

BIOREMEDIATION OF COPPER CONTAMINATED SOIL USING BACTERIA

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Received: 6th Feb. 2013 Revised: 22nd Feb. 2013 Accepted: 26th Feb. 2013

Abstract: Bioremediation is the use of living organisms (primarily microorganisms) for removal of a pollutant from the biosphere. It relies on biological processes to minimize an unwanted environment impact of the pollutants. The microorganisms in particular have the abilities to degrade, detoxify and even accumulate the harmful organic as well as inorganic compounds. Five soil samples were collected from Selaqui industrial area, from different places at a depth of 0-15 cm. These soil samples were subjected to dilution (1:10), then from these dilution 4 and 5 were used for inoculation. Nutrient agar plates were prepared to be used as media. Replica of each dilution was prepared. After 24 hours of incubation at 28 degree centigrade bacterial colonies were observed on the plates. These cultures were purified to get 10 bacterial cultures. Further these cultures were inoculated in 10ml of nutrient broths each and after dense growth were inoculated in 10gm of soil samples in petriplates and were incubated for four days and then copper was estimated by Atomic Absorption Spectrometry technique and compared with the levels of copper obtained that were not inoculated with bacterial strains. The soil samples collected are all alkaline in nature; all the 10 isolated bacteria are gram negative and are chained cocci in structure. Sample 1 and 2, both dilutions have shown reduction in the amount of copper as compared to original soil samples without bacterial inoculation. According to this research sample 1 and sample 2 have shown reduction in the copper levels as compared to the raw soil samples that is without bacterial inoculation in them.

Keywords: Bacteria, Bioremediation, Heavy metals.

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INTRODUCTION

Heavy metal pollution can arise from many sources but most commonly arises from the purification of metals, e.g., the smelting of copper and the preparation of nuclear fuels. Bioremediation is the use of organisms such as bacteria, fungi, yeast, and algae to break down and thereby detoxify dangerous chemicals present in water, soil microorganism destroy organic contaminate in the course of using their chemical for their own growth and reproduction for new cell. Bioremediation is the "use of living organisms (primarily microorganisms) for removal of a pollutant from the biosphere". It relies on biological processes to minimize an unwanted environment impact of the pollutants. The microorganisms in particular have the abilities to degrade, detoxify and even accumulate the harmful organic as well as inorganic compounds. Besides them, higher plants also been reported to remove such pollutants, primarily through their tissues.

MATERIALS AND METHODS

Soil samples were analyzed under laboratory conditions at Forest Research Institute, Dehradun to achieve the objectives of the study.

Chemicals: All the chemicals used for the preparation of growth medium for bacteria and reagents for chemicals analysis were of analytical grade (AR) and were purchased from different firms in India.

Glassware: All the glassware used in the study was of Borosil glass.

Microbial cultures: Effective strain of *bacteria* was obtained from soil samples collected from sites where industrial effluents are released in soil at Selaqui in Dehradun district of Uttarakhand.

Isolation of bacteria: One gram of the inoculant was suspended in 10 ml sterilized distilled water in a test tube and serial dilution were prepared to obtain dilutions up to 10^{-7} . One ml suspension from 10^{-5} , 10^{-6} and 10^{-7} dilutions of the inoculant was separately plated with nutrient agar medium. The characteristic bacterial colonies were selected and purified by repeated streaking on the nutrient agar medium.

Maintenance, growth and cultural characteristics of the isolates: All the obtained and isolated bacteria were maintained on medium. All these were Gram negative and formed small, round, light yellow coloured colonies on the succinate medium. The bacteria were subjected to following cultural and biochemical tests for characterization and identification.

Cultural test: The cultural characteristics like Gram reaction, shape and motility were performed as described by Benson (1990).

Gram reaction: Gram staining of the isolates was done by following standard procedure as described by Vincent (1970). The cells appearing pink coloured under microscope were taken as Gram negative and the violet coloured cells were recorded as Gram positive. Shape of the isolates cells was also recorded while recording the Gram reaction.

Colony morphology: Colony characteristics of all the isolates were studied on solid surface of the nutrient agar medium in Petri dishes. The observation on forms, margins and elevation of the colonies were recorded.

RESULTS AND DISCUSSION

Five soil samples were collected from Selaqui industrial area, from different places at a depth of 0-15 cm. These soil samples were subjected to dilution (1:10), then from these dilution no. 4 and 5 were used for inoculation. Nutrient agar plates were prepared and one drop each of dilution 4 and 5 were added to the plates. Replica of each dilution was prepared. After 24 hours of incubation at 28 degree centigrade bacterial colonies were observed on the plates. These cultures were purified to get 10 bacterial cultures. These cultures were subjected to gram analysis for identification. Further these cultures were inoculated in 10ml of nutrient broths each and after dense growth were inoculated in 10gm of soil samples in petriplates and were incubated for four days and then copper was estimated by Atomic Absorption Spectrometry technique and compared with the levels of copper obtained that were not inoculated with bacterial strains. The concentration of copper was measured for soil samples to get the initial levels of copper in the soil by the use of atomic absorption spectrometry (Table 1). Gram tests done in order to classify the bacterial strains (Table 2). The concentration of copper was measured for soil samples mixed with the concentrated solution of bacteria and incubated to get the levels of copper in the soil by the use of atomic absorption spectrometry (Table 3).

Table 1 Concentration of copper in ppm

Sample	Concentration of Cu
1	2.426
2	0.696
3	0.486
4	1.888
5	1.1

Table 2 Gram Test

Culture No.	Characteristics
Sample no. 1, Dilution 4	Gram negative, cocci
Sample no. 1, Dilution 5	Gram negative, cocci
Sample no. 2, Dilution 4	Gram negative, cocci
Sample no. 2, Dilution 5	Gram negative, rod shaped
Sample no. 3, Dilution 4	Gram negative , cocci
Sample no. 3, Dilution 5	Gram negative ,cocci in chains
Sample no. 4, Dilution 4	Gram negative, cocci

Sample no. 4, Dilution 5	Gram negative , cocci, in chains
Sample no. 5, Dilution 4	Gram negative , cocci
Sample no. 5, Dilution 5	Gram negative , cocci

Table 3 Copper concentrations (ppm)

Sample no. 1	2.426
S-1, D-4	1.696
S-1, D-5	1.234
Sample no. 2	0.696
S- 2, D- 4	0.542
S-2, D-5	0.408

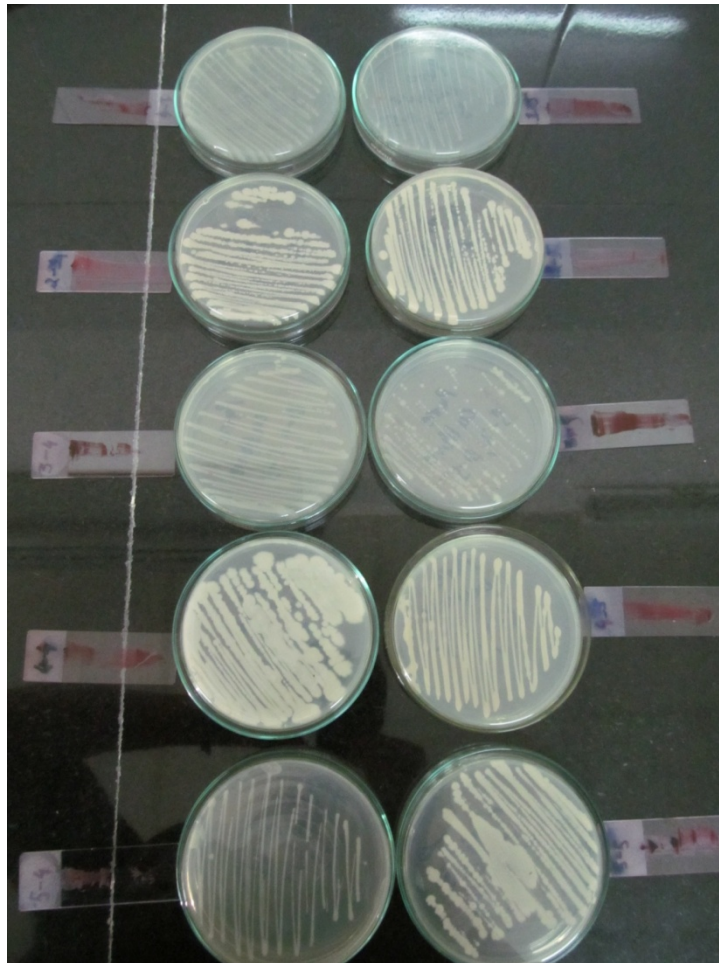


Fig. 1 shows the 10 purified strains of bacteria used for analysis

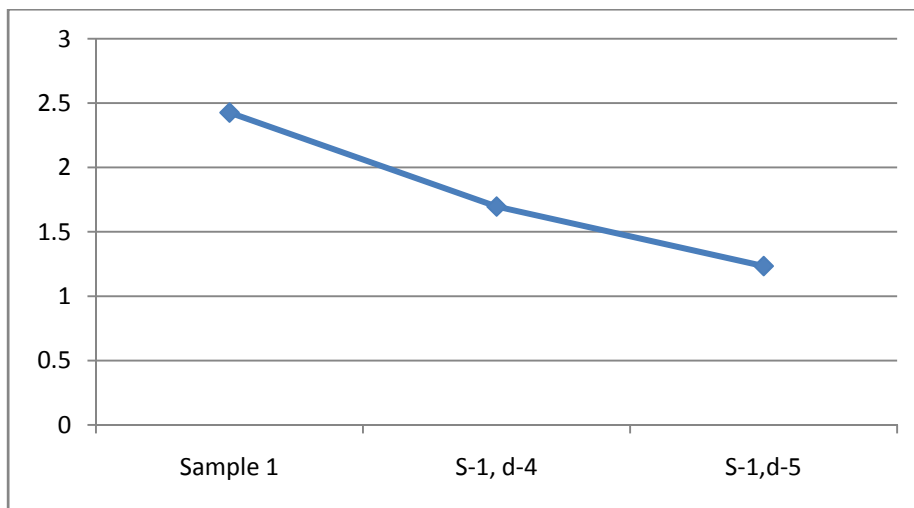


Fig.2 Soil sample 1 shows a decrease in copper levels

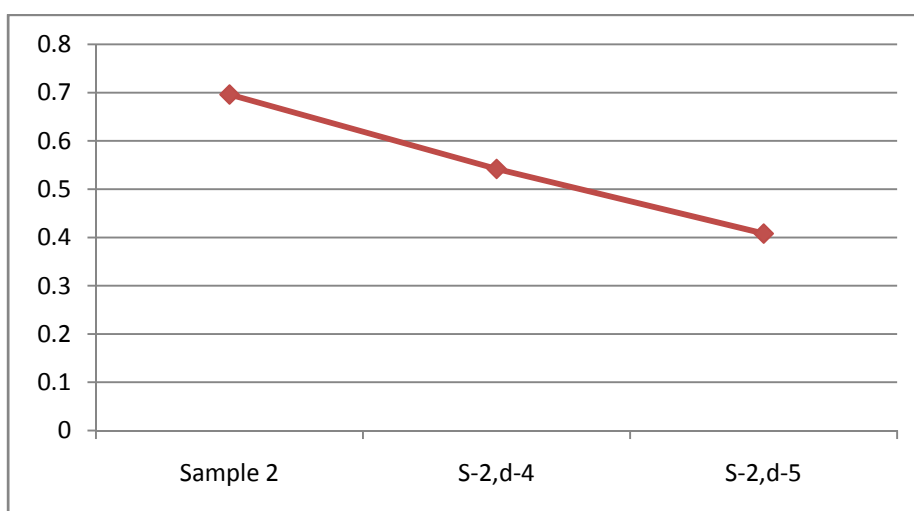


Fig. 3 Soil sample 2 shows a decrease in copper levels

CONCLUSION

The soil samples collected are all alkaline in nature; all the 10 isolated bacteria are gram negative and are chained cocci in structure. Sample no. one both dilutions have shown reduction in the amount of copper as compared to original soil samples without bacterial inoculation. Thus, biotoxic effects, when unduly exposed to them could be potentially life threatening hence, cannot be neglected. Bioremediation is a potential method for solving the problem of heavy metal pollution According to this research sample no. 1 and sample no. 2 have shown reduction in the copper levels as compared to the raw soil samples that is without bacterial inoculation in them.

Acknowledgements: Authors are thankful to Ms. Nandini Maithani for her help during sample collection and Ms. Anshu Chaudhary for proof reading of the entire research document.

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