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

SardarKhan[a,b],Brian J. Reid[c],Gang Li[a], Yong-GuanZhu[a]*

[a]Key Lab of Urban Environment and Health, Institute of Urban Environment, Chinese Academy of Sciences, Xiamen 361021, China.

[b]Department of Environmental Science, University of Peshawar, Peshawar, Pakistan.

[c]School of Environmental Sciences, University of East Anglia, Norwich, UK.

***Corresponding author**: E-mail: ygzhu@rcees.ac.cn Tel: 00865926190560; Fax: 00865926190997

Abstract

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

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

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

INTRODUCTION

Mining is considered to be one of the major anthropogenic activities that results in contamination of the environment with potentially toxic elements (PTEs), including: arsenic (As), cadmium (Cd), cobalt (Co), copper (Cu), lead (Pb), manganese (Mn) and zinc (Zn) (Khaokaew et al., 2012; Pratas et al., 2013; Williams et al., 2009).All of these PTEs represent a risk to human health. Inorganic As (iAs) is a highly toxic carcinogen that is linked to many health problems, including, infertility and cardiovascular and neurological disorders (IARC, 2004). Cd can cause numerous pathological problems such as high blood pressure, diabetes, skeletal damage and cancers (Satarug and Moore, 2004). Cd is also considered as nephrotoxicant (Horiguchi et al., 2013). Therefore, its prolong exposure can also cause renal dysfunction due to its slow release from the body (Horiguchi et al., 2013). Like Cd, Pb has been linked with abdominal pain, kidney damage, nerve damages and cancers (lungs and stomach) (Steenland and Boffette, 2000; Jarup, 2003). Pb has also been linked to anemia, memory deterioration and behavioral disturbances (Steenland and Boffette, 2000; Jarup, 2003). Polycythemia, excess red blood cell production, thyroid and coronary arteries problems have been associated with high concentration of Co present in contaminated food (Robert and Mari, 2003). The ingestion of Cu and Mn, at high concentrations, cancause neurotoxic problems including Manganism and Alzheimer's diseases (Dieter et al., 2005).Excess intake of Zn has been associated with sideroblastic anemia, cardiac arythmia and gastric disturbance (Salgueiro et al., 2000).

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

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

Accumulation ofPTEs in arable soil and their subsequent transfer into the food chain are of great concern. Crop contamination is one of the important routes for PTEs finding their way into the human body (Khan et al., 2008). Human exposure to PTEs through consumption of contaminated rice is of particular concern in mining impacted areas of China because rice is the main staple food. Previous research has shown that rice grown in contaminated paddy soil can accumulate PTEs (Ji et al., 2013; Li et al., 2011; Williams et al., 2009).Rice consumption has been recognized as a major exposure source to iAs (Li et al., 2011; Zhao et al., 2013).

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

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

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

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

2. Materials and Methods

2.1Soil and its provenance

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

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

2.2Experimental design

Soil amendments were prepared with 5%(SSBC5) and 10% (SSBC10) doses of sewage sludge biochar on dry weight basis. Soil without SSBC was also included as a control treatment (biochar preparation, cost and feasibility and characteristics are given in SI). For NPK, the basal fertilizers NH_4NO_3 (120 mg of N kg^{-1} of soil) and K_2HPO_4 (30 mg of P kg^{-1} of soil and 75.7 mg of K kg^{-1} of soil)were added to all treatments and homogenously mixed (Li et al., 2009).The amended soil (4 kg of total mass; n = 4) was put into polyvinylchloride pots (24 cm high and15 cm diameter; n=4). Rice seeds were sterilized with 30% H_2O_2 for 10 min and thoroughly washed with deionized water

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

2.3Chemical analyses

To measure the total concentration of PTEs inSSBC and soil,samples (0.5 g) were digested with aqua regia (Khan et al., 2008a), while pulverized rice plant samples were digested with a mixture (1/1 v/v) of H_2O_2 (35%) and concentratedHNO_3in a microwave accelerated reaction system (CEM-Mars,Version194A05, USA). The digested samples were filtered through 0.22 μm membrane and the filtrate made up to 50 mL with Milli-

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

To determine iAs in rice plants, powdered samples (200 mg) were placed in 50 ml polypropylene tubes and 1% (v/v) HNO_3 (10 mL)was added. Microwave assisted digestion was then used to extract the samples (Jia et al., 2012). The As speciation in the extracts was determined using HPLC-ICP-MS. Arsenic species (arsenite (AsIII), arsenate (AsV), dimethylarsinic acid (DMA) and methylarsonic acid (MMA)) were separated using an anion-exchange column (PRP X-100, Hamilton Company,USA) with the mobile phase of 10mM $(NH_4)_2HPO_4$ and 10mM NH_4NO_3 (pH 6.2). Total iAs was calculated as the sum of AsIII and AsV.The extraction efficiency of these As species ranged from 70.1-89.9% (Table 3).

2.4Quality control

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

102.1±9.4%.

2.5Dietary intake and risk assessment of PTEs

2.5.1 Daily intake of PTEs

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

$$EDI = \frac{ED \times EF \times IR_{Rice} \times C_{PTEs}}{BW \times LE}$$

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

2.5.2Hazard quotient indices

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

$$HQ = \frac{EDI}{RfD}$$

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

2.5.3Cancer risk

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

$$ILTR = \frac{ED \times EF \times IR_{Rice} \times C_{iAs}}{BW \times LE} \times SF$$

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

2.6Data analysis

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

3. RESULTS AND DISCUSSION

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

Grain yield, number of tillers and shoot biomass all increased significantly ($P\leq0.01$), while the height of tillers was significantly reduced ($P\leq0.05$)in the SSBC amended treatments (Fig. 1). Furthermore, there was no significant difference in the number of spikelets and length of panicles in the SSBC amended treatments (Figure 1).The average grain yield (7.3-8.2 g d.w) harvested from the SSBC amended soilwas greater(158-189%) than the control (2.8 g d.w) (Fig. 1A).Similarly, the number of tillers (64.1-69.2%)and shoot biomass (25.9-26.2%)were greater than observed in the control soil (Figure 1). Top leaf area was slightly increased (6.40-6.44%) in plants grown on SSBC amended soil, while second and third top leaf areas were also increased in the SSBC5 treatments, while their areas were decreased in SSBC10 amended soil (Fig 1B).

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

SSBC addition significantly ($P\leq0.05$)increased N concentration in grain (18.6-

28.4%), leaves (15.6-21.6%) and straw (72.5-92.2%)as compared to the control (Fig. 2). Similar effects of biochar(made from spruce chips)on N uptake in *Phleumpretense* plants has been reported previously (Saarnio et al., 2013).In this study, the increased N uptake could be linked to higher availability of NO_3-N (86.7-134.3%)and NH_4-N (21.9-51.7%)in SSBC amended soil compared to the control (Table 2).Saarnio et al. (2013) suggested that the higher plant uptake of N was due to lower availability of N to microbes in soil amended with biochar.

The accumulation of P in rice plants grown on SSBC amended soilwas significantly ($P \leq 0.05$) higher than in the control; increasing by: 27.1-32.6% in leaves,18.0-33.7%in stems and 14.3-35.9% in grain, respectively (Fig. 2).This increased P accumulation could be due to higher P in SSBC soil (Table 2). The biochars used in previous studies have also been reported to release PO_4-P (Hale et al., 2013), which could be available for plant uptake.

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

3.2 The influence of biochar on paddy soil PTE concentrations and availability SSBC addition changed many physico-chemical characteristics of the soil including EC, pH, TC, TN, TS, DOC, NH_4-N, NO_3-N, K, P and Na (Table 3). Further information is given in SI.

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

The addition of SSBC significantly (P=0.01) decreased available concentrations of As(13.6-22.7%),Cd (9.63-14.5%), Co (15.1-25.6%),Cu (21.1-28.9%) and Pb (24.9-30.1%), compared to the control (Table 2). Decreases in available Mn (5.71-6.95%) and Zn (6.90-7.56%) concentrations were not significantly different to the control (P>0.01). Houben et al. (2013) also reported a significant decrease in the extractable (into $CaCl_2$)fraction of Cd, Pb and Zn in the soil amended with miscanthus straw biochar. Similarly, the addition of biochars derived from other kinds of feedstock has also been reported to significantly decrease the available concentrations of PTEs (Beesley et al., 2011; Ahmad et al., 2012). These results indicate the effectiveness of SSBC in reducing the available fractions of PTEs in Mn-mining impacted soil. However, SSBC effect on available Mn (in comparison to the other PTEs assessed)was the lowest (Table 2). This could be related to its high concentration (1367 mg kg^{-1}) and its very high availability under the reducing paddy soil conditions (Führs et al., 2010).

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

3.3 The influence of biochar on metal accumulation

The concentrations of As, Cd, Co, Cu, Pb, Mn and Zn in the plant tissues were significantly ($P \leq 0.05$) reduced in SSBC amended soil compared to control. The decrease in bioaccumulation of PTEs was element-dependent and corresponded to the decreased availability of PTEs in soil (Table 3). In comparison to the control PTE accumulation in grain was significantly ($P \leq 0.05$) reduced: As(60.2-67.5%), Cd (26.5-42.0%),Co (40.6-54.7%), Cu (24.0-29.3%), Mn (36.3-42.5%), Pb (32.5-37.7%) and Zn (16.6-22.0%) concentrations in SSBC amended soils compared to the control. The difference in PTE accumulation (except for Cd) in grain was not significantly different betweenSSBC5 and SSBC10 treatments. Similarly, PTE concentrations in leaves were decreased by SSBC addition: As (76.6-86.5%), Cd (76.5-85.9),Co (35.4-51.4%), Cu (47.7-50.1%),Mn (10.6-15.4%), Pb (14.4-20.3%) and Zn (11.2-42.3%).

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

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

The addition of SSBC significantly ($P<0.01$) reduced the concentrations of As species with resepct to the control: AsIII (66.7-72.2%), AsV(46.9-62.2%) and DMA (38.6-73.6%), while MMA in the grain was below detection limit(Table3).In leaves, SSBC addition significantly ($P<0.01$) decreased the concentrations, relative to the control, of: AsIII (77.7-87.8%), AsV (74.1-80.8%) and DMA (33.3-62.4%). In keeping with observations for grains and leaves, SSBC addition also significantly ($P<0.01$) decreased the concentrations, relative to the control, of:AsIII (84.8-92.2%), AsV (51.7-63.0%) and DMA (27.4-69.4%) in stems. MMA was below detection limit in leaves and stem samples. These findings indicated that SSBC addition reduced As uptake, while biochar prepared from rice residues have been shown to increase (327%)As accumulation in rice (Zheng et al., 2012). These contrasting outcomes could well relate to the specific properties of the biochars used and the specifics of the soils and their PTE loadings. Further comparative studies are clearly needed to establish how these factors influence outcomes with respect to As phytoaccumulation.

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

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

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

3.4 Daily intake of PTEs and their health risks

In order to contextualize health risks associated with PTE intake via rice consumption, EDI and HQ were calculated (Table 4). The daily intake of PTEs was estimated using the average rice consumption by inhabitants. The average EDI was significantly ($P \leq 0.01$) reduced following the addition of SSBC (both 5% and 10%) by 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 As, Cd, Co, Cu, Mn, Pb and Zn, respectively. This estimated EDI for the control treatment was far higher than the tolerable limits or RfD, set for daily exposure to PTEs without any substantial health risk over a whole lifetime(USEPA, 2010). SSBC addition reduced EDI (except for Cu and Mn) to values close to the RfD limits instead of sever contamination of PTEs in mine impacted soil. Further research is needed in this regard to investigate long term effects of SSBC on EDI through consecutive cultivation of rice in SSBC amended soil under field conditions.

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

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

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

The food chain is one of the main pathways of human exposure to PTEs (Khan et al., 2008a,b). In China, rice is the main staple food (FAO, 2011). On account of the high capacity rice has to accumulate PTEs and its high level of consumption rice is considered to be the most important source of exposure to PTEs (Williams et al., 2009). Zhuang et al., 2009 reported dietary PTE exposure through consumption of rice to be3-11 times higher than that associated with vegetables. Ji et al. (2013) reported rice consumption to contribute more than 75%of the PTE intake for a population of village near the abandoned mine in Goseaong, Korea.

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

Given that sewage sludge is problematic waste it is suggested that its diversion into biochar production could represent a solution to this waste being disposed of to land. The results presented herein indicate that the targeted application of SSBC to PTE contaminated soil has beneficial outcomes in terms of food safety and reduced cancer risk associated with rice consumption. However, for such benefits to realized the application of SSBC to soil needs to be cost effective. Calculation, detailed in the SI, indicate SSBC production costs to vary from 0.08-0.59 USD per kg. This range reflecting pyrolysis conditions that varied from 3 to 6 h and temperatures from 400 to 600 ^{0}C. Conceptually, it would be advantageous to produce SSBC as close as possible to the locations where it is intended for application. It is submitted that the per kg SSBC production costs suggest the application of 10% SSBC to 1 hm-2 to be of the order of XXX. Assuming SSBC production close to point of application we suggest these costs to be acceptable when set against the reduction in cancer risk that could be achieved. However, further study is needed to look at the economics and life cycle analysis of biochar use in agricultural systems.

4. Conclusion

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

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

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

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Table 1

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

Properties	SSBC		Soil		SEPA[a]	Background soil[b]
pH ($CaCl_2$)	7.18		5.47		NA[d]	
EC (mS/cm)	1.76		0.79		NA	
BET Surface Area ($m^2\ g^{-1}$)	5.57		ND[c]		NA	
Pore Volume ($cm^3\ g^{-1}$)	0.015		ND		NA	
Pore Size (nm)	10.6		ND		NA	
	Total (in d.w)	Available (in d.w)	Total (in d.w)	Available (in d.w)		
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[e]	11.7	0.31[e]	NA	
Na (g/kg)	110	3.67[e]	3.31	1.74[e]	NA	
P (g/kg)	57.8	18.2[f]	2.10	16.9[f]	NA	
As (mg/kg)	10.2	0.05[g]	24.0	0.94[g]	30	5.88
Cd (mg/kg)	**4.06**	0.32[g]	3.55	0.35[g]	**0.3**	0.05
Co (mg/kg)	3.14	0.38[g]	4.12	0.20[g]	NA	
Cu (mg/kg)	**224**	6.78[g]	130	23.2[g]	**50**	19.8
Mn (mg/kg)	1367	38.7[g]	5848	1215[g]	NA	
Pb (mg/kg)	26.7	2.15[g]	1151	314[g]	250	35.6
Zn (mg/kg)	**1101**	137[g]	1473	1058[g]	**200**	79.5

[a]Maximum acceptable limits set for soil by the State Environmental Protection Administration(SEPA, 1995);

[b]Soil background values taken from Chen et al. (1992) for Fujian province, China;

[c]ND not determined;

[d]NA not allocated;

[e]NH_4OAc-extractable;

[f]Colwell P;

[g]bioavailable–EDTA (0.05 M)extracted metals

Table 2

Chemical characteristics of SSBCtreatmentsand 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_4-N (mg/kg)	151	184	229
NO_3-N (mg/kg)	28.0	52.3	65.6
DOC (mg/kg)	172	230	327
K[a] (mg/kg)	312	326	402
Na[a] (mg/kg)	1741	1880	1987
P[b] (mg/kg)	16.9	58.8	83.3
As[c] (mg/kg)	0.94	0.81	0.73
Cd[c] (µg/kg)	353	319	302
Co[c](µg/kg)	199	169	148
Cu[c] (mg/kg)	23.2	18.3	16.5
Mn[c] (mg/kg)	1215	1145	1130
Pb[c] (mg/kg)	314	236	219
Zn[c] (mg/kg)	1058	985	978

[a]NH_4OAc exchangeable, [a]vailable P (Colwell P), [c]EDTAavailable

Table 3

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

	Control			SSBC5			SSBC10		
Parameters	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)[a]	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)[b]	1.52			0.61			0.51		
ILTR[c]	2.28E-03			9.16E-04			7.68E-04		
Extraction efficiency (%)[d]	70.1	89.9	87.6	75.2	88.1	89.1	70.6	89.3	89.1

[a]The value of iAs is the sum of AsIII and AsV;

[b]Average daily intake of iAs was calculated using the equation presented in materials and methods section;

[c]ILTR was estimated using the daily intake of iAs and cancer slope factor of iAs values in the equation given in materials and methods section.

[d]Extraction efficiency for As species was calculated from the sum of As species divided by total As extracted with H_2O_2(35%) and conc. HNO_3 and multiplied by 100.

Table 4

EDI (mg/kg/d) and HQfor individual PTEsattributable tothe consumption of rice grown in mine impacted and SSBC treatedsoils. Where HQ was <1 this has been indicated in bold.

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	**0.71**
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	**0.98**	3.17E-03	**0.90**
Zn	3.21E-01	1.07	2.68E-01	**0.89**	2.50E-01	**0.83**

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

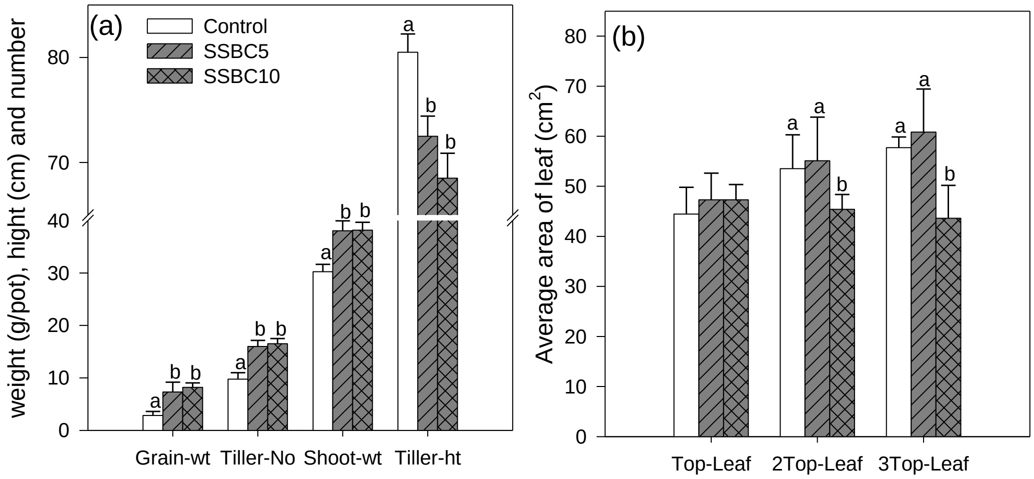

Fig.1. Effects of SSBC amendments on rice plantsgrowth:a) plant biomass (grain and shoot), tillernumber and tiller height, and b) leaf area for top three leavesgrownin the control soil (white) and soil amended with SSBC5 (dark hatched) and SSBC10 (dark cross-hatched). Error bars represent standard deviations (n=4).Different letters indicate significant difference (P≤0.01) between treatments, while similar letters and parameters without letters indicate no significantdifference.

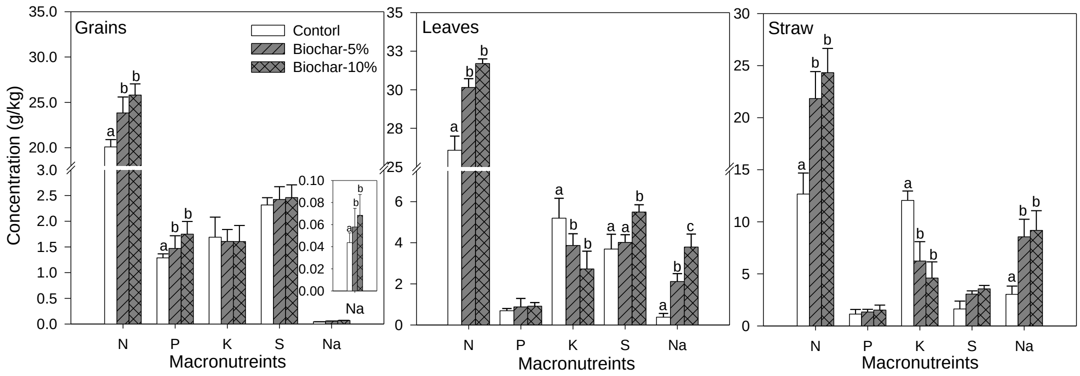

Fig.2. Nutrient concentrations in rice grains, leaves and stemsgrowninthe control soil (white)and soil amended with SSBC5 (dark hatched) and SSBC10 (darkcross-hatched). Error bars represent standard deviations (n=4). Different letters indicate significant difference (P≤0.01) between treatments, while similar letters and parameters without letters indicate no significantdifference.

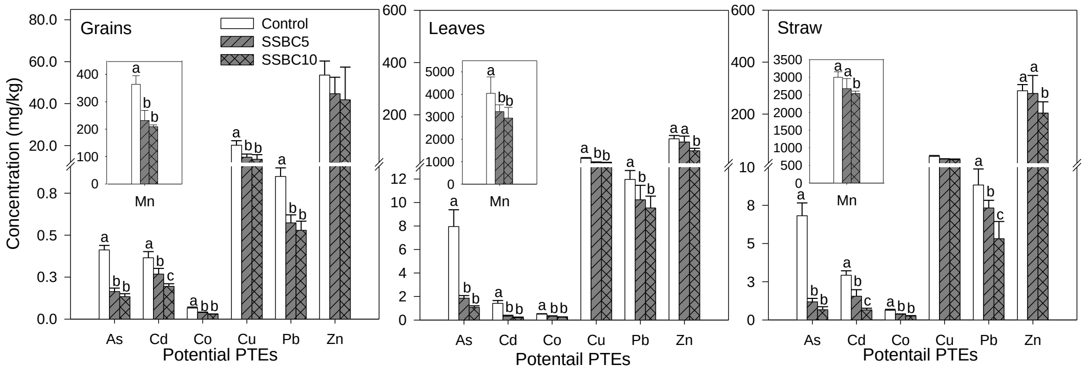

Fig.3.PTE concentrations in rice grains, leaves and stemsgrowninthe control soil (white)and soil amended with SSBC5 (dark hatched) and SSBC10 (darkcross-hatched). Error bars represent standard deviations (n=4). Different letters indicate significant difference (P≤0.01) between treatments, while similar letters and parameters without letters indicate no significantdifference.