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In-situ remediation of mine soils with biochar and evaluation of its effectiveness

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Abstract: In this report we briefly present in-situ remediation of mine soils with biochar and and evaluation of its effectiveness with *Acacia auriculiformis* plant and *Azospirillum* bacteria.

Keywords: bioremediation; mine soil; biochar; acacia auriculiformis; azospirillum

1 Introduction

The term ‘phyto’ means plant and 'remediation' means reversing of environmental damage. Phytoremediation is a generic group of technologies that use specific plants for bioremediating soils, sludges, sediments and water contaminated with organic and inorganic contaminants. It involves growing plants in a contaminated matrix (soil, water or sediments) for a required growth period, to remove or facilitate immobilization or degradation of the pollutants [1,2].Among various techniques used to remediate mine soil one effective strategy is by amendment of the mine soil with biochar. The word ‘biochar’ is a combination of ‘bio’ as in ‘biomass’and ‘char’ as in ‘charcoal’. It is a high-carbon, fine-grained residue that today is produced through pyrolysis processes like direct thermal decomposition of biomass in the absence of oxygen (preventing combustion), which produces a mixture of solids (the proper biochar), liquid (bio-oil) and gas (syngas) products [3,4].

The main purpose or motivation of the study is as follows. Mine soils in different locations around the world pose a serious problem of environmental pollution with the release and accumulationof heavy metalsand metalloids. It also leaches into neighbouring areas causing severe toxicity to local and surrounding plants, cattle, aquatic and human populations. Thus, there is need to mitigate those problems caused near mine areas as part of post mining procedures [2]. Biochar is effective at retaining both water as well as water-soluble nutrients and reduce leaching due to its hygroscopic and porous structure. It is a suitable habitat for many beneficial soil micro-organisms and it has also shown to increase soil fertility and improve disease resistance in soils [4,5].

2 Objectives

(i) To investigate the potential of biochar to remediate sample soils from Dhanbad coal mines, India in a pot experiment with *Acacia auriculiformis* plant and *Azospirillum* bacteria.

(ii) To check if there are any toxic or hazardous effects of using biochar on plants through Allium genotoxicity test.

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3 Literature survey

3.1 Bioremediation of soils with the help of biochar

Qin et al. [5] reported in their study that biochar has the potential to reduce toxicity of petroleum contaminated soils in China. It was found that the biochar made from rice straw could absorb petroleum metabolites. With the help of soil micro-cosmitit was found that biochar has the ability to degrade contaminants in soil. The degrading efficiency was significantly higher in soils amended with biochar than in soils without biochar. It was also shown that biochar did not result in negative impacts on the composition of soil microbial community.

3.2 *Acaciaauriculiformis* as a metal hyperaccumulating plant

Acacia auriculiformis is a fast growing tree in the family Fabaceae, originating from Australia and having densely matted root system which makes it suitable for stabilizing eroded land. It has been used for water and soil conservation and also to improve soil fertility in barren regions of South China. Its extraordinary drought tolerant and metal resistant capabilities along with high biomass production makes it an ideal plant species for phytoremediation [9].

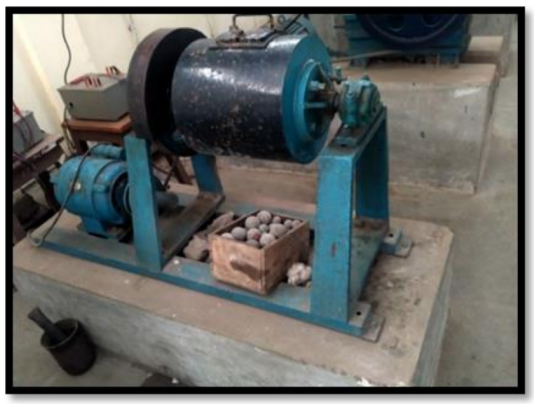
3.3 Azospirillum as a plant growth promoter in biotic stress

Azospirillum is a Gram-negative, microaerophilic, non-fermentative and nitrogen-fixing bacterial genus from the family of Rhodospirillaceae. Azospirillum bacterium fixes the atmospheric nitrogen and makes it available to plants in non-symbiotic manner that can replace 50-90 % and of the nitrogen fertilizer required by plants and enhances the plant growth. Azospirillum is also a biofertilizer and can also promote plant growth by mechanisms of tolerance of abiotic and biotic stresses [6,7,8].

4 Materials and methods

4.1 Preparation of biochar

Eucalyptus tree bark was pyrolysed at pyrolysing chamber in Department of Environmental Science & Engineering, Indian Institute of Technology, Kharagpur. Two varieties were prepared: (i) Pyrolysed at 400 °C for 45 minutes.(ii) Pyrolysed at 600 °C for 45 minutes. Biochar was then crushed to powder form with ball mill in Department of Mining Engineering, Indian Institute of Technology, Kharagpur.



Figs. 1 & 2: Pyrolysis chamber and Ball mill

4.2 Composition of soil samples

Each pot test sample carried 2kg of mine soil from coal mines of Dhanbad in the state of Chattisgarh, India. Biochar in crushed powder form was mixed uniformly with those pot test samples. Six controls were also used in the study. Two of which contained 10^8 CFU of Azospirillum in 2 kg of mine soil. Two of the positive controls C3 and C4 contained mine soils only. Other two werenegative controls C1-S and C2-S containing Azospirillum only. The remaining C - F contained fertilizer with mine soil.

Sample ID	**Biochar(gm)**	**Sample ID**	**Biochar (gm)**	**Sample ID**	**Ingredients**
0.5B1C	10	0.5B1C- NP	10	C1 - S	*Azospirillum*
1B1C	20	1B1C - NP	20	C2 – S	*Azospirillum*
2B1C	40	2B1C - NP	40	C3	Mine soil
5B1C	100	5B1C -NP	100	C4	Mine soil
10B1C	200	10B1C - NP	200	C – F	Fertilizer
0.5B2C	10	0.5B2C- NP	10		
1B2C	20	1B2C - NP	20		
2B2C	40	2B2C - NP	40		
5B2C	100	5B2C -NP	100		
10B2C	200	10B2C - NP	200		

Table 1: Composition of soil samples

Fig. 3: Soil samples with different biochar concentrations

4.3 Study design and greenhouse

A pot study was designedin which several pots for different concentrations of mine soil was amended with test material biochar. The pots were kept at a small greenhouse near the Department of Mining Engineering, Indian Institute of Technology, Kharagpur, India.

Fig. 4: Pots inside greenhouse

4.4 Estimation of plant leaf chlorophyll content

For the estimation of chlorophyll A, chlorophyll B and total carotenoid content in *Acacia auriculiformis* leaves, the standard procedure as performed by Dere et al. [10] was followed.

(i) 5 gm of leaf samples were crushed separately in 95% diethyl ether (50 ml for each gram).
(ii) Samples were homogenized & centrifuged at 2500 rpm for ten minutes & the supernatant was separated.
(iii) Absorbance was read on spectrophotometer by the formula given in Table 2.

Table 1. The formulas that used in the calculation of chlorophyll a, chlorophyll b and total caroten levels

Diethyl ether	$C_a = 10.05\ A_{662} - 0.766\ A_{644}$ $C_b = 16.37\ A_{644} - 3.140\ A_{662}$ $C_{x+c} = 1000\ A_{470} - 1.280\ C_a - 56.7\ C_b/230$
Methanol	$C_a = 15.65\ A_{666} - 7.340\ A_{653}$ $C_b = 27.05\ A_{653} - 11.21\ A_{666}$ $C_{x+c} = 1000\ A_{470} - 2.860\ C_a - 129.2\ C_b/245$
Acetone	$C_a = 11.75\ A_{662} - 2.350\ A_{645}$ $C_b = 18.61\ A_{645} - 3.960\ A_{662}$ $C_{x+c} = 1000\ A_{470} - 2.270\ C_a - 81.4\ C_b/227$

C_a = Chlorophyll a, C_b = Chlorophyll b, C_{x+c} = Total carotene

Table 2: Formulas for estimation of chlorophyll content

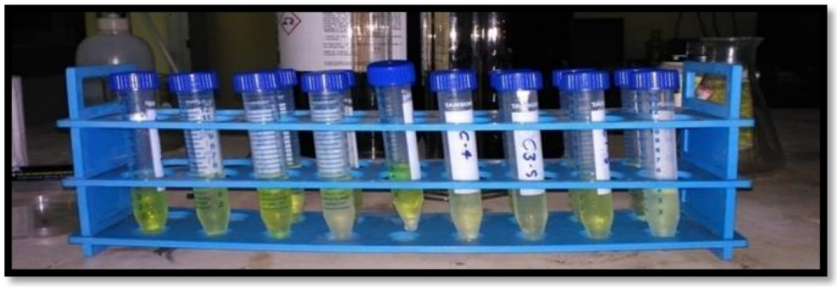


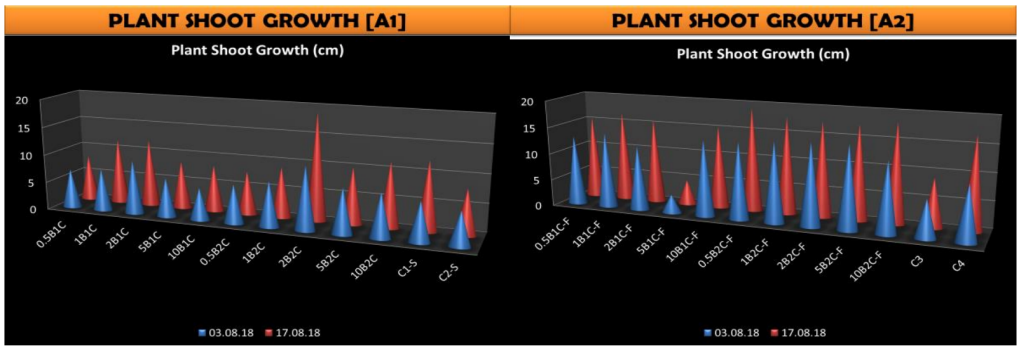
Fig. 5: Leaves homogenized in 95% Diethyl Ether

4.5 CFU count of Azospirillum bacteria

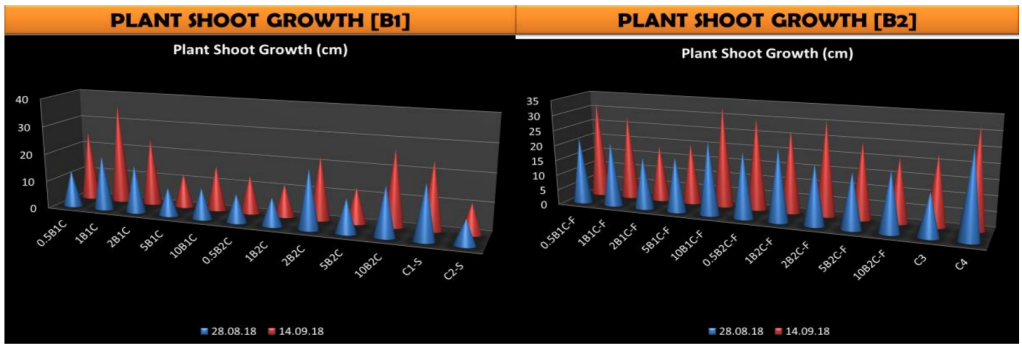
10^8 CFU/ml of Azospirillum was cultured in Azospirillium growth media in Department of Mining Engineering, Indian Institute of Technology, Kharagpur, India. In the test soil samples 1 ml of Azospirillum was taken in 50 ml of 0.85% saline distilled water and then mixed uniformly with 2 kg of test soil samples. Thus theoretically, there was 1 ml or 10^8 CFU of Azospirillum in those selected pot soils. Therefore, 1 gm of those soil should contain 50,000 CFU which can be taken as the comparable measure of Azospirillum at the starting point of the experiment. After 3 months those bacteria from pot soils were diluted upto 10^{-4} times and pour plated in selective Azospirillum medium for colony count.

5 Results and discussion

5.1 Plant shoot growth



Tables 4&5: *Acacia* plant shoot growth from 03.08.2018 to 17.08.2018



Tables 6&7: *Acacia* plant shoot growth from 28.08.2018 to 14.09.2018

From the above tables it can be seen that plant shoot growth is slightly higher in samples with both biochar and fertilizer inclusion when compared with controls and samples with only biochar amended soil.

5.2 Leaf chlorophyll content

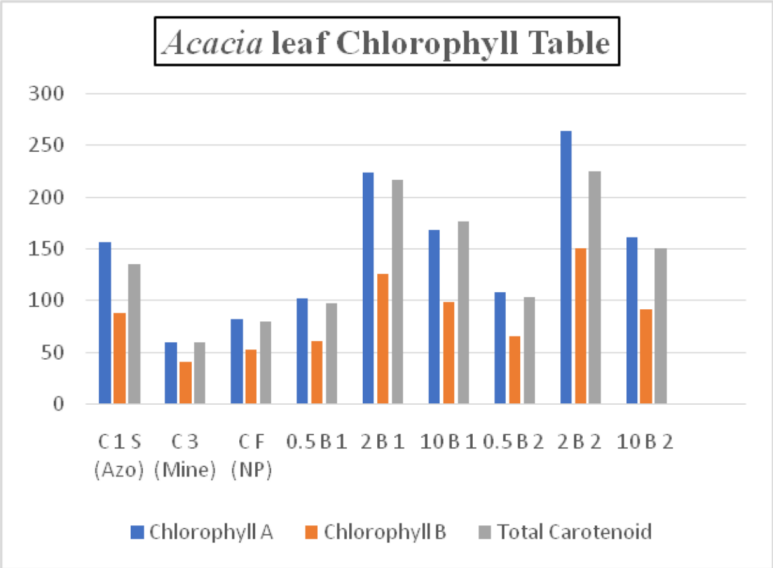


Table 8: *Acacia* leaf chlorophyll content

From the above table it can be seen that chlorophyll A, chlorophyll B and total carotenoid content was significantly higher in soil samples amended with biochar. The samples 2B1 and 2B2 which contained 40gm/kg biochar showed the most positive results.

5.3 Bacteria CFU count

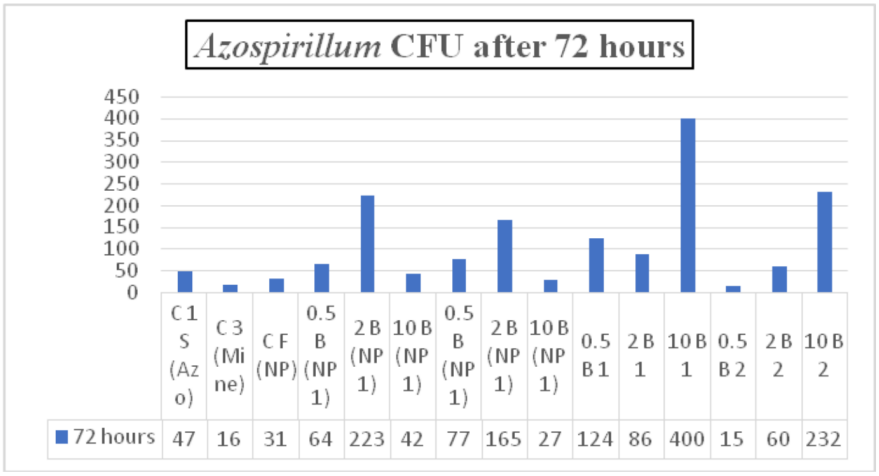


Table 9: *Azospirillum* CFU after 72 hours incubation

From the above table it can be seen that *Azospirillum* bacteria had higher viability rates in biochar amended soils. Here the soils amended only with biochar showed significantly higher bacteria viability rate than controls and fertilizer included biochar soils with 10B1 and 10B2 being the highest of them all.

Fig. 6: CFU count in petri plates

7 Conclusion

Medium dosage of biochar at 40 gm/kg of mine soil to be amended was found to possess the highest remediation potential among dosages of 10gm/kg, 20gm/kg, 40gm/kg, 100gm/kg and 200gm/kg of biochar in mine soils of Dhanbad coal dump region, state of Jharkhand, India. The remediation potential can be well proven altogether with similar patterns of data coming from plants and microbes which can be summed up as –

Acacia auriculiformis shoot growth data; *Acacia auriculiformis* leaf chlorophyll a, b and total carotenoid content; higher viability of *Azospirillum* colony forming units tested in biochar amended soil.

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