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Bioavailability of phosphorus, other nutrients and potentially toxic elements from marginal biomass-derived biochar assessed in barley (*Hordeum vulgare*) growth experiments

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Abstract

Biochars produced from marginal biomass feedstocks are a potential source of recycled nutrients for agriculture, but may also contain potentially toxic elements (PTEs) which can cause phytotoxicity. We assessed the potential for nutrient recycling from such materials against potential environmental risks in 17 biochars containing high concentrations of various PTEs and nutrients. Methods for investigating the risk of biochar-derived PTEs were developed and assessed. Short-term (21 days) growth experiments with barley (*Hordeum vulgare*) in 5% biochar/sand mixtures were used to present the 'worst-case scenario' of high dose and low pH buffering. We compared plant nutrient and PTE concentrations with amounts extracted from the same biochars using 1 M NH_4NO_3 or 0.01 M $CaCl_2$ (buffered and unbuffered, respectively) and Mehlich 3 to analyse whether such extractions could be used to predict bioavailability. The yields of barley grown with biochars "EPOCAD550", and "WLB550" were significantly higher than the control ($p < 0.05$). Total phosphorus (P) concentration in above-ground biomass was higher than the control for the EPOCAD550 treatment ($p < 0.01$). Both buffered and unbuffered 0.01 M $CaCl_2$ biochar extractions were significantly positively correlated with plant leaf concentration for six of the 18 elements investigated, more than any of the other extractions. This indicates that $CaCl_2$ extractions provide the most representative assessment of element bioavailability from marginal biochars compared to more resource-intensive growth experiments. Our results provide new insights into the bioavailability of elements in biochar and the standardisation of methods which accurately assess this attribute, which is necessary for promoting use of biochars from marginal biomass for recycling nutrients from wastewater and to agricultural production.

Keywords: Biochar, Phosphorus, Potentially toxic elements, Bioavailability, Soil application, Marginal biomass

1) Introduction

The production of biochar from pyrolysis has potential to couple organic waste management to various improvements in agricultural systems (Shackley et al., 2011). If biochar is to become widely adopted in the long term, environmental acceptability must be demonstrated in order to address the concerns of industry and environmental regulators. Realising this potential must be underpinned by robust understanding of biochar properties, including the identification and mitigation of any risks posed to the environment. Assessment of risk initially relied heavily on analysis techniques that were developed for soils and compost. Biochar is physically and chemically distinct from these materials, however, so new protocols have been developed. Examples include a modified dry ashing method to assess total elemental concentrations (Enders and Lehmann, 2012) and extended hot toluene extraction to quantify polyaromatic hydrocarbons (PAHs) (Hale et al., 2012; Hilber et al., 2012). Measuring the bioavailability of potentially beneficial elements (nutrients) and potentially toxic elements (PTEs) in biochar also needs new protocols as methods currently used have been optimised for matrices that have very different properties to biochar.

Biochar produced from high-nutrient feedstocks, such as sewage sludge and food waste digestate, and modified feedstocks as in biochar mineral complexes (BMCs), have been suggested as replacements for traditional fertilisers (Hossain et al., 2010; Joseph et al., 2010; Wang et al., 2014, 2012). Although persistence of the carbon fraction or matrix may be desirable for carbon sequestration, nutrients, such as P and potassium (K), which unlike nitrogen (N) are predominantly preserved during pyrolysis, must be leachable or reactive towards plant exudates to be plant-accessible. If nutrient reactivity is central to an agricultural application of biochar, PTE reactivity needs to be minimised.

PTEs that may be conserved during biomass pyrolysis include chromium (Cr), nickel (Ni), zinc (Zn) and copper (Cu). Such elements must remain inert in biochar, to prevent phytotoxicity or soil pollution. Estimates for the bioavailability of PTEs in biochar require a high level of confidence. PTEs are often found to be less extractable in biochar than their parent feedstock, but their measured mobility in soil is also affected by soil-specific properties (Beesley et al., 2010; Buss et al., 2016c; Farrell et al., 2013; Khanmohammadi et al., 2015; Lu et al., 2013; Luo et al., 2014). Hence, reliable methods are required for assessing PTE bioavailability in a soils context, but where results are interpreted drawing on site-specific data such as soil composition, pH and land-use.

A variety of extraction methods have been used to estimate PTE and nutrient bioavailability of biochar and biochar–soil mixes. 'Mobile' PTEs in biochar have been measured using 0.1 M $CaCl_2$ (Méndez et al., 2012), whilst 0.01 M $CaCl_2$, ultra-pure water, 1 M NH_4NO_3, 0.5 M acetic acid and 0.05 M ethylenediaminetetraacetic acid (EDTA) were compared as estimators of plant availability of biochar PTEs by Farrell et al. (2013). Diethylenetriaminepentaacetic acid (DTPA) extraction at a relatively high pH of 7.3 has also been used, prepared using 0.01 M $CaCl_2$ and a buffering agent (triethanolamine) (e.g. Fellet et al., 2011; Lu et al., 2013; Luo et al., 2014).

Many studies have reported positive correlations between 0.01 M $CaCl_2$ (pH 7.0) and 1 M NH_4NO_3 (pH 4.6) extractable PTE concentrations in soil with uptake of PTEs by plants (e.g. Meers et al., 2007; Menzies et al., 2007; Zhang et al., 2010), including a study on biochar (Farrell et al., 2013). The German Federal Soil Protection and Contaminated Sites Ordinance (1999) stipulates the use of 1 M NH_4NO_3 soil extractions to compare against legislated threshold values for available As, Cd, Cr, Cu, Ni, Pb and Zn to assess the risk of toxicity in plants and to maintain crop quality. Correlations have also been investigated between plant uptake of nutrients and PTEs and soil bioavailability assessed using the Mehlich 3 extraction (pH 2.5) which was developed to extract P, K, Na, Ca, Mg, Mn, Zn and Cu from soils using a mixture of acid, buffer and complexing components, including EDTA and NH_4NO_3 (Mehlich, 1984). Various studies exist within the literature which assess the bioavailability of PTEs and nutrients in plant growth experiments and chemical extractions (Grzebisz et al., 1983; Monterosso et al., 1999; van Raij, 1998).

The solubility of both nutrients and PTEs in soils, a factor contributing to bioavailability, varies with the pH of the soil solution. The addition of biochar (like many other inputs) often changes soil pH, and consequently, feedstock properties, pyrolysis conditions and dose will affect the impact of biochar addition on soil pH and on bioavailability. Unless biochar is added in a high dose, however, the pH change in the soil system will not be as great as in the solutions used to assess bioavailability by extraction. Temporal control of extractant pH (at a designated pH, such as 7, or the pH of the soil to which the biochar will be added) by incorporation of a buffering agent should allow more accurate comparisons and prediction of nutrient and PTE extractability.

In addition to pH control, selection of appropriate methods for analysis should take into consideration the previous validation of methods and the number of studies and/or guidelines with which experimental results can be compared. Bioavailability assessed in plant growth experiments may be regarded as more representative than chemical extractions where soil and plants are not present, but is more resource intensive.

The purpose of the present study is to draw on established knowledge of pH, bioavailability and extraction in fertilisers and phytotoxicity contexts, to identify an appropriate protocol for bioavailability assessments in biochar. As pH is suggested as a main factor in biochar metal interactions, we compared five extraction solutions which covered a range of pH, with and without buffering, to explore fully the effect of biochar pH on nutrient and PTE bioavailability. Research focused on PTEs since organic pollutants such as PAHs, when present, are very strongly sorbed to biochar and appear to have low bioavailability since they are difficult to extract, even under harsh experimental conditions (Hale et al., 2012; Mayer et al., 2016). In addition, a P-specific extraction method was tested (2% formic acid). Of the three main macronutrients required for plant growth, this study focused on P as there is no clear 'best method' for predicting the bioavailability of P in biochar. Potassium, on the other hand, is very soluble and thus highly bioavailable when present (Buss et al. 2016c) and N is mostly evaporated during pyrolysis (Antal and Grønli, 2003; Liu et al., 2014). We compared plant leaf concentrations of nutrients and PTEs grown on sand only to biochar extraction values to determine whether the low extractability of PTEs from biochar reported in the literature was also reflected in low bioavailability and whether high P biochars could act as P fertilisers in early plant growth stages. Sand was chosen as the growth medium for this study to ensure that interactions such as buffering or sorption of elements were minimal in the system. Had a soil been selected instead, comparison of the soil-free biochar extractions with plant leaf element concentrations would not have been valid.

2) Materials and methods

2.1) Biochar production and characterisation

The 17 biochars used in this study produced from nine different feedstocks were selected for their high content of different PTEs and nutrients. They were prepared at the UK Biochar Research Centre using the Stage II pyrolysis unit described in detail in (Buss et al., 2016a). Full characterisation data for 15 of the biochars can be found in Buss et al. (2016a, 2016c) and in Supplementary Information Tables 1a, 1b, 2a and 2b. Two of the biochars have not been described previously. These were prepared at 550°C and 700°C from rice husk grown on land in the vicinity of the Panipat thermal power station (Haryana, India). An overview of the biochars is provided in Table 1. Based on evaluation of the pyrolysis technology used to produce each of the biochars (Buss, 2016; Buss et al., 2016b), and data published previously, we are confident that the biochars in this study are not contaminated with organic contaminants such as PAHs.

Four of the biochars (EPAD450, EPAD550, EPOCAD450 and EPOCAD550) are modified biochars which had been exposed to a P solution, to encompass captured as well as native nutrients within the study. The P-exposed biochars were created by addition of the biochars (PAD450, PAD550, POCAD450 and POCAD550) to a 20 mg l^{-1} P solution buffered at pH 7 using 0.01 M 3-(N-morpholino)ethanesulfonic acid (MOPS), parameters defined to simulate enrichment that might be achieved in a wastewater treatment plant (Shepherd et al., submitted). Briefly, 30 g of each biochar with particles of diameter 0.25–15 mm were exposed to the P solution in a 1:20 solid to liquid ratio (m/v) and shaken for 24 h. After this time the solution was decanted and replaced with fresh P solution and this process was repeated for 6 days.

2.2) Plant growth experiments

Based on the methods of Farrell et al. (2013), spring barley (*Hordeum vulgare*) was grown in triplicate in 5% (dry mass basis) biochar/sand mixtures over 3 weeks, with five sand-only controls. The 3 week-growth period was also selected to provide barley plant tissue compatible for assessment of PTE toxicity from previous studies (Davis et al., 1978; MacNicol and Beckett, 1985). The experiment was split between two batches with different biochars and dedicated controls for each batch (Control 1, Control 2 – sand only). The experimental set-up consisted of 50 ml disposable syringe tubes containing the sand/biochar mixtures, resting in 20 ml biotite containers. Five barley seeds were placed under the surface of the biochar/sand mixture in each tube (sand only in controls) and were grown in the laboratory at 20°C under constant fluorescent light for 21 days. Plants received deionised water wicked from 10 ml aliquots in the biotite containers via cotton twine inserted into the base of the syringe tube (see Supplementary Figure 1 for a schematic diagram of the experimental set-up). This watering method was used to reduce leaching of biochar constituents out of the biochar/sand mixture, and was undertaken three times on Day 1 of the experiment as the water was taken up rapidly by the dry mixtures. Subsequently, the deionised water was replenished in the biotite containers every 2 days. At 21 days after seed planting the above ground biomass (comprising leaves only) was harvested from the tubes and rinsed in deionised water, and then oven-dried for 3 days at 80°C to determine dry biomass yield. Supplementary Figure 2 depicts a subset of samples and controls after 21 days, immediately prior to harvest.

To assess nutrient and PTE uptake, at least 40 mg of dried biomass was digested. Where less than this amount of biomass was available, replicates were combined for DW550, EPAD450, FWD550 and WHI550. The dried biomass samples and blanks were digested with 18 M H_2SO_4 and 30% w/v H_2O_2 in a heating block at 330°C for 6 h, and analysed for As, Al, B, Ca, Cd, Co, Cr, Cu, Fe, Hg, K, Mg, Mn, Mo, Na, Ni, P, Pb and Zn using a 7500ce ICP-MS (Agilent Technologies, Santa Clara, USA). Where elemental concentrations were sufficiently high (e.g. P and Ca), ICP-OES was performed using an Optima 5300DV instrument (Perkin Elmer, Waltham, USA). Standards were prepared and run during each analysis session for calibration and to check the accuracy of measurements over time. The results for digestion blanks were subtracted from the experimental results. The limit of detection for each instrument was determined as described in Buss et al. (2016a), but calculated for each sample due to the variable amounts of dry biomass produced in each replicate.

2.3) PTE and nutrient extractions

Based on a survey of the literature, two commonly used salt extractants (1 M NH_4NO_3 and 0.01 M $CaCl_2$) and one mixed component extractant (Mehlich 3) were selected. These provide relevant literature comparisons and were used to extract the 13 biochars not exposed to a P solution, i.e. all except EPAD450, EPAD550, EPOCAD450 and EPOCAD550. Buffered as well as un-buffered solutions were prepared for NH_4NO_3 (pH 4.6) and 0.01 M $CaCl_2$ (pH 7), as described in the Supplementary Information Section 3. Addition of a buffer to Mehlich 3 was not required, as it already contains a buffering agent.

The extraction solutions represent a range of pH as follows: Mehlich 3 (constantly at pH 2.5 when biochar is added), buffered 1 M NH_4NO_3 (constantly at pH 4.6), unbuffered 1 M NH_4NO_3 (starting at pH 4.6, increasing over the time of the extraction), buffered $CaCl_2$ (constantly at pH 7) and unbuffered $CaCl_2$ (starting at pH 7, increasing over the time of the extraction).Since Mehlich 3 contains a mixture of components which interact with elements via different mechanisms, factors other than pH are likely to affect the extractability of an element using this method.

For the buffered and unbuffered 1 M NH_4NO_3 and 0.01 M $CaCl_2$ extractions, 1.5 g of biochar was weighed into a 50 mL centrifuge tube and 15 mL of the relevant extractant added. The choice of this biochar:extractant ratio is explained in Buss et al. (2016c). The extractions were performed in triplicate. The tubes were laid on their side and shaken on an orbital platform shaker at 150 rpm for 2 h, then centrifuged at 3500 rpm for 30 min and the supernatant filtered using 0.45 μm syringe filters (Millipore, Watford, UK). For Mehlich 3 extractions, the same mass of biochar and volume of extractant was used, but the mixtures were only shaken for 5 min, as per the standard

Mehlich 3 procedure (Mehlich, 1984). Due to the short extraction time, rather than centrifugation, the samples were double-filtered, first using Whatman No. 1 paper filters and then using 0.45 µm syringe filters (Millipore, Watford, UK). Blanks were prepared in triplicate for each extraction and their results subtracted from those of the experimental samples. All filtrates were stored briefly at 4°C before analysis for Al, B, Ca, Cd, Co, Cr, Cu, Fe, Hg, K, Mg, Mn, Mo, Na, Ni, P, Pb and Zn by ICP-OES using an Optima 5300DV instrument (Perkin Elmer, Waltham, USA). Most elements were analysed in axial mode, except for K and Na in the salt extracts and Al, Ca, Fe, K, Mg and Na in the Mehlich 3 extracts, which were analysed in radial mode as higher concentrations of these elements were expected. Due to the different ICP-OES analysis modes and extraction ratios used, the limits of detection for individual elements differ between the different methods. More details about the analyses and the calculation of the limit of detection can be found in Buss et al. (2016a) and their values can be found in Supplementary Information Tables 3 and 4.

Since plant P uptake has previously been shown to correlate significantly with P extracted using 2% formic acid (2% FA) (Wang et al., 2012), all 17 biochars were also extracted using this method. In triplicate, 200 mg of each biochar was weighed into a 50 mL centrifuge tube and 20 mL of 2% FA was added. Reagent blanks were also prepared. The samples were shaken for 2 h, centrifuged for 30 min and syringe-filtered as described above. The extracts were analysed for soluble reactive P (SRP) by automated colorimetry (Auto Analyser III, Bran & Luebbe, Norderstedt, Germany).

2.4) Statistical analysis

Statistical analyses were performed using R Studio (R Core Team, 2015) with significance determined as $p < 0.05$. Data were tested for normality using the Shapiro-Wilk test. Where both sets of data being compared were normally distributed, Pearson's product-moment correlation coefficient was calculated, otherwise Spearman's rho was calculated to identify significant correlations. Plant element concentrations in above ground biomass were correlated against extraction concentrations for the same element. To investigate whether the extraction methods were behaving in a similar or different way, each was correlated against the other methods for each individual element.

To determine significant effects of biochar type in the plant uptake experiment, one-way ANOVA and Tukey HSD tests were performed on above ground biomass, plant P concentration and total above ground P mass for data in all treatments where at least 3 replicate results were obtained.

3) Results and discussion

3.1) Plant growth experiment

3.1.1) Above ground biomass yield

Results for above ground biomass (referred to henceforth as plant leaves) are given for all biochar treatments and controls in Table 2. Six of the biochar treatments resulted in plant leaf yields > 50% higher than the sand-only control, although the only significantly higher biomass was for WLB550 compared to its control (Control 2, $p < 0.05$). Plant leaf yield for WSI550, WHI550 and RHI700 biochars were below the relevant control, but not significantly (-24.0, -44.8 and -60.5%, respectively). The plant growth results are discussed in Section 3.1.4.

3.1.2) Uptake of potentially toxic elements into leaves

The concentration of elements in the dried leaves of barley grown in the 5% biochar/sand mixtures (Table 3a and b) were compared with “Upper critical limits” (UCL) for the PTEs As, B, Cd, Co, Cr, Cu, Hg, Mn, Mo, Ni, Pb and Zn calculated for barley plants (Davis et al., 1978; MacNicol and Beckett, 1985, see Supplementary Information Table 7). The UCL is the lowest element concentration in plant tissues before toxic effects are observed. Leaf tissue concentrations of B exceeded the UCL in PAD550, POCAD550, Control 1 and WHI550 treatments, but this does not appear to have affected the yield for PAD550 or POCAD550. Control 1 had a higher mean yield than Control 2, which suggests that it also was not negatively affected by high B or Cu content, as Control 1 also exceeded the UCL for Cu. DW550 exceeded the UCL for Mn, but again this did not appear to have an effect on yield. No other treatments resulted in leaf tissue PTE concentrations above the published UCL values. Overall, UCLs were exceeded in plants exposed to different biochars, however, this did not cause a direct effect on plant growth in this study.

According to the leaf tissue concentrations, Mn and Fe deficiency (defined as < 12 and < 30-50 mg kg^{-1} in shoots, respectively (Ohki et al., 1979; Römheld and Marschner, 1991) was observed in the WLB550 treatment, whilst Mn deficiency also occurred in the FWD550 and WSI550 treatments. The WLB550, FWD550, WSI550 and DW700 treatments all exhibited Cu deficiency (< 1-5 mg kg^{-1}) (Marschner, 1995). Given the increase in growth of barley compared to the control in both WLB550 and FWD550 treatments, it is unlikely that micronutrient deficiencies have negatively affected plant growth.

3.1.3) Uptake of phosphorus from biochar into leaves

Since a relatively large range of plant leaf yields occurred in this experiment, P concentration (in mg P kg^{-1}) and total P content (in mg P) in the plant leaves were compared to assess whether the P measured was mostly seed derived, or whether the biochar had contributed P to the plant tissues. Comparison of these two descriptors (Figure 1) shows that high leaf P concentration does not always map onto high total leaf P due to low yields in some treatments, e.g. WSI550, ADX350. This means that the leaf P concentrations give a false indication of plant P uptake when assessing the fertiliser value of biochars in this experiment.

Total leaf P mass in the EPOCAD550 treatment was significantly higher than that of the relevant control ($p < 0.05$) and was the only treatment which was significantly different to the control. The mean total leaf P mass was higher than the highest recorded value of the controls for PAD450, PAD550, POCAD450, POCAD550, EPAD550, EPOCAD450, EPOCAD550, WLB550 and DW750 (marginally), suggesting that biochar supplied P to the plants in these treatments. Notably absent from this list is EPAD450, which indicates that the P-exposure process may have resulted in less available P than for EPAD550. The plants also took up less P from EPOCAD450 compared to its 550°C-counterpart (although not significantly), which may have implications for their potential application in wastewater treatment and agriculture (Shepherd et al., 2016). Interestingly, whilst FWD550 contains very high total concentrations of P (Buss et al., 2016a) and significantly increased the length of cress (*Lepidium sativum*) shoot length compared to controls in germination tests (Buss et al., 2016c), in this experiment it did not result in higher P uptake into barley leaves compared to the control. This may be due to the way that P is bound in the biochar as, although a high concentration of P was present in FWD550, only 0.10% was 1 M NH_4NO_3 extractable (Buss et al., 2016c).

3.1.4) Overall plant response to biochar-amended sand

Comparing the plant response to biochar treatments to the controls as well as the plant leaf element composition, we can conclude that, in support of the findings of (Buss et al., 2016c), at 5% application rates in sand it is possible that some of the biochars restrict the growth of barley, most likely due to high extractable K concentrations. Root growth (indicated by % roots > 5 mm length) was significantly negatively correlated ($p < 0.001$) with biochar available K concentration in a study which included seven of the biochars investigated here (ADX350, DW550, DW750, FWD550, WLB550, WHI550 and WSI550 (Buss et al., 2016c). Elevated concentrations of PTEs in the plant leaves in some biochar treatments did not appear to be associated with lower yield, but it is not possible to say whether the edible portion of the mature plant would have met safety regulations. The biochar treatments which resulted in the highest yield increase compared to the controls were those which had moderate to low extractable K concentrations (DW550, DW750 and WLB550, from Buss et al. (2016c)), and had been exposed to P solution prior to use (EPAD550, EPOCAD550) or contained a high concentration of native P.

Overall, it is likely that the growth promoting and inhibiting effects observed in barley plants in this study can be explained by the competition between two factors, the negative effect caused by high K vs the positive effect of available P in the various biochars.

3.2) Biochar element concentrations

3.2.1) Biochar element total concentrations

Nine of the biochars investigated in this study contain one or more PTEs at concentrations exceeding the International Biochar Initiative (IBI) and European Biochar Certificate Basic (EBCB) and Premium (EBCP) threshold values for total PTE concentrations in biochar (See Supplementary Information Table 6 for threshold values; total elemental concentrations, Supplementary Tables 1a, 1b, 2a and 2b). The potential exceedance of guideline values by the P-exposed biochars (EPAD450, EPAD550, EPOCAD450 and EPOCAD550) was not assessed, as their concentrations are expected to be similar to their non-P exposed precursors (PAD450, PAD550, POCAD450 and POCAD550). The biochars containing elements present in concentrations above minimum threshold values for one or more of the guidelines are: DW750 (Cr), FWD550 (Zn) WSI550 (Mo), WLB550 (Cd, Zn), POCAD450 and POCAD550 (Cu, Mo and Zn), PAD450 and PAD550 (Cd, Cu, Mo and Zn) and WHI (Cr, Cu, Ni and Zn).

3.2.2) Potentially toxic element and nutrient extractions

The amount of element that was extractable from the biochars varied between methods, partly due to differences in pH between methods. Based on the number of biochars for which each element could be extracted for each extraction method, the elements Al, B and Co could be extracted from many of the biochars investigated above the limit of detection (LOD) using Mehlich 3 and the higher pH extractions (Table 4). Calcium, Cu, Ni and Zn were could be extracted above the LOD from more of the biochars using lower pH extractions than high pH and, with the exception of Zn, were Mehlich 3 extractable. Cadmium and Pb were only extractable for 2 of the biochars above the LOD using Mehlich 3, whilst K, Mg, Mn, Mo, Na and P could be extracted above the LOD (although with differing extraction efficiencies) using any method, except Mehlich 3 for Mo. Of the remaining elements, Cr could be extracted using the buffered and unbuffered 1 M NH_4NO_3 solutions, Fe by Mehlich 3, unbuffered 1 M NH_4NO_3 and buffered 0.01 M $CaCl_2$ solutions, and Hg by unbuffered 1 M NH_4NO_3 and buffered 0.01 M $CaCl_2$ solutions. This suggests moderately acidic to neutral pH extractions are most effective for these three elements, and that Mehlich 3 targets a specific mechanism of Fe binding in biochar that the other methods do not.Of the 13 biochars extracted following the established soil analysis method specified in the German soil ordinance (1 M NH_4NO_3), concentrations of PTEs extracted from five were higher than the recommended threshold. Arsenic was detected above threshold values from PAD450 and WHI550, Cd from POCAD550 as well as WLB550, which also exceeded threshold values for Zn. These results differ slightly to those of Buss et al. 2016c), but this is due to the low threshold values in question (0.1 mg kg^{-1}) and the relatively high Cd detection limit for the experiment. Rather than ICP-OES, ICP-MS appears to be a more suitable method for these analyses in future.

Considering that pure biochar was analysed in this study and the threshold values are referring to soil, as suggested in Buss et al. (2016c), if the biochars are applied to soil at a rate of 1% (< 20 t ha^{-1}) and the soil/biochar mixtures extracted, soil amendment with these biochars will not result in soil PTE concentrations exceeding threshold values.

3.3) Comparison of extraction methods

3.3.1) Mehlich 3, $CaCl_2$ and NH_4NO_3 extractions for potential assessment of elemental bioavailability in biochars

Despite the biochars in this study being selected for their known high concentrations of total PTEs, the quantities removed by extractions were sometimes below the experimental limit of detection. Although this limited examination of different extraction methods for assessing PTE bioavailability in biochars, it supports the findings of other studies where biochars with high concentrations of PTEs have proportionally low extractability (e.g. Buss et al., 2016c; Farrell et al., 2013; Khanmohammadi et al., 2015), indicating that soil amendment might be acceptable with a range of biochar types.

Multiple significant correlations between an element extracted from biochar with plant leaf concentrations (across methods) were revealed for ‘generally extractable’ elements, i.e. where elements were extracted from many biochars above the LOD for all (or most) extraction solutions, e.g. K, Mn, Mo and Na (Table 4). All significant correlations were positive apart from for unbuffered 1 M NH_4NO_3 where plant leaf concentrations of Ca and Zn decreased with higher concentrations extracted from the biochars. Whilst Mehlich 3 generally extracted elements at the highest concentrations and from the highest number of biochars, plant leaf concentrations were significantly correlated with these extractions only for Fe, K, Na and P, suggesting that the bioavailability of elements in biochar, apart from Fe, is not related to a chelation mechanism of extraction.

In general, both the buffered and unbuffered 0.01 M $CaCl_2$ extractions correlated well with plant leaf concentration in this study. The extracted biochar and plant concentrations were significantly positively correlated for 6 elements (all micro- and macronutrients) (Table 4), although the extracted concentrations (data not shown) were one to three orders of magnitude lower than the measured plant leaf concentrations. Plant element concentrations probably correlate well with the $CaCl_2$ extractions because the extraction pH is closest to the pH of the biochars, and in an unbuffered system the biochar is the main control of pH. Despite the large difference in the plant and extract concentration values for individual elements, it is still possible to state the relative availability of nutrients and therefore compare element bioavailability between biochars.

Correlations calculated of the total mass of the element in the leaves with the extraction methods (data not shown) did not highlight any stronger relationships than for leaf element concentrations, except for P (discussed in 3.3.2).

Comparison of the results of our study to those of Farrell et al. (2013) reveals that there are no method correlations in common. This could be due to the use of different plant species (wheat vs. barley) or number of biochars (4 vs. 7 – 17).

3.3.2) Suitability of extraction methods to determine plant P concentration

Significant correlations between P concentrations in plant tissue and biochar extractions were found for Mehlich 3, buffered and unbuffered 0.01 M $CaCl_2$ and 2% FA, however Spearman's ρ was not high (< 0.7) (Table 4). The strongest correlation was with buffered 0.01 M $CaCl_2$, ($\rho = 0.692$, $p < 0.05$).

Based on the recommendation of Wang et al. (2012) of the 2% FA method to estimate P bioavailability in high ash biochars, a curve was fitted to the plot of plant P concentration against 2% FA-extractable P (Figure 2a, $R^2 = 0.3375$). There appears to be an upper concentration limit in the plant leaves of around 11 mg P g^{-1} which could be the optimal P concentration range for barley seedling growth, with most of the values between 8 and 10 mg P g^{-1}. Of the three outliers in Figure 2a, one is due to low yield (WSI550), whilst the others appear to be related to over-estimation of P uptake by the 2% FA extraction. As previously discussed (Section 3.1.3), 1 M NH_4NO_3 extractable P from FWD550 is low relative to uptake, whilst the opposite is true for 2% FA. This suggests that the latter method overestimates the P fraction from biochar by extracting some P that is not plant available.

The comparison of total leaf P mass and 2% FA extractable P provides a better representation of the ability of the 2% FA extraction method for assessing P bioavailability from the biochars (Figure 2b). This can be explained by the fact that when the optimal P concentration in the leaves is reached, the plant does not need to take up more P and thus increase the P concentration further. However, with growth of the plant, more P is taken up by the plant to maintain optimal tissue concentration. Correlation with total leaf P mass should identify the better indicator for bioavailability. This is further emphasised by the lack of relationship between leaf P concentration and plant yield (Figure 2c) and the strong linear relationship between total leaf P mass and yield (Figure 2d, $R^2 = 0.8477$).

Figure 2d also shows the sewage sludge-derived biochars perform consistently well as sources of plant P, providing evidence to support use of biochar from sewage sludge feedstocks as a fertiliser.

3.3.3) Comparison of extraction methods: effect of pH and solution composition

Different extractant solutions have different native pH, indirectly and/or intentionally affecting the solubility of PTEs and nutrients, in addition to targeting different binding mechanisms according to their composition. It has previously been reported that acidic extractants provide a more representative assessment of element bioavailability in acidic soils, with alkaline extractants better suited to alkaline soils (Fixen et al., 1990), but this conclusion has also been questioned (Jordan-Meille et al., 2012). Thus, pH is not the only factor influencing the suitability of methods for estimating bioavailability: solution composition is also important.

Of the 13 elements for which extraction methods were significantly correlated with each other, for nine a significant correlation was found between 0.01 M $CaCl_2$ buffered and unbuffered extracted concentrations (Table 5). Conversely, significant correlations occurred between 1 M NH_4NO_3 buffered and unbuffered extracted concentrations for only 2 of the 13 elements. This is most likely related to the pH of the solutions compared to that of the biochars being extracted. The pH of the biochars were in the range 7.39 – 10.12, with most < 9 (Buss et al., 2016a; Supplementary Table 1), whilst the pHs of 1 M NH_4NO_3 and 0.01 M $CaCl_2$ are 4.6 and 7.0, respectively. The potential pH change is therefore greater for the unbuffered 1 M NH_4NO_3 extractions than for 0.01 M $CaCl_2$, for which only minor pH changes were observed upon addition of the lower pH biochars (< pH 0.5, data not shown).

The extractants with the highest number of significant correlations for element concentrations (10 elements) were buffered 1 M NH_4NO_3 and buffered 0.01 M $CaCl_2$ (Table 5). Given the different pHs of these extractants (4.6 vs. 7), pH cannot be the main factor controlling element extractions from these biochars. The most probable explanation is that since both these extractants are buffered, the extraction pH remains constant at these values, which both happen to lie just outside the pH range at which the adsorption behaviour of many elements change (pH 5-7 for Zn, Co, Ni and Mn) (Basta et al., 2004). Supporting this further is the observation that no significant correlations between these methods was found for Pb, which has a different pH range for changing adsorption behaviour (pH 3-6), which includes the pH of the buffered 1 M NH_4NO_3 extractions (4.6). Therefore, whilst buffered 1 M NH_4NO_3 and buffered 0.01 M $CaCl_2$ extract different amounts of each element, the relationship between element concentrations from the two extractions remains constant for many elements.

Predictably, the number of significant correlations was higher for Mehlich 3 and buffered 1 M NH_4NO_3 extractions (7) than for unbuffered NH_4NO_3 (3). None of the latter were in common with the former.

Elements for which significant correlations occurred in concentrations extracted from biochar by alternate methods were: Al (1), B (4), Ca (5), Cu (2), Fe (2), K (8), Mg (7), Mn (5), Mo (3), Na (7), Ni (5), P (2) and Zn (1). Insufficient data were obtained to determine whether there were correlations between the different extraction methods for Cd, Co, Cr, Hg, and Pb since extracted concentrations were generally below the detection limit, despite deliberate inclusion of high PTE-containing feedstocks. High concentrations of K, Na and Ca were extractable in most of the biochars, resulting in a higher number of data points to use for correlation analysis. Conversely, whilst Al and Fe were also present in high concentrations in many of the biochars, there were few significant correlations between extraction methods for these elements. Magnesium was not found in high concentrations in all of the biochars, but a high number of significant correlations were observed between extractable concentrations from different methods. However, extractable biochar concentrations from any of the methods were not significantly correlated with Mg leaf concentrations, so even though the extraction methods utilise similar extraction mechanisms, these do not represent those which the plant uses to access Mg from the biochars.

These observations emphasise the importance of pH for element extractability, as well as the general difficulty in determining the mechanisms controlling element extractability and thus plant accessibility of nutrients and PTEs in different biochars.

3.4) Broader context of the assessment of biochar bioavailability assessment

The results of this study contribute towards the development of standardised methods to assess bioavailability of nutrients and PTEs from biochar. Based on correlation of element concentrations in plant biomass with concentrations in biochar extracts, 0.01 M $CaCl_2$ (buffered or unbuffered) was the best estimator of element bioavailability for a range of elements. Spearman's ρ (or Pearson's r) correlation coefficient values were equal or slightly higher for all significantly correlated elements in the unbuffered solution compared to buffered 0.01 M $CaCl_2$, with the exception of P (Table 4). This suggests that methods using an extractant with pH closest to the pH of the biochar may provide the most accurate representations of element bioavailability in soils amended with biochar.

Selection of (an) appropriate method/s to assess bioavailability of nutrients and PTEs from biochar involves consideration of a number of factors, including whether values exist in the literature and legislation with which results can be compared. Identification of significant positive correlations between plant tissue concentration/contents and extracted concentrations does not necessarily mean that the extraction method gives an accurate absolute value for bioavailability, only that there is a relationship between the two sets of data. Calculations using conversion factors may need to be conducted on the extraction results to provide an estimate of bioavailability, or a ranking devised to demonstrate what constitutes a high or a low bioavailability value when plant tissue concentration/contents and extracted concentrations of an element are significantly positively correlated. Based on this observation, and in agreement with the recommendations of Farrell et al. (2013), we suggest that direct measurement of plant nutrient and PTE uptake from biochar is the most reliable method to determine bioavailability. Whilst it is more time consuming than extraction methods, it is difficult to foresee the identification of a single extraction method which will a) extract enough of each element of interest for analysis and b) also correlate with plant uptake.

A combination of nutrient and PTE leaching from biochar/soil mixtures and plant uptake studies would provide the necessary information to determine whether the biochar in question could perform well as a fertiliser and/or have the potential to cause phytotoxicity. A soil-specific leaching experiment as described in Bastos et al. (2014) might provide an appropriate measure of leachability. Reflecting on our finding (in agreement with Buss et al. (2016c)) that high K content in the 5% biochar application rate impacted negatively on plant yield, growth experiments using application rates in line with those of fertiliser (extractable or total P mass basis) should be performed to assess the suitability of biochars as P fertiliser. To provide compelling evidence, 4-5 different crop species and different soils would need to be used. Assessment of these experiments may be as simple as yield comparison, as demonstrated by the highly significant positive relationship between plant P mass and yield reported from our experiments. Furthermore, for the assessment of PTEs and general biochar toxicity, both 5% and 1% application rates could be assessed for the same range of crops in a specific soil to separate PTE and salt effects.

5) Conclusions

Concentrations of B, K, Mn, Mo, Na and P in both buffered and unbuffered 0.01 M $CaCl_2$ extractions were significantly correlated with plant uptake in barley seedlings grown in a 5% biochar/sand medium. None of the extraction methods assessed for 17 biochars correlated well with plant uptake of any of the PTEs of most concern, such as, Co, Cr, Cu, Ni, Pb or Zn. This can be explained mostly by the extractability of these elements at concentrations below the method limit of detection.

These results indicate that plant experiments used in this study are better suited for risk assessment of PTEs than extraction methods, but the method needs to be further validated with long term pot experiments. Yield inhibition compared to controls was primarily due to high K concentrations in the 5% biochar applications. The bioavailability of P was highest in post-pyrolysis P-exposed biochars made from sewage sludge feedstocks at a HTT of 550°C, indicating that these production conditions could be suitable for producing biochars with optimised characteristics for use in the wastewater and agriculture industries.

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Figure captions

Figure 1: **Plant uptake of P**. Concentration and total P mass in above ground biomass (leaves) on dry weight basis. Values are means ± 1 standard deviation, except where only one replicate was obtained (RHI700 and WSI550). Control 2 relates to WLB550, DW550, DW750, FWD550 and WSI550, whilst Control 1 relates to the rest of the treatments. Different letters symbolise significant differences between the treatments. nc = not included in statistical analysis as $n < 3$. The blue dashed line represents the highest leaf P mass measured in the controls, above which P in the plant may have been contributed by biochar.

Figure 2: **Comparison of P descriptors**. Relationships between plant leaf P mass and concentration and 2% formic acid extractable P from biochar and plant yield. White circles are the sewage sludge-derived biochars, black circles are the remaining biochars produced from various feedstocks, and grey circles in d) are controls. a) Plant P concentration and 2% formic acid extractable P from biochar. The grey fitted line includes all data points except the WSI550 and FWD550 outliers. The black fitted line also excludes the WLB550 outlier. b) Plant leaf P mass and 2% formic acid extractable P from biochar. The grey fitted line includes all data points. The black fitted line excludes WSI550 and FWD550. c) Plant P concentration and plant yield. d) Plant leaf P mass and plant yield.

Tables

Table 1: General characteristics of the biochars used in this study. HTT = highest treatment temperature, PTEs = potentially toxic elements. [A] pH measured in a 1:10 ratio (m:v) in deionised water after 1.5 h shaking on an orbital platform shaker.

Biochar	Feedstock	HTT (°C)	Post pyrolysis treatment	pH in water[A] (Mean ± 1 stdev n = 2)	Nutrients of interest (based on total concentration)	PTEs of interest (based on total concentration)	Characterised in
PAD450	Pelletised anaerobically digested sewage sludge (Edinburgh, UK)	450	None	7.49 ± 0.02	P, K	Cd, Cu, Mo, Ni, Zn	Shepherd et al., (submitted)
PAD550	Pelletised anaerobically digested sewage sludge (Edinburgh, UK)	550	None	8.25 ± 0.08	P, K	Cd, Cu, Mo, Ni, Zn	Shepherd et al., (submitted)
POCAD450	Pelletised anaerobically digested sewage sludge (Edinburgh, UK) and ochre (Fife, UK) in a 9:1 mass ratio	450	None	7.39 ± 0.05	P, K	Cu, Mo, Ni, Zn	Shepherd et al., (submitted)
POCAD550	Pelletised anaerobically digested sewage sludge (Edinburgh, UK) and ochre (Fife, UK) in a 9:1 mass ratio	550	None	7.85 ± 0.03	P, K	Cu, Mo, Ni, Zn	Shepherd et al., (submitted)
EPAD450	As for PAD450	450	Exposed to 20 mg l^{-1} P solution for 24 h x 6	-	P, K	Cd, Cu, Mo, Ni, Zn	Shepherd et al., (submitted)
EPAD550	As for PAD550	550	Exposed to 20 mg l^{-1} P solution for 24 h x 6	-	P, K	Cd, Cu, Mo, Ni, Zn	Shepherd et al., (submitted)
EPOCAD450	As for POCAD450	450	Exposed to 20 mg l^{-1} P solution for 24 h x 6	-	P, K	Cu, Mo, Ni, Zn	Shepherd et al., (submitted)
EPOCAD550	As for POCAD550	550	Exposed to 20 mg l^{-1} P solution for 24 h x 6	-	P, K	Cu, Mo, Ni, Zn	Shepherd et al., (submitted)
ADX350	Whole plant of *Arundo donax* without roots (Italy)	350	None	8.79 ± 0.44	None	Cd	Buss et al. (2016a,b)
DW550	Demolition wood (heterogeneous, glued, laminated, painted, coated or otherwise treated), (Germany)	550	None	7.65 ± 0.08	None	Cr, Cu, Pb, Zn	Buss et al. (2016a,b)
DW750	Demolition wood (heterogeneous, glued, laminated, painted, coated or otherwise treated) (Germany)	750	None	9.85 ± 0.27	None	Cr, Cu, Ni, Pb, Zn	Buss et al. (2016a,b)
FWD550	Solid residues from anaerobic digestion of food waste (UK)	550	None	8.88 ± 0.24	P, K	Cu, Zn	Buss et al. (2016a,b)
RHI550	Rice husk from plants grown on PTE contaminated land (Panipat, Haryana, India)	550	None	10.20 ± 0.15	K	Ni	n/a
RHI700	Rice husk from plants grown on PTE contaminated land (Panipat, Haryana, India)	700	None	10.40 ±0.25	K	Ni	n/a
WHI550	Water hyacinth (*Eichhornia crassipes*), whole plant, from contaminated water (New Delhi, India)	550	None	9.85 ± 0.11	P, K	Cd, Cr, Cu, Mo, Ni, Pb, Zn	Buss et al. (2016a,b)
WLB550	Willow logs with bark (*Salix* spp., species unknown) from PTE contaminated land (Belgium)	550	None	9.52 ± 0.16	None	Cd, Ni, Pb, Zn	Buss et al. (2016a,b)
WSI550	Wheat straw (*Triticum aestivum*) from PTE contaminated land (India)	550	None	10.12 ± 0.01	K	Mo, Ni	Buss et al. (2016a,b)

Table 2: Dry weight yield of above ground biomass reported in descending order of values. Results are given to 3 significant figures as means ± 1 standard deviation, unless only one replicate was obtained. [A]: Combined yield of 3 replicates, not measured separately. The grey shading indicates Control 2 and the biochars to which it relates, whilst Control 1 relates to the remainder of the biochar treatments.

Biochar	Plant yield mg ± stdev (n reps)	% difference to relevant control
EPOCAD550	86.2 ± 15.0 (3)	83.1
WLB550	84.4 ± 4.05 (3)	120.4
EPAD550	80.0 ± 30.0 (3)	69.8
DW750	75.2 ± 25.1 (3)	96.3
PAD550	74.8 ± 7.05 (3)	58.9
POCAD550	61.5 ± 9.26 (3)	30.5
PAD450	61.0 ± 3.95 (3)	29.6
DW550	60.4 ± 14.8 (2)	57.7
POCAD450	60.0 ± 1.68 (3)	27.3
EPOCAD450	59.0 ± 12.6 (3)	25.3
RHI550	57.2 ± 20.1 (3)	21.4
FWD550	56.1 ± 5.52 (2)	46.5
ADX350	50.3 ± 6.60 (3)	6.7
EPAD450	49.9 ± 9.19 (2)	5.9
Control 1	47.1 ± 11.4 (5)	N/A
Control 2	38.3 ± 17.1 (5)	N/A
WSI550	29.1 (3)[A]	-24.0
WHI550	26.0 ± 13.8 (3)	-44.8
RHI700	18.6 ± 20.3 (3)	-60.5

Table 3a: Element concentrations measured in barley leaves (mg kg^{-1}, dry matter). Values given to 3 significant figures and are means ± 1 standard deviation. n = 3 for all biochar treatments except EPAD450, for which n = 2 (for explanation see text in section 2.2). [A]: only one replicate returned a valid value from ICP-MS analysis, so no standard deviation could be calculated. Control 1 (Table 3b) is the relevant control for these data.

	PAD450	PAD550	POCAD450	POCAD550	EPAD450	EPAD550	EPOCAD450	EPOCAD550
As	4.16 ± 2.42	3.52 ± 3.04	2.24 ± 2.89	1.11 ± 0.727	0.805 ± 0.0626	2.48 ± 0.556	2.11 ± 1.13	1.19 ± 0.471
Al	61.4 ± 7.01	47.7 ± 3.61	42.8 ± 10.3	44.8 ± 17.8	53.6 ± 17.8	40.3 ± 6.42	52.5 ± 21.3	49.9 ± 6.04
B	57.6 ± 25.6	150 ± 106	32.7 ± 12.3	477 ± 366	43.0 ± 1.30	52.8 ± 10.1	61.2 ± 16.9	43.9 ± 2.66
Ca	5110 ± 631	5720 ± 279	6510 ± 460	5710 ± 605	4180 ± 335	5470 ± 1030	5380 ± 416	6170 ± 1080
Cd	0.152 ± 0.130	0.133 ± 0.0932	0.449 ± 0.619	0.0320 ± 0.0132	0.0440 ± 0.00220	0.114 ± 0.122	0.0598 ± 0.0456	0.199 ± 0.262
Co	0.324 ± 0.111	0.288 ± 0.0924	0.559 ± 0.319	0.284 ± 0.0776	0.372 ± 0.0727	0.344 ± 0.223	0.291 ± 0.113	0.250 ± 0.0607
Cr	1.17 ± 0.673	1.43 ± 0.963	1.01 ± 0.270	1.18 ± 0.225	0.751 ± 0.385	2.37 ± 1.92	1.38 ± 0.679	1.44 ± 0.569
Cu	9.72 ± 0.844	17.0 ± 5.72	8.19 ± 0.760	18.9 ± 8.88	11.6 ± 1.84	11.4 ± 1.78	11.5 ± 2.03	10.2 ± 0.641
Fe	119 ± 11.4	90.5 ± 6.67	104 ± 15.4	106 ± 21.246	118 ± 4.36	122 ± 29.2	125 ± 43.7	398 ± 372
Hg	0.0125 ± 0.0217	0.0663 ± 0.016	0.0383 ± 0.0596	0.0327 ± 0.00544	0.0376 ± 0.00562	0.0381 ± 0.0138	0.0673 ± 0.0372	0.0436 ± 0.0145
K	44600 ± 3090	46300 ± 3540	48900 ± 3990	46500 ± 5520	52900 ± 1370	41800 ± 6089	54100 ± 4330	35100 ± 2220
Mg	2390 ± 120	2200 ± 81.7	2780 ± 309	2510 ± 290	2210 ± 0.983	2650 ± 131	2400 ± 12.3	2920 ± 202
Mn	72.9 ± 11.2	86.0 ± 8.66	93.5 ± 2.52	93.5 ± 9.24	80.8 ± 21.5	90.6 ± 0.953	100 ± 19.1	101 ± 21.7
Mo	12.1 ± 3.75	12.3 ± 0.550	10.9 ± 4.73	15.3 ± 2.45	23.2 ± 3.57	27.0 ± 5.92	20.3 ± 1.51	27.3 ± 1.73
Na	9470 ± 1490	8530 ± 1140	5460 ± 494	7400 ± 2150	10000 ± 1460	8670[A]	7550 ± 2010	8420 ± 408
Ni	2.22 ± 79.9	3.05 ± 2.99	2.38 ± 0.297	2.50 ± 0.400	3.71 ± 3.69	2.11 ± 1.14	1.06 ± 0.318	0.891 ± 0.133
P	9880 ± 317	8430 ± 369	9760 ± 186	9490 ± 429	11100 ± 695	10800 ± 1350	10500 ± 584	10200 ± 236
Pb	0.167 ± 0.0546	0.961 ± 0.766	0.233 ± 0.0368	0.698 ± 0.226	0.249 ± 0.0877	0.327 ± 0.120	0.291 ± 0.250	0.193 ± 0.0596
Zn	49.4 ± 5.43	43.7 ± 3.34	41.9 ± 4.54	45.9 ± 9.46	43.2 ± 5.00	47.2 ± 4.43	43.6 ± 9.94	46.5 ± 0.453

Table 3b: Element concentrations measured in barley leaves (mg kg^{-1}, dry matter). Values given to 3 significant figures and are means ± 1 standard deviation. n = 5 for Control 1 and 2, n = 3 for all other biochar treatments except DW550 and FWD550, for which n = 2 and RHI700 and WSI550, for which n = 1 (for explanation see text in section 2.2). [B]: Only one replicate available for analysis, so no standard deviation could be calculated. < LOD: Value obtained was below the limit of detection. ND: No data was obtained for this element. Columns are shaded according to which control is relevant for each treatment i.e. white columns refer to Control 1 and grey columns refer to Control 2.

	Control 1	**Control 2**	**ADX350**	**DW550**	**DW750**	**FWD550**	**RHI550**	**RHI700[B]**	**WHI550**	**WLB550**	**WSI550[B]**
As	2.03 ± 2.39	3.66 ± 4.31	1.48 ± 0.507	0.255 ± 0.309	< LOD	< LOD	4.69 ± 5.22	4.27	1.71 ± 1.34	0.291 ± 0.166	< LOD
Al	119 ± 15.8	103 ± 6.70	24.8 ± ND	31.9 ± 7.67	23.9 ± 4.31	37.4 ± 4.50	26.8 ± 12.9	25.1	57.4 ± 24.4	32.7 ± 9.05	130
B	430 ± 221	29.4 ± 6.36	56.2 ± 10.8	ND	ND	ND	31.6 ± 14.1	23.5	297 ± 341	ND	ND
Ca	1882 ± 24.0	1750 ± 289	1670 ± 340	10500 ± 1.28	7140 ± 0.799	6050 ± 0.251	1910 ± 273	1290	1020 ± 145	6450 ± 0.227	8010
Cd	0.0268 ± 0.0109	0.131 ± 0.147	0.395 ± 0.567	0.50 ± 0.0153	0.659 ± 0.334	0.963 ± 0.139	0.0353 ± 0.0174	0.0508	0.207 ± 0.249	0.76 ± 0.0534	2.26
Co	0.416 ± 0.298	0.370 ± 0.0651	0.226 ± 0.105	< LOD	< LOD	< LOD	0.357 ± 0.0959	0.581	0.289 ± 0.0291	± 0.00461	BDL
Cr	1.02 ± 0.188	1.11 ± 0.406	0.757 ± 0.0406	0.531 ± 0.024	< LOD	0.713 ± 0.222	0.871 ± 0.243	0.857	1.80 ± 0.722	3.15 ± 5.04	0.940
Cu	23.2 ± 4.83	9.10 ± 1.62	8.32 ± 2.03	2.39 ± 0.442	1.77 ± 0.470	1.98 ± 0.0409	10.2 ± 2.21	7.50	17.9 ± 11.8	1.63 ± 0.228	1.71
Fe	60.5 ± 6.81	58.9 ± 3.82	64.8 ± 7.60	ND	ND	ND	86.7 ± 25.3	57.0	78.8 ± 10.4	14.6 ± 25.2	ND
Hg	0.049 ± 0.044	0.0248 ± 0.0211	0.17 ± 0.179	ND	ND	ND	0.0359 ± 0.0183	ND	0.00970 ± 0.0137	ND	ND
K	18500 ± 2640	20600 ± 3850	79700 ± 8730	29200 ± 5.30	55000 ± 7.12	65500 ± 4.85	63900 ± 1820	68200	86100 ± 2680	53300 ± 1.74	59300
Mg	2550 ± 180	2460 ± 266	1820 ± 402	2700 ± 0.139	2470 ± 0.509	2090 ± 0.156	2160 ± 158	1680	1270 ± 192	2040 ± 0.103	< LOD
Mn	ND	ND	ND	129 ± 0.256	113 ± 16.3	< LOD	57.0 ± 6.31	ND	ND	< LOD	< LOD
Mo	1.56 ± 1.21	1.04 ± 1.19	ND	0.502 ± 0.0153	0.659 ± 0.334	0.963 ± 0.139	1.45 ± 1.65	ND	6.34 ± 0.255	0.757 ± 0.0534	2.26
Na	1710 ± 137	1800 ± 163	769 ± 93.2	4290 ± 0.338	2960 ± 1.57	11400 ± 1.13	1070 ± 147	1280	13600 ± 847	269 ± 0.0951	14500
Ni	4.48 ± 4.54	3.06 ± 0.384	1.82 ± 0.436	ND	ND	ND	4.77 ± 2.05	3.43	5.61 ± 0.325	ND	ND
P	8930 ± 174	9390 ± 885	10500 ± 899	7580 ± 0.0907	7150 ± 1.71	8380 ± 0.147	8470 ± 786	8730	10000 ± 5.23	8540 ± 0.381	11800
Pb	1.21 ± 0.365	1.00 ± 0.176	0.136 ± 0.0501	0.185 ± 0.211	0.0431 ± 0.0402	<LOD	0.160 ± 0.148	0.454	0.620 ± 0.487	0.172 ± 0.0958	< LOD
Zn	41.9 ± 3.69	45.1 ± 5.62	44.2 ± 6.26	17.0 ± 0.424	19.5 ± 0.568	20.3 ± 0.334	46.0 ± 12.4	43.2	60.5 ± 5.55	26.8 ± 7.53	49.8

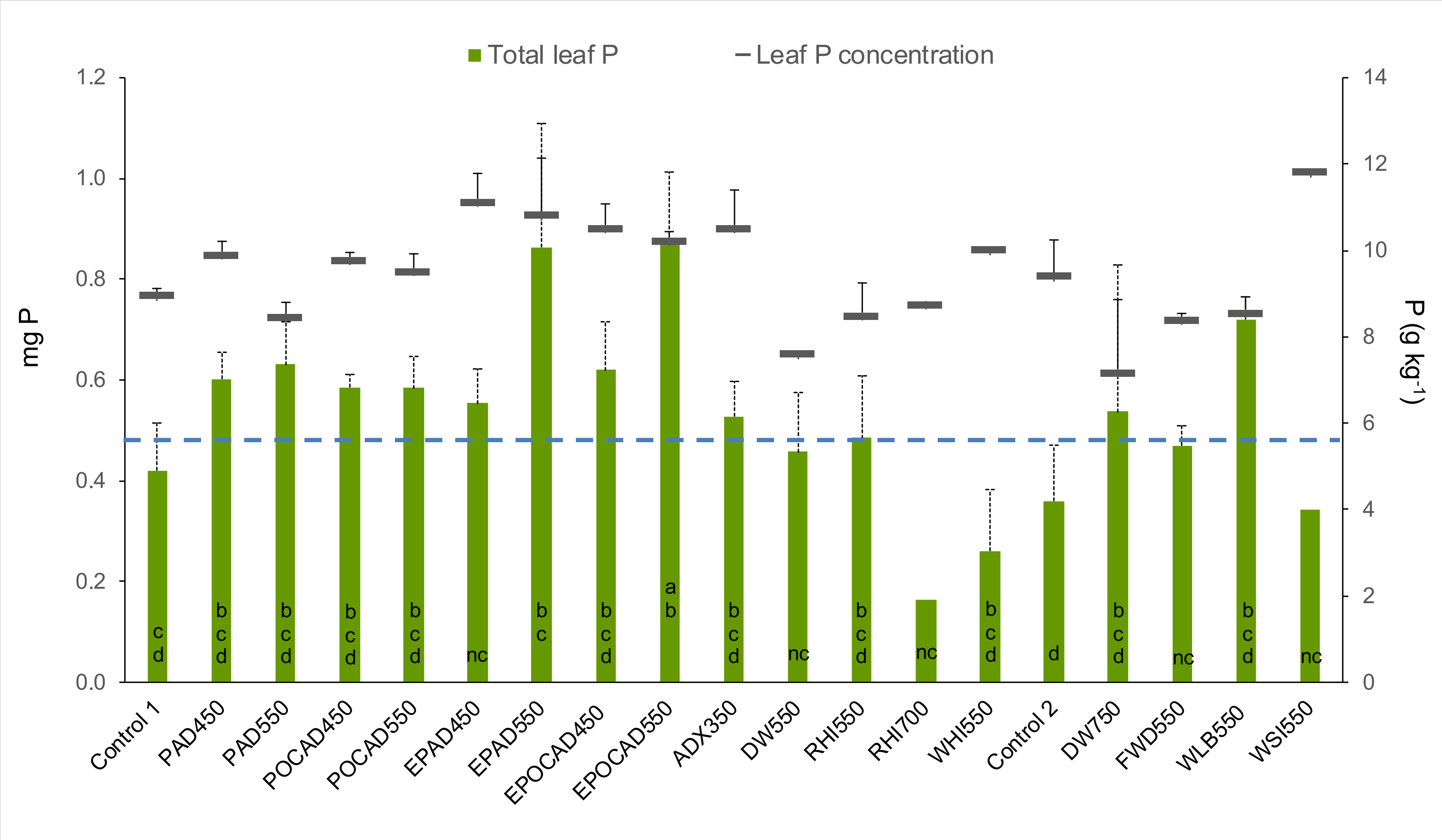
Table 4: Correlation coefficients between element concentrations measured in plant biomass from the growth experiment and those determined in biochars extracted using different methods. ICP-OES was used to determine element concentrations for all extractions except for the 2% formic acid extraction for P where P concentrations were determined by colorimetry. Values reported are Spearman's ρ, unless marked with ^{P}, where Pearson's correlation is stated. N.S. = correlation non-significant, * = $p < 0.05$, ** = $p < 0.01$, *** = $p < 0.001$. N/A = method is not applicable for that element. N.C. = not calculated as standard deviation = 0. The number in brackets indicates the number of data pairs in the dataset for which both plant and biochar extraction data were available with values above the experimental limit of detection.

	Mehlich 3		**Buffered 1 M NH_4NO_3**		**Unbuffered 1 M NH_4NO_3**		**Buffered 0.01 M $CaCl_2$**		**Unbuffered 0.01 M $CaCl_2$**		**2% formic acid**	
pH	**2.5**		**4.6**		**4.6 +**		**7.0**		**7.0 +**		**2.1**	
Al	N.S.	(12)	N.S.	(4)	N.S.	(7)	N.S.	(6)	N.S.	(8)	N/A	
B	N.S.	(6)	**0.805^{*}**	(5)	N.S.	(8)	**0.738^{*}**	(8)	**0.738^{*}**	(8)	N/A	
Ca	N.S.	(13)	N.S.	(13)	**-0.597^{P*}**	(13)	N.S.	(10)	N.S.	(7)	N/A	
Cd	N.S.	(11)	N.S.	(1)	N.S.	(2)	N.C.	(0)	N.C.	(0)	N/A	
Co	N.S.	(11)	N.S.	(1)	N.S.	(3)	N.S.	(2)	N.S.	(2)	N/A	
Cr	N.S.	(3)	N.S.	(10)	N.S.	(6)	N.S.	(2)	N.S.	(1)	N/A	
Cu	N.S.	(13)	N.S.	(13)	N.S.	(8)	N.S.	(2)	N.S.	(3)	N/A	
Fe	**0.900^{**}**	(9)	N.S.	(4)	N.S.	(8)	N.S.	(4)	N.S.	(2)	N/A	
Hg	N.C.	(0)	N.C.	(0)	N.S.	(3)	N.S.	(2)	N.C.	(0)	N/A	
K	**0.835^{***}**	(13)	**0.867^{**}**	(9)	N.S.	(13)	**0.810^{*}**	(8)	**0.929^{**}**	(8)	N/A	
Mg	N.S.	(13)	N.S.	(13)	N.S.	(13)	N.S.	(13)	N.S.	(13)	N/A	
Mn	N.S.	(10)	N.S.	(10)	**0.927*****	(10)	**0.781^{P**}**	(10)	**0.806^{**}**	(10)	N/A	
Mo	N.S.	(3)	**0.752^{**}**	(8)	N.S.	(6)	**0.758^{**}**	(7)	**0.801^{**}**	(6)	N/A	
Na	**0.892^{P***}**	(10)	N.S.	(8)	N.S.	(6)	**0.935^{P**}**	(5)	**0.943^{P***}**	(8)	N/A	
Ni	N.S.	(8)	**0.846^{**}**	(3)	N.S.	(7)	N.S.	(4)	N.S.	(3)	N/A	
P	**0.588^{*}**	(13)	N.S.	(13)	N.S.	(13)	**0.692^{*}**	(13)	**0.583^{*}**	(12)	**0.507^{*}**	(17)
Pb	N.S.	(10)	N.S.	(2)	N.C.	(0)	N.S.	(2)	N.S.	(1)	N/A	
Zn	N.S.	(13)	N.S.	(7)	**-0.566^{*}**	(9)	N.S.	(2)	N.C.	(0)	N/A	

Table 5: Significant correlations for individual elements in biochars for the extraction methods investigated (except 2% formic acid, which was only used to extract P). Correlation coefficients shown are Spearman's ρ, except indicated ^{P}, where Pearson's r is stated. Significance levels are indicated as * = $p < 0.05$, ** = $p < 0.01$, *** = $p < 0.001$.

	Mehlich 3	**Buffered 1 M NH_4NO_3**	**Unbuffered 1 M NH_4NO_3**	**Buffered 0.01 M $CaCl_2$**
Buffered 1 M NH_4NO_3	B 0.679^{*} Ca 0.703^{**} Fe -0.747^{**} K 0.917^{**} Mg 0.890^{***} Na 0.705^{*} Ni 0.571^{*}			
Unbuffered 1 M NH_4NO_3	Al 0.780^{**} Mg 0.632^{*} P 0.720^{**}	Cu 0.593^{*} Na 1^{***}		
Buffered 0.01 M $CaCl_2$	B 0.569^{*} Ca 0.569^{*} Fe -0.695^{**} K 0.881^{**} Mg 0.879^{***} Na 0.983^{P***}	B 0.663^{*} Ca 0.619^{*} K 1^{***} Mg 0.923^{***} Mn 0.841^{***} Mo 0.959^{***} Na 0.991^{***} Ni 0.739^{**} P 0.769^{**} Zn 0.662^{*}	K -0.952^{**} Mn 0.571^{*} Na 0.904^{P*}	
Unbuffered 0.01 M $CaCl_2$	Ca 0.572^{*} K 0.762^{*} Mg 0.846^{***} Na 0.984^{P***} Ni 0.645^{*}	K 0.833^{*} Mg 0.901^{***} Mn 0.665^{*} Mo 0.921^{***} Ni 0.608^{*}	K -0.833^{*} Mn 0.604^{*}	B 0.855^{***} Ca 0.904^{***} Cu 0.851^{***} K 0.833^{*} Mg 0.967^{***} Mn 0.676^{*} Mo 0.961^{***} Na 0.999^{P***} Ni 0.757^{**}

Figures


Total leaf P
Leaf P concentration
mg P
P (g kg-1)
1.2
1.0
0.8
0.6
0.4
0.2
0.0
14
12
10
8
6
4
2
0
Control 1
PAD450
PAD550
POCAD450
POCAD550
EPAD450
EPAD550
EPOCAD450
EPOCAD550
ADX350
DW550
RHI550
RHI700
WHI550
Control 2
DW750
FWD550
WLB550
WSI550
c d
b c d
b c d
b c d
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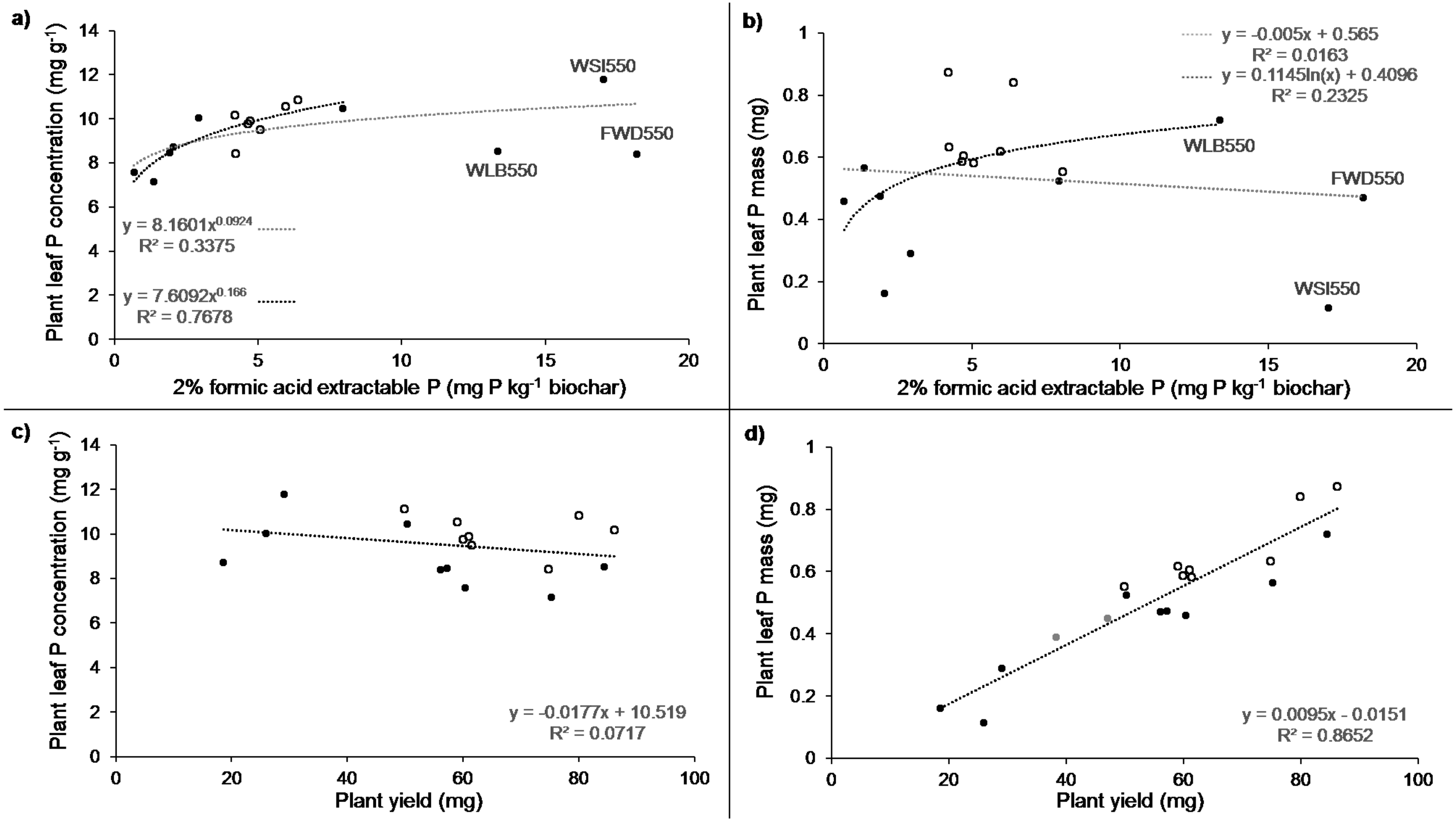
a)
Plant leaf P concentration (mg g-1)
2% formic acid extractable P (mg P kg-1 biochar)
WSI550
FWD550
WLB550
y = 8.1601x^0.0924
R² = 0.3375
y = 7.6092x^0.166
R² = 0.7678
b)
Plant leaf P mass (mg)
2% formic acid extractable P (mg P kg-1 biochar)
y = -0.005x + 0.565
R² = 0.0163
y = 0.1145ln(x) + 0.4096
R² = 0.2325
WLB550
FWD550
WSI550
c)
Plant leaf P concentration (mg g-1)
Plant yield (mg)
y = -0.0177x + 10.519
R² = 0.0717
d)
Plant leaf P mass (mg)
Plant yield (mg)
y = 0.0095x - 0.0151
R² = 0.8652