Article:
Ibrahim, M, Li, G, Chan, FKS et al. (4 more authors) (2019) Biochars effects potentially toxic elements and antioxidant enzymes in Lactuca sativa L. grown in multi-metals contaminated soil. Environmental Technology and Innovation, 15. ARTN: 100427. ISSN 2352-1864

https://doi.org/10.1016/j.eti.2019.100427

Biochars effects potentially toxic elements and antioxidant enzymes in Lactuca sativa L. grown in multi-metals contaminated soil.

Muhammad Ibrahim[a,b], Gang Li[a,c,*], Faith Ka Shun Chan[d,e], Paul Kay[e], Xiao-Xia Liu[f], Les Firbank [g], Yao-Yang Xu[a,c]

[a] Key Laboratory of Urban Environment and Health, Institute of Urban Environment, Chinese Academy of Sciences, Xiamen 361021, People's Republic of China

[b] University of Chinese Academy of Sciences, Beijing 100049, People's Republic of China

[c] Ningbo Urban Environment Observatory and Monitoring Station, Chinese Academy of Sciences, Ningbo 315830, People's Republic of China

[d] School of Geographical Sciences, University of Nottingham Ningbo Campus, Ningbo 315100, People's Republic of China

[e] School of Geography and Water @ Leeds, University of Leeds, LS29JT, Leeds, UK

[f] Zhejiang Agricultural Technology Extension Center, Hangzhou 310020, People's Republic of China

[g] School of Geography biology, University of Leeds, LS29JT, Leeds, UK

***Corresponding author**: E-mail: gli@iue.ac.cn Tel: 008657486085863; Fax: 008657486784814

HIGHLIGHTS

- Rice husk, corn cobs and peanut shells biochars were amended at 5% to multi-metal contaminated soil.
- Bioaccumulation of Ni, Cr, As, Cd and Pb reduced highly with amendment of peanut shells biochar as compared to rice husk and corn cobs biochar in Lactuca sativa L.
- Stimulation and suppression of antioxidant enzymes were biochars dependent.

Abstract

Geogenic and anthropogenic activities can leads to agriculture soil pollution and land degradation. Many cost-effective and environment friendly strategies are applied to improve soil fertility, reduce soil pollution and human health risks caused by consumption of metals contaminated vegetables. In this study we evaluate the effects of rice husk biochar (RHB), biochar from corn cob (CCB) and biochar from peanut shells (PNB) on the bioavailability of potentially toxic elements (PTEs) in soil, its bioaccumulation and antioxidant enzymes activities in Lactuca sativa L. plants.

RHB, CCB and PNB amendments significantly ($P \leq 0.05$) increased Lactuca sativa L. biomass production (39%, 65% and 100%) as well as soil fertility. Amendments of PNB, RHB and CCB significantly ($P \leq 0.05$) increased soil available phosphorous (P), cation exchange capacity (CEC), pH, total nitrogen (TN), total carbon (TC) and dissolved organic carbon (DOC) concentration, but markedly reduced bioavailable concentrations of cadmium (Cd) (31%, 20% and 22%) arsenic (As) (33%, 22% and 27%), and lead (Pb) (46%, 24% and 32%). In addition, CCB and PNB amendments significantly ($P \leq 0.01$) decreased the shoot accumulation of Pb, Cd and As, while RHB amendment increased the shoot accumulations of nickel (Ni) and chromium (Cr). The reduction in PTEs accumulation may be linked with increased sorption of PTEs by biochars. Furthermore, amendments of CCB and PNB significantly ($P \leq 0.05$) suppressed the activities of SOD (53% and 69%), POD (22%, 31%) but stimulated (38% and 31%) with amendment of RHB. However, RHB, CCB and PNB amendments significantly ($P \leq 0.05$) suppressed the activity of CAT (21%, 41% and 48%) in Lactuca sativa L. plants.

PNB was the most effective soil amendment as compared with RHB and CCB. However, to fully elucidate the effects of the tested biochars, long-term field trails are needed.

Keywords: Soil remediation, soil amendment, Lactuca sativa L.

1. Introduction

The pollution of agricultural soils by potentially toxic elements (PTEs) represents major risks to the environment and to human health. Because they are not biodegradable (Habiba et al., 2015; Bandara et al., 2017), and restrict the opportunities for future land use. Geogenic and anthropogenic activities are the major sources of PTEs particularly As, Cd, Pb, Cr and Ni globally (Pratas et al., 2013; Galuszka et al., 2016). In China, rapid economic development and industrialization in most coastal areas has led to elevated concentrations of PTEs (particularly Ni, Cr, As, Cd and Pb) in arable fields (Khan et al., 2014; Ibrahim et al., 2017). It is therefore important that methods of remediating PTEs affected soils are developed and applied quickly for China to meet its pressing needs to provide sufficient safe food. Elements such as Cd, Pb, Cr and As are essential in small amounts for normal plants growth and development (Noctor et al., 2007). However, in excess they become potentially toxic, inducing oxidative stress, toxicity and surplus accumulation of reactive oxygen species (ROS) in plants (Monteiro et al., 2009; Kim et al., 2015; Quartacci et al., 2015). This overproduction of ROS can result in DNA and RNA damage, enzyme inhibition and protein oxidation in plant cells (Chao and Seo, 2005; Lin et al., 2007). Plants have developed limited protective

mechanisms, including the production of stress response proteins and synthesis of antioxidant enzymes (includes SOD, POD and CAT).

PTEs alter the normal ecosystem functioning and induce toxicity in vegetation (Singh et al., 2013; Gill et al., 2015). Green vegetables grown in PTEs contaminated soils are the main exposure route of PTEs to humans (Khan et al., 2008; Niu et al., 2013) and food contamination with PTEs is more prevalent in urban areas of China than some other countries such as United Kingdom and United States of America (Yuan et al., 2017). The dietary intake of excessive Pb and Cd can cause lung cancer, abdominal pain, kidney failure and stomach trouble (Patrick, 2003; Meharg et al., 2013). Elevated As intake can cause cardiovascular diseases, neurological disorders and infertility (Smoke and Smoking, 2004). Many biological, physical and chemical-based remediation technologies were developed to minimize PTEs availability in metals polluted soil (Kumpiene et al., 2008; Bolan et al., 2014). In-organic minerals, compost, agricultural residues and sewage sludge were used to reduce PTEs mobility and bioavailability in metals contaminated soil by ion exchange, co-precipitation, adsorption and surface complexion (Tsang et al., 2014; Zhang et al., 2015). In-addition biodegradable cationic salts, organic acids and chelating agents have shown good effects on soil quality (Makino et al., 2008; Kim and Baek, 2015). Increased crop production and Pb precipitation was achieved with soil amendment with phosphogypsum (Anikwe et al., 2016; Yan et al., 2016) and phosphate minerals (Cao et al., 2009).

In the last few decades, biochar had been recognized as a significant element for

soil fertility, crop growth and long term carbon sequestration (Lehmann, 2007; Laird et al., 2010; Luo et al., 2014; Zhao et al., 2014; Lima et al., 2018). It is emerging that soil amendment with biochar has the potential to restore metals polluted soils due to its porous structure, feedstock type, temperature, heat transfer rate, surface area, pH and cation exchange capacity (Jiang et al., 2012; Beesley et al., 2013; Ibrahim et al., 2016; Prapagdee and Tawinteung, 2017). Biochar is usually produced in oxygen-limited conditions at different pyrolysis temperatures and is commonly used for soil fertility and sorption of in-organic and organic contaminants (Melo et al., 2015; Xu et al., 2016; Hagemann et al., 2018). Biochar amendment affects the physio-chemical properties of the soil, notably bulk density, pH, carbon concentration, water holding capacity (WHC) and CEC (Rajapaksha et al., 2016; Beiyuan et al., 2017; Li et al., 2018). There is research gap about the effects of plants based alkaline biochars on the remediation of multi-metals contaminated soil and its effects on antioxidant enzymes in vegetable plants.

Here we explore the effects of different plant based biochars amendments on multi-metals contaminated soil and the responses of the most widely consumed vegetable in China, lettuce (Lactuca sativa L.). In 2013, lettuce production reached 24.9 million tons globally, 13.5 million tons from China (FAOSTAT, 2013). Our approach was to a glasshouse study in which antioxidant enzymes were used as biomarkers for oxidative damage in plants (Sun et al., 2010; Wei et al., 2013), following previous studies (Li et al., 2013; Wu et al., 2013) that showed that PTEs accumulation can activate oxidative stress, thus promoting changes in the normal activities of antioxidant enzymes.

Glasshouse pot trails were conducted from early November to late December 2016 to determine the effects of RHB, CCB and PNB on Lactuca sativa L. 1) biomass production, 2) PTEs bioaccumulation and 3) antioxidant enzymes, including SOD, POD and CAT.

2 . Materials and methods

2.1 Site description and soil collection

Surface soil samples (0-15 cm) from agriculture field were collected with a small soil corer in triplicate from ten sites at Longyan County, Fujian Province (25 ^{0}54 ′N 118 ^{0}18 ′E), China. The climate of this experimental area is sub-tropical. The winters are mild, and the average temperature ranges from 7 ^{0}C to 10 ^{0}C, while the summers are hot with average temperatures between 21 ^{0}C and 25 ^{0}C. The average annual rainfall of 1400-2000 millimeters. This area is polluted with PTEs.

2.2.Soil characterization

The soil samples were transported to the glasshouse and spread in thin layers to be air dried. Physio-chemical characteristics such as pH, EC, TN, TC, S, DOC, O, H and particle size of the soil samples were measured using standard methods (Rayment and Higginson, 1992). Procedural details are provided in the Supportive Information (SI) section.

2.3 Biochar production and characterization

Corn cobs and rice husk waste residues were collected from agriculture fields in Xinglin Bay, while peanut shell residues were obtained from a peanut oil factory located in Siming District, Xiamen, China. After collection and transportation, these samples were sun-dried in a glasshouse. Biochars called CCB, RHB and PNB were produced from corn cobs, rice husk and peanut shell residues through pyrolysis at 500 ^{0}C for 6 h in a high performance automatic furnace (GWL-1200, Henan, China) in oxygen limited conditions under a constant flow of nitrogen (N_2). We chose this pyrolysis temperature and time adopting previous study (Uchimiya et al., 2011) that biochar produced at high temperate and time contains more micropores and larger surface area (Rajapaksha et al., 2014). CCB, RHB and PNB were characterized using a previously published method (Wei et al., 2017). Surface area and porosity of the respective biochars were measured using surface and porosity analyzer (Micromeritics, ASAP 2020, USA). For proximate analysis, biochars samples were heated in a muffle furnace at a thermal temperature of 650 ^{0}C for 6 h adopting standard method (Ahmad et al., 2013). A macroanalyzer (Vario Max CNS, Germany) was used for the measurement of TN, TC, S, H and O concentration in biochar and soil samples. Micrograph and elemental composition of RHB, CCB and PNB were measured through a scanning electron microscopy and energy dispersive x-rays spectroscopy (SEM-EDX, Carl Zeiss, Germany).

2.4 Experimental design and plant growth conditions

A complete randomized block design (CRBD) with at least four replicates for each four

treatment was adopted. 4 kg of soil was amended at 5 % of each biochar (w/w) called RHB, CCB and PNB in polyvinyl chloride (PVC) pots (14 cm of width and 25 cm of height) individually. 5% amendment ratio was chosen following our previous study (Ibrahim et al., 2016). Soil without biochar amendment called control treatment (CT) was also included. One week prior to the sowing of Lactuca sativa L. seeds, PVC pots were irrigated manually with double distilled water and incubated. Moisture content was kept at 60 % water holding capacity through proper weighing and additional supplement of water as required. After one week, 10 g of soil samples were taken with a small soil corer from each treatment and were analyzed for quantification of physio-chemical changes in biochar-amended and non-amended control soil. Lactuca sativa L. seeds were surface sterilized for 10 min with 30 % hydrogen peroxide (H_2O_2). After washing with double distilled water 5 seeds per pot were sown. The seedlings were thinned to two per pot after two weeks of growing period. The pot trial was conducted under 12/12 h day/night light conditions in a glasshouse. Daily temperature was recorded at 10 ±1 ^{0}C, night temperature 6 ±2 ^{0}C and humidity 50 ±3 %. In order to gain suitable light and temperature, PVC pots containing Lactuca sativa L. plants were randomly set at normal intervals throughout the growing period.

2.5 Chemical analysis of soil and biochar samples

The procedural method from Feng et al. (2012) was adopted for dissolved organic carbon (DOC) extraction from soil and biochar samples. To 4.0 g samples , 40 mL of 0.5M potassium sulphate (K_2SO_4) solution was added and shaken at 200 rpm (TS-2102,

Shaker, China) for 1 h. Each sample was immediately centrifuged and filtered through membrane filters of 0.22 µm. The DOC concentration in each sample was analyzed using a total carbon analyzer (TOC-VCPH Shimadzu, Japan). Colwell P (plants available P) concentration was determined in soil and biochar samples adopting a standard method (Colwell, 1963). Briefly, each sample extraction container was contained 1: 100 (w/v) soil/sodium bicarbonate ($NaHCO_3$) solution (0.5 M, pH 8.5) and shaken at 180 rpm for 16 h. The samples were centrifuged immediately. The supernatant in each sample was filtered through 0.22 µm filters membrane and available P concentration was analyzed using ICP-OES (Perkin-Elmer, Downers Grove, IL, USA). For the analysis of available concentrations of PTEs in soil samples, the EDTA-extraction fraction method was adopted. Briefly, in 50 mL centrifuge tubes, air dried soil samples (10 g) and a mixture (20 mL) containing 0.5 M of ethylene-diamine-tetra-acetic disodium (EDTA-Na_2), 0.1 M tri ethanol amine (TEA) and 0.01 M $CaCl_2$ were added. The tubes were shaken at 180 rpm for 3 h and then centrifuged immediately. The supernatant was filtered through a 0.22 µm filter membrane and stored at 4 ^{0}C for further analysis. For the extraction of total PTEs the strong nitric and perchloric acids (HNO_3 and $HClO_4$) digestion method (Wong and Li, 2004) was adopted. Total and available PTEs concentrations in biochar and soil samples were analyzed through ICP-MS. (Agilent Technologies, 7500 CX, Santa, Clara, CA, USA).

2.6 Chemical analysis of plant samples

After seven weeks of growth, Lactuca sativa L. plants were harvested and root and

shoot samples were manually separated after washing with double distilled water. Thereafter, root and shoot samples were oven-dried for 72 h at 70 ^{0}C and biomass was recorded. Shoot samples were powdered using a pestle and mortar, and 0.2 g of each sample was acid digested at a ratio of 1:1 (v/v) with 30 % H_2O_2, (GR, Sinopharm, Shanghai, China) and 65% HNO_3, (GR, Merk, Germany) using a microwave accelerated reaction system (Mars 5, CEM Crop, Matthews NC, USA). The debris was then filtered through 0.22 µm filters. Each filtrate was set to 50 mL volume in polypropylene tubes with the addition of double distilled water (Ultra-pure Water Purification System, Shanghai, China). The bioaccumulated concentration of Ni, Cr, As, Cd and Pb in shoot samples were analyzed through ICP-MS (Agilent Technologies, 7500 CX Santa, Clara CA, USA).

Antioxidant enzymes such as superoxide dismutase (SOD), guaiacol peroxidase (POD) and catalase (CAT) were extracted adopting standard method (Kazemi et al., 2010). Briefly, 1.0 g fresh shoot samples were ground in 1 mL of 50 mmol L^{-1} phosphate buffer (pH 7.5) and then centrifuged at 12,000 rpm for 10 minutes at 4 ^{0}C. Thereafter, the supernatant was filtered through 0.22 µm filters and stored at 4 ^{0}C for further analysis. SOD enzyme activity was measured by inhibition of nitro blue tetrazolium (NBT) photochemical reduction (Bai et al., 2009). Briefly, the 3 mL reaction mixture contained 100 µl enzyme aliquot in 50 mM of phosphate buffer, 0.1 mM of ethylene diamine tetra acetic acid (EDTA), 0.002 mM of riboflavin, 13 mM of methionine and 0.075 mM of NBT. The tubes contained the respective mixtures were placed for 15 minutes in a light chamber. Using a UV-6300 double beam spectrophotometer the absorbance of SOD

enzyme activity was measured at 560 nm. One unit of SOD enzyme activity was considered as the quantity of the enzyme assay mixture required to cause 50% inhibition of NBT during a chemical reaction at 560 nm. The POD enzyme activity was measured by adopting the standard method of Gorin and Heidema. (1976). The 3 mL assay mixture composed of 0.1% p-phenylenediamine, 0.05% of H_2O_2, 100 µl of enzyme aliquot and 1.35 mL (100 mM) of MES buffer (pH 5.5). The variation in the absorbance was measured at 485 nm. The POD enzyme activity was calculated by applying the extinction coefficient (26 mm^{-1} cm^{-1}) for tetra-guaiacol and was presented in µmol tetra-guaiacol min^{-1} mg $protein^{-1}$. Shoot CAT enzyme activity was determined by adopting the standard method of Singh et al. (2010). The 3 mL enzyme assay mixture was composed of 0.04 mL enzyme extract, 0.4 mL (15 mM) of H_2O_2 and 2.6 mL of phosphate buffer 50 mM (pH 7.0). Breakdown of H_2O_2 molecules was determined by absorbance at 240 nm and the enzyme activity was represented by U mg^{-1} protein (U=1 mM of H_2O_2 reduction $min^{-1}mg^{-1}$ of protein).

Plant and soil reference samples (GBW07602-GSV-1 and GBW07401-GSS-1) were purchased from the National Research Centre of Standards, China. The reference samples were incorporated in each set of plant and soil digestion for precision and accuracy.

2.7 Statistical analysis

One-way ANOVA in SPSS 11.5 and Sigma plot 12.0 were applied for the statistical and graphical analysis respectively. LSD test ($P<0.05$) was applied for significant

differences among treatments.

3. Results and discussion

3.1. Physio-chemical characteristics of tested biochar and soil samples

The physio-chemical characteristics of the tested soil and biochars are shown in Table 1. In the tested soil, the total concentrations of Pb and Cr were 7.73 and 0.19 mg kg^{-1} while that of Ni, As and Cd, were 18.41, 28.94 and 64.52 µg kg^{-1}. The soil is polluted with PTEs and the concentrations of As, Pb and Cd surpassed the maximum permissible limits (State Environmental Protection Administration, China, SEPA, 1995). The soil used in pot experiment was slightly acidic (5.21). The concentrations of PTEs such as Ni, Cd, As, Cr and Pb, in PNB samples were 0.13, 7.43, 0.87 (µg kg^{-1}), 0.01 and 0.55 mg kg^{-1} respectively while in RHB samples were 0.252, 4.84, 0.14(µg kg^{-1}), 0.03 and 0.90 (mg kg^{-1}). In CCB samples the total concentrations of Ni, As, Cd, Cr and Pb, were 0.19, 0.05, 0.02 (µg kg^{-1}), 0.02 and 0.13 mg kg^{-1} respectively (Table 1).

Soil particle size measurement gives us insight into the textural classification of the soil. In the current experiment, the tested soil had sand (44.92%), silt (49.50%) and clay (5.58%) and was classified as a silty loam. Biochars CCB, RHB and PNB showed contrasting physio-chemical characteristics such as pore volume, pore size, surface area, pH and EC. The physio-chemical properties of the biochars underpin the mechanisms of how soil fertility is improved. For any crop yield increment of soil fertility might be associated with improved water holding capacity of biochar amended soil (Jeffery et

al., 2011). The mechanism involved is such that pores present on biochar surfaces can retain water molecules thus increasing WHC and assisting in improved soil fertility. Asai et al. (2009) reported that biochar porosity retains water molecules in tiny pores and thus assisting in improvement of soil fertility through increased water holding capacity. Increased CEC is the indirect measurement of soil fertility. These variable characteristics of soil depend on the waste residues used for production of biochars. In addition, increased pH values of biochars may be also associated with the changed properties of biochars amended soil. CCB showed the highest pH (10.12) as compared with RHB (8.94) and PNB (9.33). These biochars were alkaline in nature having the potential to an increase amended soil pH. SEM-EDX micrographs investigated the elemental composition of RHB, CCB and PNB. Biochar elemental ratio (O/C and H/C) can be used as a marker of biochar polarity. O/C for CCB and RHB were higher (0.21 and 0.30) than PNB (0.15) indicating that CCB and RHB might contain high polar surfaces (Table 1). Previous study (Uchimiya et al., 2011) revealed that O/C and H/C was used to assess biochar polarity and aromaticity. Present results revealed that carbon concentration in PNB was higher (70 %) as compared with RHB (49.9%) and CCB (64.1%). SEM imaging gives us deeper insight about surface morphology of the tested biochar. PNB showed large sized pores as compared with RHB and CCB (Fig S2). Energy dispersive X-ray (EDX) spectroscopy confirmed the presence of various elements on the tested biochars surfaces on dry weight basis. Biochar RHB, EDX data showed different elemental composition and confirmed the occurrence of Oxygen (O) (20.73%), Carbon (C) (77.03%), Sodium (Na) (0.04%), Aluminum (Al) (0.11%),

Potassium (K) (0.68%), Silicon (Si) (1.21%) and Calcium (Ca) (0.22%) (Fig S1 A-B). Similarly, Fig S1 C-D of biochar CCB confirmed the concentration of O (17.31%), C (80.34%), Al (0.02%), Na (0.04%), K (2.23%), Si (0.04%) and Ca (0.02%). In addition, biochar PNB spectrum showed the presence of C (85.16%), O (12.79%), Al (0.08%), Na (0.05%), K (1.52%), Si (0.12%) and Ca (0.29%) (Fig S1 E-F). The EDX data further confirmed that O content was higher in RHB as compared with CCB and PNB. Similarly, the C content was higher in PNB as compared with RHB and CCB. This may be dependent on the type of feedstock used as a biochar material.

3.2. Comparison of biochar amended and non-amended soil

The results showed that biochars amendments altered the soil pH, EC, TC, TN, S, O, H and DOC level as compared with non-amended control. Soil pH increased from 5.21 to 6.41 units amended with PNB, 5.21 to 6.78 units amended with RHB and 5.21 to 6.82 units with CCB amendment. This rise in amended soil pH may be associated with the higher precipitation of insoluble species and sorption of PTEs to biochar surfaces (Kolodynska et al., 2012). A previous study (Wang et al., 2016) showed that tea garden soil pH increased from 3.33 to 3.63 units with amendment of biochar. Hardwood biochars prepared from Carya spp and Quercus spp increased agricultural soil pH by 1 unit (Laird et al., 2010). The increase in soil pH with biochar amendments might have changed the soil nutrients status and assisted in adsorption of nutrients on Lactuca sativa L. root surfaces. The EC of the biochars amended soil significantly ($P \leq 0.01$) elevated by up to 34%, 24% and 29% (Table 2). This rise in EC may be related to

the fact that biochar contain rich mineral compounds. Present results are in line with previous findings (Hossain et al., 2011) that with amendment of wastewater sludge biochar, soils EC significantly increased.

With amendments of the tested biochars i.e. PNB, RHB and CCB the concentration of Colwell P significantly ($P \leq 0.05$) increased by up to 50%, 26% and 33%. The highest increment of Colwell P was recorded in PNB amended soil. The TN concentration significantly ($P \leq 0.01$) elevated by up to 42%, 21% and 28% with amendment of PNB, RHB and CCB. Similarly, TC statistically ($P \leq 0.05$) increased by up to 44%, 23% and 39% with amendments of PNB, RHB and CCB. This increased concentration of TN and TC might improve the biochar amended soil fertility. The concentration of CEC remarkably increased with amendments of PNB, RHB and CCB by up to 96%, 59% and 65%. This increase in CEC values suggests improved soil fertility of biochars amended soil. Previous study (Laird et al., 2010) reported that soil CEC remarkably enhanced with amendment of biochar. Jien and Wang, (2013) investigated that biochar (prepared from Leucaena leucocephala) amendment to high weathered soil significantly ($P \leq 0.01$) elevated CEC ranged from 7.14 to 10.8 cmol kg^{-1}. Present findings showed that effects of biochars on soil physiochemical properties varied with amendment of biochar type. Overall, with biochar amendment the improved soil physiochemical characteristics may be the direct or indirect measures of decreased nutrients leaching as well as increased nutrients status which are the popular mechanisms of soil fertility.

PNB amendment reduced the available concentrations of As, Cr, Ni, Pb and Cd by

up to 33%, 33%, 40%, 46% and 31% respectively ($P \leq 0.05$). RHB amendment significantly ($P \leq 0.01$) reduced bioavailable concentrations of As, Pb, and Cd by up to 22%, 24% and 20% but increased that of Cr and Ni by up to 44% and 28%, compared to non-amended control. Similarly, CCB amendment statistically ($P \leq 0.05$) decreased the concentrations of As, Cr, Ni, Pb and Cd by up to 27%, 25%, 29%, 32% and 22%. Highest decrease in PTEs availability was recorded in the PNB amended soils as compared with RHB and CCB. The decreased available concentration of PTEs in PNB amended soils may be related to the increased sorption of PTEs by PNB, as PNB has larger pore size (10.07 nm) as compared to RHB (3.48 nm) and CCB (4.57 nm). Another, reason may be the larger surface area of PNB (12.49 m^2g^{-1}) as compared to RHB (1.85 m^2g^{-1}) and CCB (5.48 m^2g^{-1}) (Table 1). These findings are in line with Houben et al. (2013) that amendment of miscanthus straw biochar significantly ($P \leq 0.05$) decreased Cd, Pb and As bioavailability in metals polluted soil. Significant reduction in PTEs availability had also been explored with amendment of biochars produced from other waste residues (Ahmad et al., 2012). Indeed, pH is a vital parameter in PTEs sorption process therefore; the decreased PTEs availability in biochar amended soils in the current experiment may be related to the increased pH level (Smith, 1994; Zheng et al., 2015; Jelly and Najafi, 2018). In-addition, PTEs speciation and ionization processes as well as surfaces charges and chemistry of adsorbents could also be affected with increased pH levels of biochar amended soils (Kolodynska et al., 2012; Martinsen et al., 2015). In another study, Uchimiya et al. (2010) reported that amendment of broiler litter-derived biochar could decrease the adsorption of PTEs through increasing the

amended soil pH level. Soil organic matter contains a minor portion of DOC in soil but it plays an essential role in maintaining soil ecosystems due to its reactivity and mobility towards metals contaminants (Chantigny, 2003). When biochar is amended to soil it directly increases the soil organic matter concentration that subsequently alters sorption-adsorption processes of PTEs (Smernick, 2009). In the present study, the concentration of DOC increased by up to 138 %, 42 % and 68 % in PNB, RHB and CCB amended soil. Due to the natural behavior of DOC direct sorption and formation of stable complexes with PTEs, increased DOC concentration in biochars amended soil might reduce the PTEs availability (Zheng et al., 2013). Furthermore, the occurrence of aromatic and non-aromatic functional groups on biochars surfaces could also alter PTEs bioavailability in biochars amended soils (Xu et al., 2013).

3.3. Potentially toxic elements bioaccumulation and biomass production

In the current study, PNB, RHB and CCB soil amendments improved Lactuca sativa L. plant growth and biomass production. Commonly, plants exposed to high metal stress revealed toxic visible symptoms such as necrosis, chlorosis, stunted growth and lower biomass production. However, at lower metals concentration these symptoms totally disappear due to lower toxicity and oxidative damage, as a result more plant biomass is produced (Vangronsveld and Clijsters, 1992). Lactuca sativa L. roots biomass significantly ($P \leq 0.01$) increased by up to 51%, 53% and 122% with amendments of RHB, CCB and PNB as compared with non-amended control. Similarly, with amendments of RHB, CCB and PNB shoots biomass elevated by up to 39%, 65%

and 100 % as compared with non-amended control (Fig 2). This increase in biomass production may be associated with the lower PTEs phytoxicity to Lactuca sativa L. plants as well as oxidative damage. In comparison with RHB and CCB, biochar PNB amendment revealed the highest biomass production. The biochar source and type used as a feedstock may also be responsible for decreased bioaccumulation of PTEs and increased biomass production. Furthermore, the increased biomass production may be linked with improved soil fertility due to increased TC and TN concentration (44%, and 42%) in PNB amended soil as compared with RHB (23% and 21%) and CCB (39% and 28%). Therefore, the enhanced levels of TC and TN in PNB amended soil may have improved soil fertility and subsequently enhanced Lactuca sativa L. biomass production. Furthermore, phosphorus is an essential nutrient for plants growth and biomass production as well as many physiological processes such as nucleic acid and protein synthesis, cell division and formation of meristematic tissues (Parvage et al., 2013; Chintala et al., 2014). The increased concentration of available P in biochars amended soil may be another factor that improved growth and biomass production of Lactuca sativa L. plants. Current findings are in line with previous studies that with amendments of Eucalyptus sapwood and pigeon pea biochar, spinach and spring onion biomass production significantly($P \leq 0.01$) increased (Yu et al., 2009; Coumar et al., 2016). Other researchers (Hossain et al., 2010; Sun et al., 2017) reported that amendments of rice straw and waste water sludge biochars significantly ($P \leq 0.05$) increased maize and cherry tomato plant growth and biomass production remarkably

A prominent component of an ecosystem is plant which mobilizes elements from the

abiotic to the biotic environment. In the present study, bioaccumulation of PTEs was affected contrastingly with amendments of PNB, RHB and CCB in Lactuca sativa L. plant tissues. Shoot bioaccumulation of PTEs significantly ($P \leq 0.05$) decreased such as Ni (25% and 33%), Cr (24% and 36%), As (29% and 40%), Cd (26% and 31) and Pb (29% and 37%) with amendments of CCB and PNB. Similarly, shoot accumulation of As, Cd and Pb statistically decreased by up to 24%, 20% and 21% with amendment of RHB. However, accumulation of Cr and Ni significantly ($P \leq 0.05$) elevated with amendment of RHB (Fig 1A). Highest decrease in PTEs bioaccumulation was noticed with amendment of PNB as compared with RHB and CCB. The increased bioaccumulation of Ni and Cr in Lactuca sativa L. plants may be related with enhanced uptake of Ni and Cr or decreased sorption due to the smaller pore size of RHB as compared with PNB and CCB (Table 1). Another reason may be associated with higher concentration of Ni and Cr in the RHB samples. These findings are consistent with previous studies revealed that with amendment of rice bran, husk and straw biochars Cd, Pb and As bioaccumulation significantly ($P \leq 0.05$) decreased by up to 71%, 60% and 37% (Zheng et al., 2013). Similarly, Ibrahim et al. (2017) also reported reduced PTEs bioaccumulation in Phaseolus vulgaris L plants. cultivated in peanut shells biochar (PNB) and sewage sludge biochar (SSB) amended with PTEs contaminated soil. Several mechanisms could affect this decreased accumulation of PTEs in Lactuca sativa L. plants. One possible mechanism is the physical characteristics such as pore size, pore volume and surface area of amended biochar which could have reduced PTEs availability in the biochars amended soil. Furthermore, in-organic compounds as well

as exchangeable bases in soils have also been reported to increase pore volume and surface area of amended biochars, thus assisting in decreased PTEs availability in soil and subsequent bioaccumulation in plants (Kim et al., 2013). The second mechanism may be linked with the elevated pH concentration of biochars amended soil. Elevated soil pH enhances the biochar negatively-charged surface sites that directly increase the PTEs sorption capacity (Kolodynska et al., 2012). In-addition, improved DOC level in biochars amended soil could also be linked with the reduced PTEs availability in soil and subsequent accumulation in Lactuca sativa L plants. Furthermore, elevated DOC concentration creates stable complexes with PTEs molecules thus increased sorption capacity of amended biochar. A previous study (Zheng et al., 2012) explored that in biochar amended soil, DOC acts as chelator with PTEs molecules thus decreases the available concentration of PTEs. In the present findings it was investigated that in terms of PTEs sorption and biomass production, PNB was the best suitable soil amendment as compared with RHB and CCB.

3.4. Responses of antioxidant enzymes

In plant cells, adjacent to reactive oxygen species (ROS) sites, antioxidant enzymes system is present, which detoxify harmful effects of ROS (Corpas et al., 2015). Hydrogen peroxide (H_2O_2), hydroxyl radical (^{-}OH), superoxide radical (O_2^{-}), and singlet oxygen ($^1O_2$) are the major ROS, which are produced in mitochondria, chloroplast and peroxisomes. It has been shown that adverse environmental factors such as high and low light intensity, temperature, drought, salinity and heavy metals stress

rapidly secret over production of ROS in green plants (Mittler et al., 2011; Baxter et al., 2014). Therefore, against adverse environmental factors plants have developed protective mechanisms called antioxidant enzymes that play signaling role in stress conditions (Gupta et al., 2016). Over accumulation of ROS producing intensive damage to cellular proteins, nucleic acids and lipids (Sies et al., 2017). Accumulation of superoxide radicle (O_2^{-}) in chloroplast is rapidly dismutate into hydrogen peroxide (H_2O_2) by the activity of superoxide dismutase (SOD) (Wang et al., 2016). Another enzyme called peroxidase (POD) located in mitochondria can scavenge H_2O_2 into water (H_2O) and oxygen molecule (O_2) (Welinder, 1992; Passardi et al., 2007). Similarly, enzyme catalase (CAT) present in peroxisomes can eliminate over accumulation of H_2O_2 into H_2O and O_2 molecule (Reumann and Bartel, 2016).

In the present study, RHB, CCB and PNB amendments contrastingly affected the activities of antioxidant enzymes in Lactuca sativa L. plants. Activities of SOD and POD significantly ($P \leq 0.01$) stimulated by up to 38% and 31% with amendment of RHB however, declined by up to 53 %, 69 % and 22 %, 31% with additions of CCB and PNB respectively. The activity of CAT significantly ($P \leq 0.01$) declined by up to 21%, 41% and 48% with amendments of RHB, CCB and PNB as compared with non-amended control (Fig 1B).

These variations in antioxidant enzyme levels may be due to the increased or decreased PTEs bioaccumulation in Lactuca sativa L. plants. Previous studies (Mishra et al., 2006; Zhang et al., 2009) showed that Cd accumulation caused enhanced activity of SOD and enzyme encoding genes, thus resulting in elevated antioxidant enzyme pools.

Molassiotis et al. (2006) found that increased inorganic boron (B) concentration could damage the membrane and induced stress in the peroxidation of lipids, which might slow down SOD enzyme activity. Another antioxidant enzyme POD also plays a key role in oxidative stress conditions. POD enzyme can convert H_2O_2 molecules which is toxic to plants into non-toxic H_2O and O_2 molecule. Hasan et al. (2009) showed that POD enzyme could be assessed as a possible biomarker for sublethal inorganic Cd metal toxicity in various plants species. Furthermore, in plant cells enzyme CAT also play a key role in scavenging the excessive concentration of H_2O_2 and converts it into H_2O and O_2 molecules (Wu et al., 2013). Thus the enhanced CAT activity, scavenge un-necessary accumulation of H_2O_2 molecules in the peroxisomes of plant cells, thus maintaining a dynamic equilibrium between H_2O_2 synthesis and elimination. The decreased CAT activity in plant cells is caused by less oxidative stress produced by decreased metal stress, resulting in the inhibition of enzyme synthesis (Alscher et al., 1997). Furthermore, in the present study the decreased CAT activity with amendments of RHB, CCB and PNB may be due to the lower H_2O_2 synthesis by SOD enzyme during dismutation of reactive oxygen free radical O_2^- in the Lactuca sativa L. plant cells. Another, reason may be the decreased accumulations of As, Cd and Pb in plant tissues causing less oxidative stress and their higher sorption capacity by RHB, CCB and PNB in amended soils. Usually, CAT enzyme activity stimulated with elevated stress in plant cells (Gong et al., 2013).

In the current study, the bioaccumulation of Ni and Cr in RHB amended plant shoots increased, due to which the levels of antioxidant enzymes SOD and POD were also

elevated accordingly. Increased bioaccumulation of Ni and Cr caused enhanced production of ROS thus activate antioxidant enzyme system thereby, protecting Lactuca sativa L. plants from oxidative damage. It was reported previously that upregulation of antioxidant enzymes was implicated in combating oxidative stress in plant cells (Gill and Tuteja, 2010; Nadgórska-Socha et al., 2013).

4. Conclusions

In conclusion, amendments of RHB, CCB and PNB significantly ($P \leq 0.05$) decreased the bioavailable concentrations of As, Cd and Pb in soil and subsequent accumulation in Lactuca sativa L. plants. However, the concentrations of Cr and Ni significantly ($P \leq 0.01$) elevated with amendment of RHB. Activities of antioxidant enzymes such as SOD, POD significantly ($P \leq 0.05$) suppressed with amendments of CCB and PNB however, stimulated with amendment of RHB. In-addition, activity of CAT significantly ($P \leq 0.01$) declined with amendments of all three biochars. This up and down regulation in antioxidant enzymes may be biochar dependent. Highest significant effects on PTEs soil availability, plants bioaccumulation as well as biomass production were found with amendment of PNB as compared with RHB and CCB. This may be due to larger pore size and surface area of PNB. Current findings showed that how RHB, CCB and PNB reduced the available concentrations of PTEs in amended soil, its bioaccumulation in Lactuca sativa L. plants. This approach presented here can be applied to other biochar feed stocks and vegetable plants grown in multi-metals contaminated soils on global scale. Furthermore, present results proved that PNB was best soil amendment. However, applications of present outcomes require further

investigations under long-term field trials.

Acknowledgments

This research was financially supported by National Scientific Foundation of China (Grant No. 41501519, 41571130063) and the Chinese Academy of Science-The World Academy of Science (CAS-TWAS) president fellowship for the advancement of science in developing countries.

Conflict of interest

The authors declare that they have no conflict of interest.

References

Ahmad, M., Lee, S.S., Yang, J.E., Ro, H.M., Lee, Y.H., Ok, Y.S., 2012. Effects of soil dilution and amendments (mussel shell, cow bone, and biochar) on Pb availability and phytotoxicity in military shooting range soil. Ecotox. Environ. Safe. 79, 225-231.

Ahmad, M., Lee, S.S., Rajapaksha, A.U., Vithanage, M., Zhang, M., Cho, J.S., Lee, S.E., Ok, Y.S., 2013. Trichloroethylene adsorption by pine needle biochars produced at various pyrolysis temperatures. Bioresour. Technol. 143, 615-622.

Alscher, R.G., Donahue, J.L., Cramer, C.L., 1997. Reactive oxygen species and antioxidants: relationships in green cells. Physiol. Plant. 100, 224-233.

Anikwe, M., Eze, J., Ibudialo, A., 2016. Influence of lime and gypsum application on

soil properties and yield of cassava (Manihot esculenta Crantz.) in a degraded Ultisol in Agbani, Enugu Southeastern Nigeria. Soil. Tillage. Res. 158, 32-38.

Asai, H., Samson, B.K., Stephan, H.M., Songyikhangsuthor, K., Homma, K., Kiyono, Y., Inoue,Y., Shirsiwa, T., Horie, T., 2009. Biochar amendment techniques for upland rice production in Northern Laos 1. Soil physical properties, leaf SPAD and grain yield. Field. Crop. Res. 111, 81-89.

Bai, J., Gong, C.M., Chen, K., Mei-Kong, H., Wang, G., 2009. Examination of antioxidant system's responses in the different phases of drought stress and during recovery in dessert plant Reaumuria soongorica (Pall) Maxim. J. Plant. Biol. 52, 417-425.

Bandara, T., Herath, I., Kumarathilaka, P., Hseu, Z.Y., Ok, Y.S., Vithanage, M., 2017. Efficacy of woody biomass and biochar for alleviating heavy metal bioavailability in serpentine soil. Environ. Geochem. Health. 39, 391-401.

Baxter, A., Mittler, R., Suzuki, N., 2014. ROS as key players in plant stress signaling. J. Exp. Bot. 65, 1229-1240.

Beesley, L., Marmiroli, M., Pagano, L., Pigoni, V., Fellet, G., Fresno, T., Vamerali, T., Bandiera, M., Marmiroli, N., 2013. Biochar addition to an arsenic contaminated soil increases arsenic concentrations in the pore water but reduces uptake to tomato plants (Solanum lycopersicum L.). Sci. Total. Environ. 454, 598-603.

Beiyuan, J., Awad, Y.M., Beckers, F., Tsang, D.C.W., OK, Y.S., R.J., 2017. Mobility and phytoavailability of As and Pb in a contaminated soil using pine sawdust biochar under systematic change of redox conditions. Chemosphere. 178, 110-118.

Bolan, N., Kunhikrishnan, A., Thangarajan, R., Kumpiene, J., Park, J., Makino, T., Kirkham, M.B., Scheckel, K., 2014. Remediation of heavy metal (loid)s contaminated soils-To mobilize or to immobilize?. J. Harzard. Mater. 266, 141-166.

Cao, X.,Wahbi, A., Ma, L., Li, B., Yang, Y., 2009. Immobilization of Zn, Cu, and Pb in contaminated soils using phosphate rock and phosphoric acid. J. Hazard. Mater. 164, 555-564.

Chantigny, M.H., 2003. Dissolved and water-extractable organic matter in soils: a review on the influence of land use and management practices. Geoderma, 113, 57-380.

Chao, U.H., Seo, N.H., 2005. Oxidative stress in Arabidopsis thaliana exposed to cadmium is due to hydrogen peroxide accumulation. Plant. Sci. 168, 113-120.

Chen, Z., Chen, C., Liu, Y., Wu, Y., Yang, S., Lu, C., 1992. Study on soil back ground values in Fujian Province. Chin. J. Environ. Sci. 13, 70-75.

Chintala, R., Schumacher, T.E., McDonald, L.M., Clay, D.E., Malo, D.D., Papiernik, S.K., Clay, S.A., Julson, J.L., 2014. Phosphorus sorption and availability from biochars and soil/biochar mixtures. Clean. Soil. Air. Water. 42, 626-634.

Colwell, J.D., 1963. The estimation of the phosphorus fertilizer requirements of wheat in southern New South Wales by soil analysis. Aust. J. Exp. Agric. Anim. Husb. 3, 190-198.

Corpas, F.J., Gupta, D.K., Palma, J.M., 2015. Production sites of reactive oxygen species (ROS) in plants. In: Gupta D.K, Palma J.M, Corpas F.J (eds) Reactive oxygen species and oxidative damage in plants under stress. Springer. 1-22.

Coumar, M.V., Parihar, R.S., Dwivedi, A.K., Saha, J.K., Rajendiran, S., Dotaniya, M.L., Kundu, S., 2016. Impact of pigeon pea biochar on cadmium mobility in soil and transfer rate to leafy vegetable spinach. Environ. Monit. Assess. 188, 311- 331.

FAOSTAT., 2013. UN Food and Agriculture Organization, Statistics Division. "Production of Lettuce and Chicory by countries". Accessed 7 April 2017.

Feng, W., Hatt, B.E., McCarthy, D.T., Fletcher, T.D., Deletic, A., 2012. Biofilters for storm water harvesting: understanding the treatment performance of key metals that pose a risk for water use. Environ. Sci. Technol. 46, 5100-5108.

Galuszka, A., Migaszewski, Z., Duczmal, C.A., Dolegowska, S., 2016. Geochemical background of potentially toxic trace elements in reclaimed soils of the abandoned pyrite-uranium mine (south-central Poland). Int. J. Environ. Sci. Technol.13, 2649-2662.

Gill, S.S., Tuteja, N., 2010. Reactive oxygen species and antioxidant machinery in abiotic stress tolerance in crop plants. Plant. Physiol. Biochem. 48, 909-930.

Gill, R.A., Zang, L., Ali. B., Farooq, M.A., Cui, P., Yang, S., Ali, S., Zhou, W., 2015. Chromium-induced physio-chemical and ultra-structural changes in four cultivars of Brassica napus L. Chemosphere. 120, 154-164.

Gong, B., Wen, D., Vanden-Langenberg, K., Wei, M., Yang, F., Shi, Q., Wang, X., 2013. Comparative effects of Na Cl and $NaHCO_3$ stress on photosynthetic parameters, nutrient metabolism, and the antioxidant system in tomato leaves. Sci. Hortic. 157, 1-12.

Gorin, N., Heidema, F.T., 1976. Peroxidase activity in golden delicious apples as a

possible parameter of ripening and senescence. J. Agric. Food. Chem. 24, 200-201.

Gupta, D.K., Palma, J.M., Corpas, F.J., 2016. Redox state as a central regulator of plant-cell stress response. Springer

Habiba, U., Ali, S., Farid, M., Shakoor, M.B., Rizwan, M., Ibrahim, M., Abbasi, G.H., Hayat, T., Ali, B., 2015. EDTA enhanced plant growth, antioxidant defense system and phytoextraction of copper by Brassica napus L. Environ. Sci. Pollut. Res. 22, 1534-1544.

Hagemann, N., Subdiaga, E., Orsetti, S., Rosa, J.M., Knicker, H., Schmidt, H.P., Kappler, A., Behrens, S., 2018. Effect of biochar amendment on compost organic matter composition following aerobic compositing of manure. Sci. Total. Environ. 614, 20-29. https://doi.org/10.1016/j.scitotenv.2017.08.161

Hasan, S.A., Fariduddin, Q., Ali. B., Hayat, S., Ahmad, A., 2009. Cadmium: toxicity and tolerance in plants. J. Environ. Biol. 30, 165-174.

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.

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

Hossain, M.K., Strezo, V., Chan, K.Y., Ziolkowski, A., Nelson, P.F., 2011. Influence of pyrolysis temperature on production and nutrient properties of wastewater sludge

biochar. J. Environ. Manag. 92, 223-228.

Ibrahim, M., Khan, S., Hao, X., Li, G., 2016. Biochar effects on metal bioaccumulation and arsenic speciation in alfalfa (Medicago sativa L.) grown in contaminated soil. Int. J. Environ. Sci. Technol. 13, 2467-2474.

Ibrahim, M., Li, G., Khan, S., Chi, Q., Xu, Y., 2017. Biochars mitigate greenhouse gas emissions and bioaccumulation of potentially toxic elements and arsenic speciation in Phaseolus vulgaris L. Environ. Sci. Pollut. Res. 24, 19524-19534.

Jeffery, S., Verheijen, F.G., van der Velde, M., Bastos, C., 2011. A quantitative review of the Effects of biochar application to soils on crop productivity using meta-analysis. Agric. Ecosyst. Environ. 144, 175-187.

Jelly, M., Najafi, S., 2018. Effect of pH on potentially toxic trace elements (Cd, Cu, Ni, and Zn) solubility in two native and spiked calcareous soils: experimental and modeling a-review. Commun. Soil. Sci. Plant. Anal. 49, 814-827.

Jiang, T.Y., Jiang, J., Xu, R.K., Li, Z., 2012. Adsorption of Pb (II) on variable charge soils amended with rice-straw derived biochar. Chemosphere. 89, 249-256.

Jien, S.H., Wang, C.S., 2013. Effects of biochar on soil properties and erosion potential

in a highly weathered soil. CATENA. 110, 225-233.

Kazemi, N., Khavari-Nejad, R.A., Fahimi, H., Saadatmand, S., Nejad-Sattari, T., 2010. Effects of exogenous salicylic acid and nitric oxide on lipid peroxidation and antioxidant enzyme activities in leaves of Brassica napus L. under nickel stress. Sci. Hortic. 126, 402-407.

Khan, S., Cao, Q., Zheng, Y., Huang, Y., Zhu, Y.G., 2008. Health risks of heavy metals in contaminated soils and food crops irrigated with wastewater in Beijing, China. Environ. Pollut. 152, 686-692.

Khan, S., Reid, B.J., Li, G., Zhu, Y.G., 2014. Application of biochar to soil reduces cancer risk via rice consumption: A case study in Miaoqian village, Longyan, China. Environ. Int. 68, 154-161.

Kim, P., Johnson, A.M., Essington, M.E., Radosevich, M., Kwon, W.T., Lee, S.H., Rials, T.G., Labbé, N., 2013. Effect of pH on surface characteristics of switch grass-derived biochar produced by fast pyrolysis. Chemosphere. 90, 2623-2630.

Kim, H.S., Kim, K.R., Kim, H.J., Yoon, J.H., Yang, J.E., Ok, Y.S., Owens, G., Kim, K.H., 2015. Effect of biochar on heavy metal immobilization and uptake by lettuce (Lactuca sativa L.) in agricultural soil. Environ. Earth. Sci. 74, 1249-1259.

Kim, E.J., Baek, K., 2015. Enhanced reductive extraction of arsenic from contaminated soils by a combination of dithionite and oxalate. J. Hazard. Mater. 284, 19-26.

Kołodyńska, D., Wnętrzak, R., Leahy, J., Hayes, M., Kwapiński, W., Hubicki, Z., 2012. Kinetic and adsorptive characterization of biochar in metal ions removal. Chem. Eng. J. 197, 295-305.

Kumpiene, J., Lagerkvist, A., Maurice, C., 2008. Stabilization of As, Cr, Cu, Pb and Zn in soil using amendments-A review. Waste. Manage. 28, 215-225.

Laird, D., Fleming, P., Wang, B., Horton, R., Karlen, D., 2010. Biochar impact on nutrient leaching from a Midwestern agricultural soil. Geoderma. 158, 436-442

Lehmann, J., 2007. A handful of carbon. Nature. 447, 143-144.

Li, Y., Zhang, S., Jiang, W., Liu, D., 2013. Cadmium accumulation, activities of antioxidant enzymes and malondialdehyde (MDA) content in Pistia stratiotes L. Environ. Sci. Pollut. Res. 20, 1117-1123.

Li, G., Khan, S., Ibrahim, M., Sun, T.R., Tang, J.F., Cotner, J.B., Xu, Y.Y., 2018. Biochars induced modification of dissolved organic matter (DOM) in soil and its impact on mobility and bioaccumulation of arsenic and cadmium. J. Hazard. Mater. 348, 100-108.

Lima, J.R.dS., Silva, W.dM., Medeiros, E.V.d., Duda, G.P., Correa, M.M., Filho, A.P.M., Dauphin, C.C., Antonino, A.C.D., Hammecker, C., 2018. Effect of biochar on physicochemical properties of a sandy soil and maize growth in a greenhouse experiment. Geoderma. 319, 14-23.

Lin, R., Wang, X., Luo, Y., 2007. Effects of soil cadmium on growth, oxidative stress and antioxidant system in wheat seedlings (Triticum aestivum L.). Chemosphere. 69, 89-98.

Luo, F., Song, J., Xia, W., Dong, M., Chen, M., Soudek, P., 2014. Characterization of contaminants and evaluation of the suitability for land application of maize and sludge biochars. Environ. Sci. Pollut. Res. 21, 8707-8717.

Makino, T., Takano, H., Kamiya, T., Itou, T., Sekiya, N., Inahara, M., Sakurai, Y., 2008. Restoration of cadmium-contaminated paddy soils by washing with ferric chloride: Cd extraction mechanism and bench-scale verification. Chemosphere. 70, 1035-1043.

Martinsen, V., Alling, V., Nurida, N.L., Mulder, J., Hale, S.E., Ritz, C., Rutherford, D.W.,Heikens, A., Breedveld, G.D., Cornelissen, G., 2015. pH effects of the addition of three biochars to acidic Indonesian mineral soils. Soil. Sci. Plant. Nutr. 61, 821-834.

Meharg, A.A., Norton, G., Deacon, C., Williams, P., Adomako, E. E., Price, A., Zhu, Y.G., Li, G., Zhao, F. J., McGrath, S., Villada, A., Sommella, A., De Silva, P. M. C. S., Brammer, H., Dasgupta, T., Villada, A., 2013. Variation in rice cadmium related to human exposure. Environ. Sci. Technol. 47, 5613-5618.

Melo, L.C., Puga, A.P., Coscione, A.R., Beesley, L., Abreu, C.A., Camargo, O.A., 2015. Sorption and desorption of cadmium and zinc in two tropical soils amended with sugarcane-straw-derived biochar. J. Soils. Sediments. 16, 226-234.

Mishra, S., Srivastava, S., Tripathi, R.D., Govindarajan, R., Kuriakose, S.V., Prasad, M.N.V., 2006. Phytochelatin synthesis and response of antioxidants during cadmium stress in Bacopa monnieri L. Plant. Physiol. Biochem. 44, 25-37.

Mittler, R., Vanderauwera, S., Suzuki, N., Miller, G., Tognetti, V.B., Vandepoele, K., Gollery, M., Shulaev, V., van Breusegem, F., 2011. ROS signaling: the new wave?. Trend. Plant. Sci. 16, 300-309.

Molassiotis, A., Sotiropoulos, T., Tanou, G., Diamantidis, G., Therios, I., 2006. Boron-induced oxidative damage and antioxidant and nucleolytic responses in shoot tips culture of the apple rootstock EM (Malus domestica Borkh). Environ. Exp. Bot. 56, 54-62.

Monteiro, M.S., Santos, C., Soares, A.M.V.M., 2009 Assessment of biomarkers of

cadmium stress in lettuce. Ecotoxicol. Environ. Saf. 72, 811-818.

Nadgórska-Socha, A., Kafel, A., Kandziora-Ciupa, M., Gospodarek, J., Zawisza-Raszka, A., 2013. Accumulation of heavy metals and antioxidant responses in Vicia faba plants grown on monometallic contaminated soil. Environ. Sci. Pollut. Res. 20, 1124-1134.

Niu, L., Yang, F., Xu, C., Yang, H., Liu, W., 2013. Status of metal accumulation in farmland soils across China: from distribution to risk assessment. Environ. Pollut. 176, 55-62.

Noctor, G., De, P.R., Foyer, C.H., 2007. Mitochondrial redox biology and homeostasis in plants. Trends. Plant. Sci. 12, 125-134.

Parvage, M.M., Ulén, B., Eriksson, J., Strock, J., Kirchmann, H., 2013. Phosphorus availability in soils amended with wheat residue char. Biol. Fertil. Soils. 49, 245-250.

Passardi, F., Theiler, G., Zamocky, M., Cosio, C., Rouhier, N., Teixera, F., Margis-Pinheiro, M., Dunand, C., 2007. Peroxi Base: the peroxidase database. Phytochemistry. 68, 1605-1611.

Patrick, L., 2003. Toxic metals and antioxidants: Part II. The role of antioxidants in arsenic and cadmium toxicity. Altern. Med. Rev. 8, 106-128.

Prapagdee, S., Tawinteung, N., 2017. Effects of biochar on enhanced nutrient use efficiency of green bean, Vigna radiata L. Environ. Sci. Pollut. Res. 143, 1-8.

Pratas, J., Favas, P.J., Souza, R., Varun, M., Paul, M.S., 2013. Phytoremedial assessment of flora tolerant to heavy metals in the contaminated soils of an

abandoned Pb mine in Central Portugal. Chemosphere. 90, 2216-2225.

Quartacci, M.F., Ranieri, A., Sgherri, C., 2015. Antioxidative defense mechanisms in two grapevine (Vitis vinifera L.) cultivars grown under boron excess in the irrigation water. Vitis. 54, 51-58.

Rajapaksha, A.U., Vithanage, M., Zhang, M., Ahmad, M., Mohan, D., Chang, S.X., Ok, Y.S., 2014. Pyrolysis condition affected sulfamethazine sorption by tea waste biochars. Bioresour. Technol. 166, 303-308.

Rajapaksha, A.U., Chen, S.S., Tsang, D.C.W., Zhangc, M., Vithanaged, M., Mandale, S.,Bin Gaof, B., Bolane, N.S., Ok, Y.S., 2016. Engineered/designer biochar for contaminant removal/immobilization from soil and water: Potential and implication of biochar modification. Chemosphere. 148, 276-291.

Rayment, G., Higginson, F.R., 1992. Australian laboratory hand book of soil and water chemical methods. Inkata Press Pty Ltd.

Reumann, S., Bartel, B., 2016. Plant peroxisomes: recent discoveries in functional complexity, organelle homeostasis, and morphological dynamics. Curr. Opin. Plant. Biol. 34, 17-26.

SEPA., 1995. Environmental quality standard for soils. China: State Environmental Protection Administration; [GB15618-1995]

Sies, H., Berndt, C., Jones, D.P., 2017. Oxidative stress. Annu. Rev. Biochem. 86, 715-748.

Singh, B., Sharma, S., Singh, B., 2010 Antioxidant enzymes in cabbage: variability and inheritance of superoxide dismutase, peroxidase and catalase. Sci. Hortic. 124,

9-13.

Singh, H.P., Mahajan, P., Kaur, S., Batish, D.R,, Kohli, R.K., 2013. Chromium toxicity and tolerance in plants. Environ. Chem. Lett. 11, 229-254.

Smernick, R.J., 2009. Biochar and sorption of organic compounds. In: Lehmann, J.; Joseph, S.. Biochar for environmental management: science and technology. London: Earthscan. 289-300.

Smith, S.R., 1994. Effect of soil pH on availability to crops of metals in sewage sludge-treated soils. Nickel, copper and zinc uptake and toxicity to ryegrass. Environ. Pollut. 85, 321-347.

Smoke, T., Smoking, I., 2004. IARC monographs on the evaluation of carcinogenic risks to humans. IARC, Lyon. 1450-1452.

Sun, B.Y., Khan, S.H., Zhang, Y.Z., Deng, S.H., Wu, J., Yuan, H., Qi, H., Yang, G., Li, L., Zhang, X.H., Xiao, H., Wang, Y.J., Peng, H., Li, Y.W., 2010. Certain antioxidant enzymes and lipid peroxidation of radish (Raphanus sativus L.) as early warning biomarkers of soil copper exposure. J. Hazard. Mater. 183, 833-838.

Sun, C.X., Chen, X., Cao, M.M., Li, M.Q., Zhang, Y.L., 2017. Growth and metabolic responses of maize roots to straw biochar application at different rates. Plant. Soil. 416, 487-502.

Tsang, D.C.W., Hartley, N.R., 2014. Metal distribution and spectroscopic analysis after soil washing with chelating agents and humic substances. Environ. Sci. Pollut. Res. 21, 3987‑3995.

Uchimiya, M., Lima, I.M., Thomas, K., Chang, S., Wartelle, L.H., Rodgers, J.E., 2010. Immobilization of heavy metal ions (Cu II, Cd II, Ni II, and Pb II) by broiler litter-derived biochars in water and soil. J. Agr. Food. Chem. 58, 5538-5544.

Uchimiya, M., Chang, S., Klasson, K.T., 2011. Screening biochars for heavy metal retention in soil: role of oxygen functional groups. J. Hazard. Mater. 190, 432-44.

Vangronsveld, J., Clijsters, H., 1992. A biological test system for the evaluation of the phytotoxicity and immobilization by additives in metal contaminated soils. Paper presented at the Metal Compounds in Environment and Life, 4: Interrelation between Chemistry and Biology.

Wang, L., Butterly, C.R., Chen, Q., Mu, Z., Wang, X., Xi, Y., Zhang, J., Xiao, X., 2016. Surface Amendment can ameliorate subsoil acidity in tea garden soils of high-rainfall Environments. Pedosphere. 26, 180-191.

Wang, W., Xia, M.X., Chen, J., Yuan, R., Deng, F.N., Shen, F.F., 2016. Gene expression characteristics and regulation mechanisms of superoxide dismutase and its physiological roles in plants under stress. Biochemistry (Moscow). 81,465-480

Wei, J., Li, C., Li, Y., Jiang, G., Cheng, G., Zheng, Y., 2013. Effects of external Potassium (K) supply on drought tolerances of two contrasting winter wheat cultivars. PLOS . One. 8, 69737-69747.

Wei, S., Zhu, M., Song, J., Peng, P.A., 2017. Comprehensive characterization of biochars produced from three major crop straws of China. Bio. Resources. 12, 3316-3330.

Welinder, K.G., 1992. Super family of plant, fungal and bacterial peroxidases.

Curr.Opin. Struct. Biol. 2,388-393.

Wong, C.S.C., Li, X.D., 2004. Pb contamination and isotopic composition of urban soils in Hong Kong. Sci. Total. Environ. 319, 185-195.

Wu, K.H., Xiao, S.Q., Chen, Q., Wang, Q.F., Zhang, Y.N., Li, K.Z., Yu, Y.X., Chen, L.M., 2013. Changes in the activity and transcription of antioxidant enzymes in response to Al stress in black soybeans. Plant. Mol. Biol. Rep. 31, 141-150.

Xu, X., Cao, X., Zhao, L., 2013. Comparison of rice husk-and dairy manure-derived biochar for simultaneously removing heavy metals from aqueous solutions: role of mineral components in biochar. Chemosphere. 92, 955-961.

Xu, D., Zhao, Y., Zhou, H., Gao, B., 2016. Effects of biochar amendment on relieving cadmium stress and reducing cadmium accumulation in pepper. Environ. Sci. Pollut. Res. 23, 12323-12331.

Yan, Y., Qi, F., Seshadri, B., Xu, Y., Hou, J., Ok, Y.S., Dong, X., Li, Q., Sun, X., Wang, L., 2016. Utilization of phosphorus loaded alkaline residue to immobilize lead in a shooting range soil. Chemosphere. 162, 315-323.

Yuan, Z., Yao, J., Wang, F., Guo, Z., Dong, Z., Chen, F., Hu, Y., Geoffrey Sunahara, G., 2017. Potentially toxic trace element contamination, sources, and pollution assessment in farmlands, Bijie City, southwestern China. Environ. Monit. Assess. 189, 25-30.

Yu, X.Y., Ying, G.G., Kookana, R.S., 2009. Reduced plant uptake of pesticides with biochar additions to soil. Chemosphere. 76, 665-671.

Zhao, X., Wang, J.W., Xu, H.J., Zhou, C.J., Wang, S.Q., Xing, G.X., 2014. Effects of

crop-straw biochar on crop growth and soil fertility over a wheat-millet rotation in soils of China. Soil. Use. Manage. 30, 311-319.

Zhang, S., Zhang, H., Qin, R., Jiang, W., Liu, D., 2009. Cadmium induction of lipid peroxidation and effects on root tip cells and antioxidant enzyme activities in Vicia faba L. Ecotoxicology. 18, 814-823.

Zhang, D., Yuan, Z.,Wang, S., Jia, Y., Demopoulos, G.P., 2015. Incorporation of arsenic into gypsum: relevant to arsenic removal and immobilization process in hydrometallurgical industry. J. Hazard. Mater. 300, 272-280.

Zheng, R.L., Cai, C., Liang, J.H., Huang, Q., Chen, Z., Huang, Y.Z., Arp. H.P.H., Sun, G.X., 2012. The effects of biochar from rice residue on the formation of iron plaque and the accumulation of Cd, Zn, Pb, As in rice (Oryza sativa L.) seedlings. Chemosphere. 89, 856-862.

Zheng, R.L., Chen, Z., Cai, C., Wang, X., Huang, Y., Xiao, B., Sun, G., 2013 Effect of biochar from rice husk, bran, and straw on heavy metal uptake by pot-grown wheat seedling in a historically contaminated soil. Bio. Resources. 8, 5965-5988.

Zheng, R.L, Chen, Z., Cai, C., Tie, B., Liu, X., Reid, B.J., Huang, Q., Lei, M., Sun, G., Baltrėnaitė, E., 2015. Mitigating heavy metal accumulation into rice (Oryza sativa L.) using biochar amendment -a field experiment in Hunan, China. Environ. Sci. Pollut. Res. 22, 11097-11108.

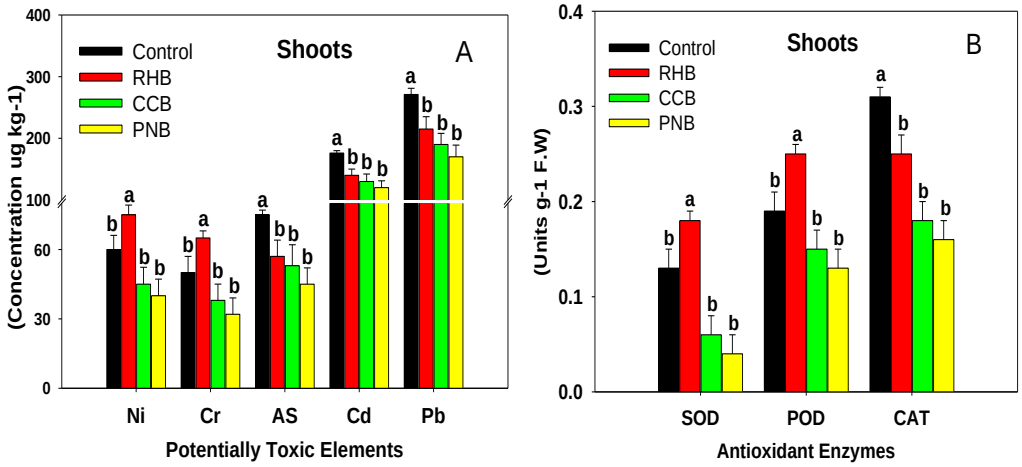

Fig. 1. (A-B) Potentially toxic elements bioaccumulation and antioxidant enzymes (SOD, POD and CAT activities, µmol tetra-guaiacol min^{-1} mg $protein^{-1}$, µmol consumed hydrogen peroxide H_2O_2 min^{-1} mg $protein^{-1}$) in Lactuca sativa L. grown in biochars amended and non-amended soil. The error bars represent standard deviations (n=4). Different letters on the bars indicate significant difference ($P \leq 0.05$), while similar letters indicate non-significant difference between treatments.

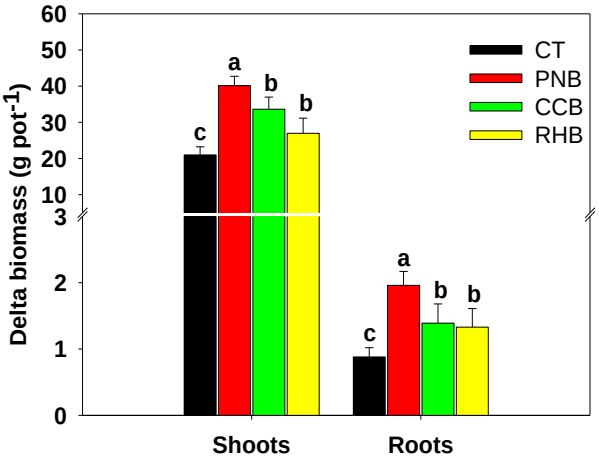

Fig. 2. Shoots and roots biomass of Lactuca sativa L. grown in biochars amended and non-amended soil. The error bars represent standard deviations (n=4). Different letters on the bars indicate significant difference ($P \leq 0.05$), while similar letters indicate non-significant difference between treatments.

Table. 1.

Physico-chemical properties of soil and biochars (n=4) and their comparison with permissible limits set for soil by the State Environmental Protection Administration (SEPA, 1995).

Parameters	Soil	PNB	RHB	CCB	Parameters	Soil	PNB	RHB	CCB	SEPA	Back ground soil
pH ($CaCl_2$)	5.21	9.33	8.94	10.12	Concentration	Total	Total	Total	Total		
EC (µS cm^{-1})	509	713	933	842	Cr (mg/kg)	0.19	0.01	0.03	0.02		
BET surface area(m^2g^{-1})	ND	12.49	1.85	5.48	Ni (µg/kg)	18.41	0.13	0.25	0.19		
Pore volume (cm^3 g^{-1})	ND	0.036	0.016	0.024	As (µg/kg)	**28.94**	0.87	0.14	0.05	0.03	5.88
Pore size (nm)	ND	10.07	3.48	4.57	Cd (µg/kg)	**64.52**	7.43	4.84	0.02	0.003	0.05
H (%)	0.74	3.22	2.79	3.68	Pb (mg/kg)	**7.73**	0.55	0.90	0.13	0.3	35.62
O (%)	13.63	10.82	15.04	13.36	TN (%)	0.14	1.45	0.63	0.55		
DOC(mg kg^{-1})	19.09	9.51	12.11	10.17	TC (%)	1.93	70.61	49.92	64.13		
Colwell P (mg kg^{-1})	30.69	4.03	4.63	2.96	S (%)	0.054	1.38	0.43	0.93		
Volatile matter (%)	ND	24.13	15.21	8.86	H/C	ND	0.04	0.06	0.05		
Fixed carbon (%)	ND	64.54	38.32	68.76	O/C	ND	0.15	0.30	0.21		
Ash content (%)	ND	6.57	18.97	12.61	(N+O)/C	ND	0.17	0.31	0.22		

An abbreviations PNB, RHB and CCB represents peanuts shell biochar, rice husk biochar and corn cobs biochar. ND No Data. Soil background value taken from Chen et al. (1992) for Fujian Province, China.

Table. 2.

Variations in the properties of biochars amended and non-amended soil after one week incubation before sowing. Mean values are shown ± standard deviation (n= 4). Different lowercase letters denote significant difference ($P \leq 0.05$) while similar letters indicate non-significant difference between treatments.

Parameters	CT	PNB	RHB	CCB
pH ($CaCl_2$)	5.21±0.14 b	6.41±0.15 b	6.78±0.16 b	6.82±0.18 a
EC (µS cm^{-1})	509.31±0.71 b	685.26±0.30 a	635.16±0.80 b	660.16±0.90 b
DOC (mg kg^{-1})	19.21±1.48 b	45.15±0.76 a	27.23±1.30 b	32.33±1.36 b
Colwell P (mg kg^{-1})	30.69±1.10 b	45.57±2.97 a	38.53±1.20 b	40.95±1.41 b
CEC (cmol kg^{-1})	1.96 ± 0.16 b	3.85± 0.12 a	3.13± 0.18 b	3.24±0.17 b
TN (%)	0.14±0.02 b	0.20±0.04 a	0.17±0.03 b	0.18±0.02 b
TC (%)	1.93±0.13 b	2.79±0.18 a	2.39±0.14 b	2.69±0.15 b
S (%)	0.054±0.01b	0.15±0.22 b	0.29±0.28 a	0.28±0.23 b
H (%)	0.74±0.16 a	0.61±0.02 b	0.63±0.03 b	0.59±0.02 b
O (%)	13.63±1.54 a	4.82±0.23 b	5.83±0.22 b	7.27±0.23 b
	Available Concentrations			
As (µg/kg)	18.94±0.56 a	12.24±0.32 b	14.14±0.31 b	13.90±0.40 b
Cr (µg/kg)	9.24±1.21 b	6.10±0.92 b	13.96±1.43 a	6.87±0.80 b
Ni (µg/kg)	12.49±0.91 b	7.37±0.74 b	14.78±1.54 a	8.76±0.72 b
Pb (mg/kg)	4.71±0.56 a	2.54±0.32 b	3.55±0.29 b	3.18±0.31 b
Cd (µg/kg)	44.54±0.90 a	30.44±1.41 b	35.73±1.35 b	34.87±1.51 b

Colwell P. Bioavailable PTEs extracted with ethylene diamine tetra acetic acid (EDTA-Na_2) (0.05 M), tri ethanol amine (TEA) (0.1 M) and $CaCl_2$ (0.01 M).

Biochars effects potentially toxic elements and antioxidant enzymes in Lactuca sativa L. grown in multi-metals contaminated soil

Muhammad Ibrahim[a,b], Gang Li[a,c*], Faith Ka Shun Chan[d,e], Paul Kay[e] , Xiao-Xia Liu [f], Les Firbank [g] , Yao-Yang Xu[a,c,]

[a]Key Laboratory of Urban Environment and Health, Institute of Urban Environment, *Chinese Academy of Sciences, Xiamen 361021, People's Republic of China*

[b] University of Chinese Academy of *Sciences, Beijing 100049, People's Republic of* China

[c] Ningbo Urban Environment Observatory and Monitoring Station, Ningbo 315830 *People's Republic of China*

[d] School of Geographical Sciences, University of Nottingham Ningbo Campus, Ningbo China, 31500, China

[e] School of Geography and Water@Leeds, University of Leeds, LS29JT, Leeds, UK

[f] Zhejiang Agricultural Technology Extension Center, Hangzhou 310020, China

[g] School of Geography biology, University of Leeds, LS29JT, Leeds, UK

***Corresponding author**: E-mail: gli@iue.ac.cn Tel: 008657486085863; Fax: 008657486784814

This section includes 3 pages containing 2 figures.

Supporting methodology

Measurement of pH and EC

Using a mixture of soil or biochar and $CaCl_2$ solution at 1:2.5 (w/v), pH and EC were measured through Accumet XL 60 pH-EC meter.

Soil particle measurement

The tested soil particle size was measured using Mastersizer 2000 (Malvern Instrument Ltd, UK).

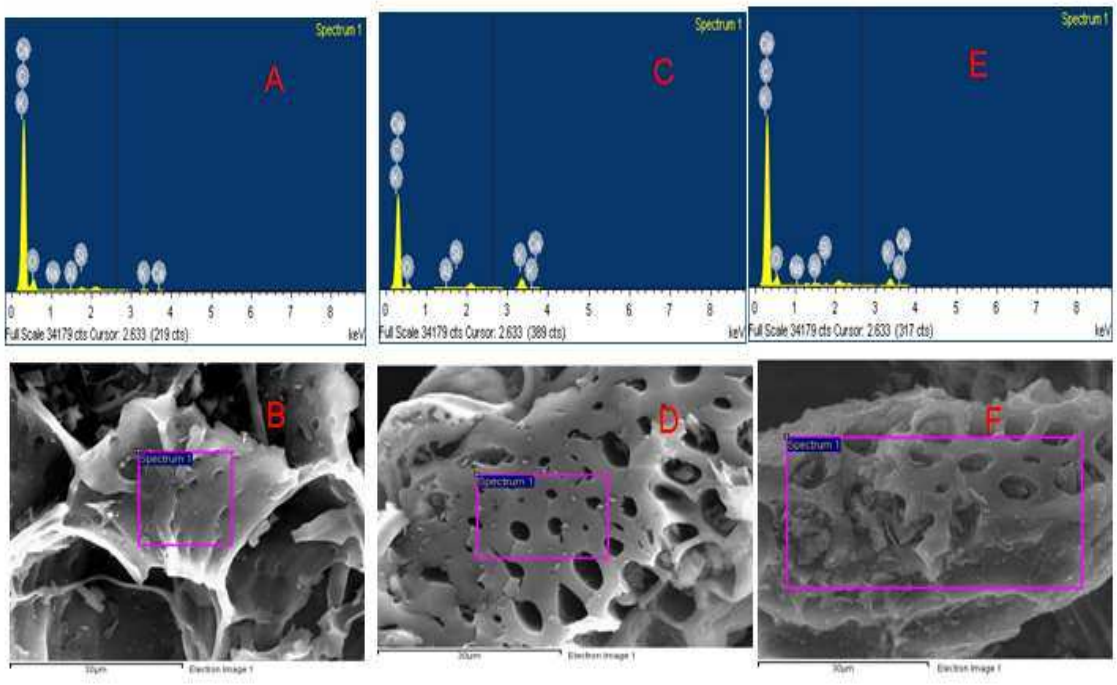

Fig.S1. Scanning electron micrograph and energy dispersive X-rays spectroscopy (SEM-EDS) of RHB (A-B), CCB (C-D) and PNB (E-F) produced at 500 ^{0}C for 6 h

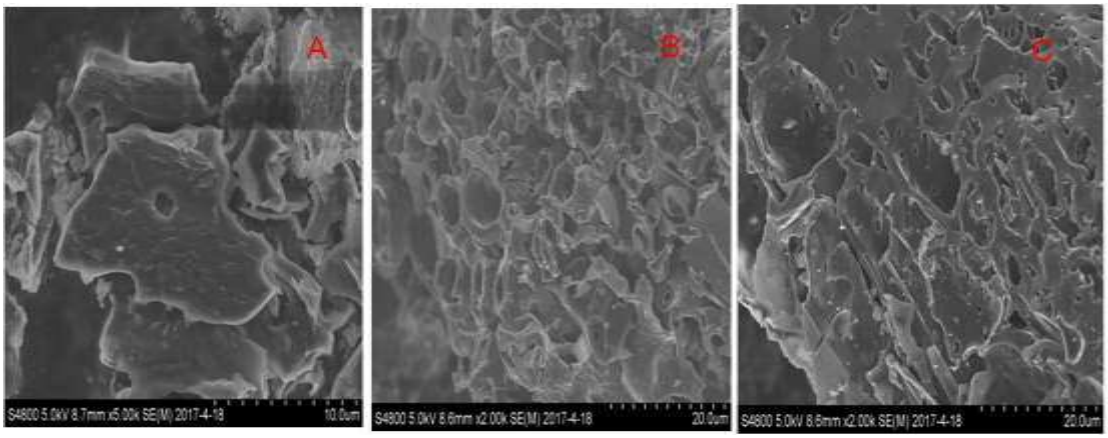

Fig. S2. Scanning electron micrographs (SEM) of RHB (A), CCB (B) and PNB (C) produced at 500^0C for 6h