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Soil Enzyme Activities in Waste Biochar Amended Multi-Metal Contaminated Soil; Effect of Different Pyrolysis Temperatures and Application Rates

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ABSTRACT

Woody biochars derived by pyrolyzing *Gliricidia sepium* at 300°C and 500°C and a waste byproduct of same biomass from a bioenergy industry (BC700) were tested for their effect on soil enzymes activities and available form of heavy metals in multi-metals contaminated soil. Pot experiments were conducted during 6 weeks with tomato (*Lycopersicon esculentum* L.) at biochar application rates, 1, 2.5, and 5% (w/w). A reduction in polyphenol oxidase with biochars produced at increasing pyrolysis temperature compared to the control whereas the maximum activity of dehydrogenase and catalase was observed in 1% BC500 and 2.5% BC300, respectively. Soil available form of Ni, Mn, and Cr were reduced by 55, 70% and 80% in 5% BC700 amended soil, respectively. The highest geometric mean of enzyme activities was observed in 2.5% BC300 treatment. Overall the application of high dosages of high temperature derived biochar masks/deteriorates soil enzyme activities but immobilizes bioavailable heavy metals and reduces toxicity.

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Catalase; phytotoxicity; polyphenol oxidase; soil amendment

Introduction

Soil biological activities have been suggested as important indicators of soil quality and the microbiological and biochemical status of a soil can be used as an early and sensitive indicator of soil ecological stress or restoration processes in both natural and agro-ecosystems (Velmourougane et al. 2013). Soil microbial activity and soil fertility are generally closely related because microbes play vital role in mineralization of the important organic elements including carbon (C), nitrogen (N), phosphorus (P), and sulfur (S) (Garcıa-Gil et al. 2000); whereas the biochemical reactions in soil are catalyzed by soil enzymes which are proteins with catalyst properties (Tabatabai 1994). Further, the soil enzymatic activities are recognized as a more sensitive bioindicator of any natural and anthropogenic disturbance (Sardar et al. 2007). In addition, soil enzymatic activity is reliable indicator reflecting the biological state of soil and it is possible to quickly obtain reliable results of pollution influence to the biological properties (Angelovicova, Bobulska, and Fazekasova 2015). There are many organic and inorganic chemicals acting as enzymes inhibitors. Fertilizer, pesticides, municipal and industrial waste are good sources of enzyme inhibition,

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which are added to the soil during soil and crop management. Among those, the presence of heavy metals exhibits a clear inhibition of soil enzymatic activities (Yang et al. 2006).

Heavy metal contamination in soil is a major environmental, agricultural and public health problem throughout the world. Total heavy metal concentrations in soil may not be directly related to soil organism toxicity due to a number of modifying factors such as organic matter content, pH, and clay content (Lee et al. 2009). Therefore, the bioavailability plays a crucial role in heavy metals toxicity for organisms. The presence of heavy metals in high concentrations in soil leads to have a significant negative impact on the soil microorganisms (Xian, Wang, and Chen 2015). Hence, various soil amendments such as biosolids, manures, and composts, rich in organic matter have been tested for the immobilization of heavy metals and reduce the mobility of contaminants in multi-metal polluted soils. However, less focus was given on simultaneous interpretations for both heavy metal immobilization and enzyme activity (Karami et al. 2011; Shaheen and Rinklebe 2015; Shaheen, Rinklebe, and Selim 2015).

The application of biochar (BC) in soils is currently becoming an increasingly universal treatment due to its wide range of properties (Oleszczuk, Jośko, and Kuśmierz 2013; Rinklebe, Shaheen, and Frohne 2016). Nevertheless, addition of BC as a soil amendment has reported contrasting data on soil enzyme activities (Awad et al., 2013; Mierzwa-Hersztek, Gondek, and Baran 2016). Demisie, Liu, and Zhang (2014) observed that BC derived from oakwood and bamboo are capable of reducing the activity of β-glucosidase with increasing BC application rates. Contrarily, Wu et al. (2013) observed an increase in β-glucosidase activity without changing the dehydrogenase activity by increasing the application rates of wheat straw biomass and its derived BC. Two year field study conducted at a paddy field in China recorded a significant increase in cellulase, urine enzyme, neutral phosphatase and sucrase by 117.4–178.3%, 31.1–37.6%, 29.7–193.8% and 36.5–328.6%, respectively, with a positive correlation of soil enzyme activities with soil pH and SOC content after biochar addition while immobilizing heavy metals (Cui et al. 2013; Yang, Yan, and Ding 2013). A recent field study on biochar application on soil enzymes demonstrated no significant influence but reduced the soil ecotoxicity (Mierzwa-Hersztek, Gondek, and Baran 2016). Most recent findings on rice straw biochar used in heavy metals rich sediments demonstrated higher biochar application (50 mg/kg) inhibiting invertase and alkaline phosphatase activity while the activity of urease and alkaline phosphatase indicated an increase at 10 mg/kg biochar (Huang et al. 2017). Therefore, the effects of BC on soil enzymatic activities are still not fully understood and hence, need more attention.

The geometric mean of enzyme activities (GMea) has proved to be a reliable index for estimating soil quality as its values are related to soil properties and to heavy metals pollution (Paz-Ferreiro et al. 2014). Hence, the present study was mainly focused on the evaluation of the effect of BC on the enzyme activities of dehydrogenase (DHA), catalase (CAT), and polyphenol oxidase (POA) and relationship with heavy metal immobilization in the serpentine soil which is rich in Ni, Cr, and Mn. The specific objectives of the study were i) to assess the effect of woody BCs produced at different temperatures, in different application rates on the enzyme activities and ii) to correlate the changes to soil available form of heavy metals and phytotoxicity in multi-metal contaminated soil to the above-mentioned enzyme activities.

Materials and methods

The multi-metal contaminated (serpentine, an entisol of the serpentinitic textural family) soil used for the experiment was characterized by Vithanage et al. (2014). Basic properties are given in Table 1. The collected soil was air dried and homogenized and sieved (<2-mm). Quickstick (*Gliricidia sepium*) biomass was used as feedstock materials. The BC300 and BC500 were prepared under laboratory condition using muffle furnace (P300, Nabertherm, Germany) at a constant temperature for 3 h, while BC700 was a byproduct of the bioenergy industry in Sri Lanka. Different BCs were characterized by their physical and chemical properties (Table 1).

Table 1. Basic properties of multi-metals contaminated soil and the biochar.

Parameter	Soil	BC300	BC500	BC700
pH (1:10 H_2O)	6.68	6.71	9.27	10.42
EC (dS/m)	0.26	0.21	0.54	1.70
CEC ($cmol^+/kg$)	27.67	4.39	4.98	5.30
Surface area ($m^2\ g^{-1}$)	–	1.02	76.30	808.00
Pore volume ($cm^3\ g^{-1}$)	–	0.001	0.01	0.89
Total metal digestion ($mg\ kg^{-1}$)				
Ni	6567	ND	ND	ND
Cr	14880	ND	ND	ND
Mn	2609	ND	ND	ND

N.D: Not detected.

Untreated soil (control) and soil amendments were prepared by mixing 250 g of soil and BC (<2 mm particle size) with a mass fraction of 1.0, 2.5 and 5.0% (w/w). Five tomato seeds (*Lycopersicon esculentum* L.) were sown in each pot and the plants were grown for six weeks in the greenhouse. Each treatment was performed in triplicate. The soil was irrigated with equal amounts of tap water (30 ml) three times per week to maintain soil moisture at 70% of the water holding capacity. The treatments consisted of a control soil without amendment (S), 300°C, 500°C, and 700°C temperature derived BCs (BC300, BC500 and BC700, respectively).

The pH and electrical conductivity (EC) of BC were measured (BC: water, 1:10) using a digital pH meter (702SM Titrino, Metrohm, Swiss) and EC meter (Orion 5 star meter, Thermo Scientific), respectively. Cation exchange capacity was analyzed using atomic absorption spectrophotometer (AAS, GBC933, Australia) after ammonium acetate extraction procedure (Anderson and Ingram 1989). The soil available form of heavy metals was extracted by using 0.01 M $CaCl_2$ following the methods of Rajapaksha et al. (2012). Specifically, 1 g of air-dried soil was extracted with 10 ml of 0.01 M $CaCl_2$. The solid solution was stirred for 2 h, centrifuged and filtered through membrane filtration (0.45 µm pore size). The supernatant was analyzed via atomic absorption spectrometer.

Soil samples were collected from each pot immediately at the end of the six weeks and DHA (Tabatabai 1994) CAT (Jin et al. 2009) and POA (Wang et al. 2013) enzymatic activities were determined in triplicates. Soil DHA was analyzed as per the standard method described by Tabatabai (1994). A 20 g of air-dried soil sample was mixed with 0.2 g of $CaCO_3$ and placed 6 g of this mixture in the test tube with three replicates. Then, 1 ml of 3% aqueous solution of 2, 3, 5- triphenyl tetrazolium chloride (TTC) and 2.5 ml of distilled water was added to the tubes, mixed thoroughly and incubated at 37°C for 24 hours. After incubation, the solution was extracted by methanol and analyzed by UV-Vis spectrophotometer (SHIMADZU – UV–160A; Shimadzu Corp., Tokyo) at a wavelength of 482 nm against methanol as blank. Triphenyl formazan (TPF) produced from TTC by DHA was estimated with reference to the calibration graph prepared from TPF standards. Dehydrogenase activity was expressed as µg TPF $g^{-1}\ h^{-1}$.

For POA, 5 g of soil was mixed with 10 ml of distilled water, 6 ml of 0.1% ascorbic acid, and 10 ml of 0.02 mol l^{-1} catechol, and then incubated for 2 min in a water bath at 30°C. Then 3 ml of 10% phosphoric acid was added and the filtrate was titrated with 0.005 mol l^{-1} iodine. The results were expressed as ml 0.005 mol l^{-1} $I_2\ g^{-1}\ h^{-1}$ (Wang et al. 2013). Catalase activity was assessed based on the rates of recovery of hydrogen peroxide. Two grams air-dried soil with 40 ml distilled water and 5 ml 0.3% H_2O_2 was shaken for 20 min (shaking velocity was 150 rpm) and filtered (Whatman 42 V) immediately. The filtrate was titrated with 0.1 mol l^{-1} $KMnO_4$ in the presence of sulfuric acid (Jin et al. 2009). The results were expressed as µmol $KMnO_4\ g^{-1}\ h^{-1}$.

The geometric mean (GMea) of the assayed enzyme activities was calculated for each sample as: $GMea = (DHA \times CAT \times POA)^{\frac{1}{4}}$ (Paz-Ferreiro et al. 2014). All results were expressed as the mean values. The differences between non-amended and BC-amended soils were analyzed by using a one-way analysis of variance (ANOVA). The Mean separation was done using Duncan's Multiple

Range Test (at P = 0.05). All statistical analyses were carried out using the Statistical software package (SAS 9.1; SAS Inc., Cary, NC).

Results and discussion

Biochar amendments on plant growth

Many studies have reported a positive influence of biochar for plant growth in the presence of heavy metals due to biochar's immobilization capacity (Herath et al. 2017, 2015). Figure 1 demonstrates that heights of plants grown in BC amended soils significantly increased ($p < 0.05$) compared to the control. Biochar may have a powerful ability to remediate such kind of heavy metals (Fellet, Marmiroli, and Marchiol 2014) and provide a favorable environment for microbial growth (Rutigliano et al. 2014). Observations indicated that metal toxicities have badly influenced on the performance of plant growth in control soil. Heavy metal immobilization ability of BC increased with increase in pyrolyzing temperature and application rate. However, 5% BC500, 2.5% BC700 and 5% BC700 amended soil showed a higher plant growth, which was a 2.7–3.1 fold increase, compared to the control (Figure 1). Moreover, BC may increase soil organic carbon content and nutrients in the soil and thus, increase in soil fertility results in better plants growth.

Enzyme activities

Soil POA enzyme mainly involves in soil carbon metabolism (Caldwell 2005) whereas CAT enzyme plays a major role in splitting hydrogen peroxide into molecular oxygen and water, and protecting cells from injury caused by reactive oxygen species (Jin et al. 2009) and in the case of DHA, it plays an essential role in the oxidation of organic matter by transferring hydrogen from the organic substrates to the electron acceptor (Balba, Al-Awadhi, and Al-Daher 1998). Compared to the control, POA was reduced significantly ($P < 0.05$) in all treatments of the pot experiment (Figure 2a). Among all BC treatments, 5% BC300 showed the highest POA while the lowest was recorded in 1% BC700 treatment, thus resulting in a reduction of 4% and 78%, respectively, compared to the control soil. However, POA significantly decreased in the incubation study with the increasing pyrolysis temperature and amendment rates compared to the

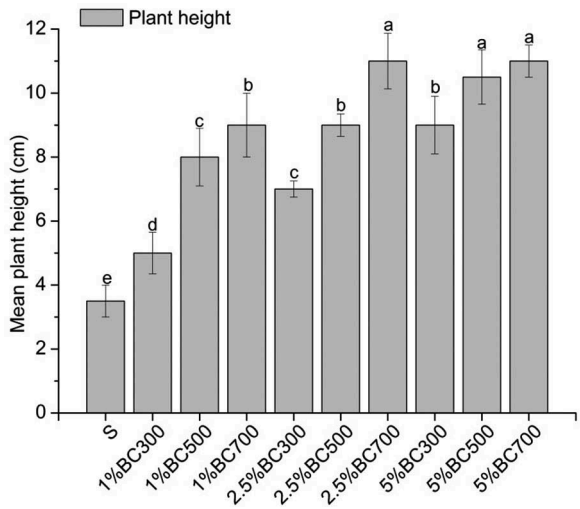

Figure 1. Mean height of tomato plant (Lycopersicon esculentum L.)in different treatments. Within a single graph, bars topped by the same letter are not significantly different ($P < 0.05$). Error bars represent standard errors of the means (n = 3).

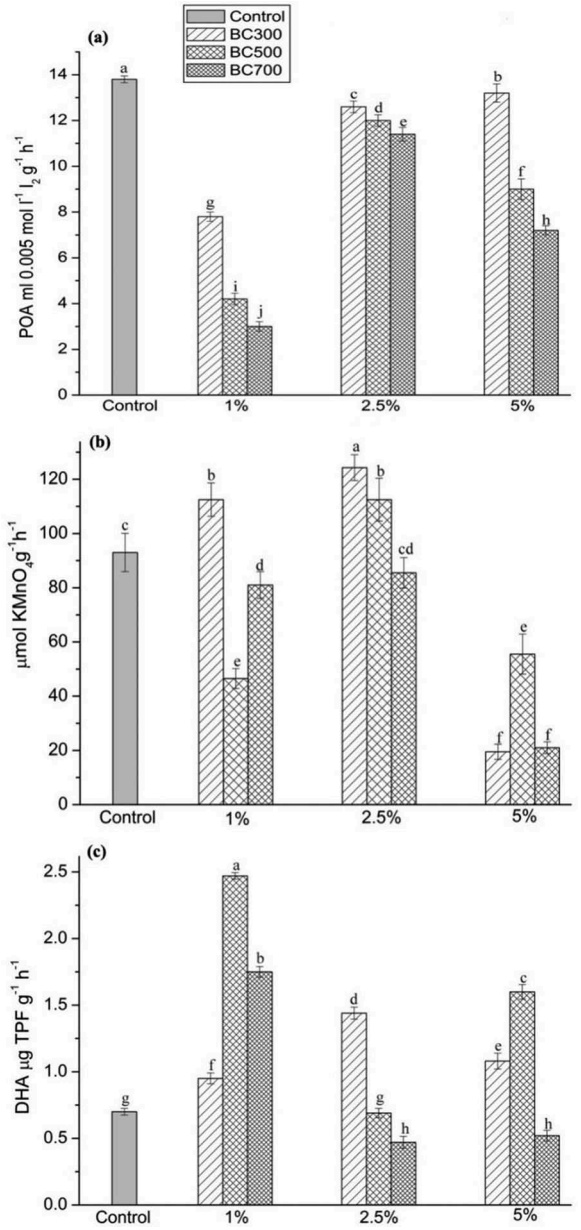

Figure 2. Enzyme activities (a) POA (b) CAT (c) DHA in soil with different treatments. Within a single graph, bars topped by the same letter are not significantly different ($P < 0.05$). Error bars represent standard errors of the means ($n = 3$).

control. Biochar has shown great capacity to sorb a broad range of organic and inorganic molecules, consequently inhibiting the diverse soil enzymes or their substrates via sorption or by blocking reaction sites and that may be the reason for the reduction that was observed (Bailey et al. 2011b; Elzobair et al. 2016; Lehmann et al. 2011).

Highest CAT activity was observed in 2.5% BC300 amended soil resulting in an increase of 34% compared to the control (Figure 2b). The soil CAT activity was higher in 2.5% amendment rate compared to the 1% and 5% amendment rates indicating optimum amendment rate is 2.5%. Soil DHA in BC amended soil showed a significant difference ($P < 0.05$) compared to the control

(Figure 2c). However, no distinct pattern was observed and the 1% BC500 amended soil showed the highest DHA that was 3.5 fold higher compared to the control soil.

Overall, the enzymatic activity reduced significantly with the BCs of higher pyrolysis temperature. These results agreed with several previous findings (Bailey et al. 2011a; Demisie, Liu, and Zhang 2014; Elzobair et al. 2016). Bailey et al. (2011a) tested on the effects of fast-pyrolysis BC (0% or 2% by wt) produced from switchgrass on the potential activity of purified enzymes, and observed decreases in glucosidase potential activity. In contrast to these findings, Elzobair et al. (2016) observed that BC amendment did not affect the potential activities of β-glucosidase, β-D-cellobiosidase, N-acetyl-β-glucosaminidase, or phosphatase, suggesting that BC cannot sorb these enzymes or their substrates/products during the enzyme assay. Wang et al. (2015) observed that extracellular enzyme activities associated with carbon transformation first increased and later decreased with BC pyrolyzed temperature. Furthermore, protease activity noticeably increased with increased pyrolysis temperatures, whereas pyrolysis temperature has limited effect on soil urease activity. Figure 3 demonstrates the calculated GMea values for BC amended and non amended soil. In the pot experiment, the highest and lowest GMea values observed in 2.5% BC300 and 5% BC700 amended soil and it was 26% increases and 46% decreases compared to the control soil. Nevertheless, in incubation experiment calculated GMea values are much greater than pot experiment except 2.5% BC300. Results indicated that the pyrolyzing temperature and BC amendment rates are crucial factors that determined the enzymatic activities in multi-metals polluted soil.

Soil available form of heavy metals and enzymatic activities

Environmental risks associated with the presence of heavy metals in soils are mainly dependent on the bioavailability of metals. The effect of the 5% BC application on the single extraction of heavy metals and soil enzymes activities is shown in Figure 4. The reduction of Ni availability increased significantly with BCs preparation temperatures. Puga et al. (2015) found a reduction of 19% and 42% for Cd, 48% and 21% for Pb and 17% and 7% for Zn, for jack beans and *Mucuna aterrima*, respectively, with 5% BC amendment rate. However, with increasing pyrolysis temperature and BC application rates, Ni availability and soil POA activity significantly reduced. Corroborative results

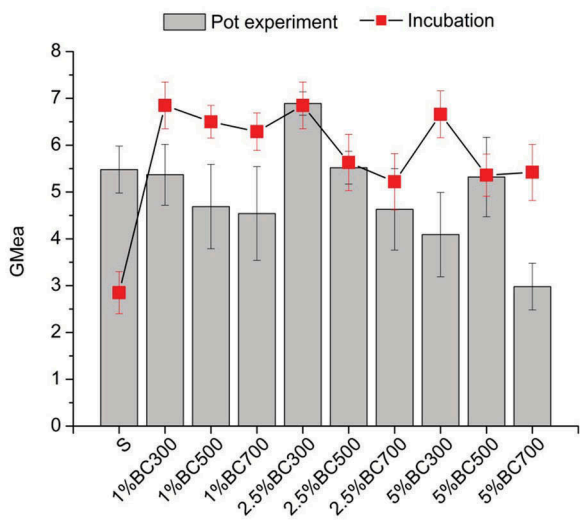

Figure 3. The variation of GMea of enzyme activities in pot and incubation experiment. Within a single graph, bars topped by the same letter are not significantly different ($P < 0.05$). Error bars represent standard errors of the means ($n = 3$).

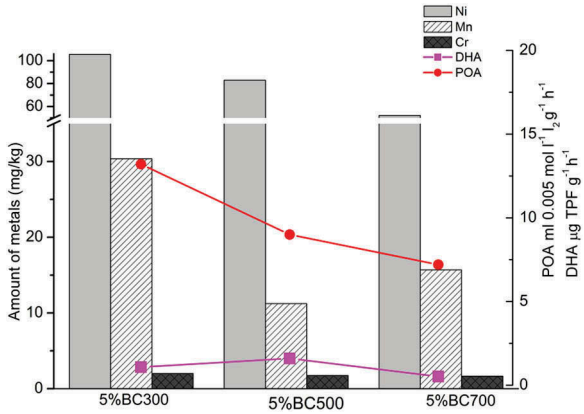

Figure 4. Relationship between bioavailable metal amounts and soil enzyme activities in the 5% BC amended soil.

were reported for invertase and alkaline phosphatase activities at high application rates of biochar (Huang et al. 2017).

Conclusions

The present study was conducted to investigate the effect of woody BC on soil quality by evaluating the enzymatic activities of POA, CAT and DHA, and availability and phytotoxicity of heavy metals in multi-metals contaminated soil. Application of BCs to the studied serpentine soil increased the growth of tomato plants associated with an increase of plant height and dry weight. Soil enzymatic activities showed a variable pattern depending on types of enzymes and BCs. An immobilization of Ni, Mn, and Cr was observed in the BC amended serpentine soil. Decrease in exchangeable fraction of metals instead of metal fractions primarily caused a reduction in their bioavailability, thus may contribute to alleviating the phytotoxicity. Overall results in the present study emphasize the importance of accurate identification of specific temperature of BC and its proper dosage that is suitable for immobilizing heavy metals and maintaining the microbial health in polluted soils. Consequently, in the case of multi-metals contaminated soil, high dosages of high temperature pyrolyzed BC should be avoided to prevent deterioration of soil enzyme activities. Further, molecular level studies are of importance to reveal the interaction mechanism of biochar with soil enzymes.

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