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1 Risks and benefits of marginal biomass-derived biochars 2 for plant growth

3 Wolfram Buss^a, Margaret C. Graham^b, Jessica G. Shepherd^{ab}, Ondřej
4 Mašek^{a*}

5 ^a UK Biochar Research Centre, School of Geosciences, University of Edinburgh, Crew Building, Alexander Crum
6 Brown Road, Edinburgh, EH9 3FF, UK

7 ^b School of Geosciences, University of Edinburgh, Crew Building, Alexander Crum Brown Road, Edinburgh, EH9
8 3FF, UK

9 *Correspondence: Ondřej Mašek, tel. 0131 6505095, fax +44 131 662 0478, e-mail: ondrej.masek@ed.ac.uk

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 NH_4NO_3 -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
22 similar pH values which is a highly important finding. Most biochars exceeded
23 German soil threshold values for NH_4NO_3 -extractable PTEs, such as Zn (by up to
24 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
29 concentration of all elements and the highest percentage availability ($47.7 \pm 19.7\%$ 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% ($>100 \text{ t ha}^{-1}$), when applied at agriculturally realistic
34 application rates ($1\text{-}10 \text{ t ha}^{-1}$), 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-
57 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
65 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
73 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

75 the 'bioavailable' fraction and, since it usually does not correlate with total elemental
76 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
89 nutrients in biochar / biochar-amended soils (Alling et al., 2014; Karer et al., 2015;
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
94 biochars have revealed that the pyrolysis process itself can immobilise various PTEs
95 already present in the feedstock; this resulted in pyrolysis being recommended for
96 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
100 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
102 which exceed soil threshold values or PTE-contaminated anthropogenic wastes, are
103 suitable 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
 120 biomass categories, including woody materials, grass, an aquatic plant and
 121 anthropogenic wastes (non-virgin feedstocks), were used for this study. As all these
 122 materials were described in detail in Buss et al. (2016), only a short description is
 123 provided in **Table 1**. Feedstock effects were studied for all 10 biomasses where
 124 pyrolysis at 550°C was used as a typical medium HTT. To study the effects of
 125 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 (<i>Saccharum</i> spp., species unknown)	SBI
winter rye straw (<i>Secale cereal</i>)	WRB
willow logs with bark (<i>salix</i> spp., species unknown)	WLB
whole plant without roots of <i>Salix purpurea</i>	SLP
whole plant without roots of <i>Paulonia tomentosa</i>	PAT
whole plant without roots of <i>Arundo donax</i>	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

131

132

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
145 0.45 µm membrane filters (Millipore, Watford, UK). Reagent blanks were prepared
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
149 the following change: K was analysed in the radial mode of the instrument whilst Al,
150 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
153 throughout the measurements (Certipur®, Merck). Limit of detections (LODs) for all
154 elements were determined for the amended method and can be found in [SI Table 1](#).
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
167 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
170 growth was not tested here as previous work showed no phytotoxic effects even for
171 heavily VOC-contaminated biochars (Buss and Mašek, 2014).

172 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**

190 After the phytotoxicity screening was performed, 9 biochars were selected for further
191 testing. 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
194 salt solution, this process was followed by addition of 25 mL of DI water and shaking
195 at 150 rpm for 2 h. Filtration was achieved using the protocol described in section 2.2
196 and the biochar samples were pre-dried in an oven overnight at 50°C. The treated
197 biochars were again tested in germination tests as described in section 2.3 to predict
198 the effect that could be expected from the biochars after they have been exposed to
199 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**

202 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
206 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
210 amount of an element extracted by NH₄NO₃ will be referred to as “available
211 concentration” when expressed on biochar mass basis (mg kg⁻¹, mg g⁻¹) (Table 2) or
212 as “percentage available” (wt%) when expressed relative to the total concentration of
213 the given element present in each biochar sample.

214 **Table 2:** NH₄NO₃-extractable (available) PTE concentrations of 19 biochars (mg kg⁻¹) as average and standard deviation (n = 3). All biochars are
 215 termed according to their feedstock as abbreviated in Table 1 and their respective production temperature (°C).

		Al	As	Cd	Co	Cr	Cu	Hg	Mo	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

* BBodSchV mg kg⁻¹

0.4

#0.1

1

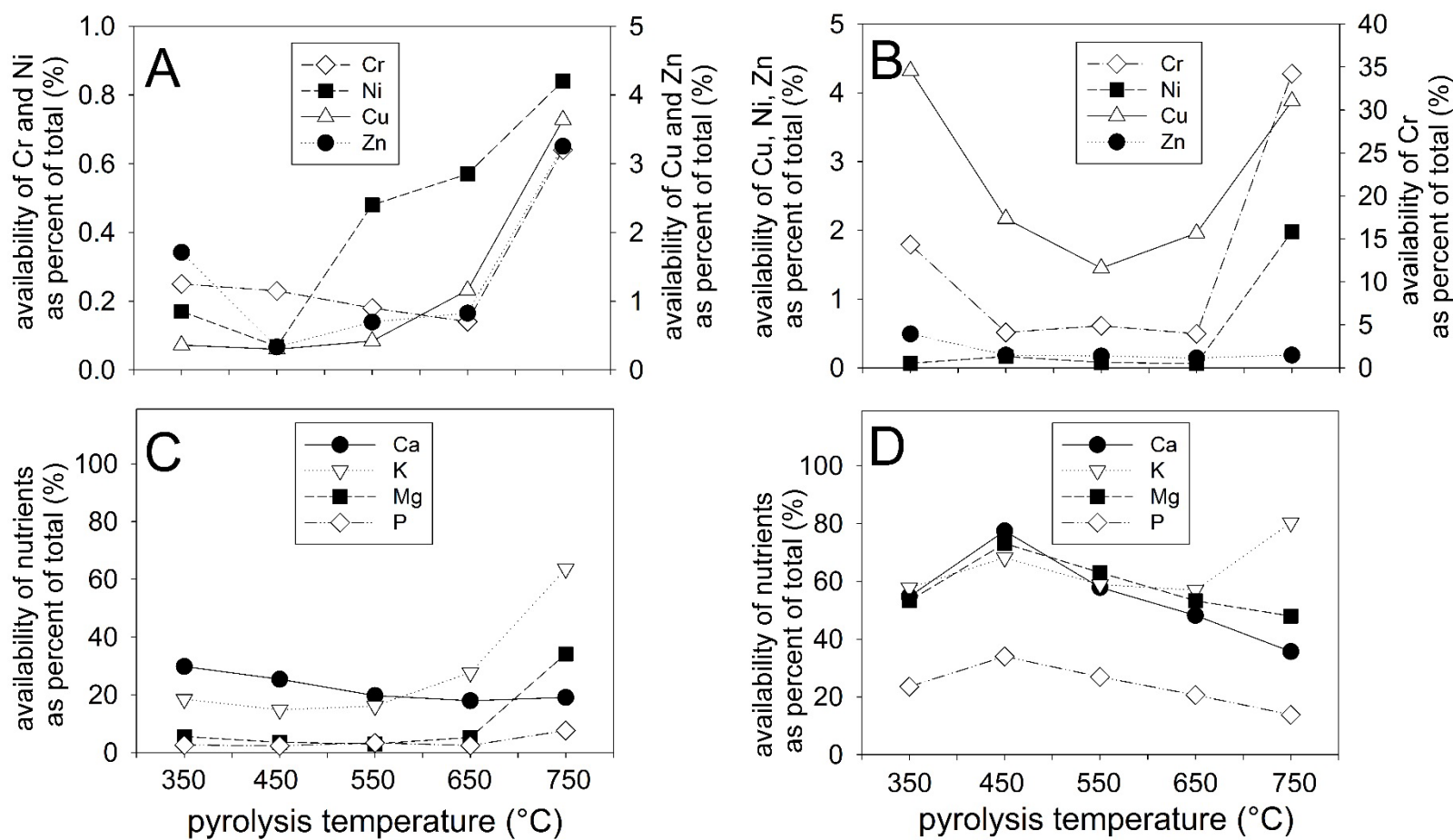
1.5

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



217

218

219

220

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). Availability was measured as percentage NH₄NO₃-extractable of the total elemental content.

221 **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
225 wood (DW) (Figure 1A) and a plant (*A. donax*, ADX) grown on contaminated soil
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 HCl
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
250 feedstocks, ADX-derived biochar showed a higher percentage available for PTEs at
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).

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

268 **Table 3:** Percentage available PTEs and nutrients in n biochars as average (AV) and
 269 standard deviation (SD) determined as NH_4NO_3 -extractable of the total elemental
 270 content. The number of biochars used for calculating the percentage availability is
 271 listed in the column with heading “n”; for biochars with total and available
 272 concentrations below the detection limit, no percentage available could be
 273 calculated.

		AV \pm SD	n
PTEs			
Al	%	0.46 \pm 0.68	19
Cr	%	4.09 \pm 8.02	19
Cu	%	1.27 \pm 1.33	19
Mo	%	23.8 \pm 23.7	16
Ni	%	0.33 \pm 0.48	19
Zn	%	1.32 \pm 1.70	19
nutrients			
B	%	13.1 \pm 16.1	19
Ca	%	28.3 \pm 19.6	19
Fe	%	0.02 \pm 0.02	19
K	%	47.7 \pm 19.7	19
Mg	%	27.2 \pm 22.2	19
Mn	%	4.30 \pm 3.10	19
P	%	10.8 \pm 10.0	19

274

275 **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 $0.02 \pm 0.02\%$ of Fe, $1.32 \pm 1.70\%$ of Zn and $4.09 \pm 8.02\%$ 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
282 and Cr was extractable with 0.005 mol L^{-1} DTPA and in Farrell et al. (2013) less than
283 1% of Ni, Cu, Cr and Zn was extractable with 1 mol L^{-1} NH_4NO_3 . Including this study,
284 typically less than 1.5% of the total concentrations of common PTEs in biochar were
285 available. Clearly, PTEs are typically strongly sorbed to biochars but to place these
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} NH_4NO_3 . 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 ± 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**

304 Threshold values for available As, Cd, Cu, Ni, Pb and Zn for soils for protecting crop
305 quality 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
308 legislation threshold, only the As concentrations for biochars WHI 550 and WSI 550
309 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
311 around 10 and the mobility of As is higher at elevated pH. This is a general problem,
312 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).

316 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
321 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
323 the 5 biochars despite the fact that the average percent availability of Zn in all
324 biochars was only $1.32 \pm 1.70\%$ (Table 3). Despite exceeding German soil threshold
325 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
327 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
331 concentrations, consequently, the exceedance of threshold values for total and
332 available concentrations were compared for two biochars. DW 750, which only
333 exceeded the threshold value for total Cr (Buss et al., 2016), exceeded the threshold
334 values for available Cu, Pb and Zn (Table 2). This might be related to the fact that
335 the metals in demolition wood were concentrated close to the surface, where paints
336 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
338 available concentrations were very low, only 2 threshold values were slightly
339 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
342 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
349 with $1 \text{ mol L}^{-1} \text{ NH}_4\text{NO}_3$ (Table 3), which is similar to what was reported in Ippolito et
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) ($300\text{-}750^\circ\text{C}$) and Singh
353 et al. (2010) ($400, 550^\circ\text{C}$). 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-

357 biochars and remained constant in the range 350-650 for DW-biochars, increasing at
358 750°C (Figure 1).

359 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
367 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
369 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
372 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**

375 Like K, Ca and Mg, P is also a plant macronutrient and is needed by plants in
376 comparatively high amounts (Kirkby, 2011). For the biochars investigated here, the
377 percentage availability of P decreased with pyrolysis HTT (Figure 1) which was also
378 reported in literature for biochar produced from swine manure (Meng et al., 2013), *A.*
379 *donax*, (Zheng et al., 2013) and biosolids (Wang et al., 2012). This was ascribed to
380 assumed structural changes and resulting stabilisation of P / transformation into a
381 less soluble form.

382 Between 0.10 and 34.0% (SI Table 3) and on average 10.8 ± 10.0% (Table 3) of the
383 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
385 deionised water, 2% citric acid, 2% formic acid, 0.5 M NaHCO₃ (Olsen-P),
386 ammonium acetate, ammonium citrate (Brod et al., 2015; Wang et al., 2012; Weber
387 et al., 2014). Although studies that used NH₄NO₃ for extracting P could not be found
388 and NH₄NO₃ is not an established method for extracting P from biochar, the
389 percentage of available P determined by NH₄NO₃ was in the same range as reported
390 for other extractants. E.g. in Singh et al. (2010) Olsen-P per total P was between not
391 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.,
401 2012) which are initially extracted by the 1 mol L⁻¹ NH₄NO₃ solution (pH of solution
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
413 (particularly those using unbuffered and non-acidic extractants), and the percentage
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⁻¹
416 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).

425 **Table 4:** NH₄NO₃-extractable (available) nutrient concentrations of 19 biochars (mg kg⁻¹) as average and standard deviation (n = 3).

		B	Ca	Fe	K	Mg	Mn	P
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

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
433 growth, root growth was also stimulated, reflected by >100% Munoo-Liisa Vitality
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-
439 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
446 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
466 simulated leaching event, nine of the biochars, including the ones showing highest
467 suppression, were washed with DI after NH₄NO₃-extraction and re-tested in the

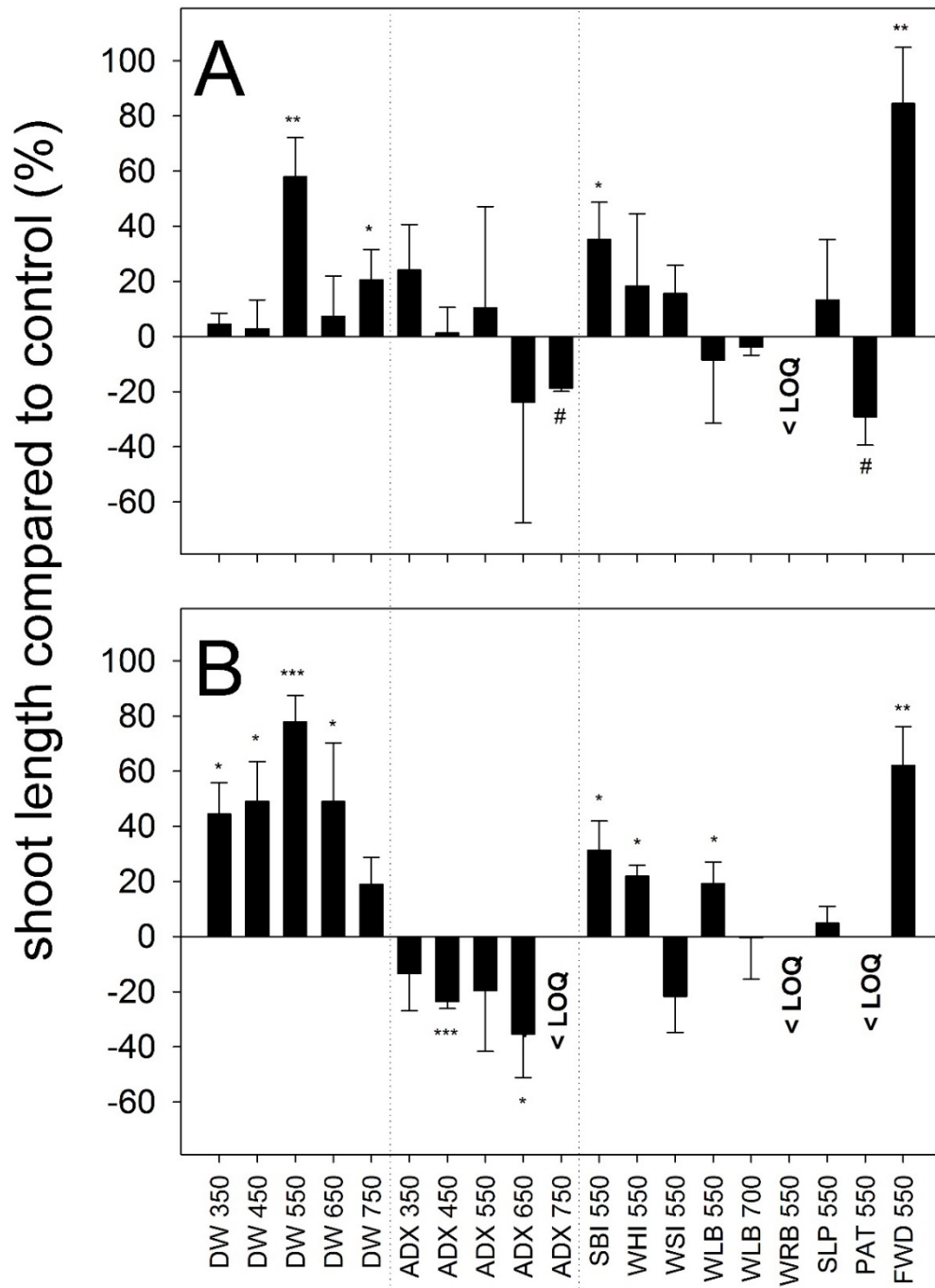
468 same germination experiment. The results revealed that for ADX 750 and WSI 550
 469 the growth suppression was alleviated (germination rate, roots >5 mm and shoot
 470 length not significantly different to control; **SI Table 2 and SI Figure 1**). On the other
 471 hand, in case of WRB 550 significant 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 NH_4^+ which caused toxicity to the
 475 roots of cress which belongs to a plant family that reacts sensitive to NH_4^+ (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
 478 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 = ***.

	leachate affected only			direct contact seeds-biochar		
	roots >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

487



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
 495 replicates showed growth and one replicate had 100% below limit of quantification
 496 (LOQ).

497

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 $<0.06 \text{ mg kg}^{-1}$ 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 $1\text{-}2 \text{ mg L}^{-1}$, 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 NH_4NO_3 -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 NH_4NO_3 -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 $\sim 1 \text{ mg L}^{-1}$
525 Mo , while in this study biochars with Mo concentrations in the NH_4NO_3 -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
529 could be a case of wrongly interpreted cause-effect relationship. The available
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).

535 Henig-Sever et al. (1996) and Singh et al. (1975) showed that solutions with pH in
536 the range 7-9 reduced germination rates in most plant species and by pH of 10-11,
537 total inhibition was observed in most cases. Singh et al. (1975) suggested that the
538 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 2nd order polynomial curve fitted very well with the plant response (Figure 3A: R² =
542 0.63, Figure 3B: R² = 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).

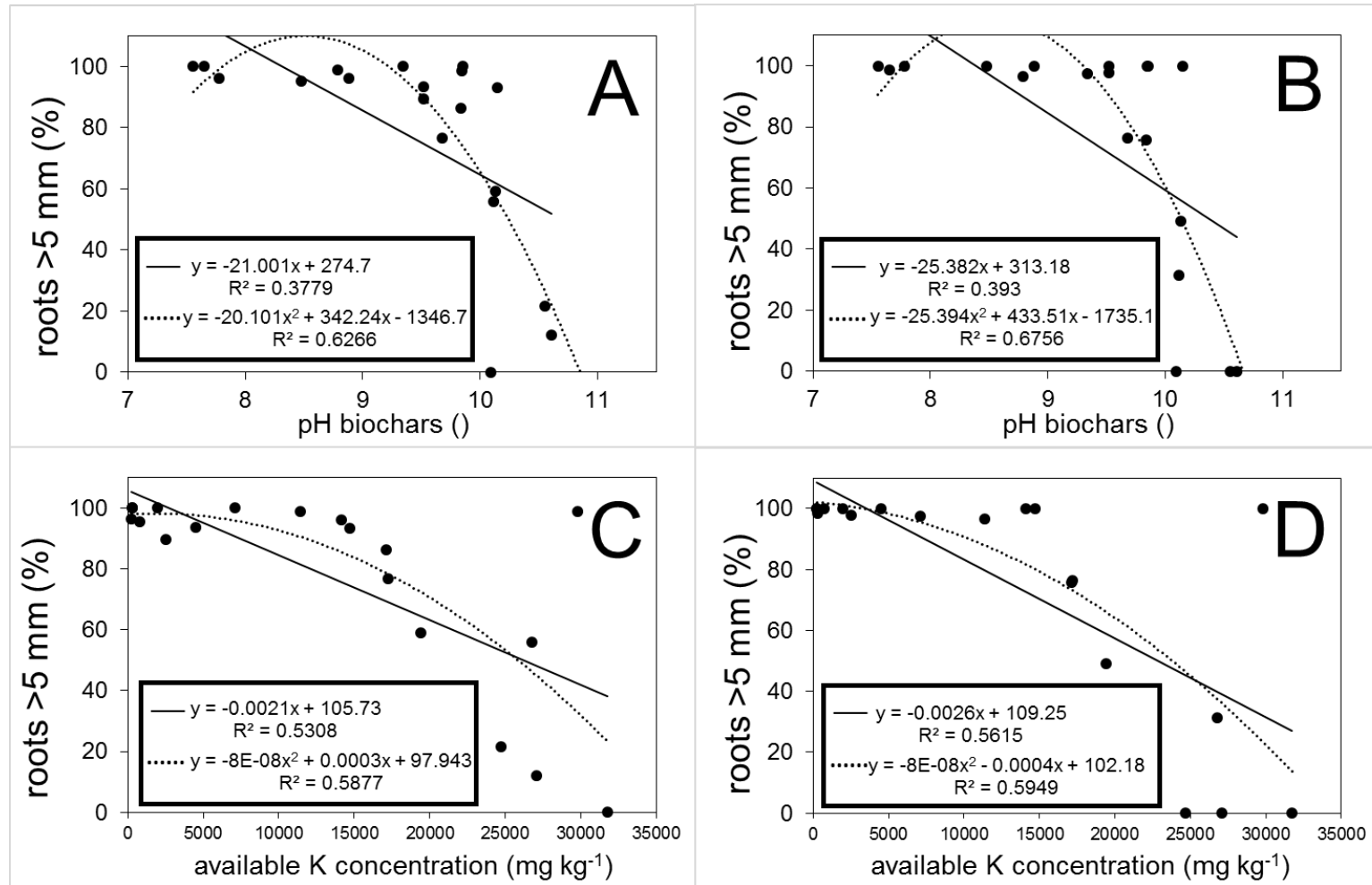
546 Consequently, K most likely caused indirect inhibition of plant growth by increasing
547 the pH in solution. Yet, the available K concentration itself shows an even higher
548 significant correlation with seedling growth than pH and a better 2nd order polynomial
549 fit, in fact available K displays 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. El-Darier and Youssef (2000) in their
556 study on effects of different salt concentrations on cress seeds, reported that due to
557 the osmotic pressure of a solution containing $>50 \text{ mmol L}^{-1}$ NaCl (100 mmol L^{-1}
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 $\sim 3,000$
560 mg L^{-1} in NH_4NO_3 -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
563 231 and 154 mmol L^{-1} , respectively, which is well in the range where reductions of
564 cress seedling growth have been reported.

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
568 (not shown) with seedling growth, much lower than that shown by the available K
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
576 which are mostly a result of dissolved K) that were the primary causes of observed
577 phytotoxicity in this study, and not the PTEs contained in the biochar.

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
 580 seedlings”) for leachate affected seeds and seeds in direct contact with sand-
 581 biochar. Only parameters with significant effect shown.

	leachate affected seeds		direct contact seeds-biochar	
	r	p-value	r	p-value
K	-0.729	***<0.001	-0.749	***<0.001
Mo	-0.660	**0.002	-0.608	**0.006
P	-0.573	*0.010	-0.478	*0.038
EC	-0.471	*0.042	-0.484	*0.036
pH	-0.615	**0.005	-0.627	**0.004

582



583

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

588 **3.8 Use of biochars from marginal biomass for amendment of soil** 589 **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
595 available cationic nutrients also contained concentrations of available PTEs which
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 $<1\%$ ($<20 \text{ t ha}^{-1}$) results
600 in a dilution of 100-fold and consequently, available PTEs would not exceed the limit.
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
605 (Rogowski et al., 1999). Therefore, although the compliance / non-compliance of
606 respective biochars with legislation would need to be decided by the responsible
607 governmental bodies, considering the available concentrations, PTEs do not seem to
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
612 stimulating effects, even in these high application rates (5 wt%, corresponding to
613 $>100 \text{ t ha}^{-1}$, 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
627 available concentrations of PTEs nor for the potential of respective biochars to cause
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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659

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