

1 **Application of biochar to soil reduces cancer risk via rice consumption:**  
2 **a case study in Miaoqian village, Longyan, China.**

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9

10 **Abstract**

11 Consumption of rice contaminated with potentially toxic elements (PTEs) is a major  
12 pathway for human exposure to PTEs. This is particularly true in China's so called  
13 "Cancer Villages". In this study, sewage sludge biochar (SSBC) was applied to soil (at  
14 5% and 10%) to suppress PTE phytoavailability and as a consequence to reduce PTE  
15 levels in rice grown in mining impacted paddy soils. Risk assessment indicates that  
16 SSBC addition (10%) markedly ( $P \leq 0.05$ ) decreased the daily intake, associated with the  
17 consumption of rice, of PTEs (As, Cd, Co, Cu, Mn, Pb and Zn by: 68, 42, 55, 29, 43,  
18 38 and 22%, respectively). In treatments containing SSBC (10%) the health quotient  
19 (HQ) indices for PTEs (except for As, Cu and Mn) were  $< 1$ ; indicating that SSBC  
20 suppressed the health risk associated with PTEs in rice. Addition of SSBC (10%)  
21 markedly ( $P \leq 0.01$ ) reduced As<sup>III</sup> (72%), dimethylarsinic acid (DMA) (74%) and  
22 As<sup>V</sup> (62%) concentrations in rice. Consequentially, following SSBC application (10%),  
23 the incremental lifetime cancer (ILTR) value for iAs (As<sup>III</sup>+As<sup>V</sup>) associated with the

24 consumption of rice was significantly( $P\leq 0.01$ ) reduced by 66%. These findings suggest  
25 that SSBC could be a useful soil amendment to mitigating PTE exposure, through rice  
26 consumption, in China's "Cancer Villages".

27

28 **Keywords:** Biochar; metals; rice; bioaccumulation; daily intake; As speciation; cancer  
29 risk

30

## 31 INTRODUCTION

32

33 Mining is considered to be one of the major anthropogenic activities that results in  
34 contamination of the environment with potentially toxic elements (PTEs), including:  
35 arsenic (As), cadmium (Cd), cobalt (Co), copper (Cu), lead (Pb), manganese (Mn) and  
36 zinc (Zn) (Khaokaew et al., 2012; Pratas et al., 2013; Williams et al., 2009). All of these  
37 PTEs represent a risk to human health. Inorganic As (iAs) is a highly toxic carcinogen  
38 that is linked to many health problems, including, infertility and cardiovascular and  
39 neurological disorders (IARC, 2004). Cd can cause numerous pathological problems  
40 such as high blood pressure, diabetes, skeletal damage and cancers (Satarug and Moore,  
41 2004). Cd is also considered as nephrotoxicant (Horiguchi et al., 2013). Therefore, its  
42 prolonged exposure can also cause renal dysfunction due to its slow release from the body  
43 (Horiguchi et al., 2013). Like Cd, Pb has been linked with abdominal pain, kidney  
44 damage, nerve damages and cancers (lungs and stomach) (Steenland and Boffette, 2000;  
45 Jarup, 2003). Pb has also been linked to anemia, memory deterioration and behavioral  
46 disturbances (Steenland and Boffette, 2000; Jarup, 2003). Polycythemia, excess red  
47 blood cell production, thyroid and coronary arteries problems have been associated with

48 high concentration of Co present in contaminated food (Robert and Mari, 2003). The  
49 ingestion of Cu and Mn, at high concentrations, can cause neurotoxic problems  
50 including Manganism and Alzheimer's diseases (Dieter et al., 2005). Excess intake of  
51 Zn has been associated with sideroblastic anemia, cardiac arrhythmia and gastric  
52 disturbance (Salgueiro et al., 2000).

53 In China, mining activities, for the most part, take place in rural areas. As a  
54 consequence, these activities (along with other industrial processes) have led to the  
55 populations of rural villages being exposed to elevated levels of PTEs (and other toxins).

56 In 2009 journalist Deng Fei published a 'Google' map indicating 100 "Cancer  
57 Villages" in China (Fei, 2010). More recently, a map published online identified 247  
58 "Cancer Villages" in China (PDO, 2013). Negative human health impacts stemming  
59 from both acute and chronic exposure to elevated levels of PTEs are extensively  
60 documented (Li et al., 2011; Niu et al., 2013). The Ministry of Environmental  
61 Protection (MEP) of China has acknowledged the existence of these "Cancer Villages"  
62 and is committed, under 12<sup>th</sup> five-year plan (2011-2015) (MEP, 2012), to controlling  
63 the risk associated with PTEs in the environment.

64 Accumulation of PTEs in arable soil and their subsequent transfer into the food  
65 chain are of great concern. Crop contamination is one of the important routes for PTEs  
66 finding their way into the human body (Khan et al., 2008). Human exposure to PTEs  
67 through consumption of contaminated rice is of particular concern in mining impacted  
68 areas of China because rice is the main staple food. Previous research has shown that  
69 rice grown in contaminated paddy soil can accumulate PTEs (Ji et al., 2013; Li et al.,

70 2011; Williams et al., 2009). Rice consumption has been recognized as a major exposure  
71 source to iAs (Li et al., 2011; Zhao et al., 2013).

72 In order to protect humans from this dietary exposure to PTEs, maximum  
73 permissible limits (MPLs) have been set for PTEs in food (SEPA, 2005; see Table  
74 1). Where food is grown in soils with elevated levels of PTEs it is desirable to suppress  
75 the transfer of PTEs from soil into food crops. This suppression can be achieved through  
76 the addition of 'safe' amendments to soil that reduce PTE availability and thereby  
77 inhibit PTE bioaccumulation into foodstuffs. A candidate amendment in this regard is  
78 biochar.

79 Biochar, is a carbon (C) rich material already recognized for its agronomic benefits  
80 and carbon sequestration potential (Woolf et al., 2010). Biochar benefits to soil have  
81 been attributed to decreases in soil bulk density, improved water dynamics and  
82 increases in soil cation exchange capacity (Glaser et al., 2002; Zhang et al., 2010; Keith  
83 et al., 2011; Quilliam et al., 2012; Khan et al., 2013a; Méndez et al., 2013; Iqbal et al.,  
84 2013). Recently, biochar safety as a soil amendment was evaluated in terms of PTE and  
85 organic compound (polycyclic aromatic hydrocarbons and dioxins) concentrations  
86 (Freddo et al., 2012; Hale et al., 2012). These reports have suggested that environmental  
87 impacts attributable to PTEs, PAHs and dioxins associated with biochar are likely to be  
88 minimal.

89 Recently, several studies have highlighted the potential for biochar materials to  
90 reduce PTE availability in soil, for example: broiler litter derived biochar (Cu, Ni and  
91 Cd) (Uchimiya et al., 2010); hardwood-derived biochar (Cd and Zn) (Beesley et al.,

92 2010); pecan-shell biochar (Zn) (Novak et al., 2009); orchard prune derived biochar  
93 (Cd, Pb and Zn) (Fellet et al. (2011). Qian et al. (2013) reported biochar derived from  
94 manure to effectively suppress aluminum availability and alleviated its phytotoxicity to  
95 wheat plants. Similarly, Ahmad et al. (2012) demonstrated biochar amendment to  
96 reduce Pb availability (in soil collected from a military shooting range). Sewage sludge  
97 derived biochar (5% and 10%) applied to acidic (but not contaminated) paddy soil has  
98 been reported to reduce the bioaccumulation of As, Cr, Co, Cu, Ni, and Pb (Khan et al.,  
99 2013b) into rice grain. While Bian et al. (2013) reported sewage sludge biochar applied  
100 ( $40 \text{ t ha}^{-1}$ ) to a range of background and contaminated paddy soils across South China  
101 to be effective in immobilizing Cd and reducing its concentrations in rice to below  
102 regulatory limits. While existing studies evidence the potential for biochar application  
103 to soil to reduce PTE transfer to crops (including rice), to date, no studies have  
104 contextualized these reductions with respect to mitigated cancer risks.

105 In this study, sewage sludge biochar (SSBC) was amended into contaminated paddy  
106 soil from a Chinese village where PTE concentrations are known to be high due to  
107 mining activities. In addition, with the rapid urbanization in China, disposal of sewage  
108 sludge (SS) is a challenge, and turning SS into SSBC and then use as a soil amendment  
109 will also be beneficial to sustainable urbanization. The aims of this research were to  
110 assess the influence of SSBC on: 1) rice crop yield, 2) PTE concentrations and  
111 availability in mining-impacted paddy soil 3) phytoaccumulation of PTEs and As  
112 speciation in different rice (*Oryza sativa* L) tissues 4) estimate daily intake (EDI),  
113 hazard quotient (HQ) and indices of cancer risk associated with iAs in rice grain.

114

## 115 **2. Materials and Methods**

116

### 117 *2.1 Soil and its provenance*

118 Miaoqian village (Liancheng County, near Longyan City, Fujian Province, China)  
119 and the surrounding area is abundant in metallic mineral resources and has a long legacy  
120 of mineral mining (Mn/Zn in particular). As a consequence of these activities the local  
121 soil is heavily contaminated with PTEs (Table 1). Rice is a staple crop grown in the  
122 village and surrounding area.

123 Triplicate soil samples (0-15 cm; 20 kg) from 10 sites were collected, air dried,  
124 sieved (2 mm mesh) and then thoroughly mixed to obtain a composite sample (600 kg).  
125 Physio-chemical characteristics including EC, pH, C, nitrogen (N), sulfur (S) and  
126 particle size were measured (Table-1). The detailed procedures for these measurements  
127 are given in supporting information (SI).

### 128 *2.2 Experimental design*

129 Soil amendments were prepared with 5% (SSBC5) and 10% (SSBC10) doses of  
130 sewage sludge biochar on dry weight basis. Soil without SSBC was also included as a  
131 control treatment (biochar preparation, cost and feasibility and characteristics are given  
132 in SI). For NPK, the basal fertilizers  $\text{NH}_4\text{NO}_3$  (120 mg of N  $\text{kg}^{-1}$  of soil) and  $\text{K}_2\text{HPO}_4$   
133 (30 mg of P  $\text{kg}^{-1}$  of soil and 75.7 mg of K  $\text{kg}^{-1}$  of soil) were added to all treatments and  
134 homogeneously mixed (Li et al., 2009). The amended soil (4 kg of total mass; n = 4) was  
135 put into polyvinylchloride pots (24 cm high and 15 cm diameter; n=4). Rice seeds were  
136 sterilized with 30%  $\text{H}_2\text{O}_2$  for 10 min and thoroughly washed with deionized water

137 (Khan et al., 2008b). These seeds were put in to a flask containing deionized water and  
138 air was supplied through an aquarium air pump (NS 750, China). After incubating at  
139 28°C for two days, the seeds were placed in clean potting soil. Deionized water was  
140 used to irrigate the pots and after 15 days two uniform seedlings were transferred into  
141 flooded (7 days before) experimental pots containing the contaminated soil or biochar  
142 treated soil. The experiment was conducted under control conditions in a greenhouse  
143 (Khan et al., 2013b). The pots were flooded (3 cm above the surface) with deionized  
144 water and regularly randomized to ensure uniform light and temperature. During the  
145 reproductive stage, the leaf length and width (1<sup>st</sup>, 2<sup>nd</sup> and 3<sup>rd</sup> top leaves) were measured  
146 to calculate leaf area (see detail in SI). Upon grain maturity (98 days after transplanting),  
147 rice plants were cut (3 cm above soil surface) and thoroughly washed with deionized  
148 water. Spikelet, panicle and tiller numbers were counted; the length of panicles and  
149 heights of tillers were also measured. Plant shoots were dried in an oven (70°C for 72  
150 h) and the dry weights recorded. The rice straw was separated into stems and leaves.  
151 Brown rice grains, leaves and stems were milled into powder and stored in paper sacks  
152 prior to chemical analysis.

### 153 *2.3 Chemical analyses*

154 To measure the total concentration of PTEs in SSBC and soil, samples (0.5 g) were  
155 digested with aqua regia (Khan et al., 2008a), while pulverized rice plant samples were  
156 digested with a mixture (1/1 v/v) of H<sub>2</sub>O<sub>2</sub> (35%) and concentrated HNO<sub>3</sub> in a microwave  
157 accelerated reaction system (CEM-Mars, Version 194A05, USA). The digested samples  
158 were filtered through 0.22 µm membrane and the filtrate made up to 50 mL with Milli-

159 Q water. To assess bioavailable concentration of PTEs in soil and treated soil, 0.05 M  
160 ethylene-diamine-tetra-acetic acid (EDTA) was added (20 ml) to dried samples (1 g) in  
161 polypropylene tubes (50 mL). The tubes were shaken (180 rpm for 3 h) and centrifuged  
162 (7500 rpm for 10 min at 25 °C), and then the supernatant was filtered through 0.22 µm  
163 membrane (Iqbal et al., 2013). As, Cd, Co, Cu and Pb concentrations were measured  
164 using ICP-MS (Agilent Technologies, 7500 CX, USA), while K, P, Na, Mn and Zn were  
165 determined with ICP-OES (Perkin Elmer Optima 7000 DV, USA).

166 To determine iAs in rice plants, powdered samples (200 mg) were placed in 50 ml  
167 polypropylene tubes and 1% (v/v) HNO<sub>3</sub> (10 mL) was added. Microwave assisted  
168 digestion was then used to extract the samples (Jia et al., 2012). The As speciation in  
169 the extracts was determined using HPLC-ICP-MS. Arsenic species (arsenite (As<sup>III</sup>),  
170 arsenate (As<sup>V</sup>), dimethylarsinic acid (DMA) and methylarsonic acid (MMA)) were  
171 separated using an anion-exchange column (PRP X-100, Hamilton Company, USA)  
172 with the mobile phase of 10mM (NH<sub>4</sub>)<sub>2</sub>HPO<sub>4</sub> and 10mM NH<sub>4</sub>NO<sub>3</sub> (pH 6.2). Total iAs  
173 was calculated as the sum of As<sup>III</sup> and As<sup>V</sup>. The extraction efficiency of these As species  
174 ranged from 70.1-89.9% (Table 3).

175

#### 176 *2.4 Quality control*

177 For accuracy and precision, reagent blanks and standard reference materials were  
178 included in each batch. Plant, soil and rice flour reference materials (GBW07603-GSV-  
179 2, GBW07406-GSS-6 and GBW10010, respectively) were obtained from the National  
180 Research Center for Standards in China. Recovery rates ranged from 90.3±8.2-



181 102.1±9.4%.

182

## 183 2.5 Dietary intake and risk assessment of PTEs

184

### 185 2.5.1 Daily intake of PTEs

186 The estimated daily intake (EDI) of PTEs through consumption of rice was  
187 determined using the following equation:

$$188 \quad \text{EDI} = \frac{\text{ED} \times \text{EF} \times \text{IR}_{\text{Rice}} \times \text{C}_{\text{PTEs}}}{\text{BW} \times \text{LE}}$$

189 where ED, EF, C<sub>PTEs</sub>, BW and LE represent the exposure duration (70 years),  
190 exposure frequency (365 days per year), PTE concentrations in rice (Fig. 3), average  
191 body weight (65 kg), life expectancy (25550 days) (values are those used in previous  
192 studies (Zhuang et al., 2009; Li et al., 2011)). Rice intake rate (IR<sub>Rice</sub>) of 398.3 g/adult  
193 person/day was taken from Zheng et al., 2007.

194

### 195 2.5.2 Hazard quotient indices

196 The hazard quotient (HQ) indices for selected PTEs were calculated using the  
197 equation detailed in USEPA (2010).

$$198 \quad \text{HQ} = \frac{\text{EDI}}{\text{RfD}}$$

199 Where RfD represents corresponding oral reference dose (0.0003, 0.001, 0.04,  
200 0.0035 0.14 and 0.3 mg/kg/d for As, Cd, Cu, Pb, Mn and Zn, respectively), as suggested  
201 by USEPA (2010). HQ for Co was not determined as its RfD value was not included in  
202 USEPA (2010).

203

### 204 2.5.3 Cancer risk

205 Incremental lifetime cancer risk (ILTR) was calculated for the iAs with the following  
206 equation (USEPA, 2010; Li et al., 2009):

$$207 \quad \text{ILTR} = \frac{\text{ED} \times \text{EF} \times \text{IR}_{\text{Rice}} \times \text{C}_{\text{iAs}}}{\text{BW} \times \text{LE}} \times \text{SF}$$

208 Where SF represents cancer slope factor (1.5 mg/kg/d) (USEPA, 2010).

## 209 2.6 Data analysis

210 The statistical package (SPSS 11.5) was used to statistically analyze the data.  
211 Figures show the mean values along with one standard deviation (n=4). The differences  
212 among treatments were tested using ANOVA, while Tukey's test (with a level of  $P < 0.05$ )  
213 was used for mean significance.

214

## 215 3. RESULTS AND DISCUSSION

216

### 217 3.1 The influence of biochar on rice crop yield and nutrient concentrations

218 Grain yield, number of tillers and shoot biomass all increased significantly  
219 ( $P \leq 0.01$ ), while the height of tillers was significantly reduced ( $P \leq 0.05$ ) in the SSBC  
220 amended treatments (Fig. 1). Furthermore, there was no significant difference in the  
221 number of spikelets and length of panicles in the SSBC amended treatments (Figure  
222 1). The average grain yield (7.3-8.2 g d.w) harvested from the SSBC amended soil was  
223 greater (158-189%) than the control (2.8 g d.w) (Fig. 1A). Similarly, the number of tillers  
224 (64.1-69.2%) and shoot biomass (25.9-26.2%) were greater than observed in the control  
225 soil (Figure 1). Top leaf area was slightly increased (6.40-6.44%) in plants grown on  
226 SSBC amended soil, while second and third top leaf areas were also increased in the  
227 SSBC5 treatments, while their areas were decreased in SSBC10 amended soil (Fig 1B).

228 Collectively these results indicate that SSBC was effective in enhancing rice plant  
229 biomass. Previously, the addition of different biochars has shown increases in the yield  
230 of rice grain, cherry tomatoes, maize and ryegrass (*Lolium perenne* L.) (Hussain et al.,  
231 2010; Kammann et al., 2012; Zhang et al., 2012; Lashari et al., 2013). The increase in  
232 rice grain yield following the addition of SSBC in this study was higher than that (16-  
233 35%) of rice grown on soil amended with carbonized rice husk and fertilizers (Maefele,  
234 2011). Improved plant growth in biochar amended soils has been attributed to numerous  
235 mechanisms that change soil physical chemical and biological characteristics (Khan et  
236 al., 2013b; Xu et al., 2013; Steinbeiss et al., 2009) and the bioavailability of nutrients  
237 such as N, P, K, S and Na (Table 3).

238 SSBC addition significantly ( $P \leq 0.05$ ) increased N concentration in grain (18.6-

239 28.4%), leaves (15.6-21.6%) and straw (72.5-92.2%) as compared to the control (Fig.  
240 2). Similar effects of biochar (made from spruce chips) on N uptake in *Phleumpretense*  
241 plants has been reported previously (Saarnio et al., 2013). In this study, the increased N  
242 uptake could be linked to higher availability of NO<sub>3</sub>-N (86.7-134.3%) and NH<sub>4</sub>-N (21.9-  
243 51.7%) in SSBC amended soil compared to the control (Table 2). Saarnio et al. (2013)  
244 suggested that the higher plant uptake of N was due to lower availability of N to  
245 microbes in soil amended with biochar.

246 The accumulation of P in rice plants grown on SSBC amended soil was significantly  
247 ( $P \leq 0.05$ ) higher than in the control; increasing by: 27.1-32.6% in leaves, 18.0-33.7% in  
248 stems and 14.3-35.9% in grain, respectively (Fig. 2). This increased P accumulation  
249 could be due to higher P in SSBC soil (Table 2). The biochars used in previous studies  
250 have also been reported to release PO<sub>4</sub>-P (Hale et al., 2013), which could be available  
251 for plant uptake.

252 Unlike N and P, K concentration was lower in the rice plants grown on SSBC  
253 amended treatments compared to the control (Table 2). The lowest decrease was  
254 observed in grain (4.90-5.00%), followed by leaves (25.5-32.6%) and then straw (48.3-  
255 61.9%) (Fig. 2). SSBC addition increased S concentrations in grain (4.68-6.23%), in  
256 leaves (8.74-48.9%) and stems (87.1-118%). SSBC addition increased Na  
257 concentrations in grain (32.2-56.2%), leaves (462-907%) and stems (182-  
258 203%); available Na was observed to increase in SSBC amended soil (Table 2). The  
259 release of macro-nutrients (including N, K, P, S and Na) from biochars has been  
260 reported to be dependent upon several factors including: types of feedstock used in their  
261 production, pyrolysis temperature and the resultant biochar pH (Kim et al., 2013; Hale  
262 et al., 2013).

263

264 *3.2 The influence of biochar on paddy soil PTE concentrations and availability* SSBC  
265 addition changed many physico-chemical characteristics of the soil including EC, pH,  
266 TC, TN, TS, DOC, NH<sub>4</sub>-N, NO<sub>3</sub>-N, K, P and Na (Table 3). Further information is given  
267 in SI.

268 The total concentrations of As, Cd, Co, Cu, Mn, Pb and Zn in the mine-

269 contaminated soil used in this study were 24.0, 3.55, 4.12, 130, 5848, 1151 and 1473  
270 mg/kg, respectively (Table 1). Concentrations of Cd, Cu and Zn exceeded the maximum  
271 permissible limits (MPL) (0.3, 50, 200 mg/kg, respectively) set for paddy soil (pH<6.5)  
272 by the State Environmental Protection Administration, China (SEPA, 1995).

273 The addition of SSBC significantly ( $P=0.01$ ) decreased available concentrations of  
274 As(13.6-22.7%),Cd (9.63-14.5%), Co (15.1-25.6%),Cu (21.1-28.9%) and Pb (24.9-  
275 30.1%), compared to the control (Table 2). Decreases in available Mn (5.71-6.95%) and  
276 Zn (6.90-7.56%) concentrations were not significantly different to the control ( $P>0.01$ ).  
277 Houben et al. (2013) also reported a significant decrease in the extractable (into  
278  $\text{CaCl}_2$ )fraction of Cd, Pb and Zn in the soil amended with miscanthus straw biochar.  
279 Similarly, the addition of biochars derived from other kinds of feedstock has also been  
280 reported to significantly decrease the available concentrations of PTEs (Beesley et al.,  
281 2011; Ahmad et al., 2012). These results indicate the effectiveness of SSBC in reducing  
282 the available fractions of PTEs in Mn-mining impacted soil. However, SSBC effect on  
283 available Mn (in comparison to the other PTEs assessed)was the lowest (Table 2). This  
284 could be related to its high concentration ( $1367 \text{ mg kg}^{-1}$ ) and its very high availability  
285 under the reducing paddy soil conditions (Führs et al., 2010).

286 The decrease in PTE availability in biochar amended soil has been linked to pH  
287 changes, which is an important parameter for PTE sorption process (Zheng et al., 2012).  
288 The pH can affect surface charges and chemistry of adsorbent and at the same time can  
289 also change the ionization and speciation processes of PTEs in soil(Kołodzyńska et al.,  
290 2012). Furthermore, changes in cation exchange capacity and dissolved organic carbon  
291 (DOC)in biochar amended soil have also been linked with the decrease in available  
292 concentration of PTEs (Zheng et al., 2012).The density of cation exchange sites on  
293 biochar surfaces has been reported to increases with pH (Harvey et al., 2011), this, in  
294 turn, promoting greater PTE adsorption. The oxygen-functional-groups of biochars also  
295 played an important role in forming complexes with metals (Uchimiya et al., 2012).  
296 Jiang and Xu (2013) have reported that these functional-groups to be particularly  
297 effective in suppressing Cu availability.

298

### 299 3.3 *The influence of biochar on metal accumulation*

300 The concentrations of As, Cd, Co, Cu, Pb, Mn and Zn in the plant tissues were  
301 significantly ( $P \leq 0.05$ ) reduced in SSBC amended soil compared to control. The  
302 decrease in bioaccumulation of PTEs was element-dependent and corresponded to the  
303 decreased availability of PTEs in soil (Table 3). In comparison to the control PTE  
304 accumulation in grain was significantly ( $P \leq 0.05$ ) reduced: As(60.2-67.5%), Cd (26.5-  
305 42.0%),Co (40.6-54.7%), Cu (24.0-29.3%), Mn (36.3-42.5%), Pb (32.5-37.7%) and Zn  
306 (16.6-22.0%) concentrations in SSBC amended soils compared to the control. The  
307 difference in PTE accumulation (except for Cd) in grain was not significantly different  
308 between SSBC5 and SSBC10 treatments. Similarly, PTE concentrations in leaves were  
309 decreased by SSBC addition: As (76.6-86.5%), Cd (76.5-85.9%),Co (35.4-51.4%), Cu  
310 (47.7-50.1%),Mn (10.6-15.4%), Pb (14.4-20.3%) and Zn (11.2-42.3%).

311 As observed for grain, the difference in leaf concentrations between SSBC5 and  
312 SSBC10 was not significantly different. PTE accumulation in stems was reduced in the  
313 SSBC amended treatments as compared to the control: As (82.6-90.2%), Cd (46.9-  
314 77.5%),Co (40.6-58.7%), Cu (27.5-34.9%), Mn (20.1-27.3%), Pb (17.0-39.8%)and Zn  
315 (3.56-29.6%).The difference in stem concentrations between SSBC5 and SSBC10 was  
316 only significant for Cd and Pb. Houben et al. (2013) reported decreases in PTE  
317 bioaccumulation in ryegrass cultivated in biochar amended soil and attributed this to  
318 lower PTE mobility in the presence of biochar.

319 The concentrations of As, Cd and Cu grown in control soil exceeded the MPLs  
320 (0.05,0.20 and 20mg/kgdw, respectively) set by SEPA (2005)for food. Following the  
321 addition of SSBC grain concentrations Cd and Cu decreased to below their respective  
322 MPLs; while grain As concentrations in SSBC amended soil were greatly reduced they  
323 did not achieve the MPL value set by SEPA (2005).

324 The addition of SSBC significantly ( $P < 0.01$ ) reduced the concentrations of As  
325 species with respect to the control: AsIII (66.7-72.2%), AsV(46.9-62.2%) and DMA  
326 (38.6-73.6%), while MMA in the grain was below detection limit(Table3).In leaves,  
327 SSBC addition significantly ( $P < 0.01$ ) decreased the concentrations, relative to the  
328 control, of: AsIII (77.7-87.8%), AsV (74.1-80.8%) and DMA (33.3-62.4%). In keeping

329 with observations for grains and leaves, SSBC addition also significantly ( $P < 0.01$ )  
330 decreased the concentrations, relative to the control, of: AsIII (84.8-92.2%), AsV (51.7-  
331 63.0%) and DMA (27.4-69.4%) in stems. MMA was below detection limit in leaves  
332 and stem samples. These findings indicated that SSBC addition reduced As uptake,  
333 while biochar prepared from rice residues have been shown to increase (327%)As  
334 accumulation in rice (Zheng et al., 2012). These contrasting outcomes could well relate  
335 to the specific properties of the biochars used and the specifics of the soils and their  
336 PTE loadings. Further comparative studies are clearly needed to establish how these  
337 factors influence outcomes with respect to As phytoaccumulation.

338 The bioaccumulation of PTEs in rice plants grown in SSBC amended soils could  
339 be affected by several mechanisms controlling the mobility and bioavailability of PTEs  
340 in soil. Among the physical characteristics of biochar, the surface area, pore volume  
341 and pore size are of vital importance to reduce metal availability in the amended soil  
342 and their subsequent uptake into plants (section 3.2). In this study, the reduced  
343 bioaccumulation of PTE in rice plants grown on SSBC amended soil could be linked  
344 with lower surface area, lower pore volume and greater pore size of SSBC (Table 2).  
345 After application of biochar to soil, exchangeable bases/inorganic compounds have  
346 been reported to increase, these changes further increased surface area and pore volume  
347 (Kim et al., 2013), and in turn, reduce PTE concentrations in soil solution. Alkaline  
348 biochars, such as the SSBC used in this research (Table 2), increase the pH of acidic  
349 soils (Table 2). PTE mobility is reduced at higher soil pH (Houben et al., 2013) and for  
350 this reason PTE phytoaccumulation in the SSBC treatments may have been lower. The  
351 number of negatively charged surface sites depends on pH. Thus, an increase in soil pH,  
352 as observed in this study, following SSBC addition may have increased these negatively  
353 charged sites and as a consequence increased sorption of PTEs (Kim et al., 2013). In  
354 this study, DOC contents were increased (33.7-90.1%) in SSBC amended soil. DOC  
355 may have acted as a chelator and reduced the availability of PTEs in soil through direct  
356 adsorption and formation of stable complexes (Zheng et al., 2012).

357 In addition, SSBC is an important P source and significantly increased available P  
358 in the amended soil (Table 2). S contents in SSBC amended soil were also increased

359 (Table 2). These two elements interact strongly with As during plant uptake, which may  
360 have reduced its bioaccumulation into rice plants. Arsenate has been reported to be  
361 accumulated from soil into plants via P transporters (Meharg and Macnair, 1992).  
362 Therefore, the increase in P in SSBC amended soil may have suppressed As  
363 accumulation in rice plants. Arsenic transport from soil to rice plant and its speciation  
364 depend on redox conditions in the soil, and AsIII is the dominant species under  
365 anaerobic conditions, while AsV is present at high concentration under aerobic  
366 conditions (Zhao et al., 2013). Addition of SSBC could induce changes in soil redox  
367 conditions (Beesley et al., 2013) which may further change As speciation. Previous  
368 studies have observed that iAs and DMA are the dominant forms in rice grain, while  
369 MMA is minor form and occasionally present (Meharg et al., 2009). In this study, iAs  
370 and DMA were also observed as dominant species and MMA was not detected in rice  
371 grain, straw and leaves (Table 3). Numerous other factors such as oxygen-functional-  
372 groups on biochar surface and changes in microbial activities could also influence PTE  
373 bioavailability in SSBC amended soil and their accumulation in rice plants (Xu et al.,  
374 2013; Steinbeiss et al., 2009).

375

#### 376 *3.4 Daily intake of PTEs and their health risks*

377 In order to contextualize health risks associated with PTE intake via rice  
378 consumption, EDI and HQ were calculated (Table 4). The daily intake of PTEs was  
379 estimated using the average rice consumption by inhabitants. The average EDI was  
380 significantly ( $P \leq 0.01$ ) reduced following the addition of SSBC (both 5% and 10%) by  
381 60.2-67.5, 26.6-41.7, 40.5-54.7, 24.2-29.4, 36.2-42.7, 32.5-37.6 and 16.5-22.1%, for  
382 As, Cd, Co, Cu, Mn, Pb and Zn, respectively. This estimated EDI for the control  
383 treatment was far higher than the tolerable limits or RfD, set for daily exposure to PTEs  
384 without any substantial health risk over a whole lifetime (USEPA, 2010). SSBC addition  
385 reduced EDI (except for Cu and Mn) to values close to the RfD limits instead of severe  
386 contamination of PTEs in mine impacted soil. Further research is needed in this regard  
387 to investigate long term effects of SSBC on EDI through consecutive cultivation of rice

388 in SSBC amended soil under field conditions.

389 Perhaps most importantly, SSBC addition also significantly ( $P \leq 0.01$ ) reduced iAs  
390 in rice grain (60.0-68.0%), leaves (77.0-86.6%) and stems (82.3-90.0%) as compared to  
391 the control (Table 3). Characteristically, iAs has higher toxicological effects compared  
392 to organic species. Therefore, it is necessary to reduce iAs concentration in the grain  
393 and also in the fodder parts of rice. EDI of iAs, associated with the consumption of  
394 rice, significantly ( $P \leq 0.01$ ) decreased in SSBC amended treatments (59.9-66.4%). The  
395 decrease of iAs in rice fodder could also be beneficial for animal health and reducing  
396 onward transfer of iAs into food stuffs derived from animals.

397 The HQ is often used for assessing potential risks and adverse health effects  
398 resulting from the ingestion of pollutants. The HQ value calculated for the control soil  
399 was higher than the level at which human health is at risk. The highest HQ was observed  
400 for Mn (14.6) and followed by As (8.23) (Table 4). This outcome was underpinned by  
401 high Mn concentrations in rice, the relatively high toxicity of As and its low RfD value.  
402 Addition of SSBC to mining impacted soil significantly ( $P \leq 0.01$ ) reduced HQ in rice  
403 compared to control (Table 4). The highest application of SSBC (10%) reduced the HQ  
404 values to values less than one (except for As, Cu and Mn), indicating PTE exposure to  
405 be reduced to less than the acceptable reference dose.

406 The value of ILTR associated with iAs was significantly ( $P \leq 0.01$ ) reduced for rice  
407 grown in the SSBC treatments was compared to the control. In this study, the calculated  
408 cancer risk was 228 per 100,000 for rice grown on the control soil, and was  
409 significantly ( $P \leq 0.01$ ) reduced to 92-77 per 100,000 for rice grown on SSBC amended  
410 soil. This decrease in ILTR (59.8-66.3%) was mostly attributable to reduced ingestion  
411 of iAs. The ILTR value for the control rice is consistent with those reported by Meharg  
412 et al. (2009) for Bangladesh rice and Li et al. (2011) for Chinese food. To our knowledge,  
413 this is the first time that the effects of biochar on reducing the ILTR for iAs in rice has  
414 been reported.

415 The food chain is one of the main pathways of human exposure to PTEs (Khan et  
416 al., 2008a,b). In China, rice is the main staple food (FAO, 2011). On account of the high  
417 capacity rice has to accumulate PTEs and its high level of consumption rice is



418 considered to be the most important source of exposure to PTEs (Williams et al., 2009).  
419 Zhuang et al., 2009 reported dietary PTE exposure through consumption of rice to be 3-  
420 11 times higher than that associated with vegetables. Ji et al. (2013) reported rice  
421 consumption to contribute more than 75% of the PTE intake for a population of village  
422 near the abandoned mine in Goseaong, Korea.

423 The findings of this study support the use of SSBC to mitigate PTE transfer to rice  
424 and thereby reducing exposure to PTEs. While this study has been limited to paddy soil  
425 contaminated primarily by Mn and Zn mines, results did suggest the applicability of  
426 biochar addition to soil to potentially mitigate cancer risks of other PTEs as well.

427 Given that sewage sludge is problematic waste it is suggested that its diversion into  
428 biochar production could represent a solution to this waste being disposed of to land.  
429 The results presented herein indicate that the targeted application of SSBC to PTE  
430 contaminated soil has beneficial outcomes in terms of food safety and reduced cancer  
431 risk associated with rice consumption. However, for such benefits to be realized the  
432 application of SSBC to soil needs to be cost effective. Calculations, detailed in the SI,  
433 indicate SSBC production costs to vary from 0.08-0.59 USD per kg. This range  
434 reflecting pyrolysis conditions that varied from 3 to 6 h and temperatures from 400 to  
435 600 °C. Conceptually, it would be advantageous to produce SSBC as close as possible  
436 to the locations where it is intended for application. It is submitted that the per kg SSBC  
437 production costs suggest the application of 10% SSBC to 1 hm<sup>2</sup> to be of the order of  
438 XXX. Assuming SSBC production close to point of application we suggest these costs  
439 to be acceptable when set against the reduction in cancer risk that could be achieved.  
440 However, further study is needed to look at the economics and life cycle analysis of  
441 biochar use in agricultural systems.

442

#### 443 **4. Conclusion**

444 It is concluded that SSBC addition to Mn-Zn mine impacted soil was effective in  
445 suppressing phytoavailable PTEs and their bioaccumulation in rice plant. Results  
446 revealed that SSBC addition facilitated PTE binding and suppressed their mobility into  
447 soil solution and then into rice. Consequently, this led to a decrease in daily intake of

448 PTEs through ingestion of rice. At high SSBC application rates (10%), the HQ values  
449 of the PTEs studied were <1 (except As, Cu and Mn) indicating that SSBC could  
450 suppress the health risk associated with rice consumption. Taken in context, the ability  
451 of SSBC to reduce iAs concentrations in rice grains (by 60.2-67.5%) is particularly  
452 significant as exposure to iAs through rice consumption is major driver of cancer in  
453 China's "Cancer Villages" (BSI, 2013; Banerjee et al., 2013).

454 While the reported results are encouraging field research is needed to explore the  
455 potential of SSBC and indeed other biochars to mitigate cancer risks in China's Cancer  
456 Villages. In addition, alternative mitigation approaches, including diversification of  
457 diets, must also be considered if cancer risks are to be reduced.

458

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461

## 462 **References**

463

464 Ahmad M, Lee SS, Yang JE, Ro HM, Lee YH, Ok YS. Effects  
465 of soil dilution and amendments (mussel shell, cow bone, and biochar)  
466 on Pb availability and phytotoxicity in military shooting range soil. *Ecotoxicol Environ*  
467 *Saf* 2012;79: 225-31.

468 Bahemuka TE, Mubofu EB. Heavy metals in edible green vegetables grown along the  
469 sites of the Sinza and Msimbazi Rivers in Dares Salaam, Tanzania. *Food*  
470 *Chem* 1999;66:63-6.

471 Banerjee M, Banerjee N, Bhattacharjee P, Mondal D, Lythgoe P R, Marti'nez  
472 M, et al. High arsenic in rice is associated with elevated genotoxic effects in  
473 humans. *Sci Rep* 2013; 3, 2195; DOI:10.1038/srep02195 (2013).

474 Beesley L, Marmiroli M, Pagano L, Pignoni V, Fellet G, Fresno T, et al. Biochar addition  
475 to an arsenic contaminated soil increases arsenic concentrations in the pore water  
476 but reduces uptake to tomato plants (*Solanum lycopersicum* L.). *Sci Total Environ*  
477 2013;454-455: 598-603.

478 Beesley L, Moreno-Jimenez E, Gomez-Eyles JL, Harris E, Robinson B, Sizmur T.  
479 A review of biochars' potential role in the remediation, revegetation and  
480 restoration of contaminated soils. *Environ Pollut* 2011;159: 3269-82.

481 Beesley L, Moreno-Jiménez E, Jose L, Gomez-Eyles JL. Effects of biochar and  
482 greenwaste compost amendments on mobility, bioavailability and toxicity of  
483 inorganic and organic contaminants in a multi-element polluted soil. *Environ*  
484 *Pollut* 2010;158: 2282-2287.

485 Bian R, Chen D, Liu X, Cui L, Li L, Pan G, et al. Biochar soil amendment as a  
486 solution to prevent Cd-tainted rice from China: Results from a cross-site field  
487 experiment. *Ecol Eng* 2013;58: 378- 383.

488 BSI, 2013. The Toxic Rice Fields of China's Cancer Villages. Blacksmith Institute<sup>475</sup>  
489 Riverside Drive Suite 860 New York, NY 10115.  
490 <http://www.blacksmithinstitute.org/blog/?p=1638> [accessed on 23 November,  
491 2013].

492 Chen Z, Chen C, Liu Y, Wu Y, Yang S, Lu C. Study on soil background values in Fujian

493 province. Chinese J Environ Sci 1992;13:70-5.

494 Dieter HH, Bayer TA, Multhaup G. Environmental copper and manganese in  
495 the pathophysiology of neurologic diseases (Alzheimer's disease and  
496 Manganism), Acta Hydroch Hydrob 2005;33:72-8.

497 FAO. Countries by commodity (Rice, paddy). Food and Agricultural Organization.  
498 Available: <http://faostat.fao.org> [accessed 12 March, 2013].

499 Fei D. Google map of China's  
500 cancer villages. <https://maps.google.com/maps/ms?hl=en&ie=UTF8&oe=UTF8&msa=0&msid=104340755978441088496.000469611a28a0d8a22dd> 2010.  
501 [accessed 12 March, 2013].

502 Fellet G, Marchiol L, Delle Vedove G, Peressotti A. Application of biochar on mine  
503 tailings: effects and perspectives for land reclamation. Chemosphere 2011; 83:  
504 1262-297.

505 Freddo A, Cai C, Reid BJ. Environmental contextualisation of potential toxic  
506 elements and polycyclic aromatic hydrocarbons in biochar. Environ Pollut  
507 2012;171: 18-24.

508 Glaser B, Lehmann J, Zech W. Ameliorating physical and chemical properties of  
509 highly weathered soils in the tropics with charcoal: a review. Biol Fertil  
510 Soils 2002;35: 219-230.

511 Hale SE, Alling V, Martinsen V, Mulder J, Breedveld GD, Cornelissen G. The sorption  
512 and desorption of phosphate-P, ammonium-N and nitrate-N in cacao shell and  
513 corn cob biochars. Chemosphere 2013; 91:1612-9.

515 Hale SE, Lehmann J, Rutherford D, Zimmerman AR, Bachmann RT, Shitumbanuma  
516 V, et al. Quantifying the total and bioavailable polycyclic aromatic hydrocarbons  
517 and dioxins in biochars. *Environ Sci Technol* 2012;46: 2830-2838.

518 Harmsen J, Naidu R. Bioavailability as a tool in site management. *JHazardMat*  
519 doi. <http://dx.doi.org/10.1016/j.jhazmat.2012.12.044>2013.

520 Harvey OR, Herbert BE, Rhue RD, Kuo L-J. Metal interactions at the biochar-water  
521 interface: Energetics and structure-sorption relationships elucidated  
522 by flow adsorption microcalorimetry. *Environ Sci Technol* 2011;45:5550-6.

523 Horiguchi H, Oguma E, Sasaki S, Okubo H, Murakami K, Miyamoto K, et al. Age-  
524 relevant renal effects of cadmium exposure through consumption of home-  
525 harvested rice in female Japanese farmers. *Environ Int* 2013;56:1-9

526 Hossain MK, Strezov V, Chan KY, Ziolkowski A, Nelson PF. Agronomic properties of  
527 wastewater sludge biochar and bioavailability of metals in production of cherry  
528 tomato (*Lycopersicon esculentum*). *Chemosphere* 2010;78:1167-71.

529 Houben D, Evrard L, Sonnet P. Mobility, bioavailability and pH-dependent leaching  
530 of cadmium, zinc and lead in a contaminated soil amended with biochar.  
531 *Chemosphere* <http://dx.doi.org/10.1016/j.chemosphere.2013.03.055>.2013.

532 IARC. Monographs on the evaluations of carcinogenic risks to humans; International  
533 Agency for Research on Cancer: Lyon, France, Vol. 84.2004.

534 Iqbal M, Bermond A, Lamy I. Impact of miscanthus cultivation on trace metal  
535 availability in contaminated agricultural soils: Complementary insights from  
536 kinetic extraction and physical fractionation. *Chemosphere* 2013; 91: 287-94.

537 Jarup L. Hazards of heavy metal contamination, *British Medical Bulletin* 2003;68: 167-  
538 82.

539 Ji K, Kim J, Lee M, Park S, Kwon H-J, Cheong H-K, et al. Assessment of exposure to  
540 heavy metals and health risks among residents near abandoned metal mines in  
541 Goseong, Korea. *Environ Pollut* 2013;178: 322-8.

542 Jia Y, Huang H, Sun G-X, Zhao F-J, Zhu Y-G. Pathways and relative contributions to  
543 arsenic volatilization from rice plants and paddy soil. *Environ Sci Technol* 2012;  
544 46: 8090-6

545 Jiang J, Xu R-K. Application of crop straw derived biochars to Cu(II)  
546 contaminated Ultisol: Evaluating role of alkali and organic functional groups in  
547 Cu(II) immobilization, *Bioresour Technol* doi:  
548 <http://dx.doi.org/10.1016/j.biortech.2013.01.161>. 2013.

549 Kammann C, Ratering S, Eckhard C, Muller C. Biochar and hydrochar effects on  
550 greenhouse gas (carbon dioxide, nitrous oxide, methane) fluxes from soils.  
551 *J Environ Qual* 2012;41:1052-66.

552 Keith A, Singh B, Singh B P. Interactive priming of biochar and labile organic matter  
553 mineralization in a smectite-rich soil. *Environ Sci Technol* 2011;45: 9611-18.

554 Khan S, Cao Q, Zheng Y M, Huang Y Z, Zhu Y-G. Health risks of heavy metals in  
555 contaminated soils and food crops irrigated with wastewater in Beijing China.  
556 *Environ Pollut* 2008a;152:686-92.

557 Khan S, Aijun L, Zhang S, Hu Q, Zhu Y-G. Accumulation of polycyclic aromatic  
558 hydrocarbons and heavy metals in lettuce grown in the soils contaminated with

559 long-term wastewater irrigation. *JHazardMat*2008b;152: 506-15.

560 Khan S, Wang N, Reid, BJ, Freddo A, Cai C. Reduced bioaccumulation of PAHs by  
561 *Lactucasativa* L. grown in contaminated soil amended with sewage sludge and  
562 sewage sludge derived biochar. *Environ Pollut* 2013a;175: 64-8.

563 Khan S, Chao C, Waqas M, Arp HPH, Zhu Y-G. Sewage sludge biochar influence upon  
564 rice (*Oryza sativa* L) yield, metal bioaccumulation and greenhouse gas emissions  
565 from acidic paddy soil. *Environ Sci Technol* DOI: 10.1021/es400554x. 2013b.

566 Khaokaew S, Landrot G, Chaney RL, Pandya K, Sparks DL. Speciation and release  
567 kinetics of zinc in contaminated paddy soils. *Environ Sci Technol* 2012;46:3957-  
568 63.

569 Kim P, Johnson AM, Essington ME, Radosevich M, Kwon W-T, Lee S-H, et al. Effect of  
570 pH on surface characteristics of switchgrass-derived biochars produced by fast  
571 pyrolysis. *Chemosphere* 2013;90: 2623-30.

572 Kołodyńska D, Wnętrzak R, Leahy JJ, Hayes MHB, Kwapiński W, Hubicki Z. Kinetic and  
573 adsorptive characterization of biochar in metal ions removal. *Chem Eng J*  
574 2012;197:295-305.

575 Lashari M S, Liu Y, Li Y, Pan W, Fu J, Zheng J, et al.  
576 Effect of amendment of biochar-manure compost in conjunction with pyroligneous  
577 solution on soil quality and wheat yield of a salt-stressed cropland from Central  
578 China Great Plain. *Field Crops Res* 2013; 144:113-118.

579 Li G, Sun GX, Williams PN, Nunes L, Zhu Y-G. Inorganic arsenic in Chinese food and  
580 its cancer risk. *Environ Int* 2011; 37:1219-1225.

581 Li RY, Stroud JL, Ma JF, Mcgrath SP, Zhao FJ. Mitigation of arsenic accumulation in  
582 rice with water management and silicon fertilizers. *Environ Sci Technol*  
583 2009;43:3778-83.

584 Meharg AA, Williams PN, Adomako E, Lawgali YY, Deacon C, Villada A, et al.  
585 Geographical variation in total and inorganic arsenic content of polished  
586 (white)rice. *Environ Sci Technol* 2009;43(5):1612-7.

587 Meharg AA, Macnair MR. Suppression of the high-affinity phosphate-uptake system-  
588 a mechanism of arsenate tolerance in *Holcus lanatus* L. *J Exp Bot* 1992; 43:519-24.

589 Méndez A, Terradillos M, Gascó G. Physicochemical and agronomic properties of  
590 biochar from sewage sludge pyrolysed at different temperatures, *J Anal Appl*  
591 *Pyrol* <http://dx.doi.org/10.1016/j.jaap.2013.03.006>. 2013.

592 MEP. Ministry of environmental protection, China. The twelfth five year plan for  
593 national environmental protection. 2012.  
594 Available: [http://gcs.mep.gov.cn/hjgh/shierwu/201210/t20121029\\_240586.htm](http://gcs.mep.gov.cn/hjgh/shierwu/201210/t20121029_240586.htm). [ac  
595 cessed on 11 June, 2013].

596 Niu L, Yang F, Xu C, Yang H, Liu W. Status of metal accumulation in farmland soils  
597 across China: From distribution to risk assessment. *Environ Pollut* 2013;176:55-  
598 62.

599 Novak J M, Busscher W J, Laird D L, Ahmedna M, Watts D W, Niandou M A S.  
600 Impact of biochar amendment on fertility of a southeastern coastal plain soil. *Soil*  
601 *Sci* 2009 ;174:105-112.

602 PDO. People's Daily Online (Shanghai version). China's mainland home to 247



603 'cancer villages'. February 5th 2013.

604 <http://english.people.com.cn/90882/8140970.html>

605 Pratas J, FavasPJC, D'SouzaR, Varun M, PaulMS. Phytoremedial assessment of flora  
606 tolerant to heavy metalsin the contaminated soils of an abandoned Pb mine in  
607 Central Portugal. Chemosphere 2013;90: 2216-25.

608 Qian L, ChenB, Hu D. Effective alleviation of aluminum phytotoxicity by manure-  
609 derived biochar. EnvironSciTechnolDOI:10.1021/es3047872. 2013.

610 Quilliam RS, MarsdenKA, Gertler C, Rousk J, DeLuca TH, Jones DL. Nutrient  
611 dynamics, microbial growth and weed emergence in biochar amendedsoil are  
612 influenced by time since application and reapplication rate. Agric Ecosyst  
613 Environ 2012;158:192-99.

614 Robert G, Mari G, Human health effects of metals, US Environmental Protection  
615 Agency Risk Assessment Forum, Washington, DC, 2003.

616 Saarnio S,HeimonenK, KettunenR.Biochar addition indirectly affects N<sub>2</sub>O emissions  
617 via soil moisture and plantN uptake. Soil Biol Biochem2013;58:99-106.

618 Salgueiro M, Zubillaga J,LysionekM,SarabiaA, CaroMI, PaoliR.Zinc as an  
619 essentialmicronutrient: A review. Nutr Res 2000;20:737-55.

620 Satarug S, Moore M.Adverse health effect of chronic exposure to low-levelcadmiumin  
621 foodstuffsandcigarette smoke. EnvironHealth Perspect2004;112:1099-103.

622 SEPA, Environmental quality standard for soils. State EnvironmentalProtection  
623 Administration, China. GB15618-1995: 1995.

624 SEPA, The Limits of Pollutants in Food. State Environmental Protection

625 Administration, China. GB2762-2005: 2005.

626 Steenland K, Boffetta P. Lead and cancer in humans: where are we now? *Am J Ind Med*  
627 2000;38:295-9.

628 Steinbeiss S, Gleixner G, Antonietti M. Effect of biochar amendment on soil carbon  
629 balance and soil microbial activity. *Soil Biol Biochem* 2009;41:1301-10.

630 Uchimiya M, Bannon DI, Wartelle LH. Retention of heavy metals by carboxyl functional  
631 groups of biochars in small arms range soil. *J Agric Food Chem* 2012;60(7):1798-  
632 809.

633 Uchimiya M, Lima IM, Klasson T, Wartelle L H. Contaminant immobilization and  
634 nutrient release by biochar soil amendment: roles of natural organic matter.  
635 *Chemosphere* 2010; 80: 935-940.

636 USEPA (United States Environmental Protection Agency). Process design manual, land  
637 application of sewage sludge and domestic septage. United States Environmental  
638 Protection Agency, Office of Research and Development, Washington, DC 20460,  
639 EPA/625/R-95/001. 1995.

640 USEPA (United States Environmental Protection Agency). Toxicological review  
641 of inorganic arsenic. Draft document. EPA/635/R-10/001. Washington, DC,  
642 USA: USEPA; 2010. p. 575.

643 Williams PN, Lei M, Sun G, Huang Q, Lu Y, Deacon C, et al. Occurrence and  
644 partitioning of cadmium, arsenic and lead in mine impacted paddy rice: Hunan,  
645 China. *Environ Sci Technol* 2009; 43:637-42.

646 Woolf D, Amonette JE, Street-Perrott FA, Lehmann J, Joseph S. Sustainable

647 biochar to mitigate global climate change. *Nat Commun* 2010;1: 1-9.

648 Xu X, Cao, X, Zhao L. Comparison of rice husk- and dairy manure-derived biochars for  
649 simultaneously removing heavy metals from aqueous solutions: Role of mineral  
650 components in biochars. *Chemosphere*  
651 <http://dx.doi.org/10.1016/j.chemosphere.2013.03.009>2013.

652 Yuan JH, Xu RK, Zhang H. The forms of alkalis in the biochar produced from crop  
653 residues at different temperatures. *Bioresour Technol* 2011;102: 3488-97.

654 Zhang A, Bian R, Pan G, Cui L, Hussain Q, Li L, et al. Effects of biochar amendment  
655 on soil quality, crop yield and greenhouse gas emission in a Chinese rice paddy: A  
656 field study of 2 consecutive rice growing cycles. *Field Crops Res.* 2012;127:153-  
657 60

658 Zhang A, Cui L, Pan G, Li L, Hussain Q, Zhang X, et al. Effect of biochar amendment  
659 on yield and methane and nitrous oxide emissions from a rice paddy from Tai Lake  
660 plain, China. *Agric Ecosyst Environ* 2010;139: 469-75.

661 Zhao F-J, Zhu Y-G, Meharg AA. Methylated arsenic species in rice: geographical  
662 variation, origin and uptake mechanisms. *Environ Sci Technol*  
663 DOI:10.1021/es304295n2013.

664 Zheng N, Wang QC, Zhang XW, Zheng DM, Zhang ZS, Zhang SQ. Population health  
665 risk due to dietary intake of heavy metals in the industrial area of Huludao City,  
666 China. *Sci Total Environ* 2007;387:96-104.

667 Zheng R L, Cai C, Liang J H, Huang Q, Chen Z, Huang Y Z, et al. The effects of biochars  
668 from rice residue on the formation of iron plaque and the accumulation of Cd, Zn,

669 Pb, As in rice (*Oryza sativa* L.) seedlings. Chemosphere 2012;7: 856-62.  
670 Zhuang P, McBrideMB, XiaH, Li N, Li Z.Health risk from heavy metals via  
671 consumption of food crops inthe vicinity of Dabaoshan mine, South China.  
672 SciTotal Environ 2009;407:1551-61.  
673

674

675 **Table 1**

676 Physical and chemical characteristics of SSBC and soil (n=3) with total and available  
 677 PTE concentrations shown alongside (SEPA, 2005) guidance values. Where total PTE  
 678 concentrations exceed guidance values this has been indicated in bold.

Properties	SSBC		Soil		SEPA <sup>a</sup>	Background soil <sup>b</sup>
	Total (in d.w)	Available (in d.w)	Total (in d.w)	Available (in d.w)		
pH (CaCl <sub>2</sub> )	7.18		5.47		NA <sup>d</sup>	
EC (mS/cm)	1.76		0.79		NA	
BET Surface Area (m <sup>2</sup> g <sup>-1</sup> )	5.57		ND <sup>c</sup>		NA	
Pore Volume (cm <sup>3</sup> g <sup>-1</sup> )	0.015		ND		NA	
Pore Size (nm)	10.6		ND		NA	
N (%)	2.34	ND	0.17	ND	NA	
C (%)	27.8	ND	2.21	ND	NA	
S (%)	5.46	ND	0.06	ND	NA	
K (g/kg)	18.3	0.51 <sup>e</sup>	11.7	0.31 <sup>e</sup>	NA	
Na (g/kg)	110	3.67 <sup>e</sup>	3.31	1.74 <sup>e</sup>	NA	
P (g/kg)	57.8	18.2 <sup>f</sup>	2.10	16.9 <sup>f</sup>	NA	
As (mg/kg)	10.2	0.05 <sup>g</sup>	24.0	0.94 <sup>g</sup>	30	5.88
Cd (mg/kg)	<b>4.06</b>	0.32 <sup>g</sup>	3.55	0.35 <sup>g</sup>	<b>0.3</b>	0.05
Co (mg/kg)	3.14	0.38 <sup>g</sup>	4.12	0.20 <sup>g</sup>	NA	
Cu (mg/kg)	<b>224</b>	6.78 <sup>g</sup>	130	23.2 <sup>g</sup>	<b>50</b>	19.8
Mn (mg/kg)	1367	38.7 <sup>g</sup>	5848	1215 <sup>g</sup>	NA	
Pb (mg/kg)	26.7	2.15 <sup>g</sup>	1151	314 <sup>g</sup>	250	35.6
Zn (mg/kg)	<b>1101</b>	137 <sup>g</sup>	1473	1058 <sup>g</sup>	<b>200</b>	79.5

679 <sup>a</sup>Maximum acceptable limits set for soil by the State Environmental Protection  
 680 Administration (SEPA, 1995);

681 <sup>b</sup>Soil background values taken from Chen et al. (1992) for Fujian province, China;

682 <sup>c</sup>ND not determined;

683 <sup>d</sup>NA not allocated;

684 <sup>e</sup>NH<sub>4</sub>OAc-extractable;

685 <sup>f</sup>Colwell P;

686 <sup>g</sup>bioavailable–EDTA (0.05 M) extracted metals

687

688 **Table 2**

689

690 Chemical characteristics of SSBC treatments and the control soil.

Properties	Treatments		
	Control (n=4)	SSBC5 (n=4)	SSBC10 (n=4)
pH	5.47	5.66	5.83
EC (mS/cm)	0.79	1.68	2.71
TN (%)	0.17	0.33	0.51
TC (%)	2.21	3.05	5.15
TS (%)	0.06	0.28	0.49
NH <sub>4</sub> -N (mg/kg)	151	184	229
NO <sub>3</sub> -N (mg/kg)	28.0	52.3	65.6
DOC (mg/kg)	172	230	327
K <sup>a</sup> (mg/kg)	312	326	402
Na <sup>a</sup> (mg/kg)	1741	1880	1987
P <sup>b</sup> (mg/kg)	16.9	58.8	83.3
As <sup>c</sup> (mg/kg)	0.94	0.81	0.73
Cd <sup>c</sup> (μg/kg)	353	319	302
Co <sup>c</sup> (μg/kg)	199	169	148
Cu <sup>c</sup> (mg/kg)	23.2	18.3	16.5
Mn <sup>c</sup> (mg/kg)	1215	1145	1130
Pb <sup>c</sup> (mg/kg)	314	236	219
Zn <sup>c</sup> (mg/kg)	1058	985	978

691

<sup>a</sup>NH<sub>4</sub>OAc exchangeable, <sup>a</sup>available P (Colwell P), <sup>c</sup>EDTA available

692

693 **Table 3**

694 Concentration of As species in rice grain(n=4) and calculated cancer risk through ingestion of

695 iAs.

Parameters	Control			SSBC5			SSBC10		
	Grain	Leaves	Stem	Grain	Leaves	Stem	Grain	Leaves	Stem
AsIII (mg/kg)	0.18	5.96	5.53	0.06	1.33	0.84	0.05	0.73	0.43
AsV (µg/kg)	77.0	1182	451	40.9	306	218	29.1	227	167
DMA (µg/kg)	34.5	11.7	6.2	21.2	7.8	4.5	9.12	4.4	1.9
MMA (µg/kg)	ND	ND	ND	ND	ND	ND	ND	ND	ND
iAs (mg/kg) <sup>a</sup>	0.26	7.14	5.98	0.10	1.64	1.06	0.08	0.96	0.60
Daily intake of iAs (µg/kg BW) <sup>b</sup>	1.52			0.61			0.51		
ILTR <sup>c</sup>	2.28E-03			9.16E-04			7.68E-04		
Extraction efficiency (%) <sup>d</sup>	70.1	89.9	87.6	75.2	88.1	89.1	70.6	89.3	89.1

696 <sup>a</sup>The value of iAs is the sum of AsIII and AsV;697 <sup>b</sup>Average daily intake of iAs was calculated using the equation presented in materials and  
698 methods section;699 <sup>c</sup>ILTR was estimated using the daily intake of iAs and cancer slope factor of iAs values in the  
700 equation given in materials and methods section.701 <sup>d</sup>Extraction efficiency for As species was calculated from the sum of As species divided by  
702 total As extracted with H<sub>2</sub>O<sub>2</sub>(35%) and conc. HNO<sub>3</sub> and multiplied by 100.

703

704

705 **Table 4**

706

707 EDI (mg/kg/d) and HQ for individual PTEs attributable to the consumption of rice grown

708 in mine impacted and SSBC treated soils. Where HQ was <1 this has been indicated in

709 bold.

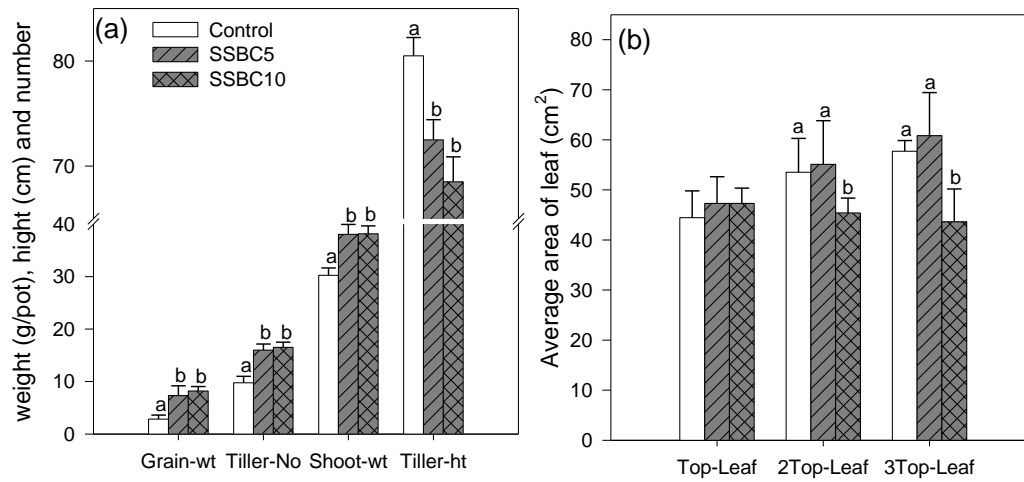
710

PTEs	Control		SSBC5		SSBC10	
	EDI	HQ	EDI	HQ	EDI	HQ
As	2.47E-03	8.23	9.83E-04	3.28	8.03E-04	2.68
Cd	2.18E-03	2.18	1.60E-03	1.60	1.27E-03	<b>0.71</b>
Co	3.95E-04	NC	2.35E-04	NC	1.79E-04	NC
Cu	1.14E-01	2.84	8.64E-02	2.16	8.05E-02	2.01
Mn	2.18E+00	14.6	1.39E+00	9.91	1.25E+00	8.95
Pb	5.08E-03	1.45	3.43E-03	<b>0.98</b>	3.17E-03	<b>0.90</b>
Zn	3.21E-01	1.07	2.68E-01	<b>0.89</b>	2.50E-01	<b>0.83</b>

711 NC\* not calculated because of no RfD values was available.

712



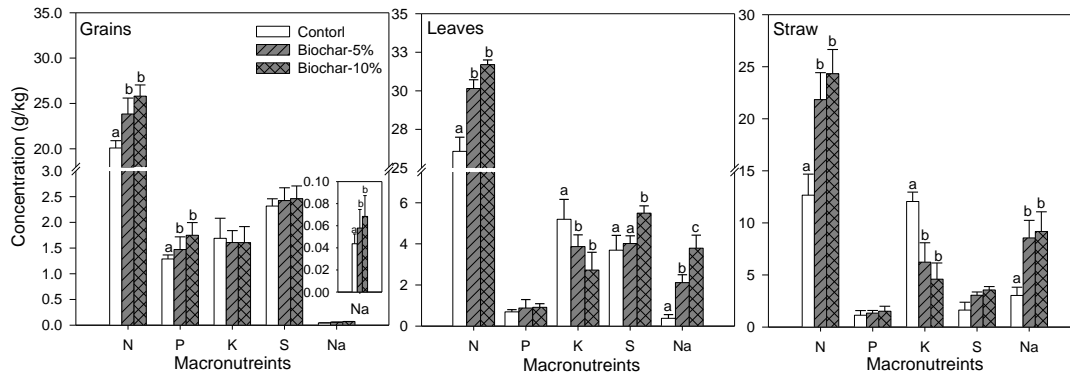


713

714 **Fig.1.** Effects of SSBC amendments on rice plants growth: a) plant biomass (grain and shoot),  
 715 tiller number and tiller height, and b) leaf area for top three leaves grown in the control soil (white)  
 716 and soil amended with SSBC5 (dark hatched) and SSBC10 (dark cross-hatched). Error bars  
 717 represent standard deviations (n=4). Different letters indicate significant difference (P < 0.01)  
 718 between treatments, while similar letters and parameters without letters indicate no  
 719 significant difference.

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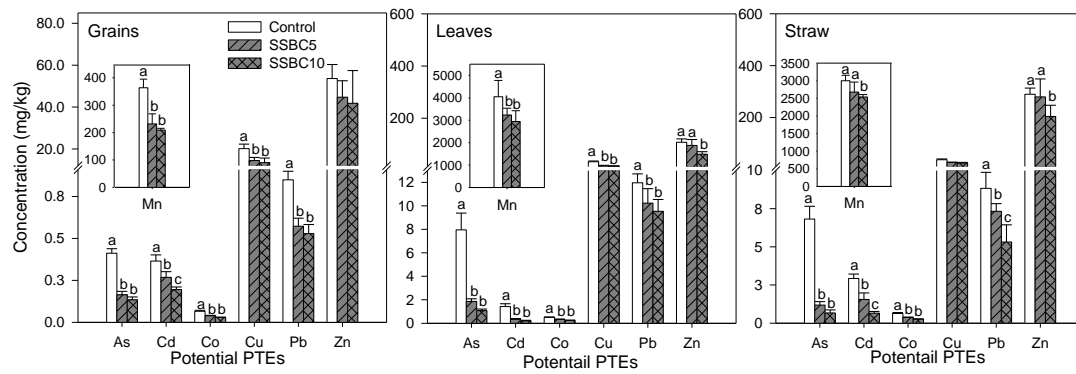
724 **Fig.2.** Nutrient concentrations in rice grains, leaves and stems grown in the control soil (white) and  
725 soil amended with SSBC5 (dark hatched) and SSBC10 (dark cross-hatched). Error bars represent  
726 standard deviations (n=4). Different letters indicate significant difference ( $P \leq 0.01$ ) between  
727 treatments, while similar letters and parameters without letters indicate no significant difference.

728

729

730

731



732

733 **Fig.3.**PTE concentrations in rice grains, leaves and stems grown in the control soil (white) and soil  
734 amended with SSBC5 (dark hatched) and SSBC10 (dark cross-hatched). Error bars represent  
735 standard deviations (n=4). Different letters indicate significant difference ( $P \leq 0.01$ ) between  
736 treatments, while similar letters and parameters without letters indicate no significant difference.