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Research article

# Phytotoxicity attenuation in *Vigna radiata* under heavy metal stress at the presence of biochar and N fixing bacteria

Mihiri Seneviratne [a, b], Lakshika Weerasundara [a], Yong Sik Ok [c], Jörg Rinklebe [d], Meththika Vithanage [a, *]

[a] Chemical and Environmental Systems Modeling Research Group, National Institute of Fundamental Studies, Kandy, Sri Lanka
[b] Department of Botany, Faculty of Natural Sciences, Open University of Sri Lanka, Nawala, Nugegoda, Sri Lanka
[c] Korea Biochar Research Center & School of Natural Resources and Environmental Science, Kangwon National University, Chuncheon 200-701, Republic of Korea
[d] University of Wuppertal, Soil- and Groundwater-Management, Pauluskirchstraße 7, 42285 Wuppertal, Germany

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ABSTRACT

This study assesses the effect of N-fixing bacteria and biochar synergism on plant growth and development of *Vigna mungo* under heavy metal stress (HM). Heavy metal stress is a worldwide problem, which causes critical effects on plant life due to oxidative stress. Application of biochar is a recent biological remediation technique, which often leads to an immobilization of heavy metals in soil. . Synergism of bacteria and biochar is a novel aspect to enhance plant growth under heavy metal stress. Woody biochar a byproduct of a dendro power industry was added as 1, 2.5 and 5% amounts combination with *Bradyrhizobium japonicum*, where mung seedlings were planted in serpentine soil rich in Ni, Mn, Cr and Co. Pot experiments were conducted for 12 weeks. The plant height, heavy metal uptake by plants, soil bioavailable heavy metal contents, soil N and P and microbial biomass carbon (MBC) were measured. The plant growth was enhanced with biochar amendment but a retardation was observed with high biochar application (5%). The soil N and P increased with the increase of biochar addition percentage while soil MBC showed reductions at 5% biochar amendment. Both soil bioavailable fractions of HM and up take of HMs by plants were gradually reduced with increase in biochar content. Based on the results, 2.5% biochar synergism with bacteria was the best for plant growth and soil nutrition status. Despite the synergism, available N was negatively correlated with the decrease of bioavailable metal percentage in soil whereas it was conversely for P.

## 1. Introduction

The deleterious effect of environmental pollution on biotic factor is a strict concern in the recent decade. Due to the industrial revolution, excess usage of fertilizer and pesticides, wastewater discharge and use in agriculture, landfill leachates and mine tailings accumulates a number of heavy metals (HMs) in soil (Järup, 2003). The available fraction of HMs can easily be interact with soil fauna, soil microbes and vegetation (Giller et al., 1998). Via the production of reactive oxygen species or the attachment of the enzymes, heavy metals can hinder the cellular biochemical reactions (Gajewska and SkŁodowska, 2010). Oxidatively damaged lipids and proteins are the major indications in oxidative stressed cells. Peroxidation of lipids enhance the permeability of cell membranes and affects the physiological and biochemicals reactions (Stark, 2005). As a result of protein carbonylation ketone or aldehyde derivatives are formed and it alters the protein structure and leads to its activity inhibition. The irreversible carbonylation is considered as one of the major destructive modifications in cellular macromolecules (Møller et al., 2007).

Heavy metal contaminated soils may not necessarily be nutrient depleted but both phenomena can occur in parallel. Such extreme environments are neither suitable for plant nor soil microbial growth (Peralta et al., 2001). In recent years biochar (BC) has received significant attention as a biological soil amendment to improve soil fertility, crop production, and nutrient retention in soil (El-Naggar et al., 2015; Hussain et al., 2016; Zhang and Ok, 2014). They are resistant to degradation and are estimated to remain in

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* Corresponding author.
*E-mail address:* meththikavithanage@gmail.com (M. Vithanage).

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soil for thousands of years (Lehmann et al., 2008). Chars are composed of numerous surface groups, such as carboxylic, phenolic, hydroxyl, carbonyl, and quinines (Cheng et al., 2006). Whereas its porous structure affects on soil cation exchange capacity (CEC), pH, and retention of water and nutrients (Glaser et al., 2002). The physical and chemical properties of chars vary significantly depending on the biomass source and pyrolyzing (Ahmad et al., 2014). In the recent past, the decrease of the bioavailable fraction of HMs and the increase in plant growth in contaminated soils have been reported in experiments conducted under greenhouse conditions (Houben et al., 2013a,b; Park et al., 2011).

Similar to the benefits, the limitations and drawbacks of the use of BC for the remediation of contaminated soils are also of particular concern specifically on the addition of BC to soil in excessive levels may cause detrimental impacts on soil structure, soil biota and pose serious consequences for the growth of crop plants. Yet, very little is known about the influence and mechanisms of biochar affects soil microbial communities. Many studies have indicated an increase or neutral action of BC on soil microbes (Lehmann et al., 2011; Xu et al., 2016), few studies have shown that high BC application may reduce or hinder soil microbial activities (Bandara et al., 2015; Dempster et al., 2012) however, those studies are limited and hence further research is essential. Application of promising soil microbe cultures may also improve soil health. Although different types of BCs exhibited successful results on reducing heavy metal stress in several crops under various conditions (Al-Wabel et al., 2015; Beesley et al., 2013; Herath et al., 2015), few studies have investigated the promising effect of combination of microbes and BCs (Bandara et al., 2015; Seneviratne et al., 2015). The ability of BCs to immobilize HMs in polluted soils may additionally lead to a favorable microenvironment for the inhabited organisms (Bandara et al., 2015).

Serpentine soil is considered as an extreme environment for plants and microbes, consists of extremely low levels of essential plant nutrients (e.g. N, P, Ca), enormously high levels of HMs, in particular Ni, Mn, Cr, and Co, and very poor water availability and retention (Vithanage et al., 2014). The crops that are grown in serpentine soil, exhibit phytotoxicity due to high content of HMs (Herath et al., 2014). The soil microbial pool may be low in serpentine soil, due to the heavy metal stress. Thereby, it hinders the effective nodulation and N fixation in legume plants (Bååth, 1989). To date, no studies have investigated the ability of N fixing bacterium and their interactions with BCs on plant growth and development. Nitrogen fixing bacteria such as *Bradyrhizobium japonicum* may provide N via nodulation where BCs offer more nutrients and immobilize heavy metals may enhance soil microbial activity and soil nutrient status in extreme soils. *Vigna radiate* is an annual legume, which is used as a food crop, especially in tropics. Under low soil N quantities legumes are able to fix atmospheric N (Rondon et al., 2007; Sawhney et al., 1990). Hence, this study assesses the synergistic effect of legume-root nodulating microbe, *Bradyrhizobium japonicum* with woody BC (produced by *Gliricidia sepium*) application on *Vigna radiate* in terms of enhancing nutrient availability, plant growth development and immobilizing heavy metals in serpentine as a model soil.

## 2. Materials and methods

### 2.1. Soil collection

Soil was obtained from Wasgamuwa (Latitude 7° 71′ 67″ N and longitude 80° 93′ 33″ E) serpentine area, Sri Lanka (Vithanage et al., 2014). Soil samples were collected within 10–15 cm from the surface after removing the surface litter layer from five random locations. The samples were sealed in polypropylene bags and transferred immediately to the laboratory and bulk soil was mixed together. The initial metal concentrations were reported in an earlier study as 6567, 2609, 14880 and 555 mg/kg of Ni, Mn, Cr and Co, respectively (Vithanage et al., 2014).

### 2.2. Microbial inoculums and biochar collection

A *Bradyrhizobium japonicum* suspension in yeast manitol broth of 0.573 at 600 nm Shimadzu UV-2450 was used as the microbial inoculums in our study. Biochar formed as a waste byproduct of a bioenergy industry (Dendro) (denotes as DBC) at Thiruppane, Anuradhapura district, Sri Lanka was used in the experiment. This DBC was produced by pyrolyzing the woody biomass (BM), *Gliricidia sepium* (Jacq.) Steud. in a closed reactor. The obtained BC was mechanically sieved to obtain <1 mm particles for ensure a homogeneous particle size. Dendro BC has been fully characterized and reported with high aromaticity with a high surface area of 714 $m^2/g$ (Herath et al., 2015). The concentration of N seemed to be very low in DBC and has reported as 0.5% from the elemental analysis (Herath et al., 2015).

### 2.3. Pot experiment

The pot experiment was conducted under the green house condition for 3 months. Mung bean (*Vigna radiata*) was selected as experimental crop. Pots were filled with soil and BC depending on different treatment types. Untreated soil was used as a control for all treatments. Dendro BCs were mixed with 250 g of soil at a mass fraction of 1.0, 2.5, and 5.0% (w/w). All treated soils were thoroughly homogenized in large plastic containers and individually prepared prior to use. Plastic pots (11.5 cm in diameter and 10.5 cm in height) were filled with DBC amended soil. Pots were placed in a dark room to equilibrate soil mixture, over 2 weeks with 70% of water holding capacity to equilibrate the soil mixture. Surface sterilized Mung bean seeds were allowed to germinate on filter papers laid in petri-dishes after overnight soaking. Germinated seeds were transplanted in prepared pots as three seeds per pot. The bacterial inoculation treatment series were inoculated with *Bradyrhizobium japonicum* culture. After inoculation, pots were placed under greenhouse conditions and allowed the plant growth over 12 weeks. Each treatment was performed in triplicate. The soil was irrigated with equal amount of tap water (20 ml) once per day to maintain soil moisture at 70% of the water holding capacity.

The plant heights were measured weekly. At the end of the 12th week plants were obtained their post reproductive stage and at that stage plants were harvested. Meteorological parameters were collected daily by weather station, installed closed to the greenhouse. During the experiment, the minimum, maximum and mean air temperatures were 22, 30 and 28 °C respectively. The mean relative humidity and light intensity were observed 75% and 210-foot candle respectively.

**Table 1**
Soil pH and EC in different treatments.

| Treatment | pH | Electrical conductivity (dS/m) |
|---|---|---|
| S | 5.26 | 0.126 |
| S + B | 5.32 | 0.129 |
| S + 1%DBC | 5.61 | 0.195 |
| S + 1%DBC + B | 5.74 | 0.158 |
| S + 2.5%DBC | 5.71 | 0.199 |
| S + 2.5%DBC + B | 5.85 | 0.122 |
| S + 5%DBC | 6.38 | 0.245 |
| S + 5%DBC + B | 6.81 | 0.228 |

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