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Influence of Biochar on Lead –Induced Oxidative Damage and Anti-Oxidative Defense Mechanisms on the Leaf of *Solanum lycopersicum* (L.) (Tomato)

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Abstract
This study examined the impact of biochar derived from kolanut pods on the growth of *Solanum lycopersicum* (tomato) and its ability to mitigate oxidative stress caused by lead (Pb) toxicity. A pot experiment was conducted in artificially Pb-contaminated soil, utilizing various levels of biochar application (1%, and 3% w/w). Tomato seedlings were cultivated under four treatment conditions for 74 days; control, soil spiked with 250 mg Pb/kg, soil spiked with 250 mg Pb /kg and treated with 1% biochar, and soil spiked with 250 mg Pb and treated with 3% biochar. Growth parameters, which include the plant height, leaf number, stem girth, leaf area, number of petiole and petiole length were monitored. The findings indicated heightened oxidative stress in plants grown in soil solely spiked with Pb. Application of 1% biochar enhanced plant growth, whereas a higher dose (3%) hindered growth. Both 1% and 3% biochar treatments reduced oxidative stress and improved antioxidant activities compared to the control and Pb-contaminated soil without biochar amendment. Consequently, the application of biochar to soil contaminated with Pb shows promise in mitigating the hazards posed by lead (Pb) toxicity in plant.

Keywords; soil contamination; soil amendment; metal immobilization; oxidative stress; antioxidant enzymes.

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Introduction

Soil contamination and land degradation pose a persistent hazard to human and ecological health (Azam, 2016). Heavy metal and metalloid intensification in the soil has increased dramatically as a result of both natural events and human activity, such as mining, farming, industrial/municipal discharge, and other activities (Sharma *et al*., 2022). Each of these practices presents a significant risk to human health and environmental preservation. Because they are not biodegradable, they can linger in the soil, enter the food chain through agricultural products, and even accumulate in people as a result of biomagnification and bioaccumulation (Gogoi *et al*., 2021).

There are 21 non-metals, 16 light metals, and 53 heavy metals among the 90 naturally occurring elements (Gholizadeh and Hu, 2021). Metals are inorganic elements with atomic densities (g•cm−3) several times higher than H_2O (1 g•cm−3), including potentially hazardous elements (Rashid *et al*., 2023). According to Buha *et al*. (2014), elements with a high atomic weight and mass and a specific density greater than 5g/cm3 are categorized as heavy metals, along with light and semi-metals. Metals have been categorized into several sub-groups based on

their physical, physiological, and chemical properties. These include: transition metals, which include elements like chromium (Cr), manganese (Mn), iron (Fe), cobalt (Co), nickel (Ni), copper (Cu), and molybdenum (Mo); post-transition metals, which include elements like aluminum (Al), zinc (Zn), cadmium (Cd), mercury (Hg), and lead (Pb); alkaline earth metals, such as calcium (Ca), magnesium (Mg), beryllium (Be), and barium (Ba); alkali metals, which include lithium (Li), sodium (Na), potassium (K), and cesium (Cs); and metalloids, which are also called semi-metals due to their metallic and non-metallic properties, such as boron (B), silicon (Si), arsenic (As), and antimony (Sb) (Pourret and Hursthouse, 2019; Naja and Volesky, 2017).

Although heavy metals are naturally found in soil, human activity raises their concentration in the surrounding environment (Abdullahi *et al*., 2021). Metal mining and smelting, burning fossil fuels, using pesticides and fertilizers in agriculture, manufacturing batteries and other metal products, disposing of municipal waste, and sewage sludge are a few of these activities (Chibuike and Obiora, 2014; Dutta and Sharma, 2019). Heavy metals are resistant to breaking down, and they can build up in the soil and linger for a very long time if they are not removed by leaching or taken up by plants (Ghori *et al.*,2019: Ali *et al.*, 2019). All living creatures are at risk when these elements' concentrations rise above the allowable limit (Shahid *et al*., 2015; Rashid *et al.*, 2023). According to Shahid *et al.* (2015) and Rashid *et al*. (2023), heavy metals can have a detrimental effect on crop health and productivity when they are present in the soil at excessive levels. For this reason, heavy metals are considered agricultural soil contaminants as much as environmental pollutants.

According to studies by Toth *et al*. (2016) and Rashid *et al*. (2023) the elements that are commonly discovered to contaminate agricultural soils and induce harmful effects at elevated levels on plants are Cd, Pb, Cr, As, Hg, Ni, Cu, and Zn. Among them, at practically all levels of contamination, Cd, Pb, As, Hg, and Cr are extremely toxic and harmful to plant health (Ghori *et al*., 2019; Ali *et al*., 2019; Rashid *et al.,* 2023). According to Villa-Achupallas *et al*. (2018), these metals can accumulate in the soil exhibiting varying degrees of availability in the plant tissue depending on the plant species. Metal pollution has become a major environmental and public health concern due to its harmful nature. Consequently, it is imperative to remove metal contamination from soils (Rathour *et al*., 2022).

Polluted soils have been cleaned up using a variety of techniques, including incineration, excavation, and chemical use. However, these techniques are too costly and do not always work—some only move the contamination from one location to another (Akcil *et al*., 2015; Wang *et al*., 2021: Yrjälä and Lopez-Echartea, 2021). One example of a physical method is excavation, which only moves the pollution from one location to another (Alori, 2015). Most of the time, they also cause secondary pollutants to be produced, which have further detrimental effects on the ecosystem (Divya *et al.,* 2015: Alori, 2015). This makes the need for a different approach, like bioremediation, more pressing and economically viable.

Compared to traditional ways of cleaning up contaminated environments, bioremediation is safer, more affordable, and more sustainable (Rodriguez-Franco and Page-Dumroese, 2021). Through careful implementation of microbial activities, this biological remediation method is a very appealing, significant, and effective substitute for cleaning, debugging, managing, and rehabilitating contaminated environments and so improving them.

The porous, carbonaceous substance known as biochar is produced when organic materials are converted by hydrothermal and thermochemical processes (such as gasification and pyrolysis) (Zhang *et al*., 2013; Paz-Ferreiro *et al*., 2014; Sajjad *et al.*, 2020: Aziz *et al*., 2020). Biochar has been used lately as a novel carbonaceous substance to adsorb metals in soil and lessen their leachability (Beesley *et al.*, 2011; Park *et al*., 2011; Sajjad *et al.*, 2020; Aziz *et al*.,

2020). Higher yields and a considerable decrease in plant metal uptake are the outcomes of the improved soil quality that is thus attained (Ippolito *et al.,* 2012a; Yu *et al*. 2019; Wang *et al.,* 2019: Kumar *et al*., 2020). The high degree of aromaticity in biochar usually results in a high pH and cation exchange capacity, as well as a very resistant character (El-Naggar *et al*., 2019; Adekiya *et al*., 2020). According to Kamali *et al.* (2020) and Mohamed *et al*. (2016), the primary mechanisms for removing organic and inorganic pollutants from soil are sorption and interactions between active functional groups on biochar (BC). The porosity of BC allows for the retention of water in its pores, which increases the soil's capacity to hold water and improves soil fertility. Typically, porous biochar has a high concentration of surface functional groups, unlike other porous carbon compounds (Mirhosseinian *et al*., 2020; Anbia *et al*., 2018). For adsorbing both organic and inorganic pollutants, biochar's porosity and surface functional groups are highly useful Deng *et al*., 2017).

According to studies (Liu *et al*., 2013; Akhrar *et al*., 2014; Paz-Ferreiro and Fu, 2014), adding biochar to soil can increase plant production by improving the soil's chemical, physical, and biological qualities as well as its ability to store water. Many processes, including surface sorption (complexation and exchange with functional groups or cations on biochar surface, physical adsorption), pH-dependent precipitation, and modification of metal redox status, may be involved in the stabilization of metals in soils after biochar application (Paz-Ferreiro *et al.,* 2014). Additionally, the type of metal present in the contaminated soil and the soil itself affect how biochar affects metal bioavailability. The physical and chemical characteristics of biochars derived from varying biomass feedstocks and pyrolysis conditions determine its ability to adsorb contaminants in soil (Beesley *et al.* 2011; Yuan and Xu, 2011; Muthusaravanan *et al.,* 2020).

Green waste biochar was more efficient in decreasing all the metals studied in indian mustard than chicken dung biochar, according to Park *et al*. (2011). However, biochar from chicken manure was effective in reducing extractable quantities of cadmium and lead (Cd and Pb), but not copper (Cu). According to a study by Jiang *et al.* (2012), rice straw biochar was more effective in immobilizing Cu and Pb than Cd. Namgay *et al.* (2010) examined the effects of activated woody biochar on the availability of As, Cd, Cu, Pb, and Zn to maize in a pot experiment. The content of arsenic (As), Cd, and Cu in maize shoots was lowered by the application of biochar. Uchimiya *et al*. (2012) examined the impact of ten biochars made from five feedstocks at two distinct temperatures on the concentrations of soil metals. Cu and Pb were shown to be relatively easy to stabilize in soil, however the amount of charcoal applied to the soil greatly affected the response of Cd and nickel (Ni).

A number of characteristics of biochar, according to Yuan *et al*. (2019), demonstrate their impact on the movement, mobilization, and precipitation of heavy metals. They also improve soil structure, release nutrients, and increase microbial diversity—all of which are beneficial to plant growth. The impact of biochar on lead-induced oxidative damage is evaluated in this study, as well as how applying biochar affects *Solanum lycoperscicum* antioxidative defense mechanisms. By the time this project concludes, the data it contains will be useful as supplementary information on green amelioration of degraded areas.

Materials and Method

Biochar Production and soil collection

Biochar and soil characterization

For the purpose of producing biochar, kolanut pods were gathered from the Oyin, Akoko North West Local Government area (7°40′24″N 5°45′24″) in Ondo State. The process of producing biochar followed the guidelines given by Jin *et al*. (2016). To keep their weight consistent, empty pods were air-dried for two months before being reduced to chips for simple pyrolysis. At the University of Ilorin Botanical Garden in Nigeria, a locally built kiln was used to pyrolyze the dried feedstock, kolanut pods. Two batches of feedstock were created. Three hours and

forty-five minutes were spent processing each batch of feedstock at 425^0 C. According to Trakal *et al.* (2011), the resultant chars were allowed to cool to ambient temperature**.**

Determination of soil and biochar pH

Using a pH/EC/TDS conductivity meter (HANNA Instruments; Model: HI 98129) in accordance with Rajkovich *et al*., 2011, the pH values of the soil and biochar were determined. To guarantee enough equilibration between solution and soil surface as well as between solution and biochar surface, one gram of each was separately weighed into a 250 ml beaker filled with 20 ml deionized water and shaken for ninety minutes. The electrode was immersed in each of the two solutions independently, and the pH meter readings were recorded.

Determination of Electrical Conductivity of soil and biochar

Using a pH/EC/TDS conductivity meter (HANNA Instruments; Model: HI 98129) and the Rajkovich et al., 2011 methodology, the electrical conductivity (EC) values of the soil and biochar were determined. For the purpose of ensuring adequate equilibration between the soil surface and the solution, one gram of each was separately weighed into a 250 ml beaker filled with 20 ml deionized water and shaken for ninety minutes. The electrode was immersed in each of the two solutions independently, and the conductivity meter readings were recorded.

Determination of Organic Matter

The Walkey-Black (1934) method was used to determine the amount of organic matter in both soil and biochar. For each sample, a 250 mL Erlenmeyer flask containing 0.5g of soil and 0.5g of biochar was weighed. 10.0 mL of 1N $K_2Cr_2O_7$ was then added, followed by 20.0 mL of concentrated H2SO4. After one minute of swirling the flasks to achieve homogenization, the samples were left motionless for thirty minutes. The next steps involved adding four drops of the 0.025 mol/L ferroin solution indicator, 200 mL of deionized water, and 10.0 mL of H_3PO_4. The specimens and control trials were titrated using a pH 0.3 solution of Fe $(NH_4)_2(SO_4)_2.6H_2O$ containing 0.5 mol/L.

Determination of the Surface area of the biochar

The surface observation of biochars was analyzed using scanning electron microscopy (SEM) (S-4800, Hitachi, Japan) and electron dispersive X-ray analysis (EDX) (7593-H, Horiba, Japan). For the observation of the biochar sample, SEM magnifications of 2.22KXwas used. The acceleration voltage used was 10 KeV, current of 10 µA, and focal length of 9-10 mm.

Determination of the oxygen containing functional groups

Using a hydraulic press operating at continuous pressure, the char was combined with KBr at a ratio of 1:200 (w/w). Fourier transform infrared transmission spectroscopy (FTIR) was then used to analyze the mixture further. Brucker Germany Alpha spectrophotometer was used to monitor the vibration of sample which also recorded the infrared spectra at room temperature (298 k) with a resolution of 1.0 cm^{-1}.

Experimental Design

Pot experiment

Soil was collected in a relatively undisturbed farm land in the Botanical garden of University of Ilorin. The soil was sieved with a mesh of 4 mm square hole and left to air dry for two weeks to maintain constant weight. After two weeks, the experimental pots with an inner diameter of 25cm and 15cm height were filled with 2kg of soil each. A total number of 12 pots were used. The potted soil was then spiked with Pb at a concentration of 250 mg / kg for every 2kg soil prior to irrigation with tap water and allowed to age for two weeks before biochar application (Ogunkunle *et al*., 2018). After two weeks of contamination,The soil was then amended (a) 250 mg Pb /kg-spiked soil + 1% biochar, (b) 250 mg Pb/kg-spiked soil + 3% biochar (c) 250 mg Pb/kg-spiked soil without biochar (d) unpolluted soil without biochar (control). Before transplanting the tomato seedlings from the nursery bed, each treatment and the control were replicated thrice and given another 28 days to agglomerate.

Thinning of the tomato seedling

Tomato seedlings were allowed to grow at one seedling per pot for 60 days to avoid competition between the seedlings before the experiment was terminated.

Plant analysis

The pot experiment was terminated after eight weeks of transplanting. The leaves were harvested and prepared for analysis of biochemical parameters.

Determination of Lipid Peroxidation

The approach outlined by Meng *et al*. (2015) was used to estimate the malondialdehyde content (MDA) by measuring the level of lipid peroxidation. Three milliliters (3ml) of 0.5% thiobarbituric acid in 20% w/v trichloroacetic acid were used to homogenize the 300 mg of fresh leaves. The homogenate was then incubated at 1000C for thirty minutes before being cooled to terminate the reaction. The samples were centrifuged at 10,000xg for ten minutes. The supernatant absorbances were measured using 721 visible spectrophotometer at 450, 532 and 600 nm.

Determination of Ascorbate peroxidase (APX)

Ascorbate peroxidase (APX) activity was determined following the method of Nakano and Asada (1981), 450 µl of 17 mM H_2O_2 and 450 µl 25 Nm ascorbate was added to the initial supernatant of each sample. The absorbance was read for three minutes at 290 nm.

Determination of Superoxide distimutase (SOD)

The method of Beyer and Fidovich (1987) was used to measure the activity of superoxide distimutase (SOD). One gram of each sample was homogenized under cold conditions in three milliliters of extraction buffer comprising one gram of PVP, fifty millimeters of phosphate (pH 7.4), and 0.5 percent (v/v) triton x -100 at 4ºC. After 20 minutes of centrifuging the samples at 10,000 ×g, the supernatant fraction was utilized for the tests. 3.5 ml of an oxygen-generating solution containing 14.3 mM methionine, 82.5 mM NBT, and 2.2 mM riboflavin should be added to 50–150 µl of enzyme extract.The extracts were adjusted to a final volume of 0.3ml with 50Mm K-phosphate (pH 7.8) and 0.1 Mm Na_2EDTA. The reaction was allowed to run for 10 minutes and then stopped by switching the light off. The change in nitroblue tetrazolium (NBT) was determined by reading the absorbance at 560 nm.

Determination of Glutathione Reductase (GR)

The Groppa and Tomaro (2001) approach was used to determine glutathione reducase. An ice-cold 300 mg sample of fresh leaves was homogenized in 3 milliliters of extraction solution containing 50 milliliters of Tris-HCl buffer (pH 7.6) and 1mM of EDTA. 100µl of the prior mixture was combined with 1 ml of a reaction mixture that contained 1 mM EDTA, 0.5 mM GSSG, 0.15 Mmnadph, 50 Mm Tris-HCl buffer (pH 7.5), and 3 mM MgCl2. For three minutes, the absorbance at 340 nm was measured.

Determination of Catalase activity (CAT)

The Aebi method (1974) was used to measure the catalase activity. Fresh leaf samples were homogenized using a homogenizer and then subjected to various treatments with liquid nitrogen. 300 mg of fresh weight plant was mixed with 0001 mL of phosphate buffer. To exclude large particles and plant debris, the homogenates were centrifuged for ten minutes at 4°C and 4000xg. After diluting the sample's supernatant with phosphate buffer and adding 334 µl of 73 Mm H_2O_2 to 666 µl of the supernatant, the absorbances were measured at 240 wavelength for three minutes. With a quartz cuvette containing a 2300 model UV/VIS spectrophotometer (Segar Scientific, Japan), all photometric measurements for the antioxidant activities were carried out.

Data Analysis

All data were analyzed using analysis of variance (ANOVA) and means separated by Duncan multiple range test (DMRT) at $p < 0.05$ using Statistical Package for the Social Sciences (IBM SPSS 25.0). Origin Pro software 7.0 and excel were used to carry out graphical work.

Results

Characteristics of biochar

The selected properties of soil and the biochar are presented in fig.1. The pH of the soil was slightly alkaline with low electrical conductivity, low ash content but high organic matter.This chart shows the baseline condition of the soil used for the experiment. On the other hand, biochar was alkaline in nature (9.33) as well as having higher electrical conductivity (6.96 dS/m) and ash content (1.85%) but low in organic matter (0.97%).

Figure 2a displays the SEM micrograph of the biochar used in the current study at a magnification of 2.22KX. This biochar's porous structure resulted from the tubular structures made of plant cells that were present during the slow pyrolysis process.

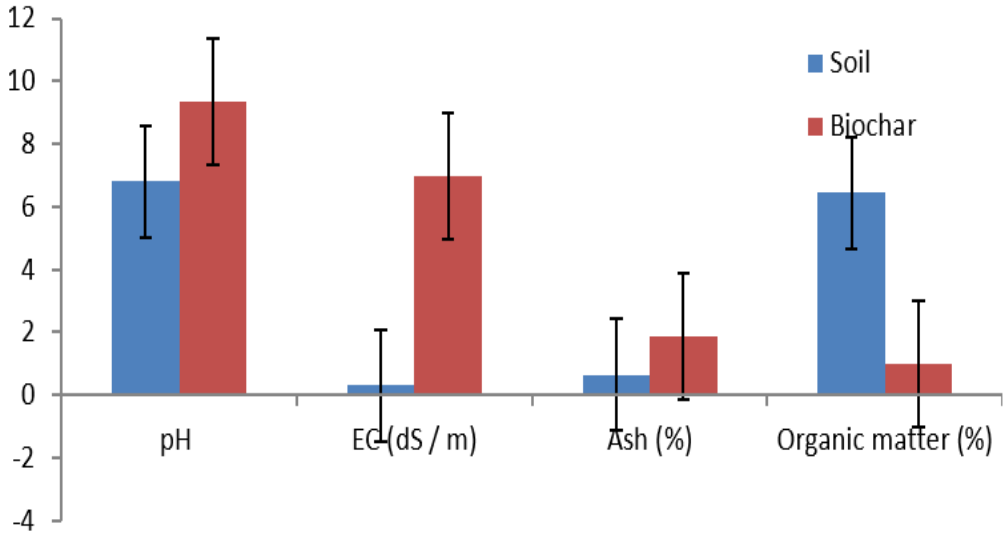
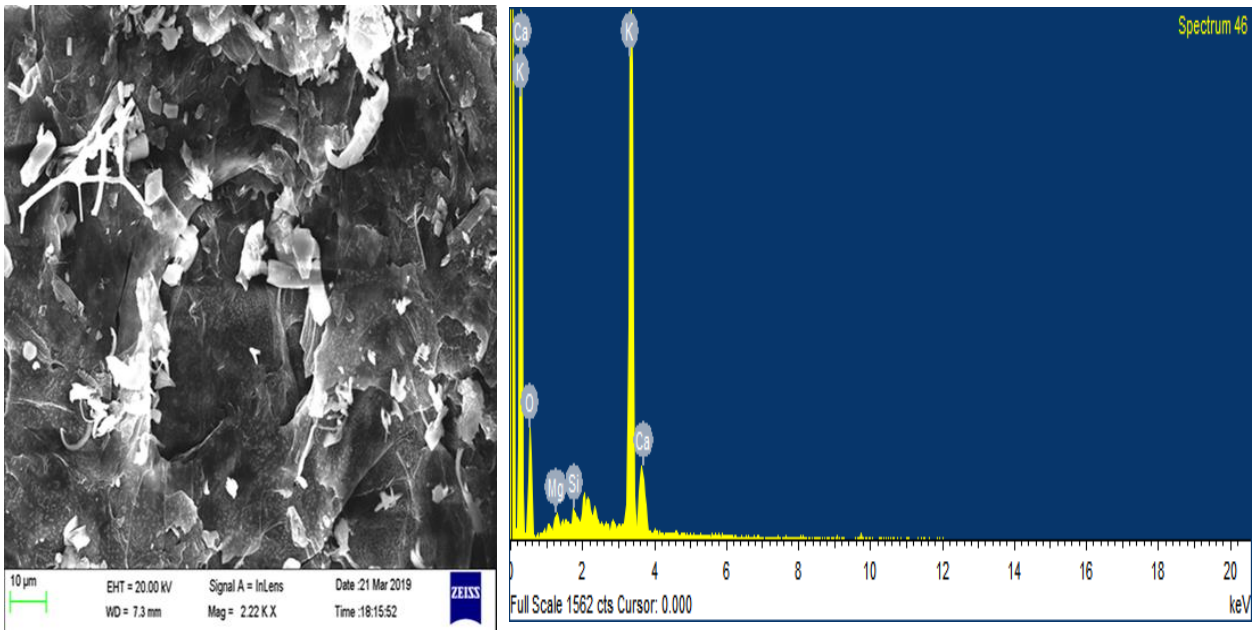


Fig. 1. Selected properties of soil and biochar used in the study



a **b**

Fig. 2(a) SEM micrograph and (b) EDAX of kolanut pod derived – biochar

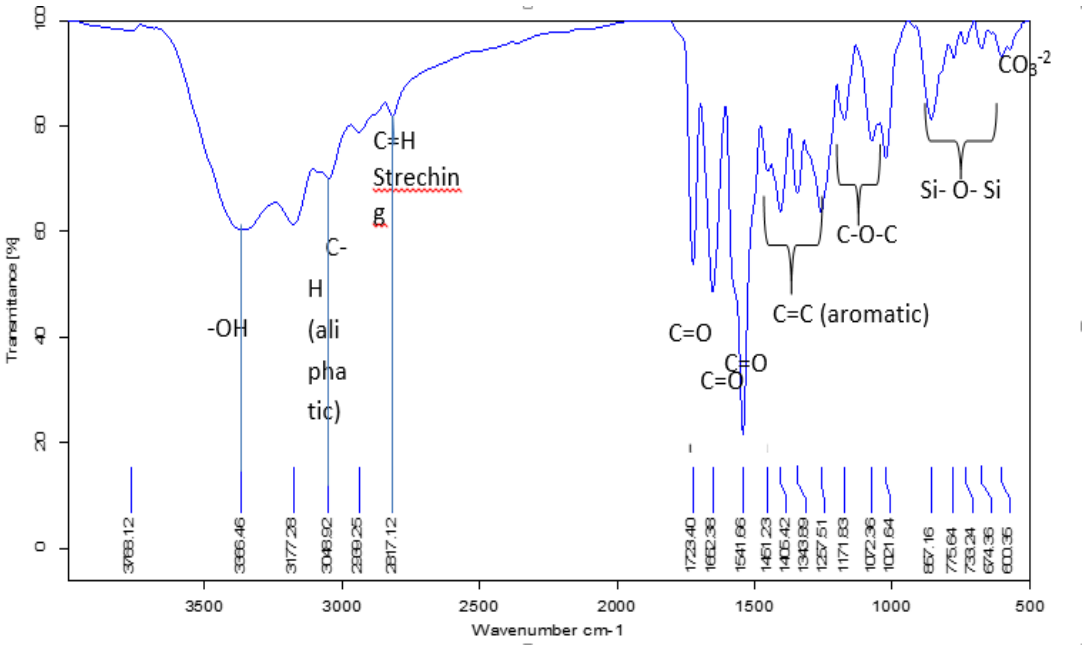


Figure 3. FTIR spectra of kolanut pod derived biochar

Table 1. Agronomic parameters at eight week of planting under different treatments

TREATMENT	Plant height(cm)	Number of leaves	Stem girth(cm)	Petiole length (cm)	Petiole number	Leaf area(cm)
Control	80.000±3.00^{a}	98.000±13.74^{ab}	1.600±0.10b	14.500±0.87^{a}	12.333±1.53^{ab}	16.067±3.52^{ab}
Pb polluted soil	74.667±7.57^{a}	93.667±8.38^{ab}	1.400±0.43^{bc}	15.500±2.18^{a}	14.333±3.06^{ab}	14.900±2.55^{ab}
1% BC +Pb polluted soil	83.333±17.00^{a}	140.667±57.0^{a}	2.333±0.29^{a}	17.667±5.58^{a}	16.333±4.93^{a}	18.833±6.93^{a}
3%+ Pb polluted soil	42.500±18.88^{b}	53.000±27.07^{b}	1.033±0.21^{c}	10.667±4.93^{a}	9.667±2.52^{b}	8.633±4.71^{b}

The values with different lower-case letters in the same column indicate significant difference ($p < 0.05$) among treatments

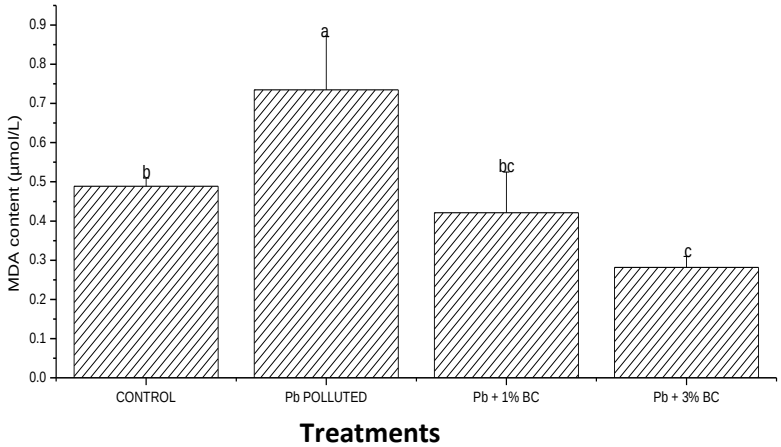


Fig. 4. Concentration of MDA in the leaves of tomato. Note: error bar represents standard deviation

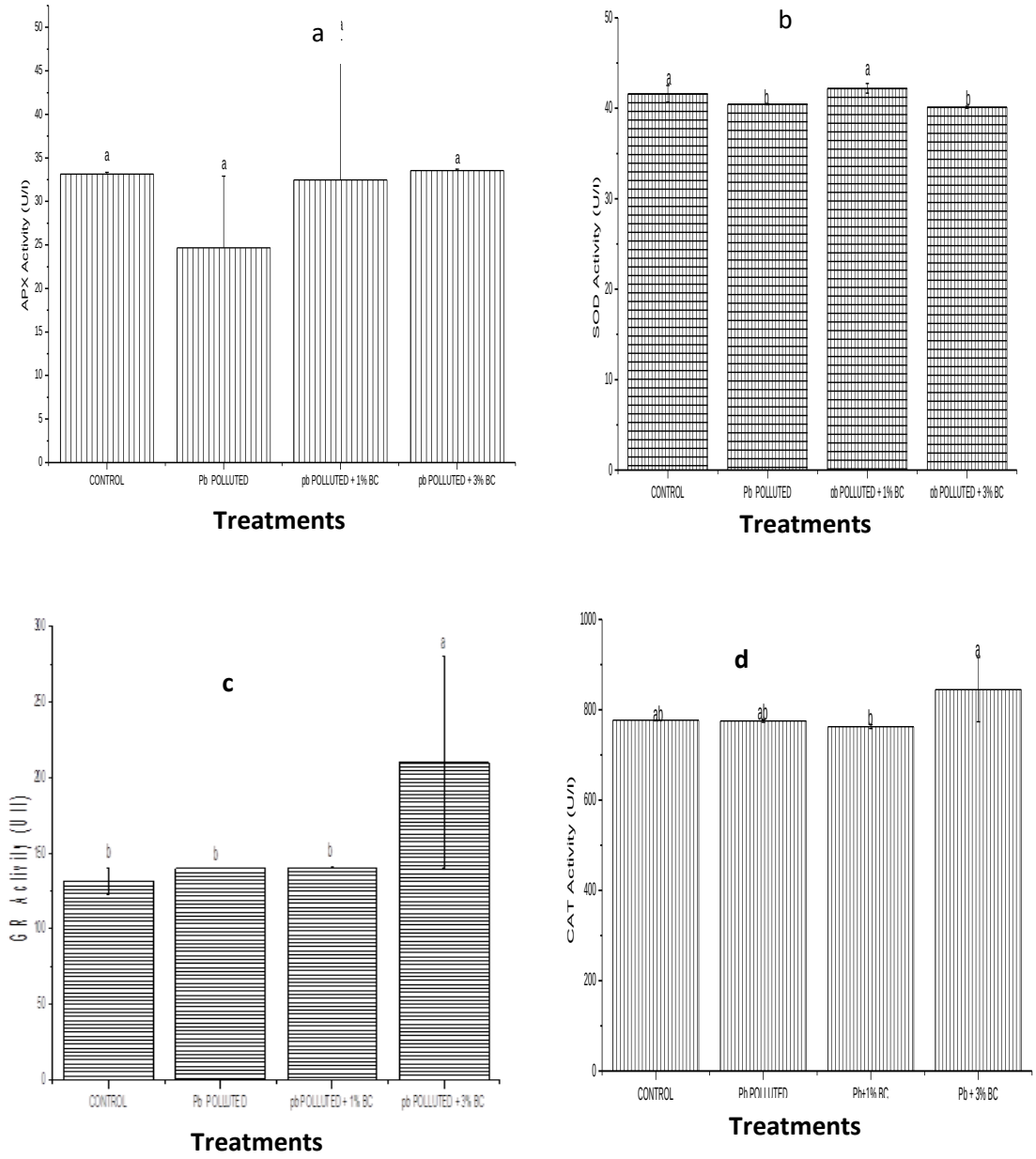


Fig. 5. Activities of (a)APX, (b)SOD, (c) CAT and (d) GR in matured leaves of tomato grown on lead (Pb) spiked soil amended with kolanut pod-derived biochar. (Control= soil without biochar and Pb, Pb polluted=soil contaminated with Pb, Pb polluted+1%BC= Lead polluted soil amended with 1% biochar, Pb polluted + 3% BC=Lead polluted soil amended with 3% biochar.

Discussion

Biochar characterisation

This biochar's surface allows it to function as a transporter that may help hold onto nutrients inside the porous structure (Brewer *et al*., 2014). Given that they are mineral nutrients for plant growth, the presence of minerals like K, Mg, Fe, Si, and Ca (Fig. 2b) in the biochar is thought to be a crucial component for its use as a soil amendment (Rivka *et al*. 2017). Additionally, these alkaline elements have the capacity to change

into carbonates, hydroxides, and oxides as well as raise the pH of the soil, all of which will undoubtedly impact plant absorption and mobility of metals (Ogunkunle *et al*., 2020).

The biochar FTIR spectrum (fig. 3), which ranges in wavelength from 500 to 3768.12 cm-1, demonstrated a greater absorbance and suggests that the biochar derived from a slow pyrolysis process contains a significant number of organic functional groups. These groups include aliphatic C=H (2939.25 - 2817.12 cm-1); stretching hydroxyl (-OH) groups are responsible for the peak at 3366.46 cm-1; and asymmetric and symmetric carbonyl/carboxyl (C=O) absorption at 1451.23 and 1541.66 cm-1 are linked to the carboxylate functional group (Guo and Chen, 2015).This kolanut pod biochar has peaks that are frequently linked to hemicellulose and cellulose, such as 3100–3178 cm-1 for C–H (Hernandez *et al*., 2017; Ogunkunle *et al*., 2020). This suggests that the temperature employed during the pyrolysis process caused these substances to breakdown. Kim *et al*. (2012) state that hemicellulose degaradation typically occurs at a temperature of 300 to 400 ºC.

Lastly, because of their ring type C=C, the peaks between 1171.83 cm-1 and 1405.42 are frequently linked to lignin (Jouiad *et al*., 2015; Ogunkule *et al*., 2020). These peaks show up to be more prominent than those in cellulose and hemicellulose; as a result, they may be linked to the lignin in kolanut pods degrading at the pyrolysis temperature (Ogunkunle *et al*., 2020; Azargohar *et al*., 2014).

The FTIR analysis revealed that although some aromatic chemicals associated to ligning were still present in the resulting biochar, the pyrolysis process resulted in the breakdown of cellulose and hemicellulose. The low pyrolysis temperature is responsible for the biochar's abundance of organic functional groups. Research by Qian *et al*. (2017), Li *et al.* (2016), and Ogunkunle et al. revealed that biochar with a slow pyrolysis temperature has more organic functional groups than one with a high pyrolysis temperature (≥ 700), which decreases the organic functional groups and increases the inorganic groups.

According to Ogunkunle *et al*. (2018), biochar generated from citrus epicarps that underwent pyrolysis at 350 ºC showed an increase in functional group. Biochar used for this research contains some significant functional groups such -OH, C=O, and C–O–C. According to Khan *et al*. (2017), biochar made from chicken dung, tomato green waste, and barley straw contains these fundamental functional groups. These functional groups—-OH, C=O, and C–O–C—are crucial to biochar (Khan *et al*., 2017) because they are responsible for the adsorption of heavy metals in the soil.

Effect on enzymes activity and oxidative stress in Pb- stressed tomato

Plants' capacity to effectively maintain a balanced antioxidant system by removing excess ROS from their cells is linked to their tolerance to environmental stress, such as that generated by metals (Ogunkunle *et al*., 2018). According to Rico *et al*. (2013), glutathione reductase (GR) produces the ascorbate required for APX to catalyze the reduction of H_2O_2, and APX then reduces H_2O_2 to H_2O. The study's predicted outcome was that Glutathione reductase (GR) activity in biochar treatment was significantly higher ($p < 0.05$) than that of the other treatments at both of the rates (1% and 3%).In comparison to the control and sole Pb-polluted treatments (250 mg Pb / kg), the activity of GR in the 3% biochar treatment (250 mg Pb / kg + 3% BC) was much higher, with an average increase of 37.5% and 33.3%, respectively (fig 5d). According to Ogunkunle *et al*. (2020), ascorbate serves as a particular electron donor in the process by which ascorbic peroxidase (APX) catalyzes the conversion of H_2O_2 into H_2O. In this experimental study, APX activity of both 1% and 3% biochar supplemented treatments was significantly higher ($p < 0.05$) than the sole Pb - spiked soil though activity of APX was more in 3% biochar treatment (fig. 5a). Such increase in activity of APX and GR was observed by Hasanuzzaman *et al*., (2021) in jute (*Corchorus olitorius*) grown on saline soil supplemented with biochar.

The activity of APX in Pb – spiked soil increased by 24 and 25.5% in 1% and 3% biochar treatments respectively in relation to sole Pb-polluted soil (250 mg Pb / kg). According to Ogunkunle *et al*.,(2018), high activity of GR and APX combats over-production of H2O2, which reduces oxidative stress in okra seedling grown on Cd- spiked soil amended with epicarp-derived biochar. This suggests that the increased enzyme activities (GR and APX) may have enhanced ROS scavenging.

Toxic peroxides are eliminated from plant cells by catalase (CAT) (Cheng *et al*., 2016). catalase functions in plant H_2O_2 degradation (Tanzeem-ul-Haq *et al.*, 2021: Sofy *et al*., 2021).In this study, application of biochar at 1% (250 mg Pb / kg + 1% BC) rate increased catalse activity in tomato plants in Pb – spiked soil, while CAT activity in 3% biochar treatment (250 mg Pb / kg + 3% BC) was not significantly different from the control and sole Pb – polluted soil (250 mg Pb/kg) (fig. 5c). Therefore biochar applied at lesser dose to Pb - spiked soil enhanced the activity of catalase in tomato leaves. Similarly , the activity of superoxidase (SOD) in 1 % biochar treatment (250 mg Pb / kg+ 1% BC) increased about 4.2% in related to the Pb - polluted treatment (250 mg Pb/kg+ 1% BC) (Fig. 5b).

According to Hasanuzzaman *et al*., (2021), SOD is essential in plants' antioxidation system and serves as a biomarker of abiotic stress (Dazt *et al.,* 2009), therefore an induce SOD activity is ascribed to a protective measure adopted by *Solanum lycopersicum* against oxidative damage caused by Pb toxicity. This result agrees with the report of Wang *et al*., (2014) that SOD activity increase in *Chlorella vulgaris* and *Malus hupehensis* by 34. 18 and 12% respectively in heavy metals polluted soil amended with smaller dose of biochar. Ali *et al.,* (2017) also reported that the SOD activity in *Brassica juncea* grown on soil amended with lesser percentage of bamboo biochar increased significantly. There was also an indication that SOD levels declined significantly in tomato leaves after increasing the dose of biochar applied in a heavy metals stress in mine polluted soil (Ali *et al.,* 2017). Thus, it can be suggested that lesser dose of Kolanut pod derived-biochar (1% biochar) showed a positive effect in reducing Pb - stress in tomato plant.

Increased generation of ROS in plants under stress usually leads to oxidative stress (Siddiqui *et al.,* 2020; Hasanuzzaman *et al*., 2018); which is always evaluated by level of membrane damage and lipid peroxidation. Therefore the level of oxidative stress in tomato after exposure to the treatments was assessed by determining the amount of MDA content. The MDA content present in the tomato leaf reduced by 62% in 3% biochar treatment (250 mg Pb / kg + 1% BC) and 43% reduction in 1% biochar treatment (250 mg Pb / kg + 3% BC) in relation to sole Pb - polluted treatment (250 mg Pb / kg) (Fig. 4).The application of biochar at 1% and 3% rates reduced the membrane damage in matured tomato plants which was caused by Pb toxicity. Therefore the significant differences observed in the content of MDA between treatments with and without biochar indicates that kolanut pod - derived biochar alleviated peroxidation of cell membrane of the tomato plants caused by lead toxicity.The results obtained so far in the current study confirm that biochar is capable of mitigating lead(Pb)-induced oxidative stress by modulating the reactive oxygen species (ROS) metabolism and antioxidant defense system to improve plant growth and physiology under heavy metal stress.

Conclusively**,** in this study, effects of biochar were evaluated on *Solanum lycopersicum.* Results showed that kolanut pod - derived biochar at 1% rate promoted plant growth while at higher doses could retard plant growth. At these two doses (1% and 3%) of application, kolanut pod derived biochar alleviated toxic effects of Pb that led to increase level of lipid peroxidation (high MDA production)resulting to oxidative stress in the tomato plant thereby reducing the toxic effects of lead. The impact may be attributable to soil biochar-induced metal immobilization that lowered the production of H_2O_2 and peroxidative damage in tomato leaves. Consequently, the use of biochar may

offer a fresh approach to cleaning up the soils affected by metals.

References

Abdelhafez A. A., Li J. H., Abbas M. H. H. (2014). Feasibility of biochar manufactured from organic wastes on the stabilization of heavy metals in a metal smelter contaminated soil.*Chemosphere*. 117: 66–71.

Abdullahi, A., Lawal, M. A. and Salisu, A. M (2021). Heavy metals in contaminated soil: source, accumulation, health risk and remediation process. *Bayero Journal of Pure and Applied Sciences*, 14(1): 1 - 12

Adekiya, A.O., Olaniran, A.F., Adenusi, T.T., Aremu, C., Ejue,W.S., Iranloye, Y.M., Gbadamosi, A. and Olayanju, A. (2020). Effects of cow dung and wood biochars and green manure on soil fertility and tiger nut (*Cyperus esculentus* L.) performance on a savanna Alfisol. *Sci Rep*. 3; 10(1): 21021

Alburquerque J. A., Salazar P., Barrón V., Torrent J., Campillo M. C., Gallardo A., Villar R (2013). Enhanced wheat yield by biochar addition under different mineral fertilization levels. *Agronomy of Sustainable Development*. 33: 475–484.

Ali A., Guo D., Zhang Y., Sun X., Jiang S. C., Guo Z. Y., Huang H.,Liang W., Li R. H., Zhang Z. Q. (2017). Using bamboo biochar with compost for the stabilization and phytotoxicity reduction of heavy metals in mine-contaminated soils of China. *Sci. Rep*. 7: 2690.

Ali, H.; Khan, E.; Ilahi, I. (2019). Environmental Chemistry and Ecotoxicology of Hazardous Heavy Metals: Environmental Persistence, Toxicity, and Bioaccumulation. *J. Chem.* 2019: 6730305.

Al-Wabel, M.I., Usman, A.R.A., El-Naggar, A.H., Aly, A.A., Ibrahim, H.M., Elmaghraby, S. and Al-Omran, A. (2014). Conocarpus biochar as a soil amendment for reducing heavy metal availability and uptake by maize plants. *Saudi J Biol Sci* 22: 503–511.

Anwaar S.A., Ali S., Ali S., Ishaque W., Farid M., Farooq, U .Najeeb, F.A, and Sharif, M.S.(2015). "Silicon (Si) alleviate cotton (*Gossypium hirsutum* L.) from zinc (Zn) toxicity stress by limiting Zn uptake and oxidative damage", *Environmental Science Pollution Research Inernational*. 22(5): 3441- 50.

Asada, K. (1994). Production and action of active oxygen in photosynthetic tissue In: (Eds): Foyer, C.H. and Mullineaux P.M. Causes of Phototooxidative Stress and Amelioration of defense System in Plants. *Boca Raton*, pp 77-104.

Awad, M., Liu, Z., Skalicky, M., Dessoky, E.S., Brestic, M., Mbarki,S., Rastogi, A., El Sabag, A. (2021). Fractionation of heavy metals in multi-contaminated soil treated with biochar using the sequential extraction procedure. *Biomolecules*, 11(3): 448, 10.3390

Beyer, W. F. and Fridovich, I. (1987). Assaying for superoxide dismutase activity: some large consequences of minor changes in conditions. *Analitical. Biochemistry*. 161: 559–566.

Blokhina O., Virolaina E. and Fagerstedt K.V. (2003). Antioxidant Oxidative damage and oxygen deprivation stress; a review. *Annals of Botany*. 91:179-194

Brandon H. C. (2014). Biochar's fitness as an amendment in bell pepper transplant and field production. *Graduate Theses and Dissertations*. 14040.

Brewer C.E., Chuang V.J., Masiello C.A. and Gonnermann H. (2014). New approaches to measuring biochar density and porosity. *Biomass and Bioenergy*. 30: 1-10

Carter, S., Shackley, S., Sohi, S., Suy, T.B, Haefele, S (2013). The impact of biochar application on soil properties and plant growth of pot grown lettuce (*Lactuca sativa*) and cabbage (Brassica chinensis). *Agronomy* 3: 404–418

Deng, Y., Zhang, T. and Wang, Q. (2017). Biochar adsorption treatment for typical pollutants removal in livestock wastewater: A Review. *Intechopen*. 68253.

Elad Y., Cytryn E., Meller H.Y., Lew B. and Grabe E.R. (2015). The Biochar Effect: plant resistance to biotic stresses. *Phytopathologia Mediterranea.* 50: 335−349

El-Naggar A., Lee S.S., Rinklebe J., Farooq M., Song H., Sarmah A.K., Zimmerman A.R., Ahmad M., Shaheen S.M., Ok Y.S. (2019) Biochar application to low fertility soils: a review of current status, and future prospects. *Geoderma*. 337:536–554.

Fatoba P.O. and Udo E.G. (2008). Effects of Some Heavy Metals on Chlorophyll Accumulation in *Barbula lambarenensis*. *Ethnobotanical Leaflets* 12: 776-83.

Gasco, G., Cely, P., Paz-Ferreiro, J., Plaza, C., and Mendez, A. (2016). Relation between biochar properties and effects on seed germination and plant development. *Biol. Agric. Hortic.* 32, 237–247.

Ghori, N.H. Ghori, T. Hayat, M.Q. Imadi, S.R., Gul, A., Altay, V. and Ozturk, M. (2019) Heavy metal stress and responses in plants. *Int. J. Envn. Sci. Technol.16*, 1807–1828.

Graber, E. R., Harel, Y. M., Kolton, M., Cytryn, E., Silber, A., David, D. R. (2010). Biochar impact on development and productivity of pepper and tomato grown in fertigated soilless media. *Plant Soil*. 337: 481–496.

Groppa, M.D., Tomaro, M.L., Benavides M.P. (2001). Polyamines as protectors against cadmium or copper-induced oxidative damage in sunflower leaf discs. *Plant Science*. 161:481-488.

Hanz, S. (2011). Initial customer interview with Hanz Sholz. Interviewed by Mike Baranick, David McElwee and Maria Zazycki.

Hasanuzzaman, M., and Fujita, M. (2022). Plant responses and tolerance to salt stress: Physiological and molecular interventions. *Int. J. Mol. Sci.* 23, 4810.

Hasanuzzaman, M.; Nahar, K.; Rohman, M.M.; Anee, T.I.; Huang, Y. and Fujita, M. (2018) Exogenous silicon protects *Brassica napus* plants from salinity-induced oxidative stress through the modulation of AsA-GSH pathway, thiol-dependent antioxidant enzymes and glyoxalase systems. *Gesunde Pflanz*. 2018, 70: 185–194.

Hasanuzzaman, M.; Raihan, M.R.H.; Khojah, E.; Samra, B.N.; Fujita, M. and Nahar, K. (2021). Biochar and Chitosan Regulate Antioxidant Defense and Methylglyoxal Detoxification Systems and Enhance Salt Tolerance in Jute (*Corchorus olitorius* L.). *Antioxidants*. 10: 2017-2025.

Hossain, M.K., Strezov, V., Chan, K.Y., Nelson, P.F., (2010). Agronomic properties of wastewater sludge biochar and bioavailability of metals in production of cherry tomato (*Lycopersicon esculentum*). *Chemosphere.* 78: 1167-1171.

International Biochar Initiative (IBI) (2014) Standardized product definition and product testing guidelines for biochar that is used in soil. Product definition Specification Standards 2.0

Houben D, Evrard L, Sonnet P (2013) Mobility, bioavailability and pH-dependent leaching of cadmium, zinc and lead in a contaminated soil amended with biochar. *Chemosphere* 92:1450–1457.

Jin, J. W., Li Y. N., Zhang J. Y., Wu S. C., Cao Y. C., Liang P., Zhang J., Wong M. H., Wang M. Y., Shan S. D. and Christie P. (2016). Influence of pyrolysis temperature on properties and environmental safety of heavy metals in biochars derived from municipal sewage sludge. *Journal of Hazardous Materials*. 320: 417 – 426.

Joseph, S., Cowie, A. L., Van Zwieten, L., Bolan, N., Budai, A., Buss, W. (2021). How biochar works, and when it doesn't: A review of mechanisms controlling soil and plant responses to biochar. *GCB Bioenerg.* 13: 1731–1764.

Kloss, S., Zehetner, F., Obergur, E., Buecker, J., Kitzler, B., Wenzel, W.W., Wimmer, B and Soja G (2014) Trace element concentrations in leachates and mustard plant tissue (*cc* L.) after biochar

application to temperate soils. Sci Total Environ 481: 498–508

Lehmann, J., Gaunt, J., and Rondon, M. (2006). Biochar sequestration in terrestrial ecosystems: A review. *Mitig. Adapt. Strateg. Glob. Change* 11: 403–427.

Liu, A., Tian D., Xiang Y. and Mo, H. (2016). Biochar Improved Growth of an Important Medicinal Plant (*Salvia miltiorrhiza* Bunge) and Inhibited its Cadmium Uptake. *Journal of Plant Biology and Soil Health.* 3(2): 6.

Mendez A., Gomez A., Ferreiro J.P., Gasco G. (2012). Effects of sewage sludge biochar on plant metal availability after application to a Mediterranean soil. *Chemosphere*. 89: 1354-1359

Mendez, A., Ferreiro , J.P and Gasco, G. (2014) Biochar from pyrolysis of drinking paper sludge and its use in the treatment of a nickel polluted soil. J Anal Appl Pyrolysis 107:46–52

Milttler, R. (2002). Oxidative stress antioxidants and stress tolerance. *Trends in Plant Science***.** 9: 490 498

Ming H., Hu C. S., Zhang Y. M. and Cheng Y. S (2007). Improved extraction method of chlorophyll from maize. *Journal of Maize Science* 15(4): 93 - 95.

Nakano Y. and Asada K. (1981). Hydrogen peroxide is scavenged by ascorbate-specific peroxidase in spinach chloroplasts. *Plant and Cell Physiology***.** 22: 867-880.

Ogunkunle C.O. Varun M., Ogundele I.G., Olorunmaiye K.S. and Paul M.S. (2018). Citrus Epicarp-Derived Biochar Reduced Cd Uptake and Ameliorates Oxidative Stress in Young *Abelmoschus esculentus* (L.) Moench (okra) Under Low Cd Stress. *Bulletin of Environmental Contamination and Toxicology*. 100(6): 827-833.

Ogunkunle, C. O., Falade, F. O., Oyedeji, B. J., Akande, F. O., Vishwakarma, V., Alagarsamy, K., Fatoba, P. O. (2020). Short-Term Ageing of Pod-Derived Biochar Reduced Soil Cadmium Mobility and Ameliorated Cadmium Toxicity to Soil Enzymes and Tomato. *Environmental Toxicology and Chemistry*. 40(12):3306-3316.

Park, J.H., Choppala, G.K., Bolan, N.S., Chung, J.W. and Cuasavathi, T. (2011). Biochar reduces the bioavailability and phytotoxicity of heavy metals. *Plant Soil*. 348: 439451.

Pourret, O. and Hursthouse, A. (2019). It's Time to Replace the Term "Heavy Metals" with "Potentially Toxic Elements" When Reporting Environmental Research. *Int. J. Environ. Res. Public Health* 16: 4446.

Qian L., Zhang W., Yan J., Han L., Chen Y., Ouyang D., Chen M (2017). Nanoscale zero-valent iron supported by biochars produced at different temperatures: synthesis mechanism and effect on Cr (VI) removal. *Environmental Pollution*. 223: 153–160

Rajkovich S., Enders A., Hanley K., Hyland C., Zimmerman A.R., and Lehmann J. (2011). Corn growth and nitrogen nutrition after additions of biochars with varying properties to a temperate soil. *Biology and Fertility of Soils.* 52: 86-97

Rashid A., Shutte B.J., Ulery A., Deyholos M.K., Sanago S., Lehnhoff E.A. and Bech L, (2023). Heavy Metal Contamination in Agricultural Soil: Environmental Pollutants Affecting Crop Health. *Agronomy* 13(16): 1521.

Rehraha D., Reddye, M.R.., Novakd, J.M., Bansodeb R.R., Schimmelf, K. A., Yuc J., Wattsd, D.W. and Ahmedna M. (2014). Production and characterization of biochars from agriculture by-products for use in soil, quality enhancement. *Journal of Analytical and Applied Pyrolysis*. 108: 301-309

Rico, C. M., Morales M. I., McCreary R., Castillo-Michel H., Barrios A.C., Hong J., Tafoya A. Lee W. Y., Varela-Ramirez A., Peralta-Videa J. R. and Gardea- Torresdey J. L. (2013). Cerium oxide nanoparticles modify the antioxidative stress enzyme activities and macromolecule composition in rice seedlings. *Environmental. Science of Technology*. 47: 14110 – 14118

Shahid, M., Khalid, S., Abbas, G., Shahid, N., Nadeem, M., Sabir, M., Aslam, M. and Dumat, C. (2015). Heavy Metal Stress and Crop Productivity. In *Crop Production and Global Environmental Issues*; Hakeem, H.R., Ed.; Springer International Publishing: Chem, Switzerland.

Siddiqui, M.H.; Alamri, S.; Alsubaie, Q.D.; Ali, H.M. (2020). Melatonin and gibberellic acid promote growth and chlorophyll biosynthesis by regulating antioxidant and methylglyoxal detoxification system in tomato seedlings under salinity. *J. Plant Growth Regul*. 39: 1488–1502.

Singh J. and Kalamdhad A. S. (2011). Effects of Heavy Metals on Soil, Plants, Human Health and Aquatic Life. *International Journal of Energy and Environmental Science*. 1: 15-21.

Sofy, M.; Mohamed, H.; Dawood, M.; Abu-Elsaoud, A.; Soliman, M. (2021) Integrated usage of arbuscular mycorrhizal and biochar to ameliorate salt stress on spinach plants. *Arch. Agron. Soil Sci.*12: 109- 116.

Tanya C. Bayen S., and Jabaji S. (2017). Biochar Amendment Modifies Expression of Soybean and *Rhizoctonia solani* Genes Leading to Increased Severity of *Rhizoctonia* Foliar Blight. *Frontier Plant Science*. 8: 221

Tanzeem-ul-Haq, H.S.; Rasool, B.; Ehtisham-ul-Haque, S.; Saif, S.; Zafar, S.; Younis, T.; Akhtar, I.; Jafri, L.; Iqbal, N.; Masood, N. (2021). Chitosan with Bentonite and Biochar in Ni-Affected Soil Reduces Grain Ni Concentrations, Improves Soil Enzymes and Grain Quality in Lentil. *Mineral*s. 11: 11-25.

Tian, D., Amei L. and Xiang Y. (2017). Effects of Biochar on Plant Growth and Cadmium Uptake: Case Studies on Asian Lotus (*Nelumbo nucifera*) and Chinese Sage (*Salvia miltiorrhiza*). *Intecopen.* 68: 52-67

Tóth, G. Hermann, T. Da Silva, M.R. and Montanarella, C. (2016). Heavy metals in agricultural soils of the European Union with implications for food safety. *Environ. Intern.* 88: 299–309.

Trakal L., Komárek M., Száková J., Zemanová V., Tlustoš P. (2011). Biochar application to metal-contaminated soil: Evaluating of Cd, Cu, Pb and Zn sorption behavior using single- and multi-element sorption experiment. *Plant, Soil and Environment.* 57(8): 372–380

Uslu, O. S., Babur, E., Alma, M. H., and Solaiman, Z. M. (2020). Walnut shell biochar increases seed germination and early growth of seedlings of fodder crops. *Agriculture* 10: 427.

Vithanage M., Rajapaksha A.U., Zhang M., Thiele-Bruhn S., Lee S.S. and Ok YS (2014). Acid-activated biochar increased sulfamethazine retention in soils. *Environ Sci Pollut Res*. 22: 2175–2186

Wang Y., Pan .F, Wang G., Zhang G., Wang Y., Chen X. and Mao Z. (2014). Effects of biochar on photosynthesis and antioxidative system of *Malus hupehensis* Rehd. Seedlings under replant conditions. *Sci Hortic*.175:9–15

Wu Z., .Wang F., .Liu S., Du Y., Li F., Du R., Wen D. and Zhao J (2016). Comparative responses to silicon and selenium in relation to cadmium uptake, compartmentation in roots, and xylem transport in flowering Chinese cabbage (*Brassica campestris* L. ssp. chinensis var. utilis) under cadmium stress. *Environmental and Experimental Bota ny*. 131: 173 – 180.

X u, D., Zhao, Y., Sun, K., Gao, B., Wang, Z., Jin J., Zhang, Z., Wang, S., YanY, L.X. and Wu, F. (2014). Cadmium adsorption on plant- and manure-derived biochar and biochar-amended sandy soils: Impact of bulk and surface properties. *Chemosphere* 11: 320–326.