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Isolation of Bacillus Sp. from Ash Dyke Area of Power Plant of Chhattisgarh

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Abstract: ***Approximately four varieties of bacteria has been isolated from the ash dyke sample of four power plants of Chhattisgarh. Bacillus being the dominant. Total 11 isolates were found to be Bacillus sp. The strains were tested for their tolerance against six different types of heavy metals dominat in the ash samples viz. Pb, Hg, Ni, Co, Cu, Mn.***

Keywords: Ash, heavy metals, MIC, *Bacillus sp.*, Bioaccumulation

1. Introduction

Microorganisms play an important role on nutritional chains that are an important part of the biological balance in the life in our planet. Soil contains a variety of microorganisms included bacteria that can be found in any natural ecosystem. Without microorganisms the decomposition process of organic matter such as straw or fallen leaves would not take place.

Soils normally contains low background levels of heavy metals. However, the areaes which are highly exploited by human hand viz. municipal or industrial waste disposal or areas involved in application of fertilizers may contain higher levels of heavy metals.

Microbial survival in polluted soils depends on intrinsic biochemical and structural properties, physiological, and/or genetic adaptations including morphological changes of cells, as well as environmental modifications of heavy metal speciation (Rajendran and Gunasekaran, 2007). Thus, levels of metal concentrations in soil can also be an important factor to affect the microbial diversity qualitatively as well as quantitatively.

This work was aimed to detect the presence of Bacillus strains capable to tolerate the heavy metal concentrations and to determine their extent of tolerance.

2. Materials and Methods

2.1 Sampling

Soil and effluent samples were obtained from Ash Dyke area of Thermal Power Plants of Chhattisgarh. The samples were collected in sterilized flasks and beakers and after sealing with parafilm, they were stored at 4˚C in the fridge.

2.2 Isolation of Microorganisms

Different bacteria were isolated from the Ash Dyke area of four different plants situated in Chhattisgarh, using serial dilution technique in Nutrient medium. Following and consulting the available literatures, Nutrient medium was selected as the best medium for the isolation of bacteria [Unaldi et al., 2005; Zolgharnein et al., 2007; Bahig et al., 2008].

1g of soil and 1ml of effluent was poured into different flasks having 9ml of saline. From these samples then, their respective serial dilution series was prepared. Out of which, $10^{-3}$and 10^{-4} dilutions were selected for soil and were spreaded onto the sterilized petriplates containing the Nutrient medium [Jiang et al., 2008; Kamala-Kanan & Lee, 2008]. Then the 25 possibly different isolates were selected randomly from the plates and were streaked by pick and patch method onto the separate petriplates containing Pb, Hg, Cu, Co, Ni and Mn in concentration 0.04mM/ml. Isolates were named as SM1 to SM25.

2.3 pH and Temperature

Selection of optimal pH and temperature for growth is done by selecting; pH 4, 6, 8, 10 and temperature 4^0C, 15^0C, 29^0C and 5^0C. Both test were performed with metal containing medium and it was found that pH 6 and temperature 35^0C was most suited for the isolated bacilli for sustaining their growth.

2.4 Bacterial Minimum Inhibitory Concentration (MIC) and Maximum Tolerance Concentration (MTC) for Heavy Metals

The minimum inhibitory concentration (MIC) has formed the basis for the selection of the potent isolates and hence forth 11 Bacilli were found. MTC of these selected isolates towards selected heavy metal has been checked in 0.08, 0.2, 0.4 and 0.6mM/ml of the six metals in Nutrient Agar medium.

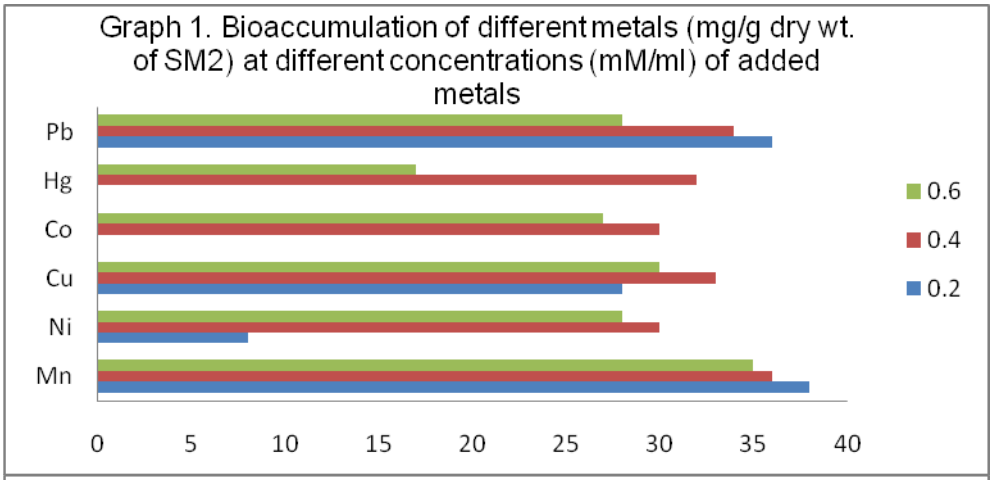
2.5 Identification of the isolates

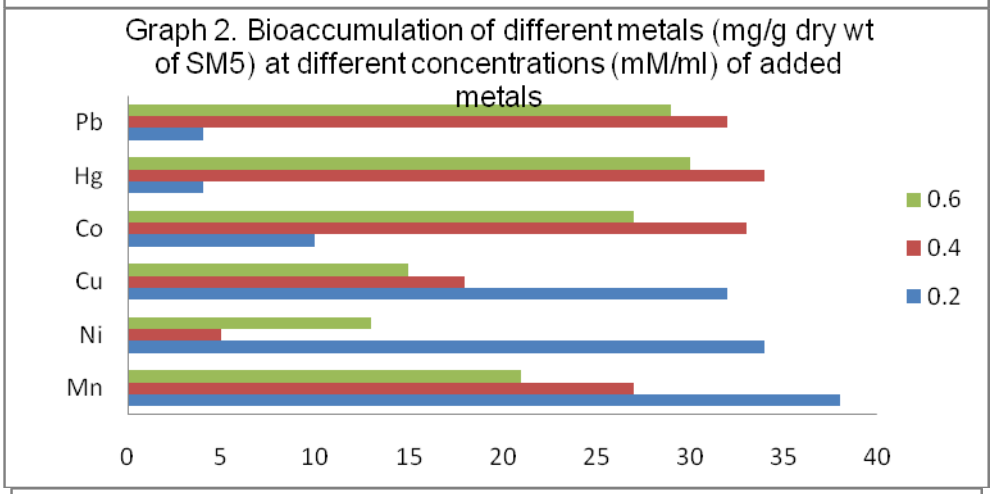
Biochemical tests formed the basis for the detection of the 11 isolates. Bacterial isolates were identified as per the standard methods following Bergeys Manual of Determinative Bacteriology. The isolates (SM 2, 5, 6, 7, 10, 15, 17, 18, 20, 22 and 23) are determined as *Bacillus sp.*

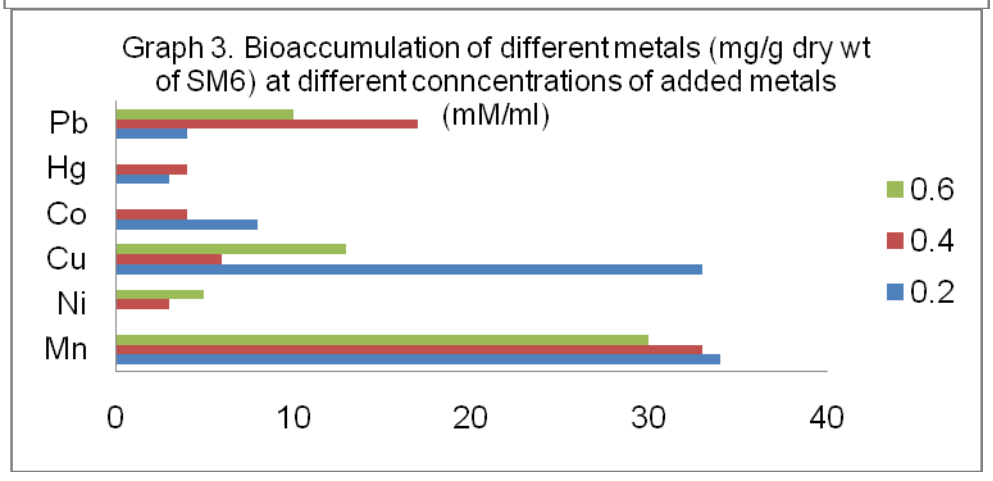
2.6 Biosorption of Metals by the Bacillus sp.

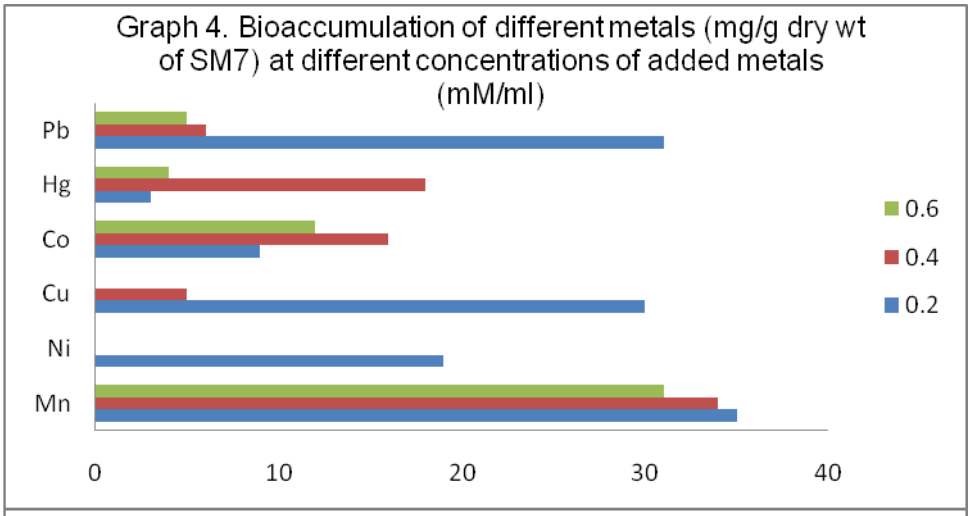
Metals were added to EG broth medium at concentration of 0.2, 0.4 and 0.6mM/ml and the 11 isolates were separately inoculated. The flasks were kept at 35'C for 48hrs at 120rpm. Then the cells were harvested by centrifugation and suspended in 1ml distilled water. The metal content of bacterial cells was determined after acid dissolution of bacterial cells using the method of Ganje and Page (1974). Metal concentration was measured by AAS.

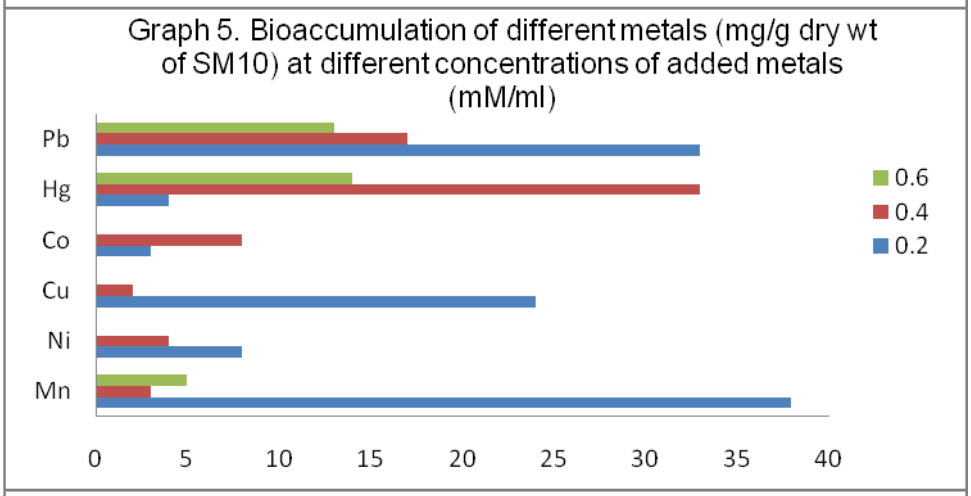
3. Result and Discussion

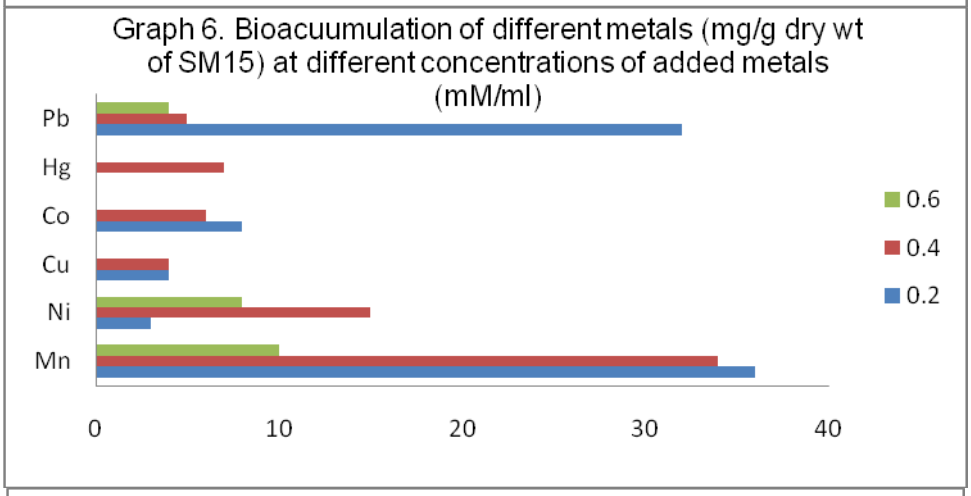


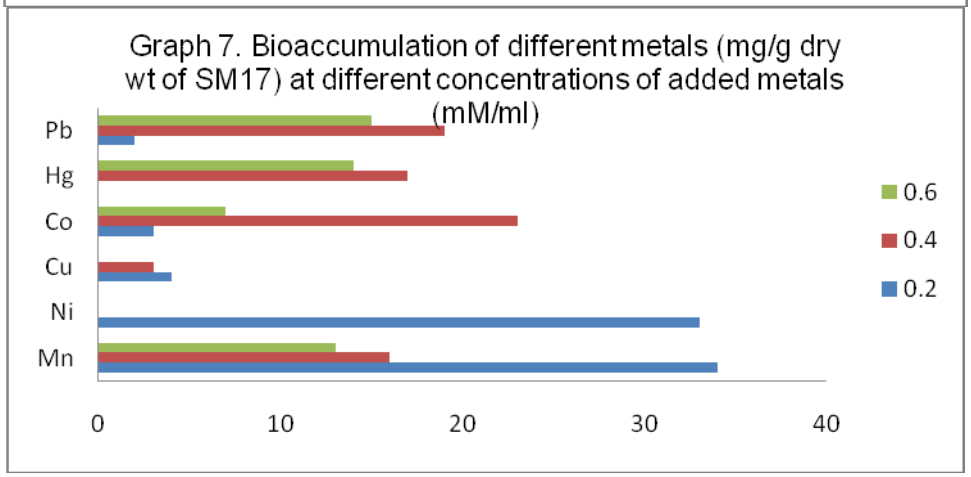


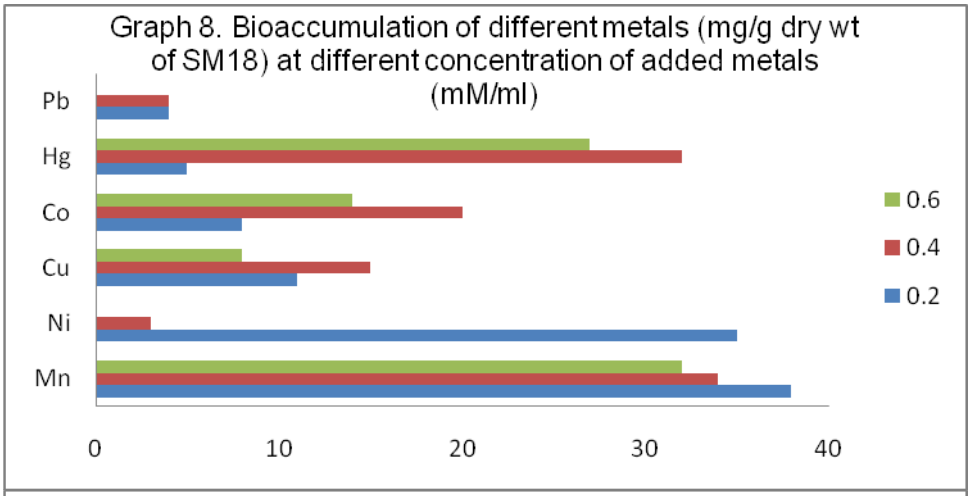


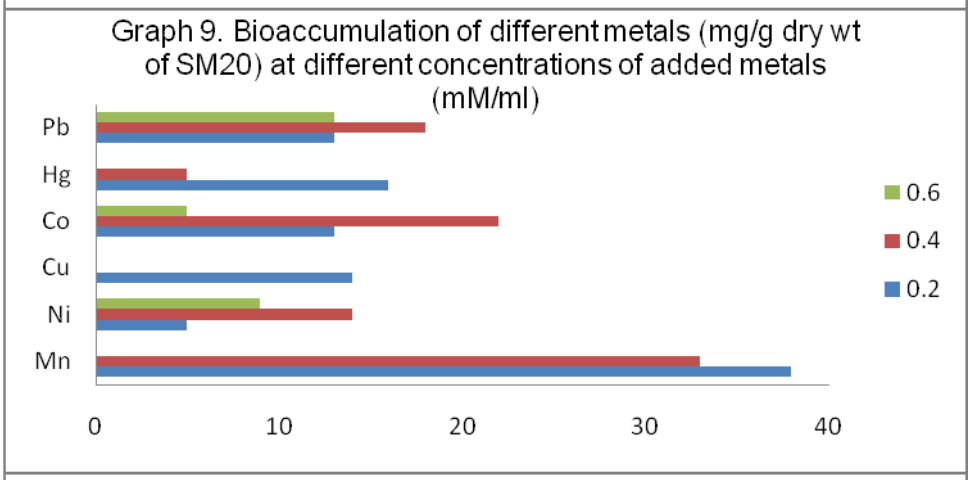


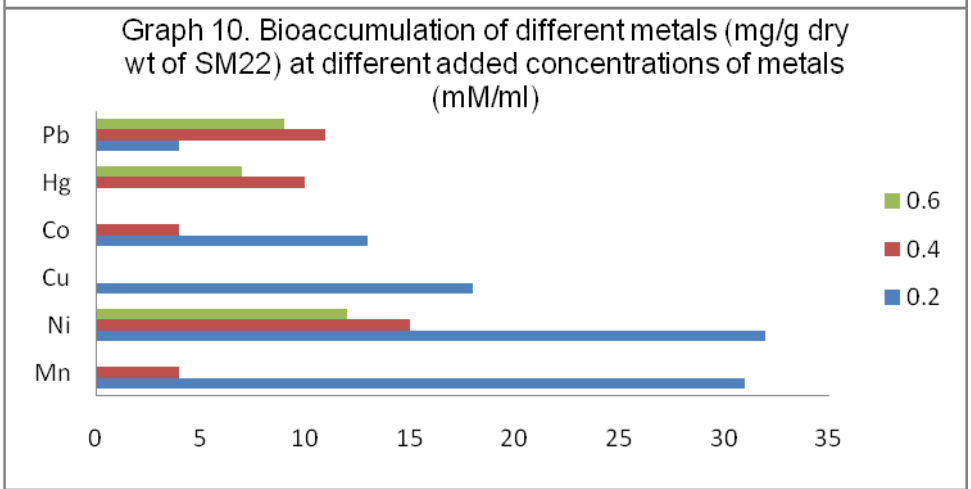


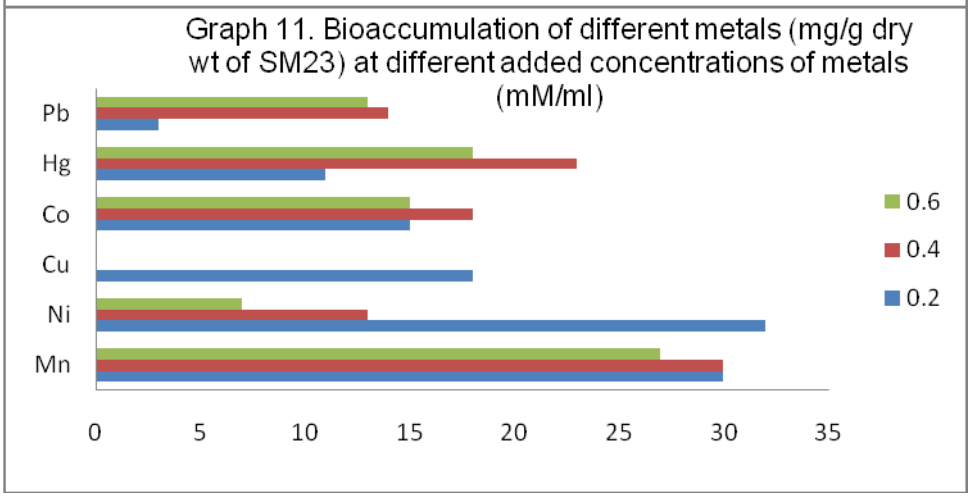












11 Bacilli isolated from the ash dyke samples of the power plants have been found to accumulate Mn, Ni, Cu, Co, Hg and Pb in good concentration (mg/g dry wt of the cells) when the added concentration of the metal in the media was low (0.2mM/ml) and was found to decrease considerably when the metal concentrations were increased to about 0.6mM/ml. SM 2, 5, 6, 7, 17, 18 and 23 were found to accumulate most of the metals even at higher concentrations (0.6mM/ml) where as SM 10, 15, 20 and 22 could accumulate 2-3 metals only at higher concentrations.

In the present study, the 11 bacilii isolated from the ash sample of power plants were selected owing to their maximum relative growth and metal accumulation ability. These results are consistent with the screening for multi-metal resistant bacteria performed in previous studies (Vullo et al. 2005; Vullo et al. 2008). Two factors can be considered to account for metal toxicity viz, at low concentrations, metals (Co, Cu and Mn) are essential for microbes as they act as vital cofactors for metalloproteins and enzymes.

In this study we have also found that for the selected six metals, the metal accumulating ability of the isolates diminished beyond the specific concentrations of the metals. This might be because of the saturation of the isolates with metals due to increased toixicity of metals at high concentrations (Kaewehai and Prasertson, 2002; Al-Garni, 2005).

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