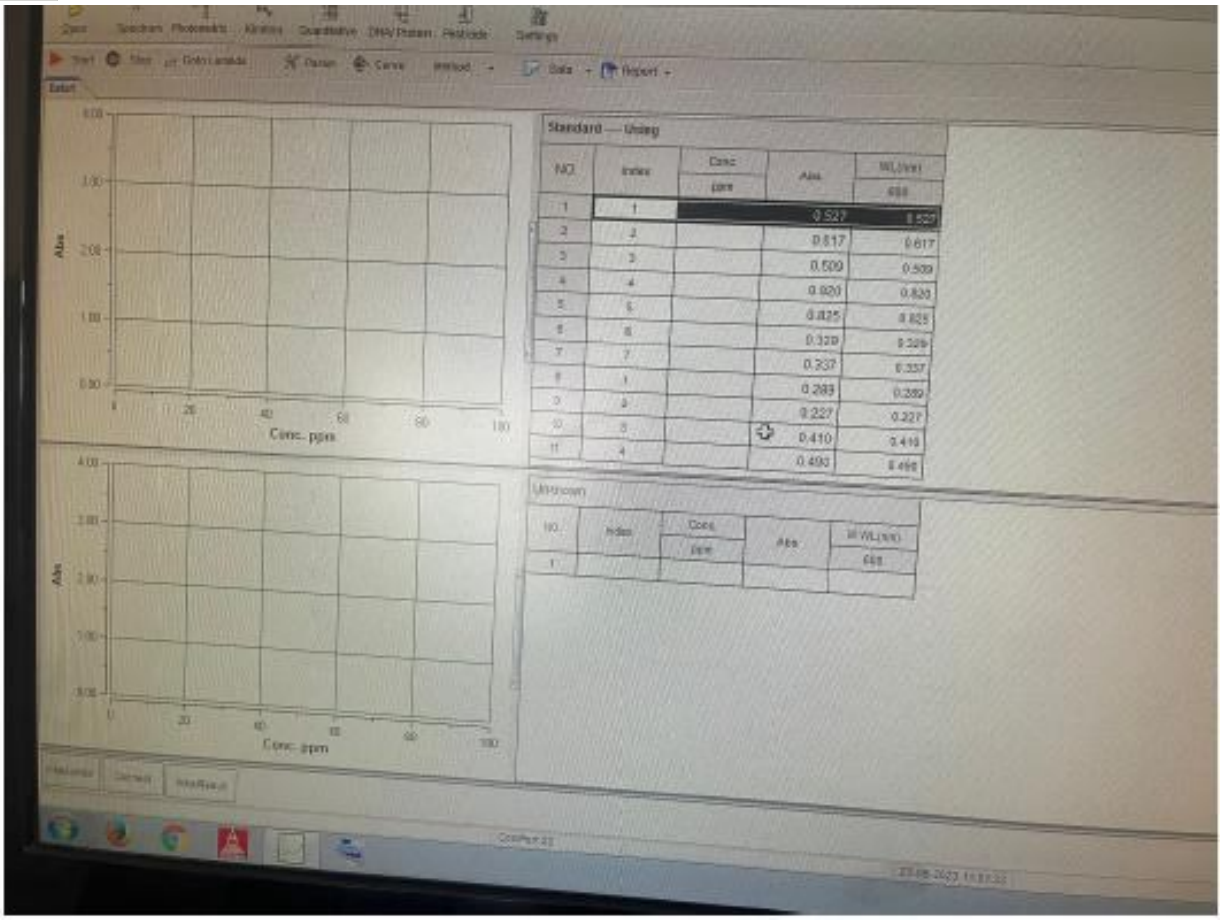
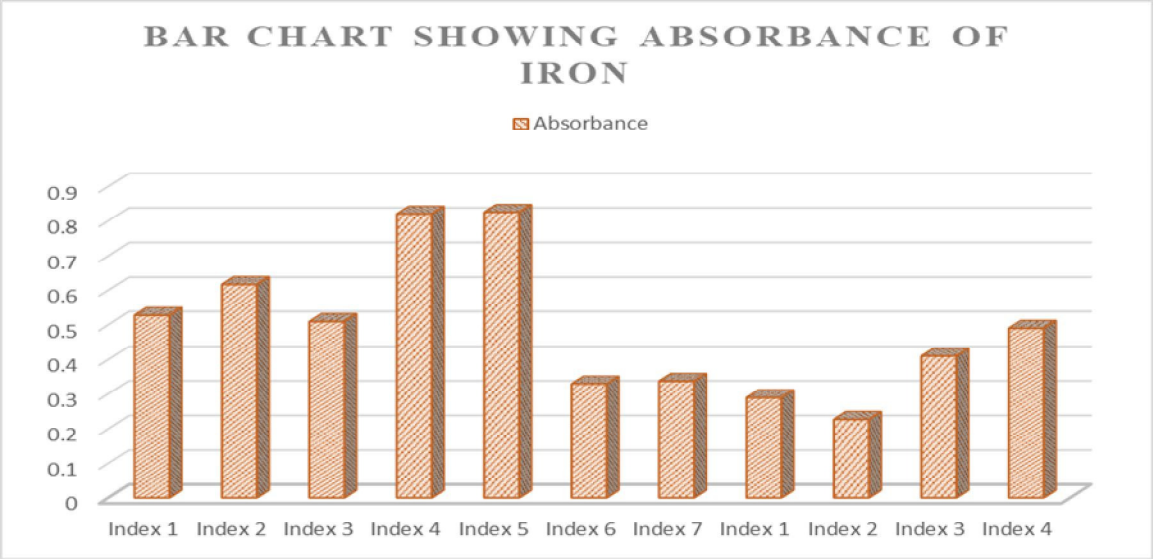


Figure 24: Results showing Absorbance values of Iron

The sequence of samples filled in the cuvette are

Table 3: Absorbance values of Iron

Index no.	Metal Induced	Colour Of Colony	Sample collection site	Absorbance
1	Fe	PURPLE	Brari Nambal	0.527
2	Fe	OFF WHITE	Habak	0.617
3	Fe	YELLOW	Hazratbal	0.509
4	Fe	PURPLE	Brari Nambal	0.820
5	Fe	YELLOW	Habak	0.825
6	Fe	OFF WHITE	Hazratbal	0.329
7	Fe	YELLOW	Brari Nambal	0.337
1	Fe	OFF WHITE	Hazratbal	0.289
2	Fe	YELLOW	Hazratbal	0.227
3	Fe	OFF WHITE	Hazratbal	0.410
4	Fe	OFF WHITE	Habak	0.490



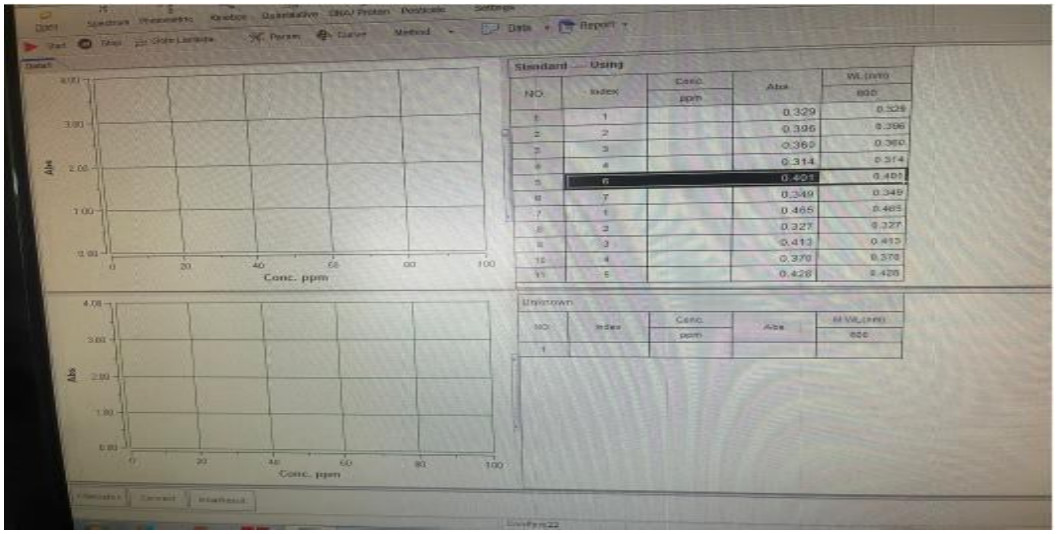
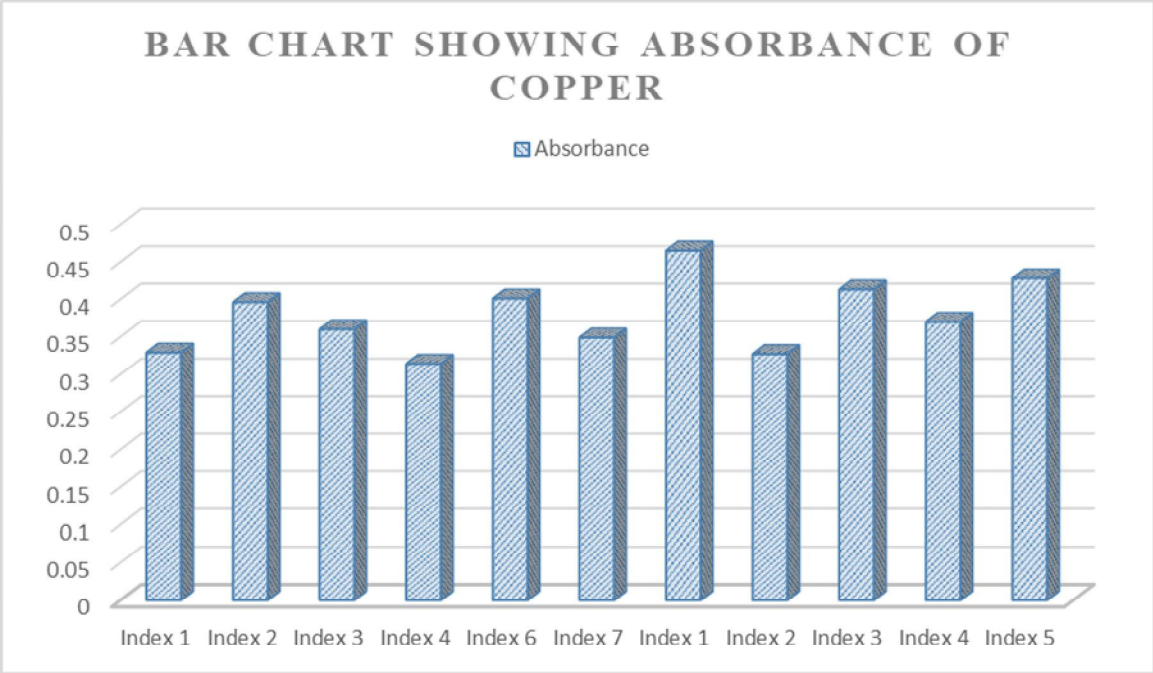


Figure 23: Results showing Absorbance values of copper

Table 4: Absorbance values of copper

INDEX NUMBER	METAL INDUCED	COLONY COLOR	SAMPLE COLLECTION SITE	ABSORBANCE
1	Cu	YELLOW	Brari Nambal	0.329
2	Cu	YELLOW	Habak	0.396
3	Cu	YELLOW	Habak	0.360
4	Cu	YELLOW	Brari Nambal	0.314
6	Cu	OFF WHITE	Brari Nambal	0.401
7	Cu	OFF WHITE	Brari Nambal	0.349
1	Cu	OFF WHITE	Brari Nambal	0.465
2	Cu	Off white	Brari Nambal	0.327
3	Cu	OFF WHITE	Hazratbal	0.413
4	Cu	OFF WHITE	Hazratbal	0.370
5	Cu	PURPLE	Brari Nambal	0.428



V. DISCUSSION

Bioremediation is a simple process many scientists use in waste treatment process for contaminated environments. The microbes that degrade the contaminant increase in numbers and release harmless products like water, carbon dioxide and cell biomass. Bioremediation has less effort, less labour usage and cost than other methods used to remove hazardous waste. It is also environment friendly, sustainable, and comparatively easy to implement. It is also beneficial for the total destruction of varied contaminants [10]. Several hazardous compounds can be converted to harmless products. Besides, bioremediation can be executed at the site of contamination itself without any major disturbance of usual activities. There is no requirement to transport huge numbers of off-site waste. There is no potential health risk, and no environmental contamination. Most of the disadvantages of bioremediation are due to it requiring a longer time to complete than other options like excavation and removing pollutants from the site. [11, 12] Also, it needs specific factors such as microbial populations, growth conditions, and quantity of nutrients and pollutants [13, 11].

The amount of UV light absorbed by a substance in a tissue depends on the concentration of the substance in the tissue. This means that spectroscopy can be used to quantify the concentration of a substance in an organism's tissues. The absorption of UV light by a substance is a measure of the strength of the interaction between the light and the substance. The stronger the interaction, the more light will be absorbed. The interaction between light and a substance is affected by the wavelength of the light. Different substances absorb different wavelengths of light. This is why it is important to use a specific wavelength of UV light when measuring the concentration of a substance using spectroscopy.

In our study, the absorption values of certain colonies for the metal Iron were quite high, signifying that a generous amount of metal was bioaccumulated. This was particularly evident in the purple colonies from STPs Brari Nambal and Hazratbal, which had absorption values that were significantly higher than the other colonies. The high absorption values for Iron in these colonies suggest that they may have been exposed to high levels of this metal.

Similarly, the absorption value for the metal copper was found to be high in the purple colonies from STP Brari Nambal. This suggests that these colonies may have been exposed to high levels of copper in their environment.

We undertook a thorough understanding of the principle, application, advantages, and disadvantages of the bioremediation technique and we were able to understand that it is an effective and sustainable method for sewage treatment.

VI. CONCLUSION

Bioremediation is a promising technology to treat sewage waste in Kashmir. It is a sustainable and cost-effective technique which can diminish the environmental impact of wastewater treatment.

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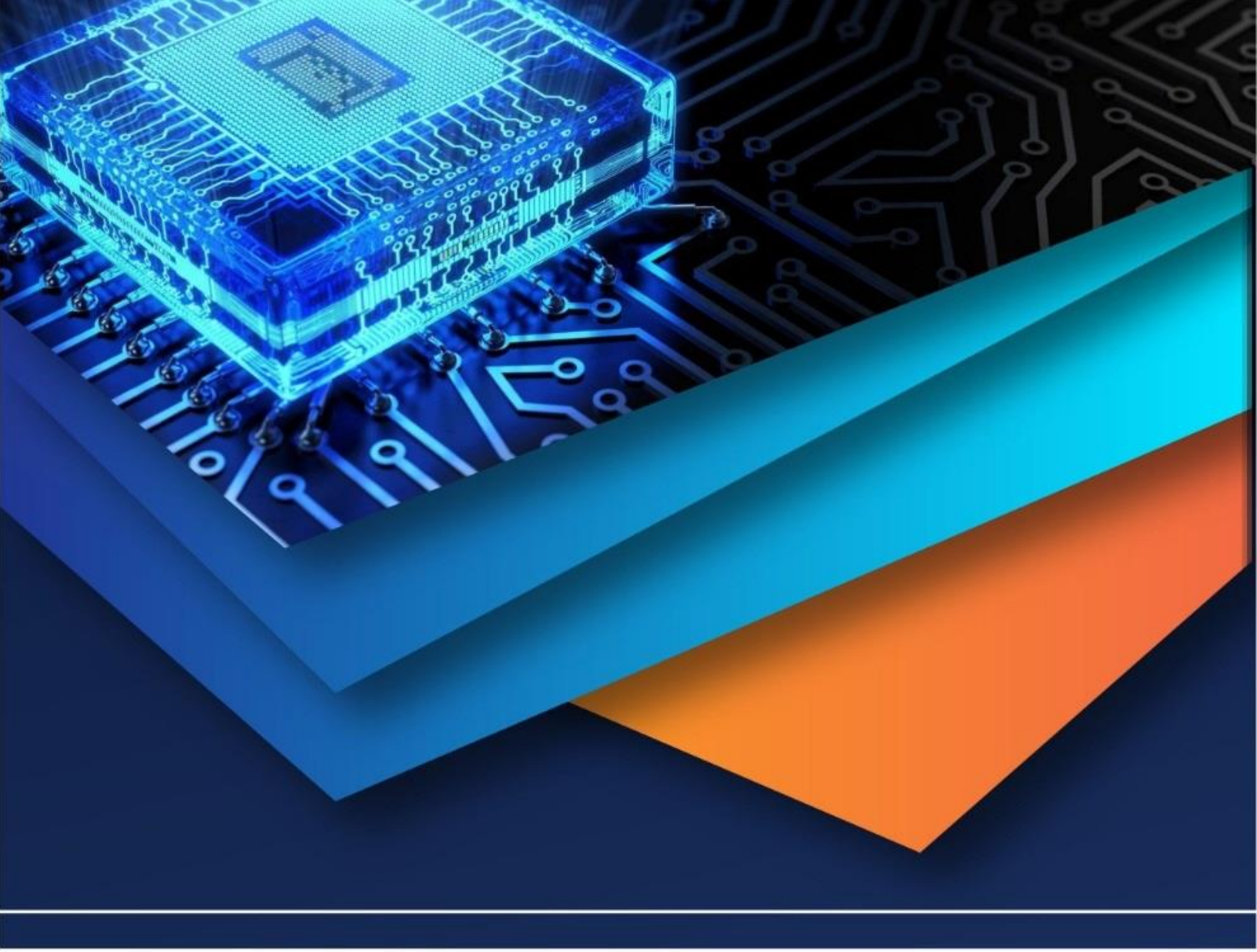

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