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Citation for published version:

Shepherd, JG, Buss, W, Sohi, SP & Heal, KV 2017, 'Bioavailability of phosphorus, other nutrients and potentially toxic elements from marginal biomass-derived biochar assessed in barley (Hordeum vulgare) growth experiments', *Science of the Total Environment*, vol. 584-585, pp. 448-457. https://doi.org/10.1016/j.scitotenv.2017.01.028

Digital Object Identifier (DOI):

10.1016/j.scitotenv.2017.01.028

Link:

Link to publication record in Edinburgh Research Explorer

Published In: Science of the Total Environment

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1 Bioavailability of phosphorus, other nutrients and potentially toxic elements from

2 marginal biomass-derived biochar assessed in barley (*Hordeum vulgare*) growth

3 experiments

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13 Abstract

14 Biochars produced from marginal biomass feedstocks are a potential source of recycled nutrients for agriculture, but may also contain potentially toxic elements (PTEs) which 15 can cause phytotoxicity. We assessed the potential for nutrient recycling from such 16 materials against potential environmental risks in 17 biochars containing high 17 18 concentrations of various PTEs and nutrients. Methods for investigating the risk of biochar-derived PTEs were developed and assessed. Short-term (21 days) growth 19 experiments with barley (Hordeum vulgare) in 5% biochar/sand mixtures were used to 20 present the 'worst-case scenario' of high dose and low pH buffering. We compared 21 plant nutrient and PTE concentrations with amounts extracted from the same biochars 22 using 1 M NH₄NO₃ or 0.01 M CaCl₂ (buffered and unbuffered, respectively) and 23 24 Mehlich 3 to analyse whether such extractions could be used to predict bioavailability. The yields of barley grown with biochars "EPOCAD550", and "WLB550" were 25 significantly higher than the control (p < 0.05). Total phosphorus (P) concentration in 26 above-ground biomass was higher than the control for the EPOCAD550 treatment 27 (p < 0.01). Both buffered and unbuffered 0.01 M CaCl₂ biochar extractions were 28 significantly positively correlated with plant leaf concentration for six of the 18 29

elements investigated, more than any of the other extractions. This indicates that CaCl₂
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

39

40 1) Introduction

The production of biochar from pyrolysis has potential to couple organic waste 41 42 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 43 44 be demonstrated in order to address the concerns of industry and environmental regulators. Realising this potential must be underpinned by robust understanding of 45 biochar properties, including the identification and mitigation of any risks posed to the 46 environment. Assessment of risk initially relied heavily on analysis techniques that were 47 48 developed for soils and compost. Biochar is physically and chemically distinct from these materials, however, so new protocols have been developed. Examples include a 49 50 modified dry ashing method to assess total elemental concentrations (Enders and Lehmann, 2012) and extended hot toluene extraction to quantify polyaromatic 51 52 hydrocarbons (PAHs) (Hale et al., 2012; Hilber et al., 2012). Measuring the 53 bioavailability of potentially beneficial elements (nutrients) and potentially toxic elements (PTEs) in biochar also needs new protocols as methods currently used have 54 been optimised for matrices that have very different properties to biochar. 55 56 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 57 58 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 59

60 may be desirable for carbon sequestration, nutrients, such as P and potassium (K),

61 which unlike nitrogen (N) are predominantly preserved during pyrolysis, must be

62 leachable or reactive towards plant exudates to be plant-accessible. If nutrient reactivity

63 is central to an agricultural application of biochar, PTE reactivity needs to be

64 minimised.

65 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 66 phytotoxicity or soil pollution. Estimates for the bioavailability of PTEs in biochar 67 require a high level of confidence. PTEs are often found to be less extractable in biochar 68 69 than their parent feedstock, but their measured mobility in soil is also affected by soilspecific properties (Beesley et al., 2010; Buss et al., 2016c; Farrell et al., 2013; 70 71 Khanmohammadi et al., 2015; Lu et al., 2013; Luo et al., 2014). Hence, reliable 72 methods are required for assessing PTE bioavailability in a soils context, but where 73 results are interpreted drawing on site-specific data such as soil composition, pH and 74 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

77 measured using 0.1 M CaCl₂ (Méndez et al., 2012), whilst 0.01 M CaCl₂, ultra-pure

water, 1 M NH₄NO₃, 0.5 M acetic acid and 0.05 M ethylenediaminetetraacetic acid

79 (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 CaCl₂ and a buffering agent

82 (triethanolamine) (e.g. Fellet et al., 2011; Lu et al., 2013; Luo et al., 2014).

83 Many studies have reported positive correlations between 0.01 M CaCl₂ (pH 7.0) and

1 M NH₄NO₃ (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

87 Contaminated Sites Ordinance (1999) stipulates the use of 1 M NH₄NO₃ soil extractions

to compare against legislated threshold values for available As, Cd, Cr, Cu, Ni, Pb and

89 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

92 extract P, K, Na, Ca, Mg, Mn, Zn and Cu from soils using a mixture of acid, buffer and

complexing components, including EDTA and NH₄NO₃ (Mehlich, 1984). Various

94 studies exist within the literature which assess the bioavailability of PTEs and nutrients

95 in plant growth experiments and chemical extractions (Grzebisz et al., 1983;

96 Monterosso et al., 1999; van Raij, 1998).

97 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 98 99 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 100 101 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 102 103 extraction. Temporal control of extractant pH (at a designated pH, such as 7, or the pH 104 of the soil to which the biochar will be added) by incorporation of a buffering agent 105 should allow more accurate comparisons and prediction of nutrient and PTE

106 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

111 extractions where soil and plants are not present, but is more resource intensive.

112 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

- 115 main factor in biochar metal interactions, we compared five extraction solutions which
- 116 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
- 120 experimental conditions (Hale et al., 2012; Mayer et al., 2016). In addition, a P-specific
- 121 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
- 123 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

125 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 126 extraction values to determine whether the low extractability of PTEs from biochar 127 reported in the literature was also reflected in low bioavailability and whether high P 128 129 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 130 elements were minimal in the system. Had a soil been selected instead, comparison of 131 the soil-free biochar extractions with plant leaf element concentrations would not have 132 133 been valid.

134

135 2) Materials and methods

136 **2.1**) Biochar production and characterisation

The 17 biochars used in this study produced from nine different feedstocks were 137 selected for their high content of different PTEs and nutrients. They were prepared at 138 139 the UK Biochar Research Centre using the Stage II pyrolysis unit described in detail in 140 (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. 141 142 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 143 144 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, 145 146 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. 147 148 Four of the biochars (EPAD450, EPAD550, EPOCAD450 and EPOCAD550) are modified biochars which had been exposed to a P solution, to encompass captured as 149 well as native nutrients within the study. The P-exposed biochars were created by 150 151 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
- 153 (MOPS), parameters defined to simulate enrichment that might be achieved in a
- 154 wastewater treatment plant (Shepherd et al., submitted). Briefly, 30 g of each biochar
- 155 with particles of diameter 0.25–15 mm were exposed to the P solution in a 1:20 solid to

156	liquid ratio (m/v) and shaken for 24 h. After this time the solution was decanted and
157	replaced with fresh P solution and this process was repeated for 6 days.

158

159 2.2) Plant growth experiments

160 Based on the methods of Farrell et al. (2013), spring barley (Hordeum vulgare) was 161 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 162 plant tissue compatible for assessment of PTE toxicity from previous studies (Davis et 163 al., 1978; MacNicol and Beckett, 1985). The experiment was split between two batches 164 165 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 166 167 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 168 169 in controls) and were grown in the laboratory at 20°C under constant fluorescent light 170 for 21 days. Plants received deionised water wicked from 10 ml aliquots in the biotite 171 containers via cotton twine inserted into the base of the syringe tube (see Supplementary 172 Figure 1 for a schematic diagram of the experimental set-up). This watering method was 173 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 174 175 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 176 177 (comprising leaves only) was harvested from the tubes and rinsed in deionised water, 178 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 179 180 harvest.

181 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,
- 183 EPAD450, FWD550 and WHI550. The dried biomass samples and blanks were
- digested with 18 M H₂SO₄ and 30% w/v H₂O₂ 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
- 186 Zn using a 7500ce ICP-MS (Agilent Technologies, Santa Clara, USA). Where
- 187 elemental concentrations were sufficiently high (e.g. P and Ca), ICP-OES was

- 188 performed using an Optima 5300DV instrument (Perkin Elmer, Waltham, USA).
- 189 Standards were prepared and run during each analysis session for calibration and to
- 190 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
- 193 variable amounts of dry biomass produced in each replicate.
- 194
- 195 2.3) PTE and nutrient extractions
- 196 Based on a survey of the literature, two commonly used salt extractants (1 M NH₄NO₃
- and 0.01 M CaCl₂) and one mixed component extractant (Mehlich 3) were selected.
- 198 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
- 200 EPOCAD550. Buffered as well as un-buffered solutions were prepared for NH₄NO₃
- 201 (pH 4.6) and 0.01 M CaCl₂ (pH 7), as described in the Supplementary Information
- Section 3. Addition of a buffer to Mehlich 3 was not required, as it already contains abuffering agent.
- 204 The extraction solutions represent a range of pH as follows: Mehlich 3 (constantly at pH
- 205 2.5 when biochar is added), buffered 1 M NH₄NO₃ (constantly at pH 4.6), unbuffered 1
- 206 M NH₄NO₃ (starting at pH 4.6, increasing over the time of the extraction), buffered
- 207 CaCl₂ (constantly at pH 7) and unbuffered CaCl₂ (starting at pH 7, increasing over the
- time of the extraction). Since Mehlich 3 contains a mixture of components which
- 209 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 NH₄NO₃ and 0.01 M CaCl₂ 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
- 216 for 30 min and the supernatant filtered using 0.45 μ m syringe filters (Millipore,
- 217 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

219 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 220 221 and then using 0.45 µm syringe filters (Millipore, Watford, UK). Blanks were prepared 222 in triplicate for each extraction and their results subtracted from those of the 223 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 224 225 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 226 and Na in the Mehlich 3 extracts, which were analysed in radial mode as higher 227 228 concentrations of these elements were expected. Due to the different ICP-OES analysis 229 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 230 the limit of detection can be found in Buss et al. (2016a) and their values can be found 231 in Supplementary Information Tables 3 and 4. 232

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).

240

241 2.4) Statistical analysis

242 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-243 Wilk test. Where both sets of data being compared were normally distributed, Pearson's 244 product-moment correlation coefficient was calculated, otherwise Spearman's rho was 245 calculated to identify significant correlations. Plant element concentrations in above 246 ground biomass were correlated against extraction concentrations for the same element. 247 To investigate whether the extraction methods were behaving in a similar or different 248 way, each was correlated against the other methods for each individual element. 249

250 To determine significant effects of biochar type in the plant uptake experiment, one-way

251 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
- 253 replicate results were obtained.
- 254

255 3) Results and discussion

256 **3.1)** Plant growth experiment

257 **3.1.1)** Above ground biomass yield

- 258 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,
- 262 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.
- 265

3.1.2) Uptake of potentially toxic elements into leaves

267 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"
- 269 (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
- 271 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
- 278 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 directeffect on plant growth in this study.

According to the leaf tissue concentrations, Mn and Fe deficiency (defined as < 12 and

 $282 < 30-50 \text{ mg kg}^{-1}$ in shoots, respectively (Ohki et al., 1979; Römheld and Marschner,

283 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 ($< 1-5 \text{ mg kg}^{-1}$) (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 plantgrowth.
- 289

290 **3.1.3**) Uptake of phosphorus from biochar into leaves

Since a relatively large range of plant leaf yields occurred in this experiment, P 291 292 concentration (in mg P kg⁻¹) and total P content (in mg P) in the plant leaves were 293 compared to assess whether the P measured was mostly seed derived, or whether the 294 biochar had contributed P to the plant tissues. Comparison of these two descriptors 295 (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 296 297 P concentrations give a false indication of plant P uptake when assessing the fertiliser 298 value of biochars in this experiment.

299 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,

303 EPOCAD450, EPOCAD550, WLB550 and DW750 (marginally), suggesting that

biochar supplied P to the plants in these treatments. Notably absent from this list is

305 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

307 compared to its 550°C-counterpart (although not significantly), which may have

- 308 implications for their potential application in wastewater treatment and agriculture
- 309 (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
- 311 (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
- 315 1 M NH₄NO₃ extractable (Buss et al., 2016c).
- 316

317 **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 318 319 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 320 restrict the growth of barley, most likely due to high extractable K concentrations. Root 321 growth (indicated by % roots > 5 mm length) was significantly negatively correlated 322 (p < 0.001) with biochar available K concentration in a study which included seven of 323 324 the biochars investigated here (ADX350, DW550, DW750, FWD550, WLB550, WHI550 325 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 326 327 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 328 329 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 330 exposed to P solution prior to use (EPAD550, EPOCAD550) or contained a high 331 concentration of native P. 332

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.

337

338 **3.2**) Biochar element concentrations

339 **3.2.1)** Biochar element total concentrations

340 Nine of the biochars investigated in this study contain one or more PTEs at

- 341 concentrations exceeding the International Biochar Initiative (IBI) and European
- 342 Biochar Certificate Basic (EBCB) and Premium (EBCP) threshold values for total PTE
- 343 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,
- 346 EPOCAD450 and EPOCAD550) was not assessed, as their concentrations are expected
- to be similar to their non-P exposed precursors (PAD450, PAD550, POCAD450 and
- 348 POCAD550). The biochars containing elements present in concentrations above
- 349 minimum threshold values for one or more of the guidelines are: DW750 (Cr), FWD550
- 350 (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).
- 352

353 3.2.2) Potentially toxic element and nutrient extractions

354 The amount of element that was extractable from the biochars varied between methods, 355 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 356 357 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, 358 359 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 3 360 361 extractable. Cadmium and Pb were only extractable for 2 of the biochars above the 362 LOD using Mehlich 3, whilst K, Mg, Mn, Mo, Na and P could be extracted above the 363 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 364 unbuffered 1 M NH₄NO₃ solutions, Fe by Mehlich 3, unbuffered 1 M NH₄NO₃ and 365 buffered 0.01 M CaCl₂ solutions, and Hg by unbuffered 1 M NH₄NO₃ and buffered 366 367 0.01 M CaCl₂ solutions. This suggests moderately acidic to neutral pH extractions are most effective for these three elements, and that Mehlich 3 targets a specific mechanism 368 of Fe binding in biochar that the other methods do not. Of the 13 biochars extracted 369 370 following the established soil analysis method specified in the German soil ordinance 371 (1 M NH₄NO₃), concentrations of PTEs extracted from five were higher than the

372 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

377 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.

382

383

3.3) Comparison of extraction methods

384 3.3.1) Mehlich 3, CaCl₂ and NH₄NO₃ extractions for potential assessment of 385 elemental bioavailability in biochars

386 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

388 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 witha range of biochar types.

394 Multiple significant correlations between an element extracted from biochar with plant leaf concentrations (across methods) were revealed for 'generally extractable' elements, 395 396 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 397 398 positive apart from for unbuffered 1 M NH₄NO₃ where plant leaf concentrations of Ca 399 and Zn decreased with higher concentrations extracted from the biochars. Whilst 400 Mehlich 3 generally extracted elements at the highest concentrations and from the highest number of biochars, plant leaf concentrations were significantly correlated with 401 402 these extractions only for Fe, K, Na and P, suggesting that the bioavailability of

403 elements in biochar, apart from Fe, is not related to a chelation mechanism of404 extraction.

In general, both the buffered and unbuffered 0.01 M CaCl₂ extractions correlated well 405 with plant leaf concentration in this study. The extracted biochar and plant 406 407 concentrations were significantly positively correlated for 6 elements (all micro- and 408 macronutrients) (Table 4), although the extracted concentrations (data not shown) were 409 one to three orders of magnitude lower than the measured plant leaf concentrations. Plant element concentrations probably correlate well with the CaCl₂ extractions because 410 411 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 412 413 concentration values for individual elements, it is still possible to state the relative 414 availability of nutrients and therefore compare element bioavailability between 415 biochars. 416 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 417 element concentrations, except for P (discussed in 3.3.2). 418

419 Comparison of the results of our study to those of Farrell et al. (2013) reveals that there 420 are no method correlations in common. This could be due to the use of different plant 421 species (wheat vs. barley) or number of biochars (4 vs. 7 - 17).

422

423 **3.3.2)** Suitability of extraction methods to determine plant P concentration

424 Significant correlations between P concentrations in plant tissue and biochar extractions

425 were found for Mehlich 3, buffered and unbuffered 0.01 M CaCl₂ and 2% FA, however

426 Spearman's ρ was not high (< 0.7) (Table 4). The strongest correlation was with

427 buffered 0.01 M CaCl₂, ($\rho = 0.692$, p < 0.05).

- 428 Based on the recommendation of Wang et al. (2012) of the 2% FA method to estimate P
- 429 bioavailability in high ash biochars, a curve was fitted to the plot of plant P
- 430 concentration against 2% FA-extractable P (Figure 2a, $R^2 = 0.3375$). There appears to
- 431 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

433 between 8 and 10 mg P g^{-1} . Of the three outliers in Figure 2a, one is due to low yield

- 434 (WSI550), whilst the others appear to be related to over-estimation of P uptake by the
- 435 2% FA extraction. As previously discussed (Section 3.1.3), 1 M NH₄NO₃ extractable P
- from FWD550 is low relative to uptake, whilst the opposite is true for 2% FA. This
- 437 suggests that the latter method overestimates the P fraction from biochar by extracting
- 438 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.
- 445 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
- 447 plant yield (Figure 2c) and the strong linear relationship between total leaf P mass and 448 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.

452

453 **3.3.3)** Comparison of extraction methods: effect of pH and solution composition

Different extractant solutions have different native pH, indirectly and/or intentionally 454 455 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 456 457 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 458 459 conclusion has also been questioned (Jordan-Meille et al., 2012). Thus, pH is not the 460 only factor influencing the suitability of methods for estimating bioavailability: solution 461 composition is also important.

462 Of the 13 elements for which extraction methods were significantly correlated with each
463 other, for nine a significant correlation was found between 0.01 M CaCl₂ buffered and

- 464 unbuffered extracted concentrations (Table 5). Conversely, significant correlations
- 465 occurred between 1 M NH₄NO₃ buffered and unbuffered extracted concentrations for
- 466 only 2 of the 13 elements. This is most likely related to the pH of the solutions
- 467 compared to that of the biochars being extracted. The pH of the biochars were in the
- 468 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 NH₄NO₃ extractions than for
- 471 0.01 M CaCl₂, for which only minor pH changes were observed upon addition of the
- 472 lower pH biochars (< pH 0.5, data not shown).
- The extractants with the highest number of significant correlations for element
- 474 concentrations (10 elements) were buffered 1 M NH₄NO₃ and buffered 0.01 M CaCl₂
- (Table 5). Given the different pHs of these extractants (4.6 vs. 7), pH cannot be the
- 476 main factor controlling element extractions from these biochars. The most probable
- 477 explanation is that since both these extractants are buffered, the extraction pH remains
- 478 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
- 481 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 NH₄NO₃
- 483 extractions (4.6). Therefore, whilst buffered 1 M NH₄NO₃ and buffered 0.01 M CaCl₂
- extract different amounts of each element, the relationship between element
- 485 concentrations from the two extractions remains constant for many elements.
- 486 Predictably, the number of significant correlations was higher for Mehlich 3 and
- 487 buffered 1 M NH₄NO₃ extractions (7) than for unbuffered NH₄NO₃ (3). None of the
- 488 latter were in common with the former.
- 489 Elements for which significant correlations occurred in concentrations extracted from
- 490 biochar by alternate methods were: Al (1), B (4), Ca (5), Cu (2), Fe (2), K (8), Mg (7),
- 491 Mn (5), Mo (3), Na (7), Ni (5), P (2) and Zn (1). Insufficient data were obtained to
- 492 determine whether there were correlations between the different extraction methods for
- 493 Cd, Co, Cr, Hg, and Pb since extracted concentrations were generally below the
- 494 detection limit, despite deliberate inclusion of high PTE-containing feedstocks. High
- 495 concentrations of K, Na and Ca were extractable in most of the biochars, resulting in a

496 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 497 498 significant correlations between extraction methods for these elements. Magnesium was 499 not found in high concentrations in all of the biochars, but a high number of significant 500 correlations were observed between extractable concentrations from different methods. 501 However, extractable biochar concentrations from any of the methods were not 502 significantly correlated with Mg leaf concentrations, so even though the extraction 503 methods utilise similar extraction mechanisms, these do not represent those which the 504 plant uses to access Mg from the biochars.

505 These observations emphasise the importance of pH for element extractability, as well

as the general difficulty in determining the mechanisms controlling element

507 extractability and thus plant accessibility of nutrients and PTEs in different biochars.

508

509 3.4) Broader context of the assessment of biochar bioavailability assessment

510 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 511 512 element concentrations in plant biomass with concentrations in biochar extracts, 0.01 M CaCl₂ (buffered or unbuffered) was the best estimator of element bioavailability for a 513 514 range of elements. Spearman's ρ (or Pearson's r) correlation coefficient values were 515 equal or slightly higher for all significantly correlated elements in the unbuffered 516 solution compared to buffered 0.01 M CaCl₂, with the exception of P (Table 4). This suggests that methods using an extractant with pH closest to the pH of the biochar may 517 provide the most accurate representations of element bioavailability in soils amended 518 519 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 527 extraction results to provide an estimate of bioavailability, or a ranking devised to 528 demonstrate what constitutes a high or a low bioavailability value when plant tissue 529 concentration/contents and extracted concentrations of an element are significantly 530 positively correlated. Based on this observation, and in agreement with the recommendations of Farrell et al. (2013), we suggest that direct measurement of plant 531 532 nutrient and PTE uptake from biochar is the most reliable method to determine 533 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 534 535 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 536 537 uptake studies would provide the necessary information to determine whether the 538 biochar in question could perform well as a fertiliser and/or have the potential to cause 539 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 540 541 agreement with Buss et al. (2016c)) that high K content in the 5% biochar application 542 rate impacted negatively on plant yield, growth experiments using application rates in 543 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, 4-5 544 545 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 546 547 significant positive relationship between plant P mass and yield reported from our experiments. Furthermore, for the assessment of PTEs and general biochar toxicity, 548 549 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. 550

551

552 **5)** Conclusions

553 Concentrations of B, K, Mn, Mo, Na and P in both buffered and unbuffered

554 0.01 M CaCl₂ 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

557 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.

559 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 560 561 validated with long term pot experiments. Yield inhibition compared to controls was 562 primarily due to high K concentrations in the 5% biochar applications. The 563 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 564 565 be suitable for producing biochars with optimised characteristics for use in the wastewater and agriculture industries. 566

567

568 Acknowledgements

569 The authors would like to thank Aditya Parmer for the provision of biochar feedstock,

570 Dr Lorna Eades and Dr Clare Peters, Francesca Gregory, Flavien Poincot and Kate

571 Shepherd for their technical assistance and Dr Ondřej Mašek for the provision of

572 pyrolysis facilities. Jessica Shepherd's PhD was supported by the University of

573 Edinburgh Principal's Career Development and Edinburgh Global Research

scholarships and the School of GeoSciences, and the research costs were funded by

research ICON (formally ACTEW Water), in Canberra, Australia. Jessica would

576 particularly like to thank Dr Chris Hepplewhite for his encouragement and support,

577 which enabled this research to be realised.

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704 Figure captions

Figure 1: Plant uptake of P. Concentration and total P mass in above ground biomass 705 (leaves) on dry weight basis. Values are means ± 1 standard deviation, except where 706 707 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 708 709 treatments. Different letters symbolise significant differences between the treatments. nc 710 = 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 711 712 contributed by biochar.

713

714

Figure 2: Comparison of P descriptors. Relationships between plant leaf P mass and 715 716 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 717 718 produced from various feedstocks, and grey circles in d) are controls. a) Plant P 719 concentration and 2% formic acid extractable P from biochar. The grey fitted line 720 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 721 722 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 723 724 mass and plant yield.

725 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	Р, К	Cd, Cu, Mo, Ni, Zn	Shepherd et al., (submitted)
PAD550	Pelletised anaerobically digested sewage sludge (Edinburgh, UK)	550	None	8.25 ± 0.08	Р, К	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	Р, К	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	Р, К	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	-	Р, К	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	-	Р, К	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	-	Р, К	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	-	Р, К	Cu, Mo, Ni, Zn	Shepherd et al., (submitted)
ADX350	Whole plant of <i>Arundo donax</i> 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	Р, К	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	К	Ni	n/a
RHI700	Rice husk from plants grown on PTE contaminated land (Panipat, Haryana, India)	700	None	10.40 ±0.25	К	Ni	n/a
WH1550	Water hyacinth (<i>Eichhornia crassipes</i>), whole plant, from contaminated water (New Delhi, India)	550	None	9.85 ± 0.11	Р, К	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 (<i>Triticum aestivum</i>) 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)	735 % 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	$50.3 \pm 6.60(3)$	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
В	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
Со	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
Р	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.

745 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	WH1550	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	$24.8 \pm \text{ND}$	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
В	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
Со	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
Р	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< th=""><th>0.160 ± 0.148</th><th>0.454</th><th>0.620 ± 0.487</th><th>0.172 ± 0.0958</th><th>< LOD</th></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

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

		Buffered		Unbuff	ered	Buffer	ed	Unbuff	2%			
	Mehlich 3		1 M		1 M	[0.01]	М	0.01 I	М	formic	
			NH4N	O 3	NH4N	O 3	CaC	12	CaC	2	acid	
pН	2.5		4.6		4.6 -	F	7.0		7.0 -	F	2.1	
Al	N.S.	(12)	N.S.	(4)	N.S.	(7)	N.S.	(6)	N.S.	(8)	N/A	
В	N.S.	(6)	0.805*	(5)	N.S.	(8)	0.738*	(8)	0.738*	0.738 * (8)		
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	
Κ	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	
Р	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.

		В	0.679*										
	Buffered 1 M NH4NO ₃	Ca	0.703**										
		Fe	-0.747**										
		K	0.917**										
		Mg	0.890***										
		Na	0.705*										
		Ni	0.571*	В	uffered 1	M N	H4NO3						
pa	1 M NH4NO3	Al	0.780**	Cu	0.593*								
uffere		Mg	0.632*	Na	1***								
lubi		D	0.720**					Unl	buffered				
		Г	0.720					1 M	NH4NO3	_			
		В	0.569*	В	0.663*	Мо	0.959***	Κ	-0.952**				
	CaCl ₂	Ca	0.569*	Ca	0.619*	Na	0.991***	Mn	0.571*				
	1 M (Fe	-0.695**	К	1^{***}	Ni	0.739**						
	ed 0.0	K	0.881**	Mg	0.923***	Р	0.769**	No	0 004 ^{p*}				
	uffer	Mg	0.879***	Mn	0.841***	Zn	0.662*	INA	0.904				
	B	Na	0.983 ^{p***}							E	Buffered 0.	01 M	CaCl ₂
_		Ca	0.572^{*}	K	0.833*			K	-0.833*	В	0.855***	Mn	0.676*
.01 M		K	0.762*	Mg	0.901***			Mn	0.604*	Ca	0.904***	Мо	0.961***
ered 0	CaCl ₂	Mg	0.846***	Mn	0.665*					Cu	0.851***	Na	0.999 ^{p***}
ŋbuff)	Na	0.984 ^{p***}	Mo	0.921***					K	0.833*	Ni	0.757**
Ŋ		Ni	0.645*	Ni	0.608*					Mg	0.967***		

Mehlich 3

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