Retention of Heavy Metals in a Typic Kandiudult Amended with Different Manure-based Biochars

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Although nutrient-rich manure biochars are expected to be an effective heavy metal stabilizer in agricultural and contaminated soils, systematic studies are lacking to predict the influence of manure variety and pyrolysis temperature on metal-binding potentials. In this study, biochars produced from five manure varieties (dairy, paved feedlot, swine solids, poultry litter, and turkey litter) at two pyrolytic temperatures (350 and 700°C) were examined for the stabilization of Pb, Cu, Ni, and Cd in a weathered, acidic Norfolk loamy sand (fine-loamy, kaolinitic, thermic, Typic Kandiudult). Equilibrium concentrations in the aqueous phase were determined for heavy metals (Cu, Ni, Cd, and Pb) and additional selected elements (Na, P, S, Ca, Mg, Al, and K); these were analyzed by positive matrix factorization to quantitatively determine the factors responsible for the biochar's ability to bind the selected heavy metals in soil. Concurrently with the greatest increase in pH and highest equilibrium Na, S, and K concentrations, poultry litter, turkey litter, and feedlot 700°C biochar exhibited the greatest heavy metal retention. In contrast, manure varieties containing disproportionately high (swine) and low (dairy) ash, P, and other elements were the least effective stabilizers. Regardless of the manure type, proton nuclear magnetic resonance analyses showed the removal of leachable aliphatic and nitrogen-containing heteroaromatic functional groups at the higher (700°C) pyrolysis temperature. Consistently greater Cu retention by the 700°C biochar indicated the mobilization of Cu by 350°C biochar-born dissolved organic carbon; however, the influence of other temperature-dependent biochar characteristics cannot be ruled out.

HEAVY METAL CONTAMINATION poses a global challenge at mining sites, shooting ranges, and industrially affected urban soils (Adams et al., 2007; Bannon et al., 2009; Monna et al., 2004). The mobility and bioavailability of heavy metal contaminants strongly depends on soil properties, especially pH, cation exchange capacity (CEC), and total organic carbon (TOC) content. There is increasing interest in the addition of stable (fixed) carbon associated with biochar (Woolf et al., 2010), which could alter the soils' existing redox status, mineralogy, and acid-base chemistry. Depending on the feedstock and pyrolysis conditions used to produce biochar (Antal and Gronli, 2003), biochar-induced changes in soil chemistry can provide additional benefits, such as the heavy metal stabilization.

For stabilization to occur, metal ions are expected to interact with the biochar organic carbon component via (i) electrostatic interactions between metal cations and the negatively charged biochar surface, (ii) ionic exchange between ionizable protons at the biochar surface and metal cations, (iii) sorptive interaction involving delocalized π electrons of aromatic biochar structure by basic char via proton exchange (Polo and Utrilla, 2002) or coordination of d-electron (Cao et al., 2009), and (iv) specific binding of metal ions by surface ligands (e.g., carboxyl, hydroxyl, phenol) (Chen and Wu, 2004) abundant in the volatile matter (VM) component of biochar. The stability of different elements in biochar-amended soils strongly depends on the release of native inorganic contents by the soils and biochars and the influence of the biochar amendment on the soil's pH and dissolved organic carbon (DOC) composition (Beesley et al., 2010). During a 60-d field exposure pot trial study, a heavy application (50 vol%) of a hardwood biochar to contaminated soil decreased the aqueous concentrations of Zn, Cd, and polycyclic aromatic hydrocarbons (Beesley et al., 2010). In contrast, aqueous Cu and As concentrations were increased by

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Abbreviations: CEC, cation exchange capacity; DDW, distilled, deionized water; DL, detection limit; DOC, dissolved organic carbon; FL, paved feedlot manure; ^{1}H NMR, proton nuclear magnetic resonance; MD, dairy manure; PCA, principal component analysis; PL, poultry litter; PMF, positive matrix factorization; SW, separated swine solids; TL, turkey litter; TMS, tetramethylsilane; TOC, total organic carbon; VM, volatile matter.

more than 30-fold by the biochar amendment, along with an increase in soil pH and DOC (Beesley et al., 2010).

Unlike wood plant–based biochar's acidic to neutral pH characteristics, manure-based biochars have a strongly alkaline nature and a high phosphorous content (Novak et al., 2009). These unique properties of manure biochars arise from ash (mineral) components commonly comprising more than 50 wt% of manure biochar (Cao and Harris, 2010; Novak et al., 2009). Broiler litter biochar exhibited a particular effectiveness for stabilizing Pb, Cu, Ni, and Cd in an acidic sandy (Norfolk) soil with low TOC and in an alkaline clay-rich (San Joaquin) soil (Uchimiya et al., 2011a). Along with the associated increase in soil pH, the ash component of biochar can stabilize heavy metals by serving as the adsorption site and by promoting the formation of solubility-limiting phases. Phosphorus-rich manure biochar is expected to effectively stabilize heavy metals, based on the reported stabilization ability of various waste-derived phosphorus amendments in contaminated soils (e.g., fly ash, bone char, biosolid compost, poultry manure, and incinerated poultry litter) (Hashimoto et al., 2009a). These phosphorus amendments were particularly effective at stabilizing Pb by the rapid formation of stable pyromorphite phases (Hashimoto et al., 2009b).

Biochar has a labile carbon VM component that is a complex mixture of phenolic and other functional groups (Deenik et al., 2010). The VM is operationally defined as the weight loss after heating a biochar sample in a covered crucible at 950°C for 7 min (D5142, 2009). The VM content of biochar can be manipulated to decrease as a function of pyrolysis temperature alongside increases in the fixed carbon content (Keiluweit et al., 2010). The VM influences the ability of biochars to sorb heavy metal (Uchimiya et al., 2011c) and organic contaminants (Chen et al., 2008). The VM also influences the microbial activity and plant growth in biochar-amended soils (Deenik et al., 2010). Complexity of manure-derived VM composition is expected from a prior report on the fast pyrolysis product of chicken manure that was composed of more than 500 compounds, including saturated and unsaturated fatty acids, N-heterocyclics, phenols, sterols, diols, and alkylbenzenes (Das et al., 2009). Compared with chicken manure feedstock, biochar had a decreasing contribution of carbohydrate and OH-containing aliphatic groups and increasing aromatic and N-heterocyclic groups (Schnitzer et al., 2007).

Depending on the soil pH and biochar type, portions of VM can be released into soil interstitial waters and enter the DOC pool through various surface chemical or physical interactions (Novak et al., 2010). Much like the organic carbon native to soil (Sposito, 1989), VM contains carboxyl, hydroxyl, phenolic, and other metal ion–coordinating functional groups. Although the immobile VM component (which stays intact on biochar surface) is likely to stabilize heavy metals by binding metal ions on the biochar surface, the labile VM component (which is released into soil interstitial water and becomes a part of DOC) can complex and mobilize metal ions in the solution phase. Dissolved organic carbon is often defined as the organic matter than passes a 0.45-µm filter (Weng et al., 2002), which can include suspended nanoparticles. Likewise, "particulate" organic carbon that is retained by the filter can be mobile in soil and serve as a carrier of otherwise immobile, particle-bound compounds.

Despite the expected utility of manure biochars as a remediation strategy for heavy metal–contaminated soils, information is lacking about how different manure varieties and pyrolysis temperatures affect the ability of biochar soil applications to stabilize heavy metals. In this study, 10 different types of manure-based biochars were tested: five different manure varieties (dairy, paved feedlot, swine solids, poultry litter, and turkey litter) at two pyrolytic temperatures (350 and 700°C for 2 h under N_2 flow). Experiments were conducted on a model system in which selected heavy metals (Pb, Cu, Ni, and Cd) were added to Norfolk soil; additional heavy metals of concern, such as Zn, will be the subject of a future study. Norfolk soil (fine-loamy, kaolinitic, thermic, Typic Kandiudult) is a highly weathered, sandy agricultural soil of Southeastern Coastal Plain region that is acidic in nature (pH <5) with low CEC (2–8 $cmol_c$ kg^{-1}) and low soil organic matter content (6.3–9.2 g kg^{-1} at 0–15 cm depth); this low organic matter content is attributed to historical inversion tillage practices accelerating the decomposition of organic matter (Novak et al., 2007). Norfolk soil is estimated to contain 740, 250, and 10 g kg^{-1} sand, silt, and clay, respectively (Novak et al., 2007). The low buffering capacity of this soil (Novak and Busscher, 2011) and its low organic matter content make the Norfolk soil an ideal soil type to test measurable changes in heavy metal stability resulting from biochar application. The specific objectives of this study were (i) to identify the oxygen- and nitrogen-donor ligands and other VM components (chloroform soluble) that can influence the retention of heavy metals, using proton nuclear magnetic resonance (^{1}H NMR); (ii) to determine the ability of biochars to stabilize the heavy metals Pb, Cu, Ni, and Cd in highly weathered Norfolk sandy loam soil; (iii) to quantify equilibrium concentrations of additional soluble elements (Na, P, S, Ca, Mg, Al, and K); and (iv) to use positive matrix factorization (PMF), a multivariate receptor model (Johnson et al., 2002), to quantitatively determine the chemical composition and contribution of the primary factors responsible for the biochar's stabilizing function in amended soils.

Materials and Methods
Chemicals

Distilled, deionized water (DDW) with a resistivity of 18 MΩ cm (Millipore, Milford, MA) was used for all procedures. Nickel (II) nitrate hexahydrate, copper (II) chloride dihydrate, lead (II) nitrate, and cadmium (II) nitrate tetrahydrate were purchased (≥99% purity) from Sigma-Aldrich (Milwaukee, WI), and individual stock solutions (0.2 mol L^{-1}) were prepared in DDW.

Biochar Preparation

Preparation of manure-based biochars was described previously (Cantrell and Martin, 2011; K.B. Cantrell, unpublished observations). Briefly, five varieties of manures were collected from commercial operations: dairy manure (MD) from Harford County, Maryland; paved feedlot manure (FL) from Sioux County, Iowa; poultry litter (PL) from Orangeburg

County, South Carolina; turkey litter (TL) from Lancaster County, South Carolina; and separated swine solids (SW) from Sampson County, North Carolina. Upon receipt, these samples were air dried and milled to >2 mm. Sample grinding was followed by overnight oven drying at 105°C before pyrolysis. The moisture content of "as pyrolyzed" material ranged between 0.31 and 6.49%.

Pyrolytic runs were performed in triplicate per manure feedstock at 350 or 700°C. The resulting biochars are herein denoted MD350, FL350, PL350, TL350, and SW350 for 350°C pyrolysis temperature and MD700, FL700, PL700, TL700, and SW700 for 700°C pyrolysis temperature. The maximum temperature was held for 2 h under an industrial-grade N_2 gas atmosphere using a flow rate of 1 L min^{-1} (equivalent to 0.04 retort chamber exchanges per minute). Pyrolysis was accomplished using a Lindburg electric box furnace equipped with a gas-tight retort (model 51662; Lindburg/MPH, Riverside, MI). The resulting biochars were allowed to cool and were removed from the retort. A composite sample based on equal weight was created from a homogeneous subsampling of each run (within a manure-temperature treatment). This was done to account for potential variability in the pyrolytic production. Table 1 shows feedstock dependency of selected biochar characteristics discussed in subsequent sections to understand the heavy metal stabilization. All analyses and sorption experiments were conducted without washing, sizing, or other post-treatments of biochar samples.

Proton Nuclear Magnetic Resonance Analysis of Chloroform Extracts

To understand the VM composition for different manure types as a function of pyrolysis temperature, chloroform extracts of biochars were obtained by shaking a biochar suspension (9.5 g L^{-1}) end-over-end at 85 rpm in chloroform for 24 h and then evaporating the chloroform from the decanted supernatant. The resulting extracts were dissolved in deuterated chloroform (CDCl$_3$) and analyzed by ^{1}H NMR (Varian Unity 400 spectrometer [400 MHz]; Varian, Inc., Palo Alto, CA) at an ambient probe temperature with tetramethylsilane (TMS) as the internal reference. The TMS signal was used to define the zero position on the δ scale (Bruice, 1998). Of various solvents available for extracting organic fractions of carbonaceous materials (Jonker and Koelmans, 2002), chloroform was selected because it is relatively unreactive, is easily evaporated, and effectively extracts basic nitrogen donor ligands.

Retention of Lead, Copper, Nickel, and Cadmium in Norfolk Soil

Batch equilibration experiments were conducted using a standard solution prepared by adding 10 mmol L^{-1} H_2SO_4 to DDW until a pH of 4.5 was attained. This solution was often used in experiments designed to simulate element leaching due to rainfall (Hooper et al., 1998). Norfolk loamy sand was obtained from the USDA-ARS Coastal Plains Soil, Water and Plant Research Center (Florence, SC) and was air dried and sieved (2 mm) before use. Separate amber glass batch reactors were prepared for Norfolk soil (20 g soil L^{-1}) with and without 10% (g biochar g^{-1} soil) MD350, MD700, FL350, FL700, SW350, SW700, PL350, PL700, TL350, and TL700. The total volume of each reactor was set to 20 mL. Reactors were pre-equilibrated for 48 h by shaking end-over-end at 70 rpm.

Subsequently, Pb(NO$_3$)$_2$, Cd(NO$_3$)$_2$·4H$_2$O, CuCl$_2$·2H$_2$O, and Ni(NO$_3$)$_2$·6H$_2$O were added together to each reactor for a final concentration of 300 µmol L^{-1} for each metal (i.e., each reactor contained 1.2 mmol L^{-1} total added metals at t_0). The pH of the soil suspension was measured after a 48 h equilibration (Orion 3-star plus benchtop pH meter; ThermoScientific, Waltham, MA). The soil suspension was then filtered (0.2 µm Millipore Millex-GS; Millipore, Billerica, MA). The filtrate was acidified to 4 vol% trace-metal grade nitric acid (Sigma-Aldrich) for the determination of soluble Cu, Ni, Cd, Pb, Na, Ca, K, Mg, S, Al, and P concentrations using inductively coupled plasma atomic emission spectrometry (ICP–AES) (Profile Plus; Teledyne/Leeman Labs, Hudson, NH). Each equilibration experiment was performed in duplicate.

Statistical Analysis: Positive Matrix Factorization

Multivariate receptor models, such as PMF, extract the principal components (factors) accounting for the majority of

Table 1. Feedstock and pyrolysis temperature influence on selected heavy metal and nutrient contents† and proximate analysis results‡ of manure-based biochars used in this study.

Feedstock	Pyrolysis temp.	Cd	Cu	Ni	Pb	Na	P	S	Ca	Mg	K	Ash	VM
	°C	——— mg kg^{-1}db ———				———————————————— g kg^{-1}db ————————————————							
Dairy manure	350	0.18	99.0	16.1	0.89	5.62	10.0	1.1	26.7	12.2	14.3	242	535
	700	BD¶	163	25.3	0.46	8.79	16.9	1.5	44.8	20.6	23.1	395	277
Paved feedlot manure	350	0.20	91.7	4.21	0.71	4.88	11.4	4.5	22.7	7.66	32.0	287	479
	700	0.02	136	6.56	0.19	7.60	17.6	4.0	35.0	12.2	49.1	440	198
Poultry litter	350	0.25	213	7.79	1.03	14.8	20.8	6.1	26.6	9.46	48.5	307	423
	700	0.11	310	11.4	1.09	22.2	31.2	6.3	40.2	14.5	74.0	462	183
Swine solids	350	0.57	1538	16.2	2.60	5.98	38.9	8.0	39.1	24.4	17.8	325	498
	700	0.23	2446	25.6	BD	9.35	59.0	8.5	61.5	36.9	25.7	529	134
Turkey litter	350	0.72	535	28.6	2.01	6.60	26.2	5.5	40.4	8.50	40.1	348	421
	700	0.73	762	39.6	BD	9.24	36.6	4.1	56.1	12.4	55.9	499	208

† ICP–AES analysis of digested biochar samples (conc. HNO$_3$ + 30% H$_2$O$_2$).

‡ All values were obtained from Cantrell et al. (unpublished observations); proximate analyses used the method of Cantrell et al. (2010).

¶ Below detection limit.

variance of the data matrix (Ramadan et al., 2000). The PMF algorism uses nonnegativity constraints (see Paatero, 1997 for a detailed mathematical description of PMF) to find unique factor compositions and contributions by least-square fitting until the difference between the original data set and solution is minimized (Henry and Christensen, 2010). Multivariate receptor models have found a wide application for determining the point source (Barabas et al., 2004) and degradation pathways (Bzdusek et al., 2006) of hydrophobic organic pollutants in sediments.

In this study, the equilibrium concentration of each element (Cu, Ni, Cd, Pb, Na, Ca, K, Mg, S, and P in mol L^{-1} units) was first normalized to the total concentration of all measured elements in each biochar amendment case. This normalization procedure allowed the recognition of the relative proportion of each element (Johnson et al., 2002). Then, normalized values below the detection limit (DL) were replaced with half the DL value (Johnson et al., 2002). Aluminum was the only element with more than 15% below DL values and was eliminated. The final data matrix contained 100 equilibrium concentrations for Cu, Ni, Cd, Pb, Na, Ca, K, Mg, S, and P for screening 10 biochar samples.

The PMF analysis was performed using software (U.S. Environmental Protection Agency, 2008) based on Paatero's PMF program (Paatero, 1997). To ensure that the numerical solutions are found at the global (and not local) minimum, 100 random starting points for each iteration were used for each run. Because there was no a priori knowledge on the number of factors (p), p was determined by repeating the PMF analysis using increasing p values until the goodness of fit (r^2) between the observed and predicted concentrations (i.e., the ability of PMF to reproduce the original data set) was 0.6 or higher for all elements in the dataset (Johnson et al., 2002).

The PMF model used in this study is given by the following equation:

$$x_{ij} = \sum_{k=1}^{p} a_{ik} f_{kj} + \varepsilon_{ij} \qquad [1]$$

where x_{ij} is the experimentally determined equilibrium concentration of element j ($j = 1\ldots m$) in sample i ($i = 1\ldots n$), a_{ik} is the contribution of factor k to the sample i, f_{kj} is the concentration of element j in factor k, and ε_{ij} represents the residual variation in the concentration of element j in sample i that is not accounted for by p factors. Positive matrix factorization iteratively finds a_{ik} and f_{kj} for each factor (Eq. [1]) until the difference between the original data and solution is minimized (Eq. [2]) (Paatero and Tapper, 1994):

$$Q = \sum_{i=1}^{n} \sum_{j=1}^{m} \left(\frac{x_{ij} - \sum_{k=1}^{p} a_{ik} f_{kj}}{s_{ij}} \right)^2 \qquad [2]$$

where s_{ij} is the uncertainty that was set to the standard deviation of the normalized concentration for each element in the data matrix (x_{ij}). In addition to PMF, principal component analysis (PCA), with varimax normalized rotation, was used using XLSTAT 2011 software (Addinsoft, 2011) to confirm the number of factors and to determine the cumulative contribution of factors.

Results and Discussion

Proton Nuclear Magnetic Resonance Analysis of Chloroform Extracts

Figures 1 and 2 present the ^{1}H NMR spectra for the chloroform extract of manure biochars formed at 350 and 700°C, respectively. The NMR spectra were obtained to understand the chemical structures present in labile VM biochar component that can mobilize heavy metals. In Fig. 1, the peaks at 0.8 to 3.4 ppm arose from the chemical shifts of the aliphatic proton groups, such as M-CH$_2$R, M-Ph, M-C(=O)R, M-C(=O)OR, M-C(=O)NR$_2$, M-NR$_2$, and M-CN (M = methyl, methylene, or methane; R = alkyl group; Ph = phenyl) (Silverstein et al., 1991). The chemical shifts in the 3.6- to 5.0-ppm region were likely due to the presence of M-OPh, M-OC(=O)R, M-OC(=O)Ph, and M-NO$_2$ (Silverstein et al., 1991), which are found in carbohydrates and their transformation products (Schnitzer et al., 2007). Sharp peaks in this region likely resulted from the cellulose components of bedding material and partially digested feed. In addition to carbohydrates, manure consists of a complex mixture of sterol, amino acid, and N-heterocyclic functional groups (Schnitzer et al., 2007) from partially digested feed as well as fermentation products such as volatile fatty acids and sulfides (Miller and Varel, 2002). The peaks from 6.5 to 8.8 ppm were attributable to the chemical shifts of protons on monosubstituted benzene rings (Silverstein et al., 1991). The peaks between 8.2 and 8.7 ppm may have occurred from the chemical shifts of protons on nitrogen-containing heteroaromatic groups such as pyridine, pyrrole, and indole (Silverstein et al., 1991). For MD350 and FL350, the broad peak at 7.0 to 7.2 ppm resulted from overlapping signals of various aromatic structures (Silverstein et al., 1991). Figure 1 suggests similarity in the relative abundance of VM functional groups for PL350 and TL350 (and to a lesser extent SW350). These spectra had sharp peaks at the 3.6 to 5.0 ppm region and at the region attributable to nitrogen-containing heteroaromatic groups (8.2–8.7 ppm). These peaks were less apparent for FL350 and MD350 (Fig. 1). These characteristics may be influenced by avian, monogastric, and ruminant digestive types (Reece, 2009).

Figure 2 presents the ^{1}H NMR spectra for the chloroform extract of 700°C manure biochars. By increasing the pyrolysis temperature from 350 to 700°C, a majority of peaks attributable to the chemical shifts of the aliphatic proton groups disappeared (0.8–3.4 ppm in Fig. 2). Similarly, the peaks at 3.6 to 5.0 ppm attributable to M-OPh, M-OC(=O)R, M-OC(=O)Ph, and M-NO$_2$ disappeared (Fig. 2). Additional peak disappearances were observed for the nitrogen-containing heteroaromatic peaks at 8.2 to 8.7 ppm (Fig. 2). In contrast, the broad peaks became more evident at 6.4 to 7.2 ppm (Fig. 2). The new broad peak centered at 6.4 to 6.5 ppm likely resulted from the overlap of aniline, N,N-dimethylaniline, and other related nitrogen-containing aromatic structures (Silverstein et al., 1991).
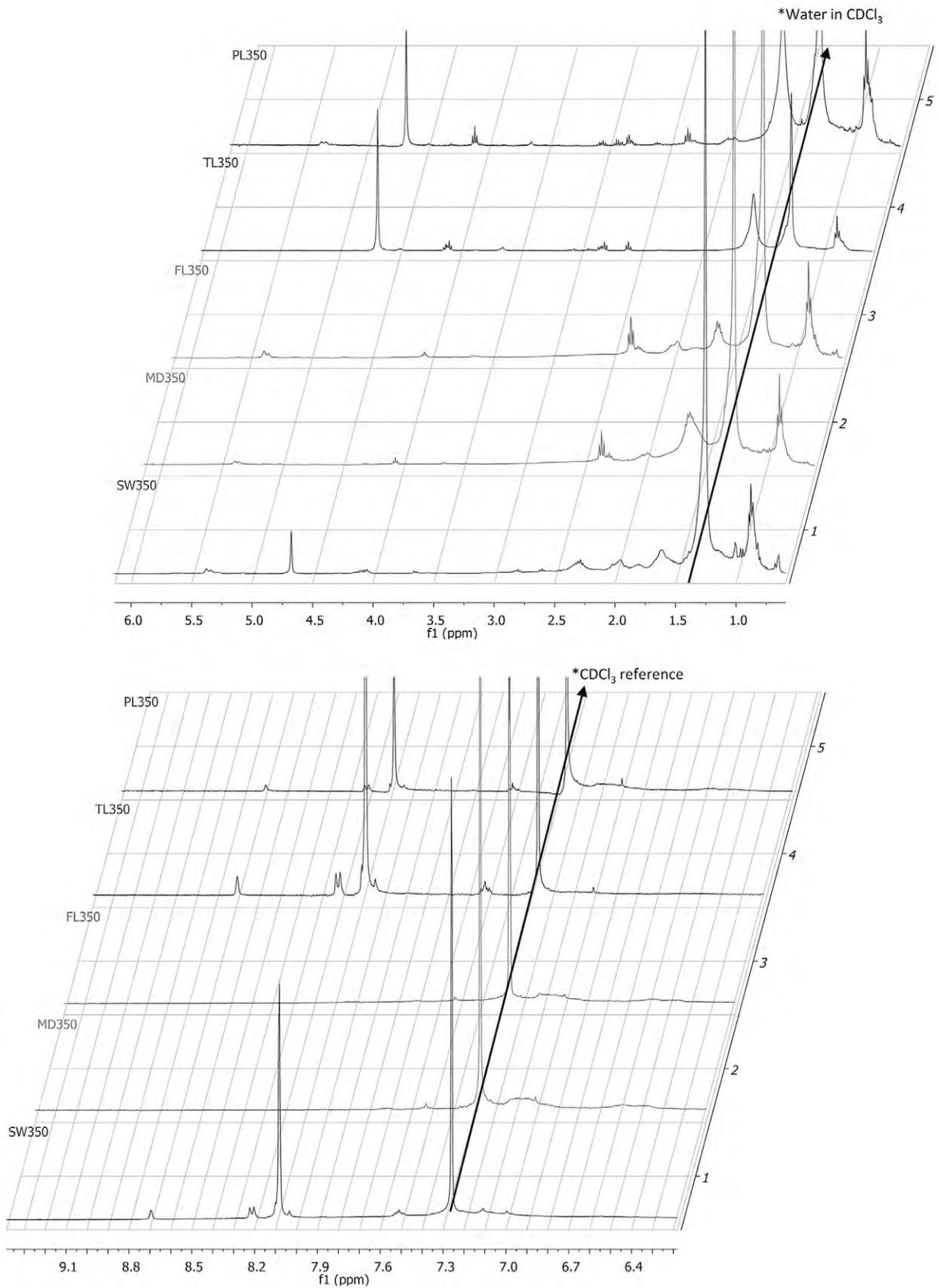
Fig. 1. Proton nuclear magnetic resonance chemical shifts (f1 in ppm) for the chloroform extracts of biochars produced at low temperature (350°C) from dairy manure (MD), paved feedlot (FL), poultry litter (PL), turkey litter (TL), and separated swine solids (SW).
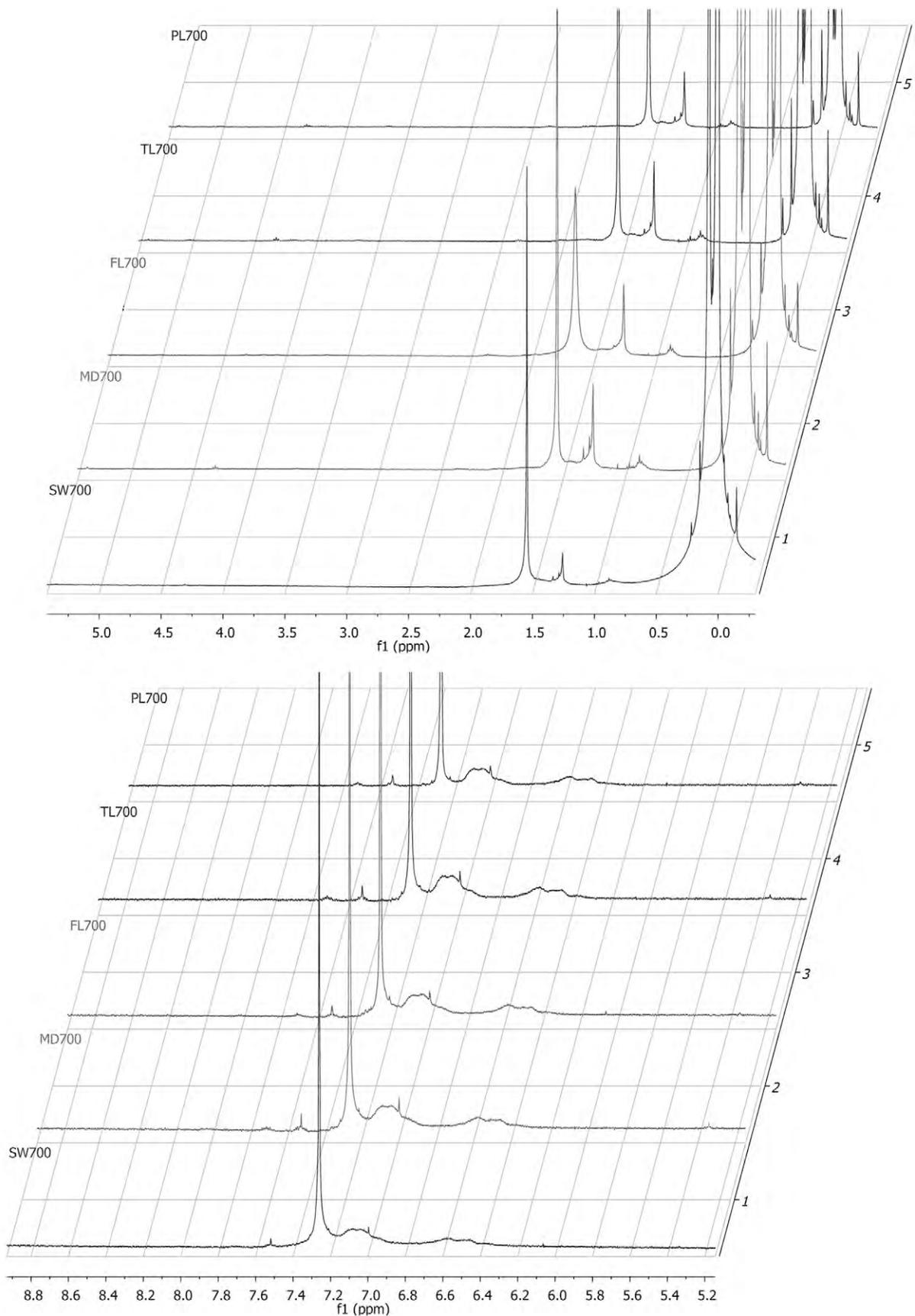
Fig. 2. Proton nuclear magnetic resonance chemical shifts (f1 in ppm) for the chloroform extracts of biochars produced at high temperature (700°C) from dairy manure (MD), paved feedlot (FL), poultry litter (PL), turkey litter (TL), and separated swine solids (SW). Reference peaks are at 0 ppm (tetramethylsilane [TMS]) and ≈7.3 ppm (deuterated chloroform [CDCl$_3$]); the sharp peak at ≈1.5 ppm corresponds to water in CDCl$_3$.

Spectral features for 700°C biochars were similar regardless of manure type (Fig. 2), and distinct groupings were no longer observed for PL350, TL350, and SW350 and for FL350 and MD350 (Fig. 1). The temperature trend from Fig. 1 to Fig. 2 was in agreement with previous reports; regardless of the feedstock type (e.g., plant, manure, and sludge), the VM content of biochar drastically decreased between 300 and 500°C (Shinogi and Kanri, 2003). This was also the case with the VM listed in Table 1. A higher pyrolysis temperature resulted in a decrease in FTIR peaks attributable to carboxyl C=O and aromatic C=C, C=O stretching of conjugated ketones and quinones for grass and wood biochars (Keiluweit et al., 2010). Likewise, phenolic GC–MS peaks were observed in acetone extracts of flash carbonization charcoal with a high VM content produced at the peak temperature of 430°C but were not observed for the corresponding charcoal produced at 650°C with a low VM content (Deenik et al., 2010). In addition, solid-state ^{13}C NMR spectra of tobacco (Sharma et al., 2002), peanut hull, switchgrass, and poultry litter biochars (Novak et al., 2009) pyrolyzed between 300 and 500°C consistently showed a sharp increase in aromatic-carbon peaks.

Screening Manure Biochars for Heavy Metal Retention

The addition of all biochars increased the acidic Norfolk soil-only (control) pH (after 48 h equilibration with added heavy metals) from 5.1 to neutral and slightly alkaline values (Fig. 3a). The pH trend suggested a strong influence of pyrolysis temperature and demonstrated the poor buffer capacity of acidic Norfolk loamy sand. For PL, TL, and FL biochars, pH was as much as 1.0 pH unit greater for biochars produced at

700°C than at 350°C, transitioning from relatively neutral to slightly alkaline. In contrast, higher pyrolysis temperature had negligible effects on pH for MD (7.1 for both temperatures) and yielded a slightly lower pH for SW (6.7 for 350°C and 6.5 for 700°C). Acidic soil conditions are known to increase the solubility and bioavailability of heavy metals (Sposito, 1989). Protons compete with metal ions for binding by ligand exchange on mineral and organic surface sites (Bohn et al., 1979). Hence, an increase in pH would cause more heavy metals to be retained in the soil–biochar system and not pass through into the filtrate.

Biochar addition, relative to the soil-only control, resulted in the heavy metal retention trend reported by others: Pb > Cu > Cd > Ni (Fig. 3b–e) (Basta and Tabatabai, 1992; Harter, 1983; Martínez and Motto, 2000). In all biochar amendment cases, heavy metal concentrations were lower than or within the error range of the soil-only control (without biochar amendment; Fig. 3). Overall, biochar amendments were most effective for decreasing Pb and Cu concentrations; even among the soil-only controls, Pb was the most adsorbed metal. Equilibrium Pb concentration decreased from 212 μmol L^{-1} for the soil-only control to 0 to 2.0 μmol L^{-1} in the presence of biochar (Fig. 3b). No general effects of temperature were noted for Pb retention. Among the different manure varieties, MD biochars were least effective for retaining Pb, with equilibrium concentrations of 0.9 and 2.0 μmol L^{-1}, respectively, for 350 and 700°C. As demonstrated by the lowest equilibrium concentration (relative to other heavy metals in Fig. 3), Pb has high affinity for (i) binding by the surface ligands of organic matter; (ii) adsorption on Fe/Mn (hydr)oxides not only as Pb^{2+} but also
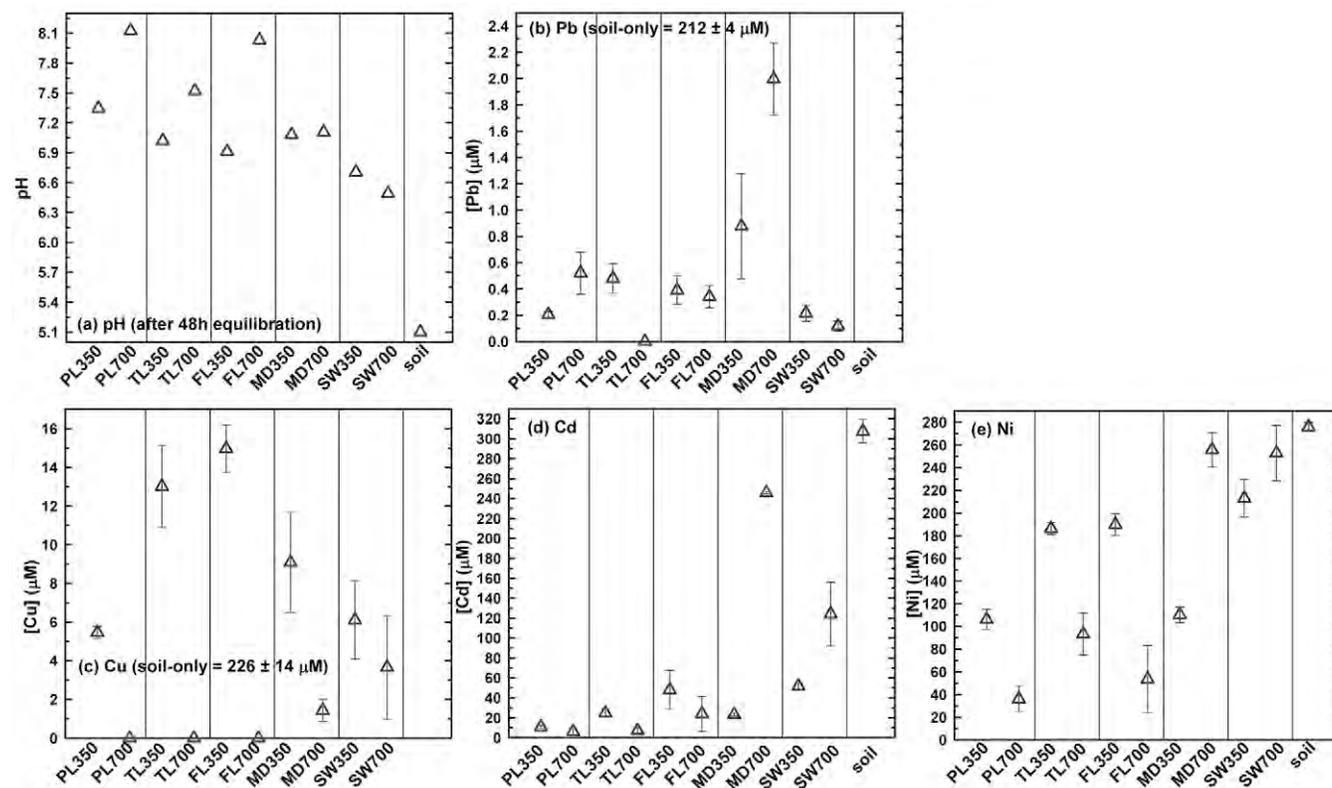

Fig. 3. Determined pH (a) and equilibrium Pb (b), Cu (c), Cd (d), and Ni (e) concentrations in Norfolk soil (20 g soil L^{-1}) with and without 10% manure-based biochar (g biochar g^{-1} soil); 300 μmol L^{-1} each metal was added together for 48 h equilibration. All values are given as mean ± SD for duplicate experiments.

as $PbCO_3$, $PbSO_4$, $PbCl^+$, and $PbPO_4$ complexes (Dijkstra et al., 2004); and (iii) formation of Pb phosphate phases at sufficiently high Pb^{2+} activity (Schröder et al., 2005).

For Cu, a clear dependence on pyrolysis temperature was observed regardless of the manure type (Fig. 3c): higher pyrolysis temperature (700°C) resulted in a greater Cu retention than lower pyrolysis temperature (350°C). The soil-only control had an equilibrium concentration of 226 μmol L^{-1} compared with the 350°C biochars ranging from 5.5 to 15 μmol L^{-1} and compared with the 700°C biochars ranging from 0 to 4.0 μmol L^{-1}. Even though 700°C biochar had consistently greater Cu content than 350°C biochar (Table 1), the equilibrium Cu concentration was lower in the presence of 700°C biochars (Fig. 3c). This was especially true for the PL, TL, and FL 700°C biochar treatments. These systems were observed to have a pH greater than 7.5, where Cu was expected to be organically complexed (Adriano, 2001). Of particular note was the SW biochars; despite an order of magnitude greater total Cu content than other manures (Table 1), the SW biochars with an average pH of 6.6 effectively decreased equilibrium Cu concentrations (Fig. 3c).

The Cu^{2+} is a d^9 metal ion and exhibits a particular stability for complex formation with nitrogen- and oxygen-donor ligands because of Jahn-Teller distortion (Wulfsberg, 2000). As a result, sorption affinity onto soil particle surfaces and complex formation with soluble ligands in soil solution are stronger for Cu^{2+} than Ni^{2+} despite the lower charge-to-radius ratio of Cu^{2+} (Wulfsberg, 2000). Consistently greater Cu stabilization ability of 700°C biochars (Fig. 3c) likely resulted from its lower leachable VM content (Fig. 1–2) and greater degree of surface interaction, relative to 350°C biochars. In clayey alkaline San Joaquin soil with inherently high heavy metal retention capacity, 350°C broiler litter biochar increased the equilibrium Cu concentration relative to the soil-only control (Uchimiya et al., 2010). This biochar was able to stabilize Cu in San Joaquin soil when pretreated by 0.1 mol L^{-1} NaOH to remove soluble organic carbon component (Uchimiya et al., 2010). In the presence of natural organic matter containing high carboxyl and phenol functional groups, Cu concentration increased (Uchimiya et al., 2010), further suggesting the mobilization of Cu by complex formation in the solution phase. Norfolk soil has negligibly low Cu retention capacity relative to San Joaquin soil (Uchimiya et al., 2011a), and 350°C manure biochars stabilized Cu (Fig. 3). However, consistently greater Cu retention by the 700°C biochars (Fig. 3) followed the temperature trend for the removal of leachable aliphatic and nitrogen-containing heteroaromatic groups (Fig. 1–2).

The stabilization of Cd followed similar trends to that of Ni. For the PL, TL, and FL biochars, higher temperature enhanced stabilization (Fig. 3d,e). However, the MD and SW biochars exhibited a reverse trend. The Cd concentration associated with MD700 was disproportionately the greatest among the biochars studied at 240 μmol L^{-1} (Fig. 3d). In contrast to Cu^{2+}, Ni^{2+}, and Pb^{2+}, Cd^{2+} is a soft acid and preferentially binds softer, sulfur-, phosphorus-, and nitrogen-donor ligands (Wulfsberg, 2000). Among other factors, the abundance of nitrogen-containing functional groups (especially PL, TL, and SW; Fig. 1) remaining intact on the 350°C biochar surface may contribute to the stabilization of Cd (Fig. 3d). However, Cd is known to

preferentially bind to DOC (Fest et al., 2005), which can result in greater filtrate concentration.

Among all the metals examined, Ni was least affected by the biochar addition. For MD700 and SW700, the equilibrium concentration of Ni was within the error range as the soil-only control; thus, no significant stabilization occurred for Ni. For the other manures (Fig. 3), the concentration of added Ni decreased relative to the soil-only control. Nickel is known to have a competitive inhibitor among the metals tested: Cu^{2+} (Adriano, 2001). Excess alkaline earth metals such as Ca^{2+} and Mg^{2+} also out-compete Ni^{2+} for binding sites (Mandal et al., 2000).

Overall, the trend for relative concentrations of each heavy metal was similar for PL and TL and, to a lesser extent, for FL; consequently, these biochar manure varieties demonstrated the greatest heavy metal stabilization ability. The equilibrium concentration trends associated with SW and MD biochars were distinctive, indicating much lower effectiveness for heavy metal stabilization (Fig. 3). For each heavy metal except Cu, MD700 was the least effective sorbent of all biochars examined in this study.

Nutrient Release

With manure-based biochars being nutrient rich (Table 1), some leaching of macro- and micro-plant nutrients, especially P, Na, and K, would be expected with biochar addition to soil for fertilization or remediation purposes. Figure 4 presents equilibrium Na, P, S, Ca, Mg, and K concentrations determined for the experiments presented in Fig. 3. Aluminum concentrations were below detection limit for all biochar treatments. Potassium concentrations were the highest of all elements considered (3.2 ± 1.3 mmol L^{-1} for 10 manure biochars); this was more than an order of magnitude greater than the soil-only case (0.26 ± 0.1 mmol L^{-1}). For all manure varieties considered in Fig. 4, equilibrium soluble Na, P, S, Ca, and Mg concentrations consistently decreased as a function of pyrolysis temperature, except for negligible differences in the following concentrations: Na for FL and MD, P for TL, and Mg for PL and TL. This was a striking contrast to the acid-digestible Na, P, S, Ca, and Mg contents of manure biochars that consistently increased as a function of pyrolysis temperature (Table 1). Equilibrium S, Na, Ca, P, Mg, and K concentrations (Fig. 4) resulted from the pre-equilibration stage and from subsequent reaction with heavy metals. Thus, equilibrium S, Na, Ca, P, Mg, and K concentrations were influenced by dissolution during the pre-equilibration period, the reaction with added heavy metals, as well as pH buffering, the formation of particulate phases, and additional processes influencing the speciation of these elements in biochar-amended soils.

Overall, there was no visible relationship between the equilibrium concentrations of added heavy metals (Fig. 3) and elements originating from biochar or soil (Fig. 4). The divalent metal cations in Fig. 3 are known to replace readily exchangeable cations (Ca^{2+} and Mg^{2+}) on soil components in the absence (Yu et al., 2002) and presence of biochar (Uchimiya et al., 2011b). At pH 5 to 6 in sandy soil without historical fertilizer application, Ca is by far the major exchangeable cation on the soil particle surfaces, followed by Mg, Al, K, and Na (Fest et al., 2005). Although equilibrium
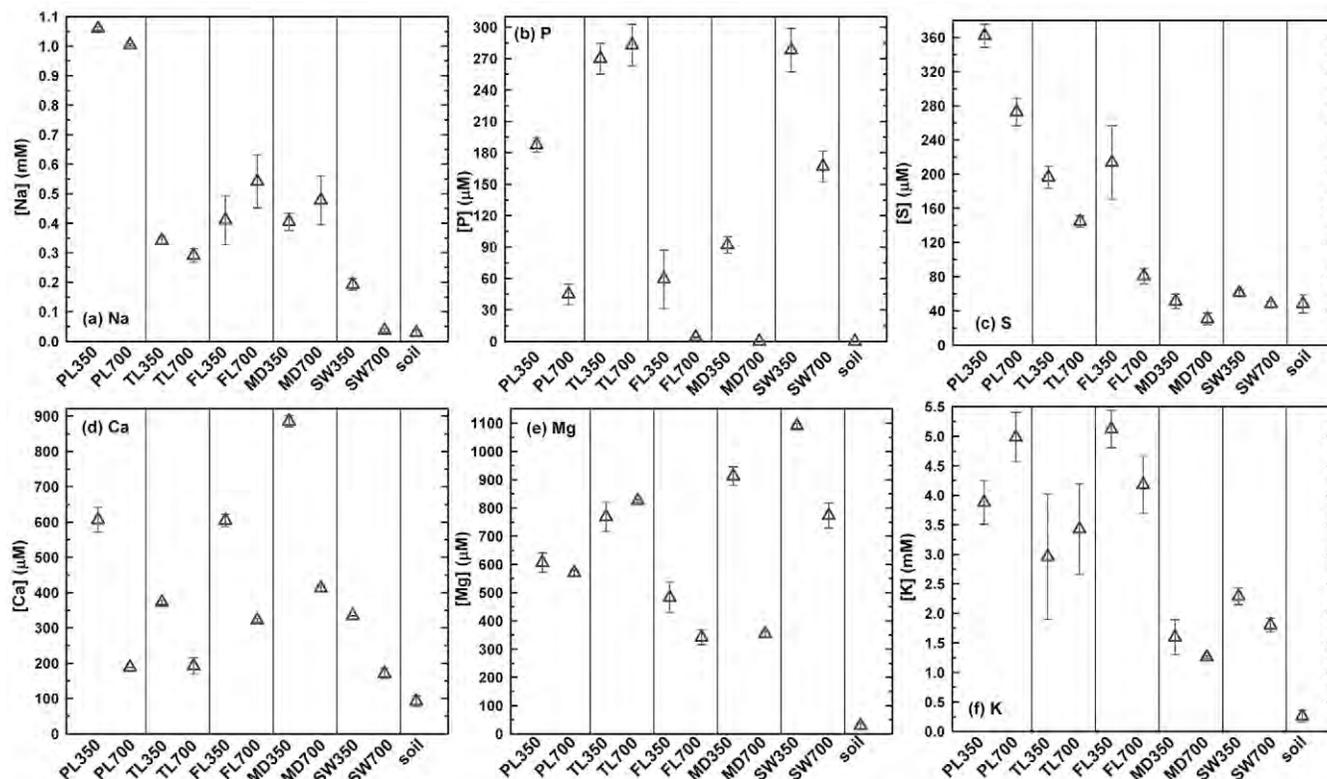
Fig. 4. Equilibrium Na, P, S, Ca, Mg, and K concentrations (corresponding to Fig. 3) in Norfolk soil amended with 10 different manure-based biochars. All values above detection limit are presented as mean ± SD for duplicate experiments. Aluminum concentration was below detection limit for all cases.

Ca and Mg concentrations consistently decreased as a function of pyrolysis temperature (Fig. 4), only Ni and Cd for MD and SW (Fig. 3) followed the trend expected if cation exchange was predominant. In Pb sorption isotherm studies on dairy manure biochars, the formation of Pb phosphate and carbonate phases was the predominant mechanism and resulted in a steady decrease in equilibrium P concentration with a higher degree of Pb stabilization (Cao et al., 2009). However, the current study did not observe an apparent relationship between Pb and P concentrations (Fig. 3 and 4).

Statistical Analyses

Table 2 presents the contribution, cumulative contribution, and characteristic elements (elements having factor loading above 0.5) obtained from the PCA of the experimental data presented in Fig. 3 and 4. The first principal component was dominated by P, Mg, and Ni and accounted for 31% of the variance (Table 2). The second principal component showed a slightly lower contribution (29%) and was dominated by Ni, Cd, and Pb. The third and fourth principal components had 17% contribution each and were dominated by Ca, Cu (third factor) and S, Na (fourth factor). Four principal components in Table 2 accounted for 94% of the variance (cumulative contribution in Table 2).

In agreement with the number of principal components in Table 2, r^2 values from the PMF analysis improved to 0.6 or higher for all elements when the number of factors was increased from 3 to 4 (Supplemental Table S1). Figure 5 presents chemical composition (Fig. 5a) and contribution (Fig. 5b) of

the four PMF factors. The chemical composition is given as % of element total (in mol L^{-1}), and the average of all contributions for each factor equaled 1.

The first factor (factor 1 in Fig. 5a) showed the highest contribution to Na (49%), K (46%), and S (57%) equilibrium concentrations and contributed negligibly to Cu, Ni, Cd, and Pb. Among 10 biochar treatments investigated, the first factor contributed significantly only to PL, TL, and FL (350 and 700°C) (Fig. 5b). Hence, factor 1 represented the heavy metal stabilization ability of biochars with high-equilibrium Na, K, and S concentrations (Fig. 3 and 4). Greater equilibrium S concentrations of PL350, PL700, TL350, TL700, and FL350 (Fig. 4c) over all other biochars were reflected in factor 1 (Fig. 5a). Exceptionally high Na concentrations in the presence of PL350 and PL700 (Fig. 4a) were also reflected in factor 1 (Fig. 5a).

Factor 2 represented the greatest proportions of Ca (60%) and Cu (62%) (Fig. 5a) and had the greatest contribution to MD350 (Fig. 5b). Examination of each manure type indicated

Table 2. Contribution, cumulative contribution, and characteristic elements of four principal components obtained from equilibrium Cu, Ni, Cd, Pb, Na, Ca, K, Mg, S, and P concentrations in Norfolk soil amended with 10 different manure-based biochars.†

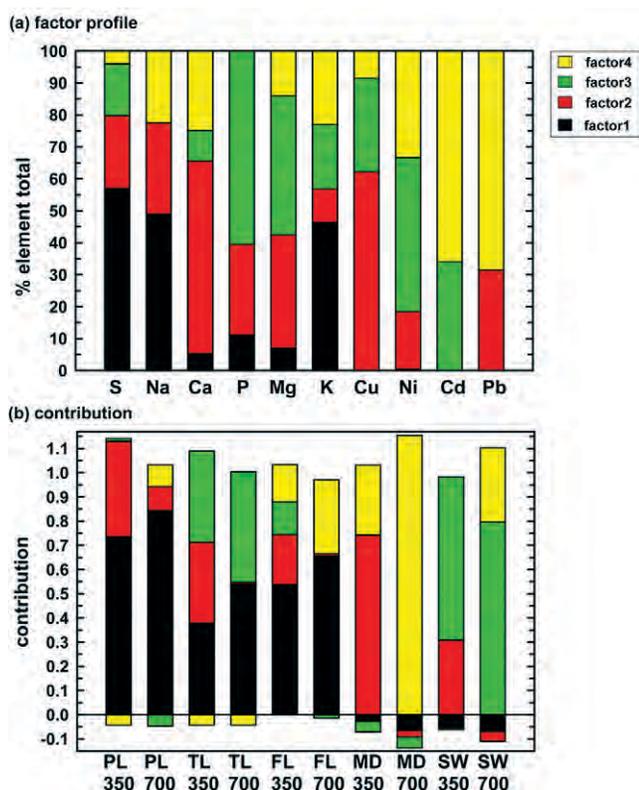| Principal component	Contribution	Cumulative contribution	Characteristic element (factor loading > 0.5)
1	31	31	P, Mg, Ni
2	29	60	Ni, Cd, Pb
3	17	77	Ca, Cu
4	17	94	S, Na

† Data shown in Fig. 3 and 4.

Fig. 5. Chemical composition (a) and contribution (b) of four positive matrix factorization–derived factors obtained from equilibrium Cu, Ni, Cd, Pb, Na, Ca, K, Mg, S, and P concentrations in Norfolk soil amended with 10 different manure-based biochars (Fig. 3–4).

a consistently greater contribution of factor 2 to the biochar produced at lower pyrolysis temperature (350°C). Among the heavy metals, a consistently lower retention ability of 350°C biochar was observed only for Cu (Fig. 3). Similarly, 350°C biochar showed consistently higher Ca concentration relative to 700°C biochar (Fig. 4d). The second factor was attributable to biochars formed at low pyrolysis temperature (350°C) having a decreased ability to retain Cu and increased release of Ca in comparison to the biochars formed at 700°C. Electrostatic interaction with readily exchangeable cations such as Ca is the weakest heavy metal retention mechanism on soil components (Sposito, 1989). Consistently greater Ca release by a less effective sorbent (350°C biochar) may indicate a progression of interaction mechanisms from cation exchange to a stronger, specific surface binding for more effective biochars, such as PL700, TL700, and FL700.

The third factor showed the greatest contribution to equilibrium concentrations of P (61%), Mg (44%), and Ni (48%). The third factor was the dominant contributor to SW biochars; this factor contributed significantly to TL biochars as well (Fig. 5b). Although SW manure biochars showed effectiveness for stabilizing Cu, Cd, and Pb, they exhibited the lowest Ni retention capacity (Fig. 3) and released the greatest amounts of P and Mg (Fig. 4). Therefore, factor 3 was attributable to high P and Mg release and lower Ni retention capacity, especially for the SW biochars (Fig. 3–4).

The fourth factor represented the greatest proportion of Cd (66%) and Pb (69%) and contributed almost exclusively to MD700 (Fig. 5). Consequently, MD700 demonstrated the lowest capacity of all biochars for heavy metal retention with disproportionately high equilibrium Pb and Cd concentrations (Fig. 3). Furthermore, equilibrium concentrations of additional elements, especially P and S, were among the lowest for MD700 (Fig. 4).

The PMF analysis resolved complex data sets into four distinct factors representative of (i) poultry and turkey litter and feedlot biochars as the most effective heavy metal stabilizer with high equilibrium Na, S, and K concentrations; (ii) lower pyrolysis temperature biochars, especially MD350, that uniquely released higher amounts of Ca and were less effective for retaining Cu; (iii) SW manure biochars that released high amounts of P and Mg and were less effective for retaining Ni and Cd; and (iv) MD manure biochar formed at 700°C that was least effective for heavy metal retention, especially Cd and Pb, of all biochars examined in this study.

Comparison of PMF (Fig. 5) and PCA (Table 2) results indicated a correspondence between the two different types of analysis. The first PMF factor (Fig. 5) had a similar chemical composition as the fourth principal component in Table 2, which is indicative of biochars that were best able to lower heavy metal concentrations (Fig. 3). The chemical composition of the second PMF factor showed a close similarity to the characteristic elements of the third principal component in Table 2. The third and fourth PMF factors corresponded to the first and second principal components, respectively, which accounted for 60% of the variance (cumulative contribution in Table 2), indicating that the majority of data variance arose from MD700 and SW biochars, with the lowest heavy metal retention capacity in Norfolk soil (Fig. 3).

Absolute contribution of each PMF factor (in molar unit) can be calculated from the chemical composition (Fig. 5a), contribution (Fig. 5b), and total concentration of all elements in each biochar amendment case. Because PMF provides solution in the unit of concentration (rather than the abstract values for PCA), PMF can be used to quantitatively assess the biochar's function as fertilizer, soil conditioner, and additional applications. It must be noted that PMF analysis requires careful data pretreatment and interpretation. The results presented in Fig. 5 pertain to the experimental conditions used in this study and do not account for the long-term changes in biochar function resulting from processes such as aging and transport.

In conclusion, feedstock manure variety and pyrolysis temperature strongly influenced the heavy metal stabilization ability of manure biochar in weathered, acidic, low TOC, and low CEC Norfolk soil. Apart from FL biochars, PL and TL (avian) biochars exhibited similar VM composition, increased pH the most, and were the most effective heavy metal stabilizers. For TL, PL, and FL, higher pyrolysis temperature (700°C) resulted in higher soil pH, lower chloroform-extractable VM content, and greater stabilization capacity. The lowest effectiveness of MD700 may be attributable to the uniquely low ash and total S and P contents of dairy manure biochars (Table 1). Among other factors, the lower availability of softer ligands may have caused the decreased ability of MD700 to stabilize Cd. Similarly to MD biochars, SW biochars containing disproportionately high total Cu, Mg, and P (Table 1) did not show a clear

relationship between the heavy metal stabilization ability and the pyrolysis temperature. Overall, the elemental composition and ash content of manure feedstock (Table 1) can provide a preliminary estimate for the relative effectiveness of biochars as heavy metal sorbents in soil. Of all heavy metals investigated, Cu showed the most consistent dependence on pyrolysis temperature and chloroform-extractable VM composition across manure types (i.e., enhanced stabilization). This was likely due to the drastic reduction in leachable carbon fractions (represented by chloroform-extractable VM) and increased surface binding on the aromatic, ligand, and ash components of the higher-temperature biochars, regardless of manure type. The observed relationship between pyrolysis temperature and Cu stabilization ability does not make Cu a "model heavy metal" for understanding metal stabilization by biochars in soil. Compared with Cu and Pb, Ni and Cd showed much lower tendency to be stabilized in soil amended with biochar (Fig. 3), and there was also larger variability in the ability of biochars to stabilize the latter two elements. Before a large-scale field application of biochars to stabilize heavy metals, laboratory characterization of biochar function should be conducted with a particular emphasis on less easily stabilized metals such as Ni and Cd.

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