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Abstract

This study evaluated the feasibility of using biochars produced from three types of crop residues for immobilizing Pb and As and their effects on the abundance of microbial community in contaminated lowland paddy (P-soil) and upland (U-soil) agricultural soils. Biochars were produced from umbrella tree [*Maesopsis eminii*] wood bark [WB], cocopeat [CP], and palm kernel shell [PKS] at 500 ˚C by slow pyrolysis at a heating rate of 10 ˚C min^{-1}. Soils were incubated with 5% (w w^{-1}) biochars at 25 ˚C and 70% water holding capacity for 45 d. The biochar effects on metal immobilization were evaluated by sequential extraction of the treated soil, and the microbial community was determined by microbial fatty acid profiles and dehydrogenase activity. The addition of WB caused the largest decrease in Pb in the exchangeable fraction (P-soil: 77.7%, U-soil: 91.5%), followed by CP (P-soil: 67.1%, U-soil: 81.1%) and PKS (P-soil: 9.1%, U-soil: 20.0%) compared to that by the control. In contrast, the additions of WB and CP increased the exchangeable As in U-soil by 84.6% and 14.8%, respectively. Alkalinity and high phosphorous content of biochars might be attributed to the Pb immobilization and As mobilization, respectively. The silicon content in biochars is also an influencing factor in increasing the As mobility. However, no considerable effects of biochars on the microbial community abundance and dehydrogenase activity were found in both soils.

Keywords: Black carbon; Slow pyrolysis; PLFA; Soil enzymes; Toxic metals

1. Introduction

A large amount of crop residues is generated worldwide, and their proper use as an initial feedstock for many applications is very desirable because of the carbon-rich composition and renewability of the crop residues (Colantoni et al., 2016). The production of global crop residues has reached >3.7 Pg y^{-1}, and its potential increase can be >1.3 Pg y^{-1}. The environmentally benign practices of crop residues in the form of biochars are widely considered for soil carbon sequestration or soil quality improvement (Ahmad et al., 2014b; Kim et al., 2015; Rajapaksha et al., 2015).

Biochars, a carbon-rich mixture of in/organic compounds, are generated as a byproduct in pyrolysis of feedstocks at limited oxygen conditions (Lehmann and Joseph, 2009). The feedstock properties such as density, particle size, particle shape, thermal conductivity, and permeability, and the intrinsic properties (*i.e.*, lignin, cellulose, and hemicelluloses contents, composition of inorganic compounds, moisture content, etc.) are the important factors for determining the properties of biochars (Joseph et al., 2009). In addition to the feedstock properties, the pyrolytic conditions also determine the physicochemical properties of biochars (Ahmad et al., 2014b). On the basis of these results, research studies were conducted with various pyrolytic conditions (*i.e.*, slow/fast pyrolysis, gasification, etc.) to generate biochars (Manyà, 2012; Poucke et al., 2016). The chemical performance of biochars is dependent on its physiochemical properties, including surface area, porous structure, surface functional groups, ash content, crystalline and amorphous carbon structures, and elemental composition (Ahmad et al., 2013; Inyang et al., 2016; Qian et al., 2015; Rajapaksha et al., 2014). An increase in biochar surface area mainly results from the liberation of volatile matter from the pore spaces with increasing pyrolysis temperature (Ahmad et al., 2014a). The reported biochar surface area ranged from 0.1 to >900 m^2 g^{-1} (UC Davis

Biochar database, 2015). Generally, slow pyrolyzed biochars have a large surface area and high carbonization degree because low heating rates and long holding times facilitate the removal of volatile matter and the systematic arrangement (*i.e.*, grapheme-like structures) of organic carbon structures (Manyà, 2012). Therefore, the slow pyrolyzed biochars have properties favorable for soil amendment, soil fertility improvement, and contaminant immobilization, in addition to its benefits in soil carbon sequestration (Gómez et al., 2016; Pandey et al., 2016).

Although biochars have been known as soil amendments to effectively immobilize soil heavy metals, the efficacy of slow pyrolyzed biochars on soil microorganisms has not been well investigated yet (Ahmad et al., 2014a, 2016a,b; Anderson et al., 2011; Lehmann et al., 2011; Luo et al., 2013; Oleszczuk et al., 2014). Scientists have reported contrasting observations in microbial communities following biochar application to soils mainly because of the differences in biochar and soil properties and biochar application rates (Luo et al., 2013). The readily available carbon and nutrients, large surface area, and porous structures of the biochars are considered as the favorable factors for soil microbial growth (Lehmann et al., 2011). Among these factors, the readily available carbon and nutrients are reported as the most important factor for improving the microbial community abundance within a short term (Kolb et al., 2009). Biochars produced at a low temperature contain a high amount of carbon, which is readily available (Ahmad et al., 2014b). However, the experimental evidence associated with soil microbial community abundance and mass transportation (*i.e.*, carbon and nutrient) from biochars to microorganisms is not fully established (Lehmann et al., 2011). In addition, the role of biochars in microbial abundance in metal-contaminated soils remains largely unknown. The present study hypothesizes that the high metal adsorption capacity of biochars because of their large surface area and high aromaticity could lower the biotoxicity of metals in contaminated soils, thereby improving the soil microbial community abundance in soil within a short term. Reduced biotoxicity of metals also helps in *in-situ* biogeochemical processes for organic matter decomposition and nutrient cycling in the soil. To evaluate our hypothesis, we produced biochars at 500 ˚C by slow pyrolysis to increase the surface area and aromaticity and tested their effectiveness in Pb and As immobilizations and microbial community abundance in contaminated agricultural soils. Three types of crop residues containing large amounts of lignin were used as the biomass for producing slow pyrolized biochars to obtain high aromaticity.

The objectives of this study are to evaluate (1) the efficacy of immobilization of heavy metals in contaminated agricultural soils by using biochars produced from umbrella tree (*Maesopsis eminii*) wood bark (WB), cocopeat (CP), and palm kernel shell (PKS), (2) the changes in chemical properties of heavy metal-contaminated agricultural soils with the incorporation of three biochars, and (3) the microbial community abundance and activity in heavy metal-contaminated agricultural soils with the incorporation of three biochars, using laboratory incubation. Sequential extraction of metals was used to analyze the metal immobilization by biochars. The fatty acid methyl ester (FAME) analysis and the dehydrogenase activity were used to evaluate the microbial community and the activity in heavy metal-contaminated soils treated with biochars, respectively.

2. Materials and methods

2.1. Biochars

Biochars were produced from three crop residues collected from Indonesia: umbrella tree (*M. eminii*) WB, CP, and PKS, as reported in a previous study by Lee et al. (2013). Slow pyrolysis was performed at a heating rate of 10 ˚C min^{-1} from ambient temperature to 500 ˚C and holding it at 500 ˚C for 1 h to produce biochars. A complete anaerobic condition was maintained inside the furnace by N_2 gas at a purging rate of 1.5 L min^{-1}. The biochar properties are listed in Table 1 (Lee et al., 2013). The graphitized carbon structures and the surface functional groups of biochars were characterized by Raman spectrophotometry (ARAMIS, Horiba, Japan) and Fourier transform infrared spectroscopy (FT-IR; Frontier, PerkinElmer, UK), respectively.

2.2. Soil collection and characterization

Contaminated agricultural soils were collected from a lowland paddy field (P-soil), which is located near the closed Seoseong mine at Seosan-si (36.78˚ N, 126.45˚ E) in Chungnam-do, Korea, and from an upland fallowed agricultural field (U-soil), which is located near the Tancheon mine at Gongju-si (36.44˚ N, 127.12˚ E) in Chungnam-do, Korea.

Soils were air dried and screened using a 2-mm sieve. Soil texture (by the pipette method), pH and electrical conductivity (1:5 soil to deionized water), exchangeable cations (Ca^{2+}, K^+, Mg^{2+}, and Na^+), exchangeable Pb (ammonium acetate at pH 7; ICP-OES, Optima 7300 DV, Perkin-Elmer, USA), and total As and Pb (MARS, HP-500 plus, CEM Corp., NC, USA) were determined (Ahmad et al., 2016a; Smith and Mullins, 1991; USEPA, 2007).

2.3. Soil incubation experiment

A short-term laboratory incubation study was conducted to evaluate the biochar effects on As and Pb immobilizations and soil microbial community abundance. Biochar incorporation could increase the soil microbial community abundance in short term because of its volatile matter supplement (Lehmann et al., 2011). A mixture of 100 g soil and 5% (w w^{-1}) biochar was placed in a 600-mL high-density polyethylene bottle. The water content in the bottle was adjusted to

70% water holding capacity and incubated at 25 ˚C in dark for 45 d in an incubator (MIR-554, SANYO Electronic, Co., Ltd., Tokyo, Japan). Each treatment was performed in triplicates. The bottles were opened every 3 d to maintain the water content and avoid anaerobic condition. Once the incubation was completed, the soil samples were collected and stored at 4 ˚C for microbial analysis on the next day. Soil chemical properties and dehydrogenase activity of the air-dried samples were determined.

2.4. Soil characterization

2.4.1. Chemical properties

Exchangeable cations (Ca^{2+}, K^{+}, Mg^{2+}, and Na^{+}), total As and Pb contents, pH, and EC were determined, as described in section 2.2. The water-soluble anion and cation concentrations were determined by using an ion chromatograph (IC; Metrohm Compact IC-861, Switzerland) and an inductively coupled plasma optical emission spectrometer (ICP-OES; Optima 7300 DV, Perkin-Elmer, USA), respectively, as described by Rajapaksha et al. (2015). The total carbon and nitrogen contents were determined using an elemental analyzer (Eurovector, EA, Italy).

2.4.2. Sequential extraction

The sequential extraction procedure explained by Tessier et al. (1979) was used to evaluate the geochemical metal(loid) fractions in soils. One gram of air-dried soil was used for the sequential extraction of metal(loid) fractions, and the concentrations of As and Pb were analyzed in each consecutive supernatant using the ICP-OES. Different solutions at different pH values were used to extract five geochemical metal(loid) fractions: exchangeable, bound to carbonates, bound to Fe and Mn oxides, bound to organic matter, and residual as explained by Tessier et al. (1979), and the concentrations of As and Pb were analyzed in each consecutive supernatant by using the ICP-OES.

The quantification accuracy of As and Pb fractions was calculated, as described by Antić-Mladenović et al. (2011).

Recovery % = $\frac{\sum five\ fractions\,(mg kg^{-1})}{TMC\ (mg kg^{-1})} \times 100$

where TMC is the total metal(loid) content obtained from the soil digestion (USEPA, 2007). The average recoveries of As and Pb in soils were 87.16% and 92.25%, respectively.

2.4.3. Geochemical modeling

Geochemical modeling by visual MINTEQ ver. 2.6 software was used to predict the possible precipitation of Pb compounds. Water soluble cations (Ca^{2+}, Mg^{2+}, Na^{+}, K^{+}, Mn^{2+}, Al^{3+}, Fe^{3+}, and Pb^{2+}) and anions (Cl^{-}, SO_4^{2-}, PO_4^{3-}, and NO_3^{-}) were used as the input parameters. A temperature of 25 ˚C, CO_2 pressure of $10^{-3.4}$ atm, and the pH of aqueous suspensions were used as the fixed parameters (Cao et al., 2008). The possibility of mineral precipitation was identified from the saturation index (SI) values of Pb minerals.

$$SI = \log IAP - \log K_{sp}$$

where IAP and K_{sp} are the ion activity product and solubility product constant, respectively. The SI values < 0 and > 0 indicate the status of undersaturation and supersaturation, respectively (Hashimoto et al., 2009).

2.4.4. Microbial fatty acids and dehydrogenase activity

The FAME analysis was performed to extract the microbial fatty acids from soils, as described by Schutter and Dick (2000). The FAMEs were recognized with the retention times and equivalent chain lengths of standards (Microb analyser sample kit, Agilent Technologies).

The identified FAMEs were designated as the biomarker profiles of various microbial groups according to the literature. Bacteria (*i.e.*, 14:0, 15:0, 16:0, 17:0, and 16:1ω9c) (Bååth et al., 1992; Langer and Rinklebe, 2011; Mual et al., 2016), Gram-negative bacteria (GNB) (*i.e.*, 18:1ω5c, cy17:0, Sum In Feature 3 [16:1ω7c/16:1ω6c], Sum In Feature 5 [18:0 ante/18:2ω6,9c], and Sum In Feature 8 [18:1ω7c]) (Frostegård and Bååth, 1996; Jindal et al., 2013; Langer and Rinklebe, 2011; Moche et al., 2015), Gram-positive bacteria (GPB) (*i.e.*, i15:0, a15:0, i16:0, i17:0, and a17:0) (Federle, 1986; Frostegård et al., 1993; Langer and Rinklebe, 2011; White et al., 1976; Zelles, 1997), actinomycetes (*i.e.*, 10Me18:0) (Frostegård et al., 1993), arbuscular mycorrhizal fungi (AMF) (*i.e.*, 16:1ω5c) (Olsson, 1999), and fungi (*i.e.*, 18:1ω9c) (Olsson, 1999) were used as biomarkers to identify the respective groups of microorganisms.

Dehydrogenase activity of the air-dried was determined using 2, 3, 5-triphenyltetrazolium chloride (TTC) as a substrate, as described by Casida (1964). Triphenyl formazan produced by the hydrolysis of TTC was analyzed using a UV-visible spectrophotometer (UV-1800 Spectrophotometer, Shimadzu, Japan) at the wavelength of 490 nm (Camiña et al., 1998).

2.5. Statistical analysis

Data are expressed as the mean of three replicates, and the variability among the replicates was stated in standard deviation. One-way analysis of variance (ANOVA) and Pearson correlation (*r*) were performed using Statistical Analysis System ver. 9.3 (SAS, Cary, NC, USA). Tukey's honestly significant difference (HSD) test was conducted to elucidate the significant differences between different treatments at a significance level of 0.05. The strength of *r* was categorized as follows: <0.20 very weak; 0.20–0.39 weak; 0.4–0.69 modest; and >0.69 strong correlations, according to Fowler et al. (2006). The principal component analysis (PCA) of microbial biomarkers was performed using Minitab 16 Statistical Software.

3. Results and discussion

3.1. Biochars and soils

Graphite-like structures were formed in biochars (Fig. 1a). There were two main bands at around 1354 cm^{-1} (D band) and 1594 cm^{-1} (G band)in Raman spectra of all biochars due to sp^2 sites (Ferrari and Robertson, 2001). The G band corresponds to the bond stretching of all pairs of sp^2 atoms in ring and carbon chain structures, and the D band represents the breathing modes of sp^2 atoms in carbon ring structures (Ferrari and Robertson, 2001). The ratio of D and G band intensities (I_D/I_G) is known to be an indicator of the degree of graphitization or systematic arrangement of carbon in biochars, and a small value of I_D/I_G implies a high degree of systematic arrangement of carbon (Wei et al., 2016). The values of I_D/I_G ratios of WB, CP, and PKS were 0.82, 0.81, and 0.71, respectively. All three biochars showed very low I_D/I_G ratio, thus having relatively high degrees of graphitization, and the highest graphitization was observed in PKS. The relatively high pyrolysis temperature at 500 ˚C might stimulate the formation of carbon ring structures and arrangement of carbon structures more systematically in all studied biochars.

The systematic arrangement of carbon structures is further supported by the results of FT-IR spectra (Fig. 1b). Aliphatic surface functional groups at the wavenumber regions of 2800–2980 cm^{-1} and 1000–1320 cm^{-1} totally disappeared in all biochars and exhibited enrichment of aromatic -C-H stretchings at the wavenumber region of 750–885 cm^{-1}. Biochar surface functional groups containing -O were not observed in the FT-IR spectra. This is likely because of the heat sensitivity of O at the relatively high pyrolysis temperature of 500 ˚C (Uchimiya et al., 2010).

The P-soil contained large amounts of fine particles (22.39% silt and 18.15% clay) compared to those in the U-soil (9.24% silt and 10.85% clay) (Table 2). The topography of P-soil showing the low-lying terraces might influence the accumulation of fine particles in the top soil layer. Comparatively, the U-soil having a high elevation enhanced the domination of coarse soil particles. The pH of P-soil and U-soil were neutral (pH 6.96) and acidic (pH 5.01), respectively. The As contents (P-soil 52.58 mg kg^{-1}; U-soil 1940.92 mg kg^{-1}) and the Pb contents (P-soil 1259.58 mg kg^{-1}; U-soil 1445.00 mg kg^{-1}) of the soils were extremely higher than the warning limits specified by the Korean standard of soil contamination (As 25 mg kg^{-1} and Pb 200 mg kg^{-1}; Ministry of Environment Korea, 2016).

3.2. Incubation study

3.2.1 Soil chemical properties

Two alkaline biochars (*i.e.*, WB [pH 9.6] and CP [pH 10.3]) increased the soil pH of both P-soil and U-soil (Fig. 2a, b). Even though the PKS is neutral (pH 6.9), the soil pH of P-soil was increased by 0.24 units compared to that of the control, probably because of the reactions among soil buffering capacity and biochar properties (Ahmad et al., 2012). At the end of incubation period, the pH in the soils treated with biochars was between 5.4 and 7.6. Hence, the addition of studied biochars may not be a considerable factor to increase the soil pH to a harmful level of >8 (Brady and Weil, 2014). The different buffering capacities might be a reason for the dissimilarity in the pH increase in both the soils. Soil buffering capacity relies on the soil clay content and mineralogy, oxide and carbonate contents, initial pH, weatherable mineral contents, and so on (Bowman et al., 2008). The clay content, initial soil pH, and amount of basic cations were higher in the P-soil than in the U-soil as shown in Table 2. These factors might be effectively facilitated to buffer the pH changes with regard to the biochar additions in the P-soil compared to those in the U-soil.

A significant enhancement of soil EC was observed in soils treated with CP (Fig. 2c, d). The very high K^+ (22960 mg kg^{-1}) and Na^+ (13710 mg kg^{-1}) contents in CP might be a reason for the significant increase in soil EC. The CP treatment showed the highest exchangeable K^+ and Na^+ in the soils after the incubation period (Table S1). However, CP did not increase the soil EC to a harmful level (*i.e.*, EC > 2 dS m^{-1}) for plant growth and microbial activity (Brady and Weil, 2014). The addition of CP also enhanced the total exchangeable basic cations (*i.e.*, Ca^{2+}, Mg^{2+}, and K^+) in both the soils, following the addition of WB; however, the addition of PKS did not increase the total exchangeable cations (Fig. 2e, f). The large contents of basic elements (Table 1) in WB and CP compared to those in the PKS might have increased the total exchangeable basic cations in the soils. Because basic cations are considered as essential plant nutrients (Brady and Weil, 2014), the additions of WB and CP to soil can be beneficial for plant growth.

3.2.2. Geochemical fractions of metals

The geochemical fractions of exchangeable, carbonate-bound, Fe and Mn oxide-bound, organic matter-bound, and residual metal(loid)s were identified from the sequential extraction (Tessier et al., 1979). The proportions of Pb in these five fractions of P-soil and U-soil were 0.21, 4.84, 74.49, 2.34, and 18.11% and 1.18, 0.20, 4.11, 0.59, and 93.93%, respectively, in the same order as mentioned above (Table S2). The additions of WB and CP to the P-soil significantly reduced the exchangeable fraction of Pb, whereas the addition of PKS was not effective (Fig. 3a). In the U-soil, all the biochars significantly reduced the exchangeable Pb fraction while showing the highest reduction by WB, similarly to that in the P-soil (Fig. 3b). A significant increase in the carbonate-bound Pb fraction was observed in the U-soil following the WB and CP additions, and Fe- and Mn-bound Pb fraction increased by all biochars. Moreover, the addition of CP increased the organic matter-bound Pb fraction in the U-soil. All biochars enhanced the formation of more stable Pb compounds in the U-soil. Hence, the efficacy of biochars on Pb immobilization was better in the U-soil compared to that in the P-soil.

The increased soil pH by biochar showed a positive effect for the immobilization of the exchangeable Pb fraction, as observed in the Pearson correlation analysis (r = -0.91, p < 0.0001; Table S3). Lead tends to be stable under alkaline conditions by the formation of stable minerals (Moon et al., 2015). The release of K^+, Na^+, OH^-, PO_4^{3-}, and Cl^- ions from soils under alkaline conditions can facilitate the formation of stable compounds of Pb (Ahmad et al., 2014a; Ahmad et al., 2016a; Yan et al., 2016).

This result was further confirmed by soil extraction using NH_4OAc. The exchangeable Pb was reduced significantly by all biochars in both the soils (Fig. S1a and b). The WB, CP, and PKS decreased NH_4OAc extractable Pb by 44.10%, 18.90%, and 14.60%, respectively, in the P-soil, whereas this decrease had higher values of 66.14%, 51.54%, and 19.28%, respectively, in the U-soil. One of the possible reasons for the high efficacy of WB and CP in Pb immobilization in both the soils is the high P content (Ahmad et al., 2014a; Almaroai et al., 2014; Rajapaksha et al., 2015). The P contents in WB, CP, and PKS were 485, 302, and 274 mg kg^{-1}, respectively, and these values corresponded with the exchangeable Pb reductions by the biochars. Similarly, Cao et al. (2011) observed the formation of stable Pb compounds following the application of P-rich-manure biochars.

The geochemical modeling by visual MINTEQ also revealed the possible formation of stable Pb compounds in P-soil and U-soil (Table S4). Stable complexes on negatively charged biochar surface can be formed with cationic metals through π interaction (Uchimiya et al., 2010). The precipitation of Pb on the biochar surface with cations K^+, Na^+, Ca^{2+}, and Mg^{2+} and anions OH^-, Cl^-, CO_3^{2-}, PO_4^{3-}, and SO_4^{2-} was identified previously as the preliminary mechanism of biochars in immobilization of Pb (Xu et al., 2013). Even though the -O containing functional groups on the biochar surface aid the formation of stable Pb complexes (Ahmad et al., 2016a, b; Wang et al., 2015), they might not enhance the immobilization of Pb in our study because the relatively high pyrolysis temperature of 500 ˚C reduced the -O containing functional groups in the studied biochars, as proved in the FT-IR surface analysis.

There was no exchangeable As in the P-soil, and it was the same even after biochar application. However, in the U-soil, exchangeable As was significantly increased by the WB addition (Fig. 3d). On the basis of the results from the two soils, it could be concluded that the biochars may transform only the exchangeable As fraction. According to the observation of Michálková et al. (2016), the pH enhancement by adding alkaline biochars in acidic soils leads to increase in As mobility. In the present study, WB that showed the highest soil pH increase significantly increased As in the exchangeable fraction. The increase in exchangeable As resulted from the ion competition between OH^- and $HAsO_4^{2-}$ to the limited anionic binding sites on the biochar surface. Subsequently, this competition resulted in an increase in mobile As (Inyang et al., 2010; Mukherjee et al., 2011; Yin et al., 2016). Therefore, it could also be a reason for the higher As bioaccumulation in plants, as reported previously (Shakoor et al., 2016; Zheng et al., 2012). The Pearson correlation also strongly supports this result that the soil pH and the exchangeable fraction of As had a strong positive correlation (r = 0.81, p <0.005; Table S3). In addition, the biochars influence the reduction of As(V) to As(III) by acting as electron donors (Beesley et al., 2014). The biochar surface composed of functional groups (e.g., phenolic, alcoholic, and carboxylic) donated electrons to the As(V), and biochar carbon materials were oxidized by abiotic reactions (Beesley et al., 2014). Choppala et al. (2016) reported similar observation following the application of chicken-manure biochar. The As(III) species are highly soluble, mobile, and toxic than As(V) (Mascher et al., 2002). Additionally, there is a possibility for the decline in the comparatively stable As fractions (*i.e.*, carbonate-bound fraction, iron and manganese oxide-bound fraction, and organic matter-bound fraction) and the residual As fractions because of the increase in the soil pH by alkaline biochars (Table S3).

The available P content in the biochars might also negatively affect the As immobilization. PO_4^{3-} is chemically analogous to As(V); hence, the increase in PO_4^{3-} induces the release of As from the soil colloids to the soil solution (Ahmad et al., 2016c; Beesley et al., 2014; Lim et al., 2016). This might be a reason for the highest exchangeable As observed when WB was added to the U-soil. The P content was highest in WB followed by those in CP and PKS, and the As mobility also had the same order as the P content in the studied biochars.

SiO_4^{4-} is the second effective competitor for As adsorption on Fe-(hydr)oxide next to PO_4^{3-} (Garnier et al., 2011). Under alkaline conditions, the formation of Fe-(hydr)oxide complexes with As might be further inhibited by the high Si contents in WB and CP. Even though the Si content in PKS is very high, the incapability of PKS to enhance the soil pH might be resulted from the formation of stable As–Fe-(hydr)oxide complexes (Yin et al., 2016).

3.2.3. Microbial community and dehydrogenase activity

The microbial communities in soils were assessed from the 18 fatty acids identified in the FAME profiles. The whole fatty acid profile was found in the P-soil treated with all studied biochars or control P-soil, but not in the U-soil (Fig. S2). In the control U-soil, the bacterial biomarkers 15:0, 17:0, and 16:1ω9c, GNB biomarker 18:1ω5c, and actinomycete biomarker 10Me18:0 were absent with CP and PKS; however, the bacterial biomarker 15:0 appeared (Fig. S2b). The high levels of metals or sand contents in the U-soil provide unfavorable conditions to the soil microorganisms, thereby reducing the fatty acid biomarkers. A similar observation was reported by Langer and Rinklebe (2011) who conducted a similar experiment with sandy textured soil contaminated with metals.

The magnitudes of the total FAME and that of each microbial group in the P-soil remained the same with no significant difference after incubation with biochars (Fig. 4a). For the U-soil, only PKS showed significant enhancement of the total FAME compared to that in the control (Fig. 4b). With the additions of studied biochars, the improvement of soil chemical properties and the reduction of bioavailable Pb fractions did not affect the increase in microbial communities in the soils. As explained by Kolb et al. (2009), the addition of biochars can enhance soil microbial communities within a short period if biochars supply usable carbon substrates or enhance the degradation of existing organic carbon. Previous studies have reported that the volatile matters on the biochar surfaces act as readily available carbon sources and boost the microbial growth and their functions in a month (Steiner et al., 2007). The decomposition of soil organic carbon can be expedited by adding biochars because of their increased surface areas for microbial growth (Hamer et al., 2004). The volatile matter contents (WB: 18.14%, CP: 14.30%, and PKS: 12.29%) and the surface areas (WB: 13.6 m^2 g^{-1}, CP: 13.7 m^2 g^{-1}, and PKS:

191 m^2 g^{-1}) of the tested biochars were fairly low. Even though PKS had the largest surface area among the tested biochars in this study, it may not be beneficial for microbial growth because of ~10 nm or smaller pore size (Lee et al., 2013). Lehmann et al. (2011) insisted that the biochar pores should be larger than 2 μm to provide a suitable environment for soil microorganisms. Therefore, the effective surface area of PKS for microbial growth might be similar to those of WB and CP, and the microbial colonization on RPS surface might not be significantly high compared to those on WB and CP surfaces. Even in long term, the high surface area of RPS may not be helpful in the microbial colonization because of very low pore size (Lehmann et al., 2011).

The PCA of the identified fatty acids clearly distinguished the two soils. The PCA results revealed that the additions of biochars did not affect the initial microbial community structures. For the U-soil, the significant increase in the total FAMEs by PKS was not vital to differentiate its microbial community from those in the WB- and CP-treated soils including the controls (Fig. 5a). The results from the PCA of the fatty acid biomarkers of the specific microbial groups revealed the predominant microbial biomarkers responsible for the differentiation of the two soils (Fig. 5b). The principal components PC1 and PC2 explained 94.1% and 4.4% variations, respectively. PC1 was responsible for more than 95% of the total variations explained by the first two principal components. The bacterial biomarker 16:0, GNB biomarkers Sum In Feature 3 (16:1ω7c/16:1ω6c), Sum In Feature 5 (18:0 ante/18:2ω6,9c), and Sum In Feature 8 (18:1ω7c), GPB biomarker i15:0, and fungi biomarker 18:1ω9c obtained the highest positive PC1 loadings. These were determined by the clear-cut separation of the microbial communities in the P-soil and those in the U-soil. It is comprehensible that these six biomarkers are highly sensitive to the heavy metal(loid) contaminations and unfavorable soil conditions, as they were highly abundant in the P-soil control, which is less contaminated than the U-soil.

The dehydrogenase activity did not show any significant difference among all tested biochars in P-soil and U-soil (Fig. S3a and S3b). Biochars could not increase the dehydrogenase activity in soils as they were inefficient to enhance the total FAME. It was confirmed by the very strong positive correlation (r = 0.98, p < 0.0001) between the total FAME and the dehydrogenase activity by the Pearson correlation analysis. The comparatively very low dehydrogenase activity in the U-soil might be because of the high level of metal(loid) contamination in the U-soil than that in the P-soil (Ahmad et al., 2012; Lee et al., 2013).

Dehydrogenases are intracellular enzymes commonly found in all soil microorganisms, and their activity is considered as a reliable indicator of the overall microbial metabolic activity in the soils (Oliveira and Pampulha, 2006). The dehydrogenases primarily govern the biological oxidation of organic compounds, and there are numerous dehydrogenases that are highly responsible for the numerous oxidation reactions occurring in the soil environments (Tabatabai, 1994). The substantial release of metal stress because of the application of biochars did not enhance the microbial community size or their activity in both the soils. Because all geochemical processes including the nutrient cycling are primarily mediated by the soil microorganisms (Langer and Rinklebe, 2011), the tested biochars were not effective to increase the overall soil quality.

4. Conclusion

Biochars produced from three different crop residues at 500 ˚C by slow pyrolysis were applied to heavy metal(loid)-contaminated agricultural lowland and upland soils. Pb was immobilized in the U-soil by all biochars, and by WB and CP in the P-soil; however, As was mobilized in the U-soil by WB and CP. Feedstock type, alkaline pH, and high P content of the biochars affected the

Pb immobilization and As mobilization, and the Si content of the biochars was also seemed to be another influencing factor in increasing the As mobility. Moreover, biochars showed increased As mobility if the soil was composed of an exchangeable As fraction. Hence, the application of tested biochars might not be harmful to be used in soils, which do not consist an exchangeable As fraction. The total FAME, GPB, GNB, fungi, actinomycetes, and AMF were not affected by the biochar application to the P-soil, and only PKS increased the total FAME in the U-soil, which might be because of the largest surface area of PKS. However, the PAC results clearly showed that none of the biochars were effective to increase the microbial community of heavy metal(loid)-contaminated soils in the short term. It could be because of the low volatile matter content and the low effective surface area for microbial growth in the tested biochars. Furthermore, the biochar efficacy to increase the dehydrogenase activity is negligible in the considered soils. Therefore, the reduced biotoxicity of Pb by the biochars might not improve the microbial parameters in soils in a short term. We suggest further studies with slow pyrolyzed biochars at different temperatures for immobilization of heavy metal(loid)s and improving the microbial parameters in soils within a short term.

Acknowledgment

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660 Table 1: Biochar properties (adapted from Lee et al. (2013))

		WB	CP	PKS
Proximate analysis (%)	Moisture	0.36	2.55	0
	VM	18.14	14.30	12.29
	FC*	68.66	67.25	80.85
	Ash	12.84	15.90	6.86
	VM/FC	0.26	0.21	0.15
Ultimate analysis† (%)	C	84.84	84.44	87.85
	H	3.13	2.88	2.91
	O*	10.20	11.67	8.14
	N	1.83	1.02	1.11
Elements (mg kg^{-1})	Al	6588	3436	4275
	Ca	19730	2667	392
	Fe	4736	2088	21380
	K	6470	22960	1219
	Mg	1111	554	131
	Mn	221	33	35
	Na	30	13710	534
	P	485	302	274
	Si	7604	11590	10310
	Ti	615	507	230
Surface area‡ (m^2 g^{-1})		13.6	13.7	191
Average pore diameter		109.9 nm	24310 nm	57.2 nm

pH	9.6	10.3	6.9

WB, Wood bark biochar 500 ˚C

CP, Cocopeat biochar 500 ˚C

PKS, Palm kernel shell biochar 500 ˚C

VM, Volatile matter; FC, Fixed carbon

*By difference

†Moisture- and ash-free basis

‡N_2-BET area

Table 2: Physicochemical properties of soils

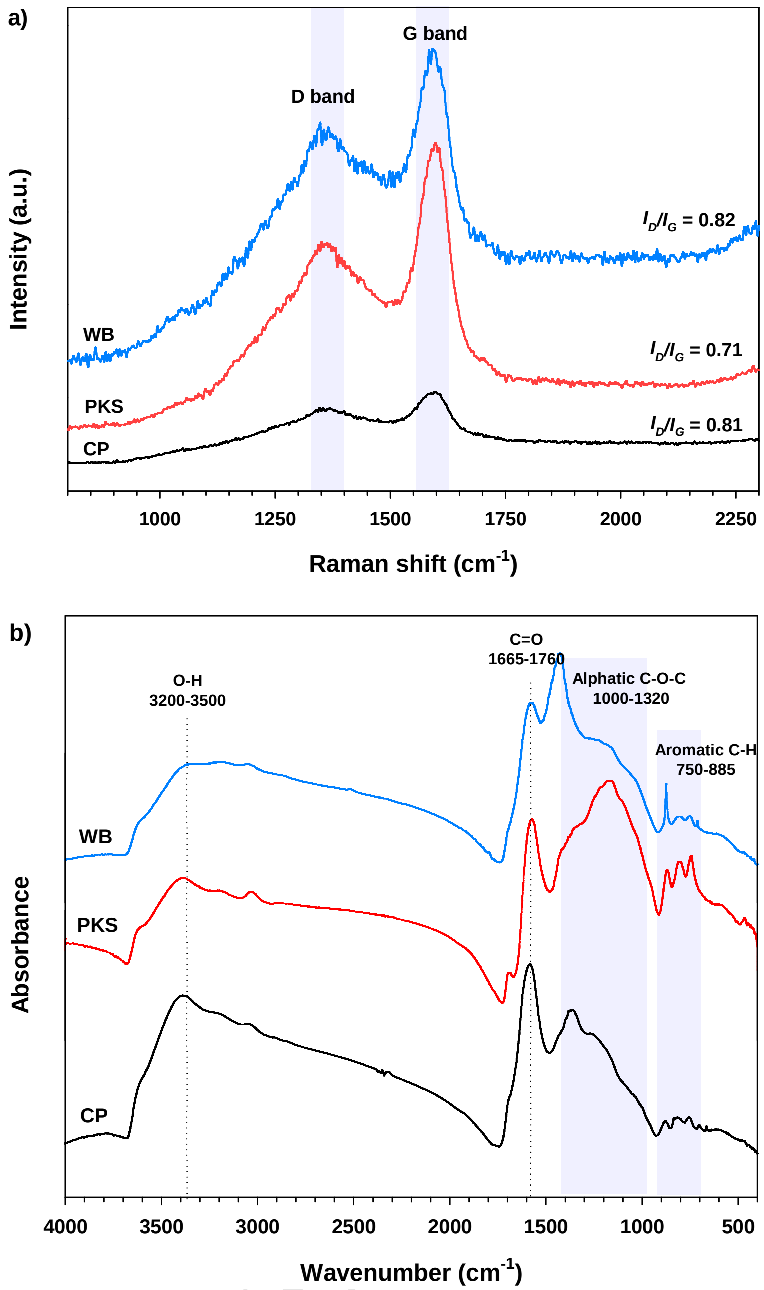
									Exchangeable cations				Total	
Soil	Land use	Sand	Silt	Clay	Soil texture	OC	pH*	EC*	Ca	K	Mg	Na	As	Pb
		%	%	%		%		dS m^{-1}	$cmol_{(+)}$ kg^{-1}	$cmol_{(+)}$ kg^{-1}	$cmol_{(+)}$ kg^{-1}	$cmol_{(+)}$ kg^{-1}	mg kg^{-1}	mg kg^{-1}
P-soil¶	Lowland paddy field	59.46	22.39	18.15	Sandy loam	2.14	6.96	0.31	8.76	0.29	3.10	0.07	52.58	1259.58
U-soil¶	Upland fallowed agricultural field	79.92	9.24	10.85	Sandy loam	5.76	5.01	0.11	1.63	0.44	0.61	0.03	1940.92	1445.00
Korean standard of soil contamination warning limits †													25	200

OC, Organic carbon

*1:5 soil to deionized water ratio

¶Collected from agricultural lands located adjacent to closed mining areas of Korea

†Ministry of Environment Korea (2016)



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675 Figure 1. Raman spectra (a) and FT-IR spectra (b) of biochars. WB, PKS, and CP represent the

676 wood bark, palm kernel shell, and cocopeat, respectively. The biochar production temperature

677 was 500 ˚C.

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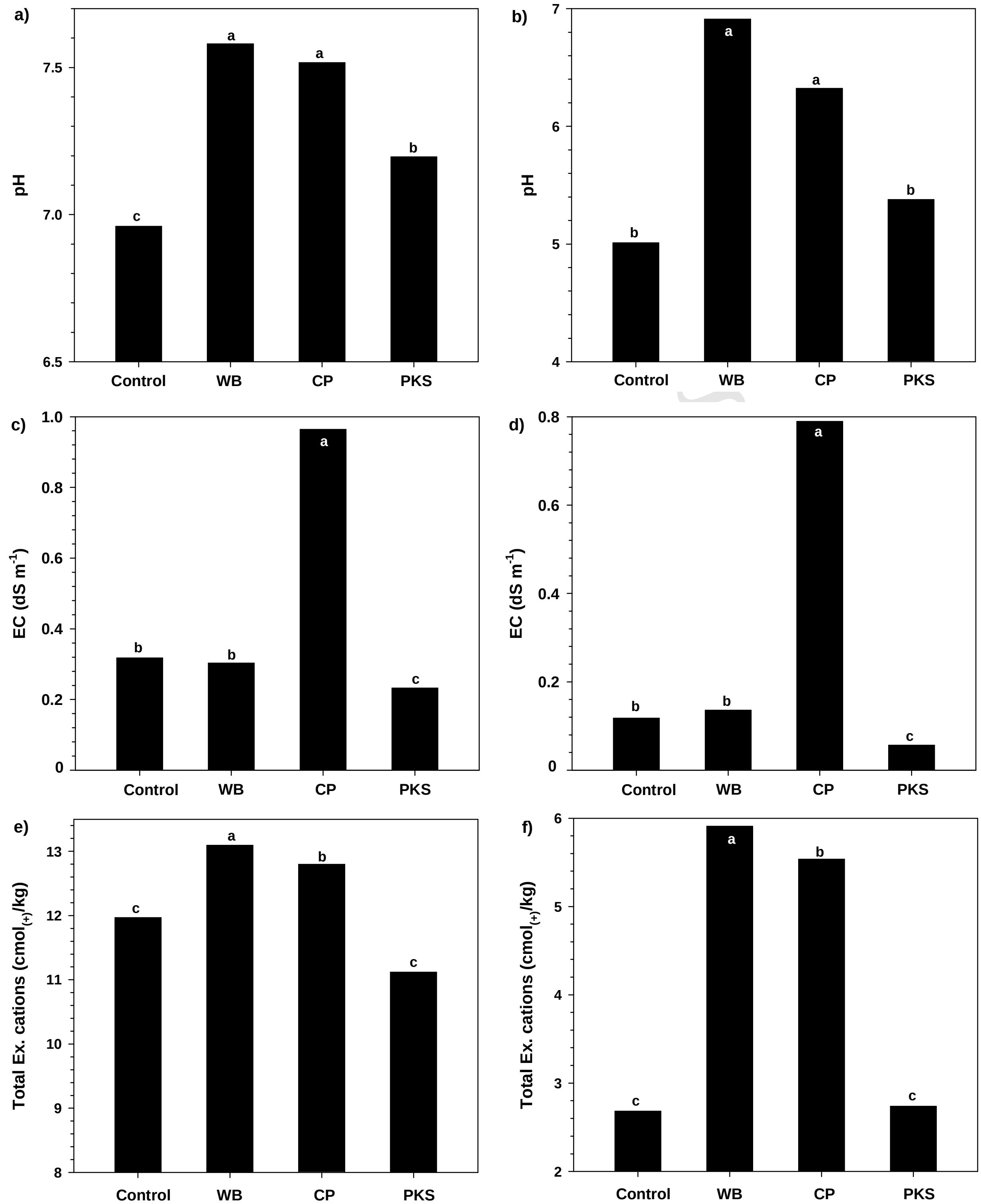

Figure 2: pH of P-soil (a) and U-soil (b), EC of P-soil (c) and U-soil (d), and the total exchangeable basic cations (sum of Ca, Mg, and K) in P-soil (e) and U-soil (f) after the

incubation period. WB, CP, and PKS represent the wood bark, cocopeat, and palm kernel shell, respectively. The biochar production temperature was 500 ˚C. Different letters above the vertical bars indicate the statistically significant difference at $p < 0.05$ (Tukey's HSD test).

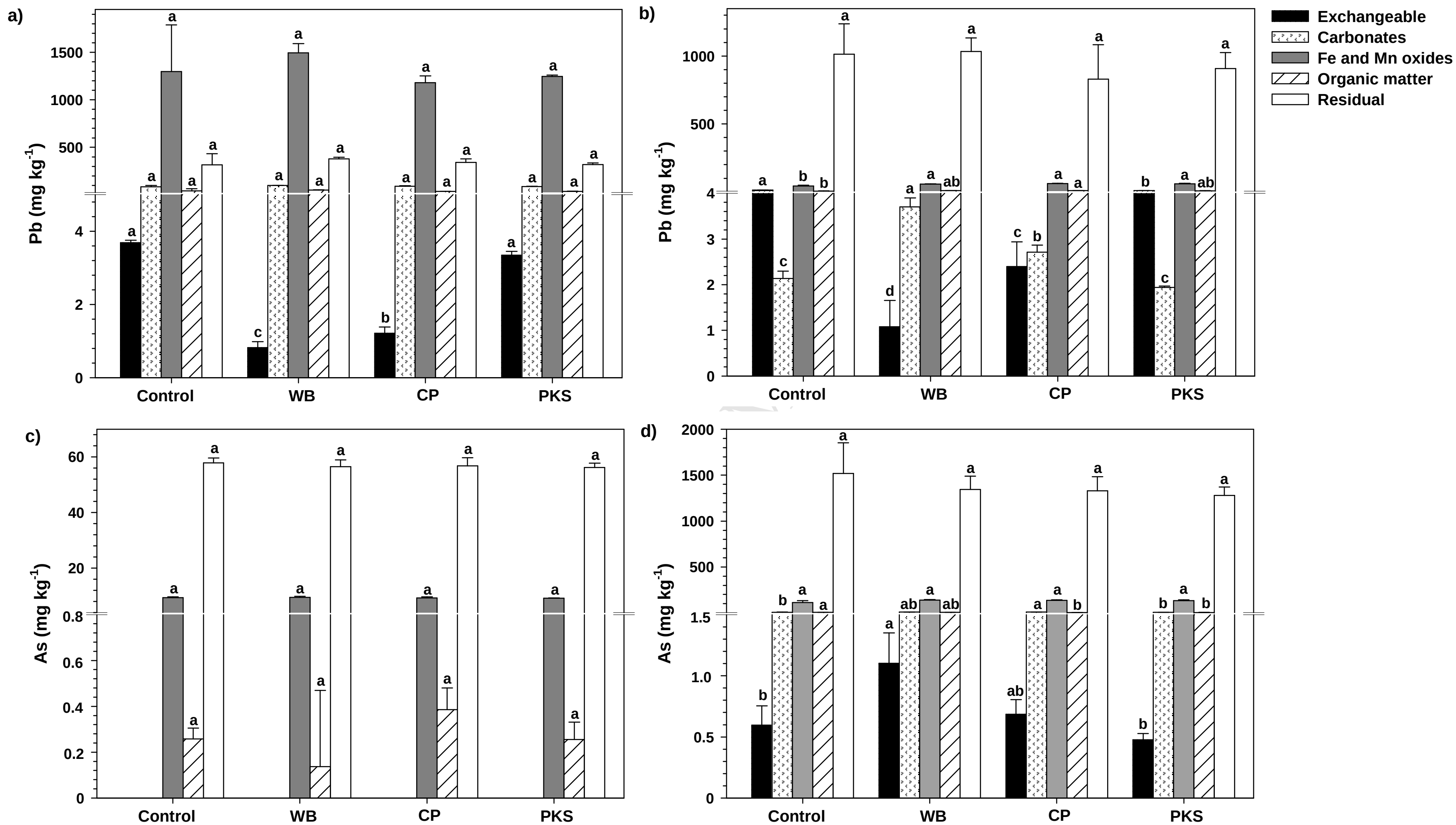

Figure 3: Geochemical fractionation of metals in soils after the incubation period, obtained by sequential extraction procedure; Pb in P-soil (a), Pb in U-soil (b), As in P-soil (c), and As in U-soil (d). WB, CP, and PKS represent the wood bark, cocopeat, and palm

kernel shell, respectively. The biochar production temperature was 500 ˚C. Different letters above the vertical bars indicate the statistically significant difference at $p < 0.05$ (Tukey’s HSD test).

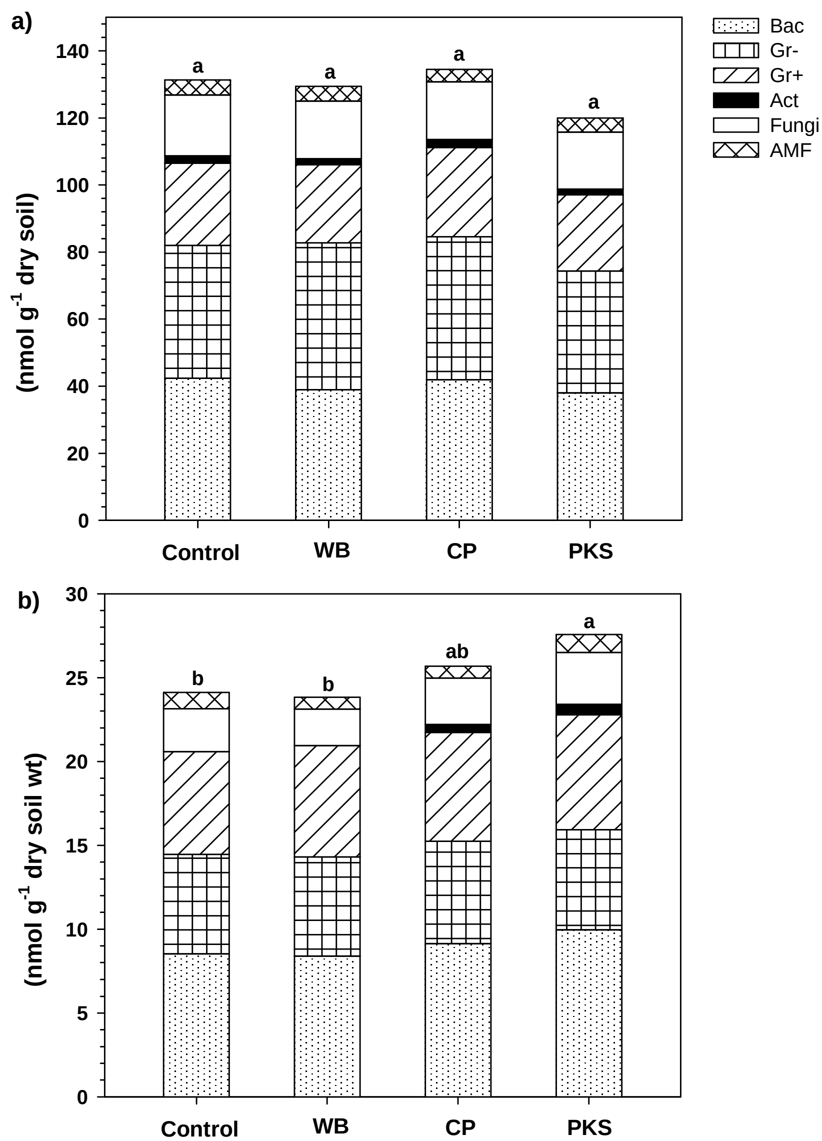

Figure 4: Absolute abundance of the total FAME and the specific microbial groups categorized on the basis of the identified fatty acid biomarkers in the soils after the incubation period; P-soil (a) and U-soil (b). WB, CP, and PKS represent the wood bark, cocopeat, and palm kernel shell, respectively. The biochar production temperature was 500 ˚C. Different letters above the vertical bars indicate the statistically significant difference at p <0.05 (Tukey's HSD test). GNB, GPB, Act, and AMF are Gram-negative bacteria, Gram-positive bacteria, actinomycetes, and arbuscular mycorrhizal fungi, respectively.

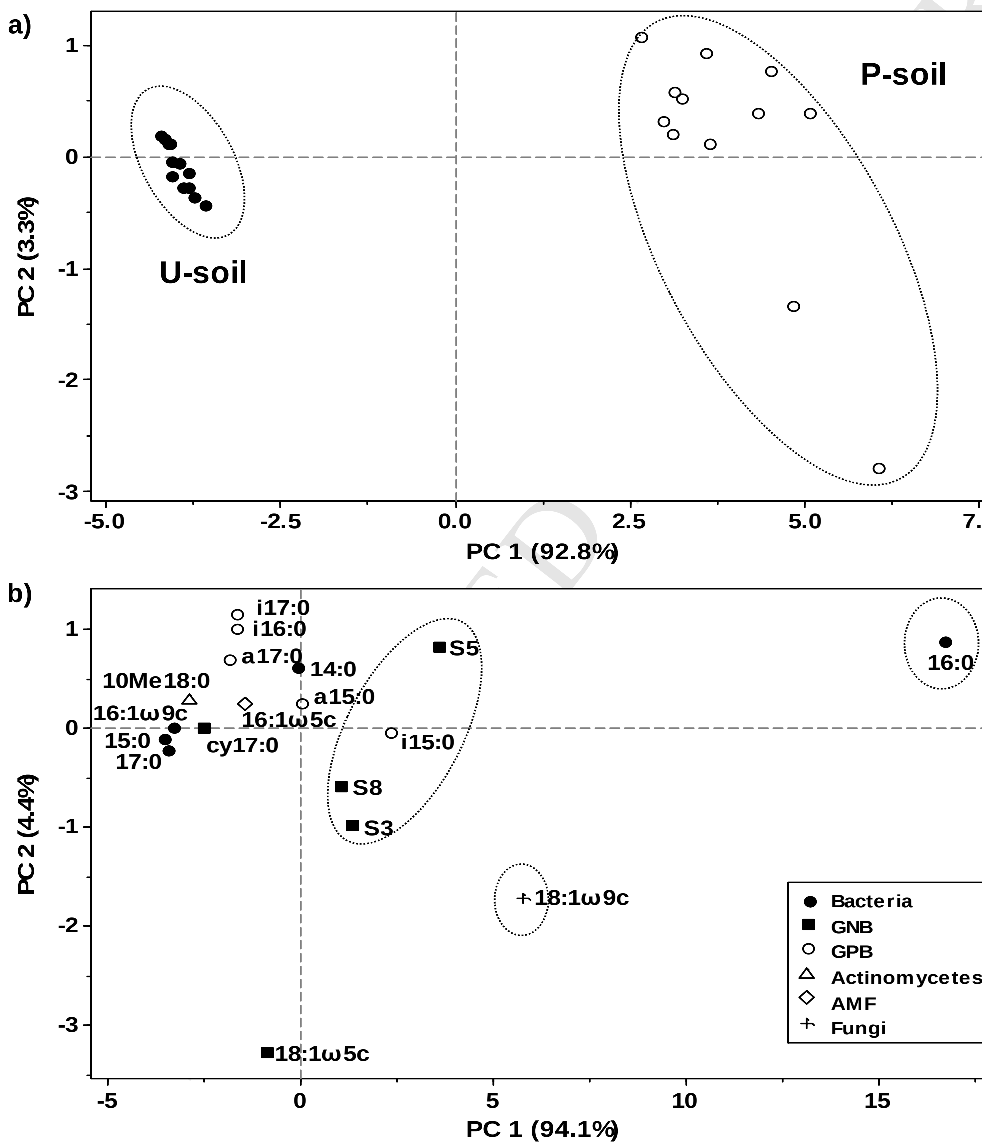

Figure 5: Ordination plot of the principal component analysis based on the FAME profiles of different treatments (a), ordination plot of the principal component analysis based on the

biomarker FAME profiles of the specific microbial groups to identify the responsible microbial communities that make the distinction between two sites (b). S3, S5, and S8 are Sum In Feature 3 (16:1ω7c/16:1ω6c), Sum In Feature 5 (18:0 ante/18:2ω6,9c), and Sum In Feature 8 (18:1ω7c), respectively. GNB, GPB, and AMF are Gram-negative bacteria, Gram-positive bacteria, and arbuscular mycorrhizal fungi, respectively.

Highlights

- Slow pyrolyzed biochars from three crop residues immobilized Pb in soils.
- Biochars were efficient in improving soil chemical properties.
- Biochars did not enhance As immobilization in soils.
- Biochars were not beneficial for soil microbial community abundance.
- Biochars were not beneficial for increase in soil dehydragenase activity.