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Risks and benefits of marginal biomass-derived biochars for plant growth

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10 Abstract

11 In this study, 19 biochars from marginal biomass, representing all major biomass

- 12 groups (woody materials, grass, an aquatic plant, anthropogenic wastes) were
- 13 investigated regarding their content of available potentially toxic elements (PTEs)
- 14 and nutrients (determined by NH₄NO₃-extractions) and their effects on cress
- 15 (*Lepidium sativum*) seedling growth. The objective was to assess the potential and
- 16 actual effects of biochar with increased PTE content on plant growth in the context of
- 17 use in soil amendments and growing media. It showed that the percentage of
- 18 available PTEs was highest for biochars produced at the highest treatment
- 19 temperature (HTT) of 750°C. On average, however, for all 19 biochars, the
- 20 percentage availability of Cu, Cr, Ni and Zn (<1.5% for all) was similar to the
- 21 percentage availability reported in the literature for the same elements in soils at
- similar pH values which is a highly important finding. Most biochars exceeded
- German soil threshold values for NH₄NO₃-extractable PTEs, such as Zn (by up to 25-fold), As and Cd. Despite this, cress seedling growth tests with 5% biochar in
- 25 sand did not show any correlations between inhibitory effects (observed in 5 of the
- 26 19 biochars) and the available PTE concentrations. Instead, the available K
- 27 concentration and biochar pH were highly significantly, negatively correlated with
- 28 seedling growth (K: p<0.001, pH: p=0.004). K had the highest available
- concentration of all elements and the highest percentage availability (47.7±19.7% of
- 30 the total K was available). Consequently, available K contributed most to the osmotic
- 31 pressure and high pH which negatively affected the seedlings. Although a potential
- 32 risk if some of these marginal biomass-derived biochar were applied at high
- 33 concentrations, e.g. 5% (>100 t ha⁻¹), when applied at agriculturally realistic
- 34 application rates (1-10 t ha⁻¹), the resulting smaller increases in pH and available K
- 35 concentration may actually be beneficial for plant growth.

36 Keywords

- 37 Potentially toxic elements, contaminants, heavy metals, biochar, availability,
- 38 phytotoxicity

39 Abbreviations¹

40 **1** Introduction

41 Biochar can improve soil chemical properties (e.g. pH, cation exchange capacity 42 (CEC)), soil biological properties (e.g. stimulate microbial growth) and soil physical 43 properties (e.g. water holding capacity) (Lehmann and Joseph, 2015) and in 44 addition, supply nutrients directly to the soil (Ippolito et al., 2015). Consequently, 45 among other things, biochar is being tested for plant growth promotion in agriculture, 46 horticulture and viticulture. However, inhibiting effects caused by biochar could 47 negate any positive effects and so biochar should not contain contaminants which 48 pose a risk to plant growth.

- 49 The contaminants in biochar which have been reported to be present at sufficient
- 50 concentrations to affect plant growth are: polycyclic aromatic hydrocarbons (PAHs),
- 51 volatile organic compounds (VOCs) and potentially toxic elements (PTEs). These
- 52 can originate from the feedstock (predominantly PTEs) and/or the production
- 53 process itself (VOCs, PAHs and some metals) (Buss et al., 2015, 2016; Hale et al.,
- 54 2012; Hilber et al., 2012). While process conditions can be adjusted and pyrolysis
- 55 units can be built to minimise contamination resulting from the production process 56 (Buss et al., 2016; Hale et al., 2012), contaminants in the feedstock are source-
- (Buss et al., 2016; Hale et al., 2012), contaminants in the feedstock are sou
 dependent, and therefore, careful selection of biomass is necessary.
- 58 From an economic and sustainability perspective, the ideal feedstock for biochar
- 59 production is biomass or organic waste that would otherwise be landfilled or
- 60 incinerated (Shackley et al., 2011). However, these materials are likely to contain
- 61 contaminants, e.g. originating from the soil or water bodies in which the biomass was
- 62 grown or from direct anthropogenic influences (e.g. wood from demolition sites,
- 63 sewage sludge and food waste). Such material of limited economic value is
- 64 henceforth referred to as "marginal biomass". Biochars produced from marginal
- biomass containing organic contaminants, e.g. PAHs or dioxins, have been shown to
- 66 pose a low risk as such contaminants tend to be largely destroyed or evaporated
- 67 during pyrolysis (Wijesekara et al., 2007; Zielińska and Oleszczuk, 2015).
- 68 PTEs, on the other hand, mostly remain in the solids (feedstock / biochar) during
- 69 biochar production and only a few are partially evaporated (Buss et al., 2016).
- 70 Consequently, guideline values for total concentrations of PTEs have been
- 71 introduced and biochars can be tested for compliance against these guidelines
- 72 (EBC, 2012; International Biochar Initiative, 2011). However, when biochar is applied
- to a soil or a plant growth medium, only a fraction of the PTEs (and nutrients) are
- 74 present in forms which can be taken up by plants. This proportion is usually termed

¹ PTE, Potentially toxic elements; LOD, limit of detection; LOQ, limit of quantification; SD, standard deviation; MLV-index, Munoo-Liisa-Vitaility index; CEC, cation exchange capacity; HTT, highest treatment temperature

the 'bioavailable' fraction and, since it usually does not correlate with total elemental

content (Ippolito et al., 2015), methods to assess the extent of PTE availability have

77 been developed.

78 Numerous chemical extraction methods using a wide range of extractants including 79 deionised (DI) water, salt solutions, complexing agents or weak acids have been 80 used to approximate the bioavailable fraction of PTEs (and nutrients) in soils and 81 biochar (Farrell et al., 2013; McLaughlin et al., 2000; Monter Roso et al., 1999; van 82 Raij, 1998). BS ISO 19730:2008 (2008) describes soil extraction with 1 mol L⁻¹ 83 NH₄NO₃ for assessing the fraction of trace elements able to interact and affect crop 84 growth and was used to establish German legislation threshold values for PTEs for 85 protecting plant growth and crop quality (German Federal Soil Protection and 86 Contaminated Sites Ordinance, 1999). In addition to extraction of PTEs in soil, the 87 method has also been tested and recommended for extractable cationic nutrients 88 (Schöning and Brümmer, 2008; Stuanes et al., 1984) and for extracting PTEs and nutrients in biochar / biochar-amended soils (Alling et al., 2014; Karer et al., 2015; 89 90 Kim, 2015; Kloss et al., 2014b; Park et al., 2011). The proportion recovered by such 91 extractants has been described using various terms; in this study, the term

92 "available" will be used throughout.

93 Previous studies determining the available concentration of PTEs in feedstocks and

biochars have revealed that the pyrolysis process itself can immobilise various PTEs

- already present in the feedstock; this resulted in pyrolysis being recommended for
 waste treatment prior to landfilling (Farrell et al., 2013; Hwang and Matsuto, 2008;
- 97 Khanmohammadi et al., 2015; Liu et al., 2014; Meng et al., 2013). The
- 98 immobilisation was reported to result from different binding of PTEs to the carbon
- 99 lattice after pyrolysis and through increase in pH of the material when converted into

biochar (Gu et al., 2013; Liu et al., 2014). Yet, it remains unclear if biochars resulting

- 101 from feedstocks that are heavily contaminated with PTEs, e.g. plants grown on soil
- which exceed soil threshold values or PTE-contaminated anthropogenic wastes, aresuitable for amendment of soil and growing media.

104 In a previous study, the total concentrations of nutrients and PTEs were analysed in 105 19 marginal biomass-derived biochars and PTE concentrations were tested for 106 compliance with threshold values for total PTEs (Buss et al., 2016). In the current 107 study, cress germination and early seedling growth tests were conducted to assess 108 the risk of PTEs in biochar for plant growth. Furthermore, available PTEs were 109 determined using NH₄NO₃ and compared to German legislation threshold values. To 110 complete the risk-benefit analysis of application of marginal biomass-derived biochar 111 to soil and growing media, the availability of nutrients were determined to assess the 112 potential fertiliser value. In addition, the effect of highest treatment temperature 113 (HTT) and feedstock on percentage available of total PTEs and nutrients was 114 examined. Ultimately, the available elemental content of the biochars (and biochar 115 pH and EC values) were correlated with phytotoxic effects to identify the parameter

116 with the greatest potential to affect plant growth adversely.

117 2 Materials and Methods

118 **2.1 Biochars**

119 Nineteen biochars produced from 10 marginal biomass feedstocks from all major

biomass categories, including woody materials, grass, an aquatic plant and

121 anthropogenic wastes (non-virgin feedstocks), were used for this study. As all these

- materials were described in detail in Buss et al. (2016), only a short description is
- provided in Table 1. Feedstock effects were studied for all 10 biomasses where
 pyrolysis at 550°C was used as a typical medium HTT. To study the effects of
- pyrolysis at 550°C was used as a typical medium HTT. To study the effects of
 temperature, 2 feedstocks (ADX, DW) were pyrolysed at HTTs of 350, 450, 550, 650
- 126 and 750°C and 1 (WLB) was pyrolysed at 550°C and 700°C. In all cases, the
- 127 biochars were produced using the continuous screw pyrolysis unit described in Buss
- 128 et al. (2016). All biochars are termed according to their feedstock as abbreviated in
- 129 Table 1 and their respective production temperature (°C).
- 130 Table 1: Ten biomass feedstocks used for biochar production in this study.

feedstock	abbreviation
7 materials from contaminated land	
wheat straw (<i>Triticum aestivum</i>)	WSI
sugarcane bagasse (Saccharum spp., species unknown)	SBI
winter rye straw (Secale cereal)	WRB
willow logs with bark (salix spp., species unknown)	WLB
whole plant without roots of Salix purpurea	SLP
whole plant without roots of Paulonia tomentosa	PAT
whole plant without roots of Arundo donax	ADX
1 material from contaminated water water hyacinth (whole plant) (<i>Eichhornia crassipes</i>), originated from a waste water drain was sourced from close to Bhalswa Landfill Site (New Delhi, India)	WHI
2 non-virgin biomass	
solid residues from anaerobic digestion of food waste	FWD
demolition wood (heterogeneous, glued, laminated, painted, coated, or otherwise treated wood)	DW

133 2.2 Ammonium nitrate (NH₄NO₃) extractions

134 According to BS ISO 19730:2008 (2008) the recommended soil-to-NH4NO3-solution 135 ratio is 1:2.5 (m/v); however, due to its low bulk density and high water sorption 136 capacity, the ground biochar did not mix well with the small amount of water and the 137 mixture was too viscous to ensure proper extraction. Different solid-to-solution ratios 138 were tested and thorough mixing of the sample was ensured by using a ratio of 1:10 139 (m/v). In short, representative samples were taken from each biochar container by 140 taking sub-samples, grinding those with mortar and pestle and taking triplicate 141 aliquots. Next, the samples were weighed into 50 mL centrifuge tubes and 142 suspended in 1 mol L⁻¹ NH₄NO₃ (Fisher Scientific, laboratory reagent grade) using a 143 bench-top shaker (150 rpm for 2 h). Afterwards, the samples were centrifuged for 30 144 min at 3500 rpm and passed through Whatman No. 1 filter papers and then through 0.45 µm membrane filters (Millipore, Watford, UK). Reagent blanks were prepared 145 146 using the same procedure.

- 147 The extracts and reagent blanks were analysed by ICP-OES (Perkin Elmer Optima
- 148 5300DV). Details on the analytical method are as stated in Buss et al. (2016) with
- the following change: K was analysed in the radial mode of the instrument whilst AI,
- As, B, Ca, Cd, Co, Cr, Cu, Fe, Hg, Mg, Mn, Mo, Ni, P, Pb, Se and Zn were analysed
- 151 in the axial mode. ICP multi-element standard solution IV was used for the
- 152 calibration and ICP multi-element solution VI as independent calibration check
- throughout the measurements (Certipur[®], Merck). Limit of detections (LODs) for all
 elements were determined for the amended method and can be found in SI Table 1.
- 154 elements were determined for the amended method and can be found in ST rable
- 155 Details on how the LODs were calculated are stated in Buss et al. (2016).
- 156 Concentrations of elements in reagent blanks were subtracted from those for the 157 sample solutions and the data were expressed as the mass of available element 158 relative to mass of solid biochar (i.e. mg kg⁻¹ or g kg⁻¹ for elements present at high 159 concentration). The data were also converted to percentage availability using the
- 160 total elemental concentration data for the same biochar samples (Buss et al., 2016).
- 161 Details on the calculation can be found in the SI file.

162 2.3 Germination tests

- 163 Biochar phytotoxicity screening was performed according to Buss and Mašek (2014)
- 164 using 7-day 'all exposure routes' cress (*Lepidium sativum*) seed germination tests.
- 165 Each biochar sample was ground and incorporated in sterilised sand (sterilisation at
- 166 500°C for ~2 h) to give a 5% w/w biochar-sand mixture, the control was sterilised
- sand only. Cress seeds were either in direct contact with the biochar-sand mixture or
- 168 only exposed to the solution leaching through the mixture (set-up done in triplicates).
- 169 The effect of volatile organic compounds (VOCs) from the biochars on seedling
- growth was not tested here as previous work showed no phytotoxic effects even for
- 171 heavily VOC-contaminated biochars (Buss and Mašek, 2014).
- As in other studies (El-Darier and Youssef, 2000; Jones-Held et al., 1996),
- 173 germination was defined here as cracking of seed coating and visibility of root

174 growth. Seedling growth is reported to be more sensitive to PTEs than seed 175 germination which can lead to seeds with emerged radicle (root) but no growth of the 176 embryo (Li et al., 2005) and consequently, an intermediate stage between 177 germinated seeds and readily developed seedlings was distinguished, termed 178 "stunted seedlings". Stunted seedlings were defined as seeds with visible roots but a 179 root length of <5 mm (which was also used as the limit of quantification (LOQ)); this 180 has also been used by the US EPA (1996) as the threshold for "active growth by an 181 embryo". For all seeds with root length >5 mm (here called "healthy, non-stunted 182 seedlings"), shoots and roots were measured using image analysis (ImageJ) and the 183 difference compared to the sand-only control was calculated. Germination rate and 184 root growth was summarised in one parameter by calculating the Munoo-Liisa-185 Vitality index (MLV-index) which gives the percentage difference of the parameters 186 to performance of the seedlings in the sand only control (European Standard, 2011)

187 (for seedlings with roots <LOQ, 0.5 * LOQ was used).

188 2.4 Removal of available elements from biochar samples prior to 189 germination tests

After the phytotoxicity screening was performed, 9 biochars were selected for furthertesting. These included biochars which caused growth stimulation, growth

- 192 suppression and no effects (selected biochar can be found in the SI). The biochars
- 193 were extracted with 1 mol L^{-1} NH₄NO₃ as described in section 2.2. To remove excess
- salt solution, this process was followed by addition of 25 mL of DI water and shaking
- at 150 rpm for 2 h. Filtration was achieved using the protocol described in section 2.2
- and the biochar samples were pre-dried in an oven overnight at 50°C. The treated
- biochars were again tested in germination tests as described in section 2.3 to predict
- the effect that could be expected from the biochars after they have been exposed to
- the environment, e.g. after extractable nutrients and PTEs were removed by natural
- 200 leaching processes shortly after biochar application.

201 2.5 Data analysis

Available concentrations of 19 elements (if <LOD, 0.5 * LOD was used), pH and EC

- 203 (pH and EC both from Buss et al. (2016)) were correlated with percentage of healthy,
- 204 non-stunted seedlings using Pearson correlation (r) in R studio (Version 0.99.484,
- 205 https://www.rstudio.com/) and R² in excel. P-values were calculated and stated as
- following: p <0.05 are indicated as *, p <0.01 as ** and p-values <0.001 as ***.

207 3 Results and Discussion

- 208 In this study, the availability of 19 elements (PTEs and nutrients) in 19 biochars was
- 209 determined using 1 mol L^{-1} NH₄NO₃ -extractions followed by elemental analyses. The
- $\label{eq:210} amount of an element extracted by NH_4NO_3 will be referred to as "available$
- 211 concentration" when expressed on biochar mass basis (mg kg⁻¹, mg g⁻¹) (Table 2) or
- as "percentage available" (wt%) when expressed relative to the total concentration of
- 213 the given element present in each biochar sample.

Table 2: NH_4NO_3 -extractable (available) PTE concentrations of 19 biochars (mg kg⁻¹) as average and standard deviation (n = 3). All biochars are termed according to their feedstock as abbreviated in Table 1 and their respective production temperature (°C).

		AI	As	Cd	Со	Cr	Cu	Hg	Мо	Ni	Pb	Se	Zn
DW 350	mg kg ⁻¹	< 0.11	< 0.10	< 0.16	< 0.01	0.09 ± 0.05	0.12 ± 0.02	< 0.02	< 0.06	0.02 ± 0.01	< 0.04	< 0.23	2.01 ± 0.16
DW 450	mg kg ⁻¹	< 0.11	< 0.10	< 0.16	< 0.01	0.11 ± 0.05	0.10 ± 0.05	< 0.02	< 0.06	< 0.01	< 0.04	< 0.23	0.50 ± 0.04
DW 550	mg kg ⁻¹	< 0.11	< 0.10	< 0.16	< 0.01	0.10 ± 0.03	0.15 ± 0.06	< 0.02	< 0.06	0.06 ± 0.06	< 0.04	< 0.23	1.16 ± 0.11
DW 650	mg kg ⁻¹	< 0.11	< 0.10	< 0.16	< 0.01	0.14 ± 0.01	0.54 ± 0.08	< 0.02	< 0.06	0.22 ± 0.02	< 0.04	< 0.23	1.95 ± 0.16
DW 750	mg kg ⁻¹	2.60 ± 0.45	< 0.10	< 0.16	< 0.01	0.52 ± 0.24	1.93 ± 0.21	0.34 ± 0.04	0.18 ± 0.11	0.14 ± 0.02	0.13 ± 0.23	0.34 ± 0.10	3.45 ± 0.42
ADX 350	mg kg ⁻¹	2.75 ± 0.49	< 0.10	< 0.16	< 0.01	0.87 ± 0.44	0.29 ± 0.07	0.02 ± 0.02	< 0.06	< 0.01	< 0.04	0.63 ± 0.05	0.21 ± 0.09
ADX 450	mg kg ⁻¹	0.49 ± 0.17	< 0.10	< 0.16	< 0.01	0.10 ± 0.09	0.13 ± 0.01	< 0.02	0.06 ± 0.04	< 0.01	< 0.04	< 0.23	< 0.14
ADX 550	mg kg ⁻¹	0.88 ± 0.33	< 0.10	< 0.16	< 0.01	0.28 ± 0.12	0.09 ± 0.00	< 0.02	0.20 ± 0.01	< 0.01	< 0.04	< 0.23	< 0.14
ADX 650	mg kg ⁻¹	0.96 ± 0.11	< 0.10	0.21 ± 0.03	< 0.01	0.34 ± 0.06	0.15 ± 0.02	< 0.02	0.25 ± 0.05	< 0.01	< 0.04	< 0.23	< 0.14
ADX 750	mg kg ⁻¹	2.17 ± 0.01	< 0.10	0.36 ± 0.03	< 0.01	0.98 ± 0.02	0.29 ± 0.01	0.35 ± 0.09	0.35 ± 0.02	0.08 ± 0.04	< 0.04	0.66 ± 0.04	< 0.14
SBI 550	mg kg ⁻¹	1.39 ± 0.21	< 0.10	< 0.16	< 0.01	0.44 ± 0.04	< 0.02	< 0.02	0.18 ± 0.08	< 0.01	< 0.04	< 0.23	< 0.14
WHI 550	mg kg ⁻¹	1.95 ± 0.27	0.82 ± 0.35	0.27 ± 0.04	< 0.01	0.86 ± 0.10	0.18 ± 0.02	0.09 ± 0.07	0.79 ± 0.01	0.02 ± 0.01	< 0.04	0.69 ± 0.05	1.06 ± 0.25
WSI 550	mg kg ⁻¹	1.28 ± 0.43	0.62 ± 0.32	< 0.16 ±	< 0.01	0.59 ± 0.20	0.14 ± 0.01	0.13 ± 0.02	2.01 ± 0.16	< 0.01	< 0.04	0.99 ± 0.15	< 0.14
WLB 550	mg kg ⁻¹	< 0.11	< 0.10	< 0.16	< 0.01	< 0.03	0.17 ± 0.01	0.02 ± 0.04	< 0.06	0.12 ± 0.02	< 0.04	0.44 ± 0.20	24.28 ± 0.81
WLB 700	mg kg ⁻¹	1.34 ± 0.27	< 0.10	< 0.16	< 0.01	0.41 ± 0.10	0.14 ± 0.02	< 0.02	< 0.06	0.32 ± 0.11	0.48 ± 0.84	< 0.23	51.48 ± 0.97
WRB 550	mg kg ⁻¹	< 0.11	< 0.10	< 0.16	< 0.01	0.03 ± 0.03	0.16 ± 0.01	0.12 ± 0.05	4.54 ± 0.34	< 0.01	< 0.04	0.76 ± 0.11	46.19 ± 2.96
SLP 550	mg kg ⁻¹	1.01 ± 0.27	< 0.10	< 0.16	< 0.01	0.22 ± 0.12	0.17 ± 0.03	< 0.02	0.27 ± 0.01	< 0.01	< 0.04	< 0.23	7.47 ± 0.74
PAT 550	mg kg ⁻¹	0.64 ± 0.04	< 0.10	< 0.16	< 0.01	0.20 ± 0.05	0.14 ± 0.00	< 0.02	0.50 ± 0.05	< 0.01	< 0.04	0.42 ± 0.19	23.77 ± 1.64
FWD 550	mg kg ⁻¹	0.55 ± 0.34	< 0.10	0.24 ± 0.02	< 0.01	0.26 ± 0.23	0.21 ± 0.15	< 0.02	0.16 ± 0.02	0.03 ± 0.05	< 0.04	1.52 ± 0.14	0.66 ± 0.08
* 00 - 10 - 14	/		0.4	#0.4									•

* BBodSchV mg kg \cdot^1 0.4 #0.1 1 1.5 0.1 2 * German Federal Soil Protection Ordinance; Trigger values in agriculture for As, Cu, Ni and Zn in regards to growth inhibition of crops (Annex 2.4) and Cd, Pb in regards to crop quality (Annex 2.2), using NH₄NO₃ extraction

[#] Action value, if the plant species accumulates Cd strongly, a lower value of 0.04 mg kg⁻¹ is defined



Figure 1: Percentage available of the total PTE content (Cr, Ni, Cu and Zn) (Figure A, B) and nutrient content (Ca, K, Mg and P) (Figure C, D) as

- a function of pyrolysis temperature (highest treatment temperature) for biochars produced from demolition wood (A, C) and *A. donax* (B, D).
- 220 Availability was measured as percentage NH4NO3-extractable of the total elemental content.

3.1 Effect of pyrolysis HTT on percentage of PTE available

222 The effects of pyrolysis HTT on percentage availability (available concentration as 223 percentage of total elemental concentration in biochar) of typical PTEs (Cr, Cu, Ni 224 and Zn) and nutrients (Ca, K, Mg and P) were studied using biochars from demolition wood (DW) (Figure 1A) and a plant (A. donax, ADX) grown on contaminated soil 225 226 (Figure 1B). For biochars from both feedstocks, the percentage available of Cr, Ni, 227 Cu and Zn increased sharply when the HTT was increased from 650 to 750°C 228 (Figure 1A, B). Using the data for total and available elemental contents in 229 Khanmohammadi et al. (2015), Meng et al. (2013) and Yachigo and Sato (2013) to 230 calculate the % availability, confirms this trend. Khanmohammadi et al. (2015) 231 observed the same behaviour of Cr, Cu, Ni and Zn in sewage sludge biochars 232 pyrolysed at 5 temperatures between 300 and 700°C; the highest % availability was 233 detected at 700°C and it increased in particular when the HTT increased from 600 to 234 700°C. In Meng et al. (2013), Cu and Zn showed a higher percentage of availability 235 in biochars produced at 700°C compared to those produced at 400°C (DTPA 236 extraction) and, in Yachigo and Sato (2013), Cd and Zn showed higher % availability 237 in biochar produced at 800°C compared with that produced at 300°C (0.1 M HCI 238 extraction).

239 The influence of HTT on external metal sorption behaviour of biochar has previously 240 been explained as follows: biochars produced at low HTT possess more negative 241 surface charges and functional groups (higher CEC) which are reported to sorb 242 external cations strongly (chemisorption). For biochars produced at higher HTT, 243 however, chemisorption is reduced (due to reduced CEC) and external cations are 244 attached to biochar through electrostatic bonds which are weaker (Beesley et al., 245 2015). The same mechanisms responsible for sorption of external PTEs onto biochar 246 might also explain the sharp increase in the percentage availability of inherent PTEs 247 within biochar produced at 750°C. More mechanistic studies are needed to confirm 248 this hypothesis.

249 The curve of percentage availability with HTT displays a different shape in the two feedstocks, ADX-derived biochar showed a higher percentage available for PTEs at 250 251 a HTT of 350°C which was not visible in DW-biochar (Figure 1). This could be 252 related to the fact that the feedstock particle size of ADX prior to pyrolysis was bigger 253 (<30 mm) than for DW (<5 mm) (details on feedstock and biochar production in Buss 254 et al. (2016)) which, due to the relatively short residence time of 20 min, might have 255 resulted in only partial pyrolysis of ADX at 350°C. Indeed, the comparatively high 256 char yield and volatile matter content of ADX 350 compared to ADX 450 (Buss et al., 257 2016) and the generally higher H/C ratios of the ADX biochars compared to the DW 258 biochars (H/C ratio, SI Table 2) indicate a less complete carbonisation. Concluding 259 from this, it seems that ADX 350 behaved similarly to unpyrolysed material which 260 generally exhibits a higher percentage availability of PTEs compared to the resulting 261 biochar (Farrell et al., 2013).

In summary, it was shown that using medium HTTs is most suitable for production of biochars from contaminated feedstocks as the percentage availability of PTEs was lower than that observed at higher and lower HTTs. However, to further assess the risks posed by PTEs in biochar, their availability needs to be compared with the percentage availability obtained for other biochars and soils and, where they exist, with legislative threshold values.

Table 3: Percentage available PTEs and nutrients in n biochars as average (AV) and standard deviation (SD) determined as NH₄NO₃-extractable of the total elemental content. The number of biochars used for calculating the percentage availability is listed in the column with heading "n"; for biochars with total and available concentrations below the detection limit, no percentage available could be calculated.

		AV ± SD	n
PTEs			
AI	%	0.46 ± 0.68	19
Cr	%	4.09 ± 8.02	19
Cu	%	1.27 ± 1.33	19
Мо	%	23.8 ± 23.7	16
Ni	%	0.33 ± 0.48	19
Zn	%	1.32 ± 1.70	19
nutrients			
В	%	13.1 ± 16.1	19
Ca	%	28.3 ± 19.6	19
Fe	%	0.02 ± 0.02	19
K	%	47.7 ± 19.7	19
Mg	%	27.2 ± 22.2	19
Mn	%	4.30 ± 3.10	19
Р	%	10.8 ± 10.0	19

3.2 Average percentage availability of PTEs in all biochars

276 In relation to the total elemental content only $1.27 \pm 1.33\%$ of Cu, $0.33 \pm 0.48\%$ of Ni, 277 0.02 ± 0.02% of Fe, 1.32 ± 1.70% of Zn and 4.09 ± 8.02% of Cr was available (when 278 the 5 biochars from feedstock A. donax are not taken into account only $1.18 \pm 0.68\%$ 279 of the total Cr was available) (Table 3). Two recent studies on total and available 280 PTEs in various biochars obtained comparable results to those in this study: in 281 Khanmohammadi et al. (2015) 0.5-1.4% of the total concentration of Cu, Fe, Ni, Zn and Cr was extractable with 0.005 mol L⁻¹ DTPA and in Farrell et al. (2013) less than 282 283 1% of Ni, Cu, Cr and Zn was extractable with 1 mol L⁻¹ NH₄NO₃. Including this study, 284 typically less than 1.5% of the total concentrations of common PTEs in biochar were available. Clearly, PTEs are typically strongly sorbed to biochars but to place these 285 286 results in a wider context, further comparison must be made with the average 287 percentage availability of PTEs present in soils.

- 288 In Liebe et al. (1997), 335 soil samples from North Rhine-Westphalia (Germany) 289 from different land use types containing comparable total PTE concentrations to the 290 biochars in our study were extracted with 1 mol L⁻¹ NH₄NO₃. The pH of the soils 291 varied widely, while the biochar samples in our study all had pHs >7.5 (Buss et al., 292 2016). Elevated pH decreases the percentage availability of Cu, Cr, Ni and Zn and 293 consequently, the average percentage availability in soils with pH >7.5 was 294 calculated from Liebe et al. (1997). The availability (%) of Cu and Ni in 23 soils with 295 pH >7.5 (average pH 7.93 \pm 0.65, organic carbon content 2.94 \pm 1.93%) was not 296 significantly different to the average percentage available in the 19 marginal 297 biomass-derived biochars (p = 0.206, p = 0.108; two-sample, two-tailed t-test) and 298 the availability (%) of Cr and Zn was even significantly lower in soils (Cr: p = 0.037, 299 Zn: p = 0.012). From this it was concluded that biochars do not sorb PTEs more 300 strongly than soils do at similar pH values and confirms that the effect of biochar on 301 Cu, Cr, Ni and Zn immobilisation in soil can be mostly attributed to pH increase, e.g.
- 302 as shown in Houben et al. (2013).

303 3.3 Exceedance of threshold values for available PTEs in biochar

Threshold values for available As, Cd, Cu, Ni, Pb and Zn for soils for protecting crop guality and crop growth were established in the German Federal Soil Protection and

306 Contaminated Sites Ordinance (1999) (Table 2). Comparing the available

307 concentrations of As (mg kg⁻¹) for the biochars in this study with the German

- legislation threshold, only the As concentrations for biochars WHI 550 and WSI 550
 exceed the limit (Table 2). Both of these biochars showed very high availability of As
- 310 (close to 100%). This can be explained by the fact that both biochars have a pH of
- around 10 and the mobility of As is higher at elevated pH. This is a general problem,
- as addition of biochar, and subsequent increase of soil pH, could mobilise As that is
- 313 already present in the soil. This can lead to increased leaching of As into
- 314 groundwater and increased uptake by plants (Beesley et al., 2015; Kloss et al.,
- 315 2014a).

- The threshold value for available Cd (0.1 mg kg⁻¹) was exceeded by four biochars
- 317 (ADX 650, ADX 750, WHI 550 and FWD 550) by a factor of 2-3 (Table 2). However,
- 318 the biochars derived from plant biomass from Cd, Zn and Pb contaminated sites
- 319 (WLB 550, WLB 700, WRB 550, SLP 550 and PAT 550) and which significantly
- 320 exceeded biochar guideline values for total Cd (Buss et al., 2016), did not show
- detectable concentrations of Cd in NH_4NO_3 extracts (LOD 0.16 mg kg⁻¹). The
- 322 available Zn concentrations (in mg kg⁻¹), however, were far above the limit values for
- the 5 biochars despite the fact that the average percent availability of Zn in all
 biochars was only 1.32 ± 1.70% (Table 3). Despite exceeding German soil threshold
- biochars was only $1.32 \pm 1.70\%$ (Table 3). Despite exceeding German soil threshold values for available Zn, application of Zn-rich biochar as soil amendment can be
- 326 beneficial for plant growth in Zn-deficient soils as Zn is a micronutrient and is
- intentionally added to some fertilisers (see section 3.8) (Beesley et al., 2010;
- 328 Evangelou et al., 2014; Rogowski et al., 1999).
- 329 The concentration of available PTEs is relevant when effect on plants are concerned,
- 330 yet, legislation and guideline threshold values are mostly based on total
- concentrations, consequently, the exceedance of threshold values for total and
- available concentrations were compared for two biochars. DW 750, which only
- exceeded the threshold value for total Cr (Buss et al., 2016), exceeded the threshold
- values for available Cu, Pb and Zn (Table 2). This might be related to the fact that
- the metals in demolition wood were concentrated close to the surface, where paints
- and other coatings were applied and therefore were easy to extract. WHI 550, on the
- 337 other hand, had the highest values for total concentrations for most PTEs but the
- available concentrations were very low, only 2 threshold values were slightly
 exceeded (As, Cd). These two examples confirm that total concentrations in biochar
- 340 do not relate to available concentrations and highlight the need to investigate the
- 341 availability in biochars from different feedstocks separately. For risk assessment, the
- available concentrations are to be determined, therefore, threshold values should
- 343 also be based on available concentrations.

344 3.4 (Percentage) availability of K, Ca and Mg in biochar

- 345 Besides PTEs, biochars contain potentially beneficial elements, such as the 346 macronutrients K, Ca and Mg. For assessing the value of biochar as fertiliser, the
- 347 concentration of available nutrients is of primary importance.
- 348 K was the most available of all elements; $47.7 \pm 19.7\%$ of the total K was extractable with 1 mol L⁻¹ NH₄NO₃ (Table 3), which is similar to what was reported in Ippolito et 349 350 al. (2015) for various biochars and extraction techniques. The percentage availability 351 of K increased when the HTT was increased from 650°C to 750°C in both feedstocks 352 (Figure 1). A similar effect was observed by Wu et al. (2011) (300-750°C) and Singh 353 et al. (2010) (400, 550°C). Around 30% of the Mg and Ca in biochar were available 354 (Table 3), however the availability showed different trends with HTT. While the 355 percent of available Ca decreased slightly with increasing HTT in biochars from both 356 feedstocks, the percent of available Mg decreased with increasing HTT in ADX-

biochars and remained constant in the range 350-650 for DW-biochars, increasing at
750°C (Figure 1).

- Available K, Ca and Mg concentrations on biochar mass basis were between 0.3-30
- 360 g kg⁻¹ (highest for WHI 550, WSI 550 and WRB 550), 1.3-5.6 g kg⁻¹ and 0.03-1.2 g
- 361 kg⁻¹, respectively (Table 4) which is in a similar range to cow manure and poultry
- 362 litter biochars (K 14-18 g kg⁻¹, Ca 0.5-2.5 g kg⁻¹ and Mg 0.5-1.3 g kg⁻¹) (Singh et al.,
 363 2010).
- 364 Ippolito et al. (2015) calculated the application rate of different biochars needed to
 365 satisfy the K and P demands of corn plants based on concentrations of available
 366 nutrients in biochar ("medium soil", 67 kg ha⁻¹ K₂O and P₂O₅), which was between 20
- t ha⁻¹ (turkey litter biochar) and 145 t ha⁻¹ (softwood pellets biochar) for P and 1.8 t
- 368 ha⁻¹ (papermill waste biochar) and 41.4 t ha⁻¹ for K (hazelnut biochar). Applied to the
- biochars from this study, this would correspond to an application rate of only 1.2 to
- 370 2.6 t ha⁻¹ of ADX 650/750, WSI 550, WHI 550, SLP 550, PAT 550 and FWD 550 to
- 371 satisfy the K demands of the same corn plants and these biochars would also
- provide high amounts of available Ca and Mg. This emphasises the suitability of
- 373 marginal biomass-derived biochars for provision of cationic nutrients to plants.

374 **3.5 (Percentage) availability of P in biochar**

- Like K, Ca and Mg, P is also a plant macronutrient and is needed by plants in comparatively high amounts (Kirkby, 2011). For the biochars investigated here, the percentage availability of P decreased with pyrolysis HTT (Figure 1) which was also reported in literature for biochar produced from swine manure (Meng et al., 2013), *A. donax*, (Zheng et al., 2013) and biosolids (Wang et al., 2012). This was ascribed to assumed structural changes and resulting stabilisation of P / transformation into a less soluble form.
- 382 Between 0.10 and 34.0% (SI Table 3) and on average 10.8 ± 10.0% (Table 3) of the
- total P was available. In the literature, various methods have been tested for
- 384 measuring the available content of P in biochars and waste products, such as
- deionised water, 2% citric acid, 2% formic acid, 0.5 M NaHCO₃ (Olsen-P),
- ammonium acetate, ammonium citrate (Brod et al., 2015; Wang et al., 2012; Weber
- 387 et al., 2014). Although studies that used NH_4NO_3 for extracting P could not be found
- and NH₄NO₃ is not an established method for extracting P from biochar, the
- 389 percentage of available P determined by NH_4NO_3 was in the same range as reported
- for other extractants. E.g. in Singh et al. (2010) Olsen-P per total P was between not detectable to 40% (wood, leave, poultry litter), water soluble of total concentrations in
- 392 (composted) swine manure biochars were between 0.3-25.5% (Meng et al., 2013)
- 393 and for numerous other biochars, available of total P was between 0.4-34%
- 394 determined by various extraction methods (Ippolito et al., 2015).
- 395 FWD 550 was the biochar with the highest total P concentrations by far (Buss et al.,
- 396 2016), but the available concentration was only 20 mg kg⁻¹ (Table 4), which
- 397 corresponded to 0.10% of the total P, by far the lowest percentage of P available in

398 all biochars (SI Table 3). FWD 550 also had the lowest percentage of available Ca 399 (SI Table 3). A plausible explanation for this is as follows: it was reported that P is 400 mostly bound as Ca-phosphates in biochar (Bridle and Pritchard, 2004; Wang et al., 2012) which are initially extracted by the 1 mol L^{-1} NH₄NO₃ solution (pH of solution 401 402 4.6). However, with the gradual increase in solution pH due to the high pH of the 403 biochars, we suggest that Ca-phosphates increasingly precipitated (Goss et al., 404 2007) and were filtered from the solution during preparation for analysis, which was 405 also observed in Xu et al. (2013). Generally, at high concentrations of Ca and P 406 (FWD had the highest total concentrations of P and Ca), more ions are present in 407 solution to react to form Ca-phosphates. This resulted in a very low measured 408 percentage availability of P and Ca in FWD 550. The same would not necessarily 409 occur when total biochar Ca and P concentrations are low, as there would be less 410 present to extract and therefore fewer ions in the extraction solution to react and 411 precipitate, resulting in more reliable analysis results. This phenomenon could also 412 be responsible for the generally low measured availability of P in other biochars (particularly those using unbuffered and non-acidic extractants), and the percentage 413 414 of available P not having exceeded 40% in numerous studies. 415 While WRB 550 had the highest available P concentrations by far and only 16.6 t ha 416 ¹ would need to be applied to satisfy the P requirements in a "medium soil" (Ippolito 417 et al., 2015), 1458 t ha⁻¹ of FWD 550 would be needed to provide sufficient available 418 P. In contrast, only 2.6 t ha⁻¹ of FWD 550 would be needed to supply K (FWD 550

419 available concentrations: 14 g kg⁻¹ K, 5.6 g kg⁻¹ Ca, 10 g kg⁻¹ Mg and 0.02 g kg⁻¹ P).

- 420 Despite generally comparatively low concentrations of available P in biochar, some
- 421 studies did show that certain biochar can be used as P-fertiliser with high agronomic
- 422 efficiencies, in some instances even performing better than mineral fertilisers, which
- 423 indicates that the extraction techniques used so far might not reflect the amount of P
- 424 available to plants (Wang et al., 2012; Weber et al., 2014).

		В	Са	Fe	К	Mg	Mn	Р
DW 350	mg kg ⁻¹	< 0.02	1911.81 ± 131.84	0.12 ± 0.04	264.04 ± 4.40	36.42 ± 1.57	6.09 ± 0.16	15.36 ± 0.37
DW 450	mg kg ⁻¹	< 0.02	1981.25 ± 83.59	< 0.01	247.41 ± 4.08	26.76 ± 1.21	6.47 ± 0.06	14.28 ± 0.13
DW 550	mg kg ⁻¹	< 0.02	1828.74 ± 174.81	< 0.01	308.62 ± 14.90	26.54 ± 1.75	10.29 ± 0.63	8.47 ± 0.48
DW 650	mg kg ⁻¹	< 0.02	1627.32 ± 52.09	< 0.01	754.54 ± 6.38	49.83 ± 3.29	8.87 ± 0.38	13.21 ± 0.48
DW 750	mg kg ⁻¹	6.80 ± 1.14	1731.43 ± 90.58	< 0.01	1945.05 ± 88.55	333.39 ± 22.24	29.52 ± 1.41	4.34 ± 0.23
ADX 350	mg kg ⁻¹	1.56 ± 0.57	1566.53 ± 83.58	0.80 ± 0.05	11396.19 ± 567.74	468.17 ± 25.09	3.63 ± 0.40	267.61 ± 17.12
ADX 450	mg kg ⁻¹	< 0.02	2625.05 ± 70.22	0.69 ± 0.03	17119.33 ± 222.85	810.66 ± 18.10	1.41 ± 0.04	455.52 ± 9.35
ADX 550	mg kg ⁻¹	0.42 ± 0.38	2381.03 ± 92.87	0.59 ± 0.06	17214.35 ± 380.96	841.69 ± 23.84	2.35 ± 0.07	427.48 ± 15.59
ADX 650	mg kg ⁻¹	< 0.02	2150.60 ± 47.24	0.38 ± 0.02	19409.80 ± 202.94	768.22 ± 11.50	2.74 ± 0.05	357.60 ± 8.68
ADX 750	mg kg ⁻¹	1.16 ± 0.48	1524.37 ± 29.54	0.17 ± 0.06	27071.02 ± 886.79	661.31 ± 4.82	2.26 ± 0.05	230.84 ± 5.73
SBI 550	mg kg ⁻¹	0.27 ± 0.47	1323.06 ± 35.58	3.19 ± 0.36	7123.26 ± 242.66	1156.43 ± 98.67	8.59 ± 0.23	390.23 ± 27.71
WHI 550	mg kg ⁻¹	6.90 ± 0.16	5118.32 ± 143.48	< 0.01	29827.20 ± 647.63	973.44 ± 37.05	9.08 ± 0.17	158.27 ± 11.68
WSI 550	mg kg ⁻¹	3.87 ± 0.15	2109.14 ± 89.68	0.12 ± 0.01	26794.53 ± 461.14	805.64 ± 29.67	1.86 ± 0.04	107.34 ± 0.48
WLB 550	mg kg ⁻¹	2.01 ± 0.29	2830.15 ± 163.09	0.25 ± 0.01	2524.05 ± 364.50	228.01 ± 10.12	0.96 ± 0.05	241.54 ± 13.06
WLB 700	mg kg ⁻¹	5.58 ± 0.03	2732.63 ± 86.83	0.88 ± 0.76	4511.81 ± 115.88	494.04 ± 13.68	1.68 ± 0.05	212.53 ± 6.22
WRB 550	mg kg ⁻¹	6.32 ± 0.51	1496.45 ± 76.67	1.84 ± 0.09	31751.74 ± 715.76	77.51 ± 4.15	0.79 ± 0.04	1759.49 ± 82.86
SLP 550	mg kg ⁻¹	12.38 ± 1.08	4608.27 ± 192.06	0.83 ± 0.15	14721.49 ± 790.28	1115.42 ± 65.59	2.73 ± 0.01	238.55 ± 15.11
PAT 550	mg kg ⁻¹	14.10 ± 0.66	3794.41 ± 130.56	2.76 ± 0.06	24696.58 ± 719.37	1240.34 ± 27.39	1.91 ± 0.09	70.09 ± 1.94
FWD 550	mg kg ⁻¹	2.72 ± 0.26	5582.42 ± 351.53	< 0.01	14123.90 ± 378.96	996.97 ± 49.30	2.96 ± 0.13	20.06 ± 1.40

425 Table 4: NH_4NO_3 -extractable (available) nutrient concentrations of 19 biochars (mg kg⁻¹) as average and standard deviation (n = 3).

427 **3.6 Effect of biochars on germination and early seedling growth**

428 **3.6.1 Growth promoting effects of biochars**

- 429 Of the 19 biochars tested, 8 showed significant shoot growth-promoting effects on
- 430 cress seedlings in direct contact with the biochar-sand mixture (Figure 2B). In 4
- 431 treatments, cress seedlings only exposed to the solution leaching through biochar-
- 432 sand mixtures also displayed significantly longer shoots (Figure 2A). Besides shoot
- growth, root growth was also stimulated, reflected by >100% Munoo-Liisa Vitality
 indices (MLV-indices) which takes into account root growth and germination rate
- 434 Indices (MLV-Indices) which takes into account root growth and germination rate
- 435 (Table 5).
- 436 Improvements of physical soil properties by biochar can mostly be excluded as the
- 437 reason for the stimulation of seedling growth, because seedlings also showed
- 438 improved growth when only exposed to the solution leaching through the biochar-
- sand-mixture. Although nutrients may have been partially responsible for the growth
- 440 promoting effects, these cannot explain effects observed in the case of DW biochars.
- 441 Four of the five DW-biochars significantly increased shoot length, despite having
- 442 comparatively low available nutrient concentrations (Table 4) and in particular, DW
- 443 550 showed striking stimulation of shoot growth, which cannot be associated with
- 444 available nutrients.
- 445 Overall, DW 550, SBI 550 and FWD 550 increased shoot length significantly in
- seedlings in either direct contact with biochar-sand or exposed to biochar leachate.
- 447 FWD 550 and DW 550 stimulated the growth by 60-80% in the 7-day cress test
- 448 compared to the control (Figure 2A, B). While the biochars from demolition wood
- 449 produced at 5 HTTs showed strong growth promoting effects which peaked at
- 450 medium HTT, ADX-derived biochars inhibited seedling growth with increasing HTT
- 451 (in ADX 350 seedlings could fully develop, while in ADX 750 100% of the seedling
- 452 showed stunted growth, Table 5).

453 **3.6.2 Growth suppression effects of biochars**

- 454 Germination rate (cracked seed coatings and visible roots) was barely affected by 455 any of the biochars; it was ~100% in almost all cases, with the exception of WRB 456 550 and PAT 550 where germination rate was only 80-90% (SI Table 4). As also 457 observed in Li et al. (2005), however, early root growth extension was significantly 458 inhibited by five of the 19 biochars, all of which were derived from biomass from 459 PTE-contaminated land (ADX 650 / 750, WSI 550, WRB 550 and PAT 550). This 460 resulted in a reduction of healthy seedlings (roots >5 mm) to only 0-60% when in 461 direct contact with biochar-sand or when exposed to biochar-sand leachate (Table 462 5). Seedlings were able to germinate but their further development was immediately 463 and strongly impeded, and the seedlings that did grow further showed reduced shoot
- 464 (Figure 2) and root growth (MLV-indices, Table 5).
- 465 To test the nature and persistence of the growth-suppressing effects after a
- simulated leaching event, nine of the biochars, including the ones showing highest
- 467 suppression, were washed with DI after NH_4NO_3 -extraction and re-tested in the

468 same germination experiment. The results revealed that for ADX 750 and WSI 550

- the growth suppression was alleviated (germination rate, roots >5 mm and shoot
 length not significantly different to control; SI Table 2 and SI Figure 1). On the oth
- 470 length not significantly different to control; SI Table 2 and SI Figure 1). On the other
 471 hand, in case of WRB 550 significant inhibitive effects remained, ~50% of the
- 472 seedlings were stunted and the shoot growth was reduced by around 40%.
- 473 Generally, the MLV-index was lower in the biochar treatments than in the sand only
- 474 controls most probably resulting from residues of NH₄⁺ which caused toxicity to the
- 475 roots of cress which belongs to a plant family that reacts sensitive to NH₄⁺ (Britto and
- 476 Kronzucker, 2002). Overall, it can be concluded that leaching which would occur
- 477 under natural conditions does alleviate some, but not all, of the toxic effects caused
- by the investigated biochars. The next step was to find out what caused the inhibition
- 479 of growth of cress seeds in the samples in the first place.
- 480
- 481 Table 5: Percentage of seedlings with roots >5 mm ("healthy, non-stunted
- 482 seedlings") as average and standard deviation, and Munoo-Liisa-Vitality-Index (%) of
- 483 19 biochars tested in 'all exposure routes' germination tests. Seeds were only
- 484 affected by leachate from biochar-sand or were in direct contact with the mixture.
- 485 Results for biochars were compared to the control using two sample, two tailed t-
- 486 tests. P-value: <0.05 = * , <0.01 = ** , <0.001 = ***.

	leacha	te affecte	ed only	direct contact seeds-biochar			
	roots >5	5 mm	MLV-index	roots >5 mm	MLV-index		
	%	%	%	% %	%		
DW 350	100.00	± 0.0	131.1	100.0 ± 0.0	119.8		
DW 450	96.3	± 6.4	107.1	100.0 ± 0.0	117.2		
DW 550	100.0	± 0.0	158.5	98.6 ± 2.4	111.0		
DW 650	95.3	± 4.8	101.6	100.0 ± 0.0	114.5		
DW 750	100.0	± 0.0	142.8	100.0 ± 0.0	96.7		
ADX 350	99.0	± 1.8	142.1	96.5 ± 3.3	172.8		
ADX 450	* 86.4	± 7.1	55.3	75.8 ± 16.2	53.2		
ADX 550	76.6	± 26.5	42.6	76.5 ± 31.0	58.6		
ADX 650	* 59.1	± 24.0	26.2	*** 49.0 ± 3.2	21.2		
ADX 750	*** 12.2	± 10.8	6.5	*** 0.0 ± 0.0	7.5		
SBI 550	100.0	± 0.0	160.1	97.5 ± 4.3	108.8		
WHI 550	98.7	± 2.2	89.5	100.0 ± 0.0	109.3		
WSI 550	55.9	± 33.2	25.7	** 31.4 ± 18.8	18.5		
WLB 550	89.6	± 15.1	81.8	97.8 ± 1.9	101.8		
WLB 700	93.5	± 2.8	58.5	100.0 ± 0.0	85.0		
WRB 550	*** 0.0	± 0.0	3.7	*** 0.0 ± 0.0	7.0		
SLP 550	93.2	± 7.8	51.0	100.0 ± 0.0	93.2		
PAT 550	** 21.7	± 21.4	6.3	*** 0.0 ± 0.0	7.7		
FWD 550	96.0	± 4.2	123.2	100.0 ± 0.0	117.0		



488

489 Figure 2: Shoot length of cress seedlings compared to control (%) after exposure to 490 5% biochar in sand for 7 days. (A) shows the results from seeds only being affected 491 by leachate from the mixture and (B) shows the seeds which were exposed to 492 biochar-sand. Results for biochars were compared to the control using two sample, 493 two tailed t-tests. LOQ = limit of quantification, * significant difference with p < 0.05, 494 ** with p <0.01, *** with p <0.001, # not statistically tested because only two of the replicates showed growth and one replicate had 100% below limit of quantification 495 496 (LOQ).

498 3.7 Correlating plant response with biochar characteristics 499 (available elemental concentrations, pH and EC)

500 Measuring the concentrations of available PTEs and conducting plant tests is a 501 means of risk assessment; to be able to take appropriate risk management 502 measures to avoid the toxic effects of biochar, however, the underlying reasons need 503 to be understood. Consequently, the performance of biochars in cress germination-504 and growth tests (percentage of healthy, non-stunted seedlings) was correlated with 505 the available elemental concentrations of all 19 elements and with biochar pH and 506 electrical conductivity (EC) (determined in Buss et al. (2016)) to identify the 507 parameter that most likely affected the cress seedling growth adversely.

508 In the plant tests, the phytotoxicity of the ADX-biochars increased linearly with HTT 509 (Table 5, Figure 2). The availability of most PTEs in ADX-biochar, however, did not 510 increase with HTT, except for Mo which increased from <0.06 mg kg⁻¹ in ADX 350 to 511 0.35 mg kg⁻¹ in ADX 750 (Table 2). Indeed, correlating the percentage of healthy, 512 non-stunted seedlings with available Mo concentrations for the whole set of 19 513 biochars showed a significant negative, linear correlation (Table 6). It is reported that 514 phytotoxic effects caused by Mo are very uncommon (Gupta and Gupta, 1998; 515 Kabata-Pendias, 2011; Kaiser, 2005; MacNicol and Beckett, 1985), yet, Mo-related 516 inhibitions were observed in some studies: the lowest concentration that showed 517 toxic effects on pea plants and in various other plants in solution was 0.96 mg L⁻¹ Mo 518 (0.01 mmol L⁻¹) and 1-2 mg L⁻¹, respectively (Kevresan et al., 2001; McGrath et al., 519 2010). In the germination tests conducted in our study, a water-to-biochar ratio of 520 1:14 was used, while the extractions were performed with a ratio of biochar-to-521 NH₄NO₃-solution of 1:10 and consequently, the Mo concentrations to which the 522 seeds were exposed were comparable to the concentrations detected in our 523 NH₄NO₃-extracts (concentrations in the raw extracts 10 fold lower than in Table 2). In 524 Kevresan et al. (2001) and McGrath et al. (2010) inhibitory effects started at ~1 mg L⁻ 525 ¹, while in this study biochars with Mo concentrations in the NH₄NO₃-extracts of 526 0.035 mg L⁻¹ (ADX 750) totally inhibited early seedling growth in direct contact with 527 biochar. In conclusion, while it cannot be entirely excluded that Mo has contributed to 528 the total inhibition of early seedling growth, it seems highly unlikely. Instead this could be a case of wrongly interpreted cause-effect relationship. The available 529 530 concentration of Mo is not the cause for the toxicity but it is a symptom of the high 531 pHs of these biochars. Therefore, it is the elevated pHs that caused the observed 532 growth suppression effects. Indeed, biochar pH (determined in Buss et al. (2016)) 533 showed a similarly high negative correlation with healthy, non-stunted seedlings as 534 the available Mo concentration observed in this study (Table 6).

Henig-Sever et al. (1996) and Singh et al. (1975) showed that solutions with pH in
the range 7-9 reduced germination rates in most plant species and by pH of 10-11,
total inhibition was observed in most cases. Singh et al. (1975) suggested that the
germination rate-response to pH followed a 2nd order polynomial curve, and

539 therefore, a linear correlation (Pearson) does not describe the relationship between

- 540 pH and growth response appropriately. Tested on our data set, we found that indeed 541 a 2^{nd} order polynomial curve fitted very well with the plant response (Figure 3A: $R^2 =$ 542 0.63, Figure 3B: $R^2 = 0.68$). Investigation of the causes of relatively high pH of the 543 biochar used in this study showed that it can be attributed mainly to potassium salts, 544 e.g. potassium carbonate, as potassium was the element with by far the highest 545 available elemental concentration in all biochars (Table 2, Table 4).
- 546 Consequently, K most likely caused indirect inhibition of plant growth by increasing 547 the pH in solution. Yet, the available K concentration itself shows an even higher significant correlation with seedling growth than pH and a better 2nd order polynomial 548 549 fit, in fact available K displays the best fit of all parameters tested (r = -0.728, p 550 <0.001) (Table 6, Figure 3C, D). However, the only direct, adverse effect reported for 551 K excess is reduced uptake of other nutrients, which should not affect the early 552 seedling growth, where nutrients are mostly provided by the seed itself (Butnan et 553 al., 2015; Hawkesford et al., 2011). Consequently, the most likely mechanism 554 responsible for growth inhibition caused by available K, as for pH, is an indirect 555 mechanism, an increase in osmotic pressure. EI-Darier and Youssef (2000) in their study on effects of different salt concentrations on cress seeds, reported that due to 556 557 the osmotic pressure of a solution containing >50 mmol L¹ NaCl (100 mmol L¹ 558 active ions) the shoot and root length were significantly reduced. In the current study, 559 the 4 biochars that caused the highest inhibition had concentrations of K of ~3,000 560 mg L⁻¹ in NH₄NO₃-extracts (concentrations in the raw extracts 10 fold lower than in 561 Table 4) which corresponds to 77 mmol L⁻¹. Assuming K dissolution as potassium 562 carbonate or chloride, the active concentrations of ions resulting from this would be 231 and 154 mmol L⁻¹, respectively, which is well in the range where reductions of 563 cress seedling growth have been reported. 564
- 565 As electric conductivity (EC) is often used as a proxy for osmotic potential of a 566 solution, we assessed it as a potential indicator of plant response. Statistical analysis 567 showed that EC showed a comparatively low Pearson correlation (Table 6) and R² (not shown) with seedling growth, much lower than that shown by the available K 568 569 concentration. This is attributed to the fact that, while ions in solution contribute to 570 EC to different extents, depending on type of ion and its charge, in case of osmotic 571 potential / pressure, which is the actual factor affecting seedling growth, only the 572 quantity of solute per unit volume of solution (molarity) is relevant (Richards, 1954). 573 Consequently, EC is not necessarily a good predictor for the inhibition of germination 574 and early seedling growth, while molarity of the solution is. In conclusion, we showed 575 that it was the osmotic potential of the solution and partially the high pH (both of which are mostly a result of dissolved K) that were the primary causes of observed 576 phytotoxicity in this study, and not the PTEs contained in the biochar. 577

578 **Table 6**: Pearson correlation coefficient (r) of available elemental content, pH and EC 579 of 19 biochars with percentage of seedlings with roots >5 mm ("healthy, non-stunted

579 of 19 biochars with percentage of seedlings with roots >5 mm ("healthy, non-stur 580 seedlings") for leachate affected seeds and seeds in direct contact with sand-

581 biochar. Only parameters with significant effect shown.

	leachai s	te affected eeds	direct contact seeds- biochar		
	r	p-value	r	p-value	
K	-0.729	***<0.001	-0.749	***<0.001	
Мо	-0.660	**0.002	-0.608	**0.006	
Р	-0.573	*0.010	-0.478	*0.038	
EC	-0.471	*0.042	-0.484	*0.036	
pН	-0.615	**0.005	-0.627	**0.004	





Figure 3: Regression of available K concentration and pH of 19 biochars with percentage of roots >5 mm ("healthy, non-stunted seedlings"). Biochar pH (determined in solution in liquid-to-solid ratio of 20:1) is shown with (A) seedlings affected by biochar-sand leachate and (B) seedlings in direct contact with biochar-sand. Available K concentration in biochar (determined by NH₄NO₃ extraction) is depicted with (C) seedlings affected by biochar-sand leachate and (D) seedlings in direct contact with biochar-sand.

3.8 Use of biochars from marginal biomass for amendment of soil or as ingredients in growing media

590 For the use of biochar for amendment of soil and in growing media, biochar has to 591 comply with environmental, health and safety legislations and cannot pose a threat 592 for plant growth. On the contrary, it needs to offer beneficial properties, such as the 593 provision of nutrients.

594 Overall, in this study, all biochars with agronomically viable concentrations of available cationic nutrients also contained concentrations of available PTEs which 595 596 exceed the soil threshold values for protection of crop growth of the German Federal 597 Soil Protection and Contaminated Sites Ordinance (1999). However, the threshold 598 values are limits for soil and not soil amendments. Consequently, where pure 599 biochars exceeded threshold values, incorporation in soil at <1% (<20 t ha⁻¹) results in a dilution of 100-fold and consequently, available PTEs would not exceed the limit. 600 601 Furthermore, comparing the total PTE concentrations to commercially available 602 fertiliser products shows that the concentrations of As, Cd, Cr and Ni are much 603 higher in inorganic fertilisers than in the biochars investigated here and Zn is even 604 added intentionally to inorganic fertilisers to supply Zn for Zn-deficient soils (Rogowski et al., 1999). Therefore, although the compliance / non-compliance of 605 606 respective biochars with legislation would need to be decided by the responsible governmental bodies, considering the available concentrations, PTEs do not seem to 607 608 be of any concern. More importantly, the phytotoxic effects observed in this study 609 could not be correlated with available PTEs concentrations.

610 Five of the 19 biochars did adversely affect growth in germination tests (linked to 611 high pH and high content of available K), while 8 showed significant growth stimulating effects, even in these high application rates (5 wt%, corresponding to 612 613 >100 t ha⁻¹, depending on soil and application type). Consequently, some of the 614 tested biochars would not be suitable for application in high concentrations, e.g. in 615 growing media, without causing phytotoxic effects. However, the application rates 616 used in this work were unrealistically high from the perspective of agricultural 617 application (these were selected intentionally high to exacerbate negative effects of 618 PTEs), and therefore application in lower, practically relevant application rates (1-10 619 t ha⁻¹) would result in smaller increases in pH and lower additions of K, and would 620 therefore most likely result in growth stimulating effects. This application rate would 621 also not elevate the available PTE concentrations in soil above the threshold values.

622 4 Conclusions

623 In this study, 19 biochars produced from marginal biomass feedstocks, representing 624 all major biomass categories, were investigated to assess their content of available 625 PTE and nutrients, focussing on any plant growth promoting or suppressing effects. 626 The study confirmed that total concentrations of PTEs are not reliable predictors for available concentrations of PTEs nor for the potential of respective biochars to cause 627 628 adverse plant effects. Furthermore, it was concluded that in the investigated biochar 629 set inherent Cu, Cr, Ni and Zn were bound to biochar with similar strength to that of 630 soil at a similar pH (>7.5). This new finding has significant implications for designing 631 biochars for immobilisation of PTEs in soil. The study also showed that only the 632 highest HTT used, 750°C, increased the availability of most PTEs and decreased the 633 availability of several nutrients, meaning that even biomass with high PTEs content 634 could safely be processed in a wide range of temperatures. In terms of plant 635 responses, eight of the 19 biochars studied significantly increased early seedling 636 growth, while 5 biochars suppressed growth. The phytotoxic effects showed only 637 poor correlation with available PTEs, but a strong correlation with pH and available K 638 concentration. We hypothesised that available K increased the osmotic pressure 639 causing plant growth inhibition. Consequently, in this study, where relatively high 640 biochar application rates were used, the high available K concentrations and the high 641 pH were responsible for seedling growth inhibitions. However, we concluded that, 642 were such biochars used at lower application rates, both factors (available K and pH) 643 would contribute to growth promoting effects and would be among the most 644 important assets of these biochars. Although much more research on short and long-645 term effects of PTE-rich biochars on other plants and soil organisms and in a variety 646 of soils is needed, this study showed that most marginal biomass-derived biochars 647 have good potential to be used as nutrient source for plants. Most importantly, it 648 showed that they have low potential to cause adverse effects despite increased 649 content of PTEs. Based on this we suggest revision of guidelines for application of 650 biochar and other materials to soil, to reflect the true risks posed by different 651 materials, and not simply base such judgments on the total content of PTEs.

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