

## Evaluating cadmium bioavailability in contaminated rice paddy soils and assessing potential for contaminant immobilisation with biochar

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### Abstract

Cadmium (Cd) contaminated soils from the Mae Sot district in northwest Thailand, a region in which rice Cd concentrations often exceed health limits (0.4 mg/kg) set by the World Health Organisation, were examined for isotopically exchangeable Cd (Cd E values using a <sup>111</sup>Cd spike) to determine how this rates as a predictor of rice grain Cd in comparison with soil total Cd and solution extractable Cd (using the commonly applied BCR scheme and, in an attempt to distinguish carbonate bound forms, the Tessier soil sequential extraction scheme reagents). Step 1 of the BCR scheme (0.11 M CH<sub>3</sub>COOH) and step 1 of the Tessier scheme (1M MgCl<sub>2</sub>) showed the highest R<sup>2</sup> values in regressions with rice Cd (91% and 90%, respectively), but all predictors were strongly linked to rice Cd (p<0.001) and could be used for prediction purposes. One soil, of the six tested, was an exception to this, where all predictors over-estimated grain Cd by a factor of 2.5 to 5.7, suggesting that rice grain Cd had been restricted here by the differing flooding regime and subsequent changes to redox conditions. E values and Tessier step 1 extractions were closely related, indicating that these measurements access similar pools of soil Cd. Separately, the isotopic exchangeability (representing bioavailability) of Cd was also assessed in two soils amended with rice husk and miscanthus biochars (0, 1, 5, 10, 15 and 20% w/w) in order to assess the utility of the biochars as a soil amendment for immobilising Cd in-situ. One soil showed significant reductions in Cd E value at 5% rice husk biochar addition and at 15% miscanthus biochar addition however, based on the E value-rice grain Cd regression relationship previously established, the E values in the amended soils still predicted for a rice Cd concentration above the health limit. In the second soil, neither of the biochars successfully reduced the Cd E value. This indicates that further work is needed to customise biochar properties to suit specific soil and contaminant situations if they are to be used successfully for remediation of metal contaminated soils.

**Keywords:** cadmium, soil, contamination, remediation, biochar; rice

### 1. Introduction

Metal contamination of soils is a long recognised and persistent environmental problem in many parts of the world. Indeed, ecological and/or human health concerns linked with elevated soil metal concentrations have been reported in every continent (e.g. Basta and Gradwohl, 2000; de Lima Neto et al., 2017; Farmer et al., 2011; Flynn et al., 2002; Mungai et al., 2016; Pereira et al., 2017; Taylor et al., 2010; Zhao et al., 2015). Contamination can arise from many potential sources, including mining and smelting activities, the use of rock phosphate or sewage sludge in agriculture, gaseous and particulate emissions from coal combustion, other industrial releases, and vehicular emissions (e.g. Atkinson et al., 2011; McGrath et al., 1988; McLaughlin et al., 1999; Santos et al., 2015). Transfer from soil to plants and so entry into food chains is a primary concern in relation to soil metal content and, while any metal can become toxic at elevated concentrations, cadmium (Cd) is a particular problem because it is a non-essential element that can accumulate in plants at levels that are harmful to humans and other animals before becoming phytotoxic (Prince et al., 2002). Rice is known to accumulate Cd when grown on contaminated soils and human health impacts, particularly

kidney disease and associated conditions, have been conclusively linked to Cd exposure via rice consumption (Cai et al., 1990; Chaney, 2015; Swaddiwudhipong et al., 2012).

One avenue of research that is receiving increasing attention is the use of soil amendments to bind Cd (and other contaminants) in situ and so restrict bioavailability and assimilation into plants. Biochar, a charcoal-like material created via thermal decomposition (or pyrolysis) of organic biomass under limited supply of oxygen (Lehmann and Joseph, 2009), has shown promise in this regard with several studies having highlighted the capacity of biochars made from various feedstocks to restrict mobility and availability of metals in soils (Beesley and Marmiroli, 2011; Fellet et al., 2014; Houben et al., 2013). Such studies have shown decreased concentrations of metals (e.g. Cd, Pb and Zn) in the porewaters and vegetation of mining or otherwise metal contaminated soils following treatment with biochar (Puga et al., 2015). However, other studies have reported mixed results, including cases where biochar additions were found to lower the water soluble Cu and Cd concentrations in soil but left unchanged or enhanced the concentrations in earthworms (Gomez-Eyles et al., 2011). The contaminant binding capacity of biochars and their use in environmental management is therefore a growing and evolving area of research. It is understood that the structure of biochar, being porous with high surface area and reactive surfaces, is what leads to this high potential binding capacity (Lehmann and Joseph, 2009; Sohi et al., 2009). Its alkalinity, i.e. typically pH 7-9 (Beesley et al., 2011), also likely contributes to its binding potential. However, the nature of the feedstock and the pyrolysis conditions during biochar production will have an influence on the properties of the end product (Buss et al., 2016) and so research is needed to establish the efficacy of various types of biochars for this application. Furthermore, in order to be of practical use in a soil remediation situation, the feedstock materials need to be available locally or regionally and so this must also be considered when trials or treatments with biochars are conducted.

The Tak province of northwest Thailand has areas of Cd contaminated soils where health and environmental problems have been identified hence numerous studies have investigated the Cd levels in soils and rice of the region as well as the health effects (Akkajit and Tongcumpou, 2010; Kosolsaksakul et al., 2014; Simmons et al., 2005; Swaddiwudhipong et al., 2012). The aims of the current study were to further examine the mobility and bioavailability of Cd in soils from one important area within this region of Thailand by employing isotopic exchange techniques and comparing them with previous assessments based on single and sequential extraction methods. Isotopic exchange techniques have been described as a rigorous and direct way of assessing the lability of metals in soil (e.g. Huang et al., 2011; Nakhone and Young, 1993; Smolders et al., 1999) and, as outlined by Degryse et al. (2011), they function by discriminating between isotopically exchangeable ('labile') and nonexchangeable ('non-labile') metal pools in soils. The technique involves adding a small amount of an isotopic tracer of the analyte of interest to the soil and determining the degree to which the native element within the soil can exchange with it between solid and solution phases. Moreover, the study aimed to examine properties (surface functional groups) of biochars produced from locally available materials (rice husk and miscanthus) and evaluate the feasibility of using them as a soil amendment to restrict the mobility, and thus bioavailability, of Cd.

## **2. Methods**

### **2.1 Sampling sites**

Soils (top 20 cm) and rice grain were collected from sites within a set of 18 rice paddy fields located in a Cd contaminated region in the Mae Tao watershed, Mae Sot District, Tak Province, Thailand (Figure 1), the location and contamination history of which has been fully described in our previous paper (Kosolsaksakul et al., 2014). In brief, the area supports regionally important cascade-irrigated rice paddy fields that are linked to the Mae Tao Creek (the main water supply) through a system of canals. The soils within the set of 18 fields examined in this study have pH ranging 6.8-7.7, organic matter between 1.1 and 3.7% and a loamy texture with 16-27% clay (Kosolsaksakul et al., 2014). These soils have been found to

have Cd concentrations from 2.5 to 88 mg/kg (Kosolsaksakul et al., 2014), while other soils in the nearby region were found to have even higher Cd levels, e.g. up to 284 mg/kg at Ban Pha Te (Simmons et al., 2005). The elevated Cd observed in many soils of this area is likely linked to the close proximity (<5 km in places) of large scale Zn mining operations (Padaeng deposit – a major Zn mineralisation with substantial levels of Cd as an impurity) (Kosolsaksakul et al., 2014). The general area is recognised as being Cd contaminated and has been the focus of multiple soil remediation and human health investigations (e.g. Akkajit and Tongcumpou, 2010; Khaokaew et al., 2011; Khaokaew and Landrot, 2015; Simmons et al., 2009; Simmons et al., 2005; Swaddiwudhipong et al., 2012).

Details of soil and rice grain collection from each of the 18 fields and the sample processing methods employed (i.e. air-drying and grinding to <2 mm for soils; rice grains were rinsed in de-ionised water, dried at 60°C and ground to a powder) were described previously, as were details of total Cd determinations following *aqua regia* (3:1 HCl:HNO<sub>3</sub>, trace analysis grade) digestion and ICP-OES analysis (Optima 5300 DV instrument, Perkin Elmer, UK) (Kosolsaksakul et al., 2014). Soil samples from selected fields (Table 1), chosen in order to give a spread of the total Cd concentrations observed across the site, were used in the Cd bioavailability and sequential extraction procedures.

## 2.2 Soil extractions

For our previous study (Kosolsaksakul et al., 2014), soils were subjected to the European Communities Bureau (BCR) sequential extraction method (Ure et al., 1993), the first step of which is an extraction with 0.11 M CH<sub>3</sub>COOH at pH 2.85 (targeting the 'exchangeable' fraction, i.e. nominally that bound to carbonates as well as loosely held elements). The soils were also subjected to the first two steps of the Tessier sequential extraction scheme (Tessier et al., 1979) in an attempt to distinguish easily exchangeable forms of Cd (i.e. Cd extractable by 1M MgCl<sub>2</sub> at pH 7; Tessier 1) from those nominally bound to carbonates (i.e. extractable by 1M CH<sub>3</sub>COONa at pH 4.5; Tessier 2). The extract solutions generated were analysed via ICP-OES (Optima 5300 DV instrument, Perkin Elmer, UK) using reagent-specific calibration standards. Further details of the extraction scheme methods, including all quality control measures employed, are provided in our previous paper (Kosolsaksakul et al., 2014). The extractable element concentrations of soils for the first step of the BCR (BCR-1), and first and second step of the Tessier (Tessier-1 and Tessier-2, respectively) schemes determined in that previous study are presented in Table 1 to facilitate comparison with the isotopically exchangeable Cd concentrations determined in the present study.

Table 1. Soil properties and rice grain Cd previously determined for the fields (F) selected for study (mean ± standard error) (Kosolsaksakul et al., 2014)

| Soil | pH        | OM (%)    | % Clay | Soil Cd mg/kg | BCR-1 <sup>a</sup> mg/kg | Tessier-1 <sup>b</sup> mg/kg | Tessier-2 <sup>b</sup> mg/kg | Rice grain Cd mg/kg |
|------|-----------|-----------|--------|---------------|--------------------------|------------------------------|------------------------------|---------------------|
| F1   | 7.68±0.02 | -         | 26     | 4.67±0.08     | 1.97±0.05                | - <sup>c</sup>               | -                            | 0.07±0.02           |
| F4   | 7.52±0.07 | 4.15±0.35 | 22     | 16.78±0.47    | 10.61±0.50               | 7.58±0.15                    | 7.62±0.52                    | 0.90±0.00           |
| F7   | 7.27±0.03 | 4.55±0.25 | 27     | 3.70±0.06     | 0.635±0.01               | -                            | -                            | 0.12±0.02           |
| F10  | 7.70±0.03 | 4.75±0.05 | 16     | 83.69±0.44    | 72.03±2.05               | 20.37±0.36                   | 60.6±0.61                    | 4.03±0.04           |
| F14  | 7.60±0.00 | 1.25±0.15 | 24     | 35.44±4.34    | 22.13±0.23               | 10.12±0.02                   | 16.4±0.38                    | 0.37±0.02           |
| F18  | 7.70±0.02 | 3.60±0.90 | 16     | 81.91±4.74    | 68.24±0.94               | 17.81±0.28                   | 58.1±0.33                    | 2.77±0.03           |

<sup>a</sup> BCR-1: extractable element content of soil considering step 1 of the BCR scheme (0.11 M CH<sub>3</sub>COOH at pH 2.85, targeting the readily 'exchangeable' fraction of elements).

<sup>b</sup> Tessier-1: extractable element content of soil considering step 1 of the Tessier scheme (1M MgCl<sub>2</sub> at pH 7, targeting readily 'exchangeable' fraction of elements but excluding carbonate bound forms).

<sup>c</sup> Not determined.

### 2.3 Isotopically exchangeable Cd

Following the stable isotope exchange method described in Sterckeman *et al.* (2009), an enriched  $^{111}\text{Cd}$  solution (IES-Cd111, Innovative Solutions in Chemistry;  $10.224 \pm 0.029$  mg Cd/kg, isotopic abundances  $^{106}\text{Cd}$  0.008%,  $^{108}\text{Cd}$  0.008%,  $^{110}\text{Cd}$  0.351%,  $^{111}\text{Cd}$  96.167%,  $^{112}\text{Cd}$  2.004%,  $^{113}\text{Cd}$  0.487%,  $^{114}\text{Cd}$  0.867% and  $^{116}\text{Cd}$  0.108%; matrix 2% v/v  $\text{HNO}_3$ ) was used to spike samples with  $^{111}\text{Cd}$ . For calculations of isotope mixing and exchange  $^{114}\text{Cd}$ , the most abundant naturally occurring isotope (28.73% abundance), was used as the other isotope in calculations of isotopic ratio, i.e.  $^{114}\text{Cd}/^{111}\text{Cd}$ . The spike solution was prepared such that the added Cd represented ~0.5-1% of the total Cd already present in the contaminated soils (Table 1), thus meeting the requirement for reliable isotopic exchange studies that the added spike does not appreciably disturb the equilibrium established between solid and solution phases (as discussed by Degryse *et al.* (2011) and Young *et al.* (2006). A 20-fold dilution of the IES-Cd111 solution, using 2% v/v Aristar  $\text{HNO}_3$ , was performed to generate a working spike solution. Then, 1.4 ml of spike solution (0.7  $\mu\text{g}$  Cd) was added to a suspension of 1.00 g soil in 20 ml 0.01 M  $\text{MgCl}_2$  that had been agitated for the previous 48h (mechanised wrist-action shaking) to ensure that it was fully mixed prior to spiking. Chloroform (60  $\mu\text{l}$ ) was also added to inhibit microbial activity (Lombi *et al.*, 2003; Oliver *et al.*, 2006), with the samples then placed on a shaker for one week to ensure isotopic exchange processes reached equilibrium (Ayoub *et al.*, 2003). Samples were then centrifuged at 7000 rpm (6793 g) for 15 minutes, with supernatant solutions filtered (0.22  $\mu\text{m}$  Millex® Syringe Filters) in preparation for Cd isotope analysis via Inductively Coupled Plasma-Mass Spectrometry (ICP-MS; Agilent 7500ce). All soils in Table 1 were subjected to this isotopic exchange measurement in duplicate (n=2). Isotopically exchangeable Cd (or the Cd E-value) was calculated according to Equation 1 (Nolan *et al.*, 2004; Oliver *et al.*, 2006):

$$E \text{ value (mg/kg)} = R \times \frac{AW_{\text{nat}}}{AW(^{111}\text{Cd})} \times \frac{IR_{\text{sp}} - IR_{\text{mes}}}{IR_{\text{mes}} - IR_{\text{nat}}} \times (IR_{\text{nat}} + 1) \quad \text{Eq.1.}$$

where;

|                       |   |  |
|-----------------------|---|--|
| E value               | = | isotopically exchangeable Cd (mg/kg),  |
| R                     | = | total concentration of $^{111}\text{Cd}$ in the spike ( $\mu\text{g}$ ) / soil weight (g), |
| $AW(^{111}\text{Cd})$ | = | atomic weight of $^{111}\text{Cd}$ isotope,  |
| $AW_{\text{nat}}$     | = | atomic weight of natural Cd (112.411)  |
| $IR_{\text{nat}}$     | = | the $^{114}\text{Cd}/^{111}\text{Cd}$ ratio based on natural abundances                    |
| $IR_{\text{sp}}$      | = | the $^{114}\text{Cd}/^{111}\text{Cd}$ ratio in the spike solution                          |
| $IR_{\text{meas}}$    | = | measured $^{114}\text{Cd}/^{111}\text{Cd}$ ratio in the soil solution after equilibration  |

Quality control was achieved through checking Cd isotope measurement stability ( $^{106}\text{Cd}$ ,  $^{108}\text{Cd}$ ,  $^{111}\text{Cd}$  and  $^{114}\text{Cd}$ ) in standard solutions (0.5-100  $\mu\text{g/l}$ ), with a high degree of linearity in instrumental response having been maintained (i.e.  $R^2$  values of 0.999 or better obtained for each Cd isotope, see supp. Information). Stability within analytical runs was verified by determining the  $^{114}\text{Cd}/^{111}\text{Cd}$  ratio in the 5 and 10  $\mu\text{g/l}$  Cd standard solutions after every 5 or 6 samples (example values of  $2.32 \pm 0.004$  and  $2.35 \pm 0.003$ , respectively, observed). The  $^{114}\text{Cd}/^{111}\text{Cd}$  ratio was also determined in spiked blanks (i.e. no soil, just 20 ml 0.01 M  $\text{MgCl}_2$ ) and were found to be, in two separate batches,  $0.01291 \pm 0.0002$  and  $0.01183 \pm 0.0003$ , which are in close agreement with the calculated  $^{114}\text{Cd}/^{111}\text{Cd}$  ratio of the pure enriched  $^{111}\text{Cd}$  spike. In addition, the natural  $^{114}\text{Cd}/^{111}\text{Cd}$  ratio was directly determined in unspiked soil samples ( $2.34 \pm 0.005$ , n=11) and was used as the natural abundance ratio ( $IR_{\text{nat}}$ , Eq.1) in all calculations of isotopically exchangeable Cd. This measured natural abundance compares well with the theoretical natural abundance ratio of 2.25. A final quality control measure was to determine the  $^{114}\text{Cd}/^{111}\text{Cd}$  ratio in a certified reference soil (CRM/SRM1643, certified Cd  $6.568 \pm 0.073$  mg/kg), determined as  $2.33 \pm 0.007$  (n=10), in good agreement with the isotopic ratios obtained for the test soils.

Because the isotopically exchangeable Cd (E values) closely matched the extractable Cd determined by step 1 of the Tessier scheme (see results section 3.1), a further experiment was conducted in which the isotopically exchangeable Cd was determined on the residues of samples following Tessier-1 extraction. The aim was to evaluate whether the E value and Tessier-1 procedures did indeed access the same pool of Cd in the test soils.

#### 2.4 Assessing Cd immobilisation via biochar additions

Two types of biochar were trialled for their capacity to immobilise Cd in contaminated soils and thereby restrict Cd bioavailability as measured by isotopic exchangeability (E values). The biochars were made at the Biochar Research Centre (University of Edinburgh) by pyrolysis of rice husk and miscanthus grass feedstocks (separately) at 350°C. Rice husk and miscanthus were chosen because they reflect raw materials available to farmers in the Mae Sot district. The biochars were characterised in terms of cation exchange capacity (CEC) and surface functional groups (Fourier Transform Infra-red spectroscopy, FTIR) in order to provide insight into possible Cd binding processes. CEC was determined following Sumner and Miller (1996), where 1.5 g finely ground solid ( $n=3$ ) was treated three times in succession with 33 ml 0.2 M  $\text{CaCl}_2$  - 0.0125 M  $\text{CaSO}_4$  solution (15 minute shaking, centrifuged for 15 minutes at 7000 rpm (6793 g) and supernatant discarded) and then washed repeatedly with deionised water to remove excess Ca (until Ca concentrations were  $<1$  mg/l, verified by ICP-OES). Then, three times in succession, 33 ml 0.2 M  $\text{Mg}(\text{NO}_3)_2$  was added to displace Ca, samples were shaken for 15 minutes, centrifuged for 15 minutes (7000 rpm, 6793 g), and supernatants filtered (Whatman 42) into a 100 ml flask, after which the volume was made to 100 ml with deionised water. Displaced Ca (and thus CEC) was measured by ICP-OES. To facilitate FTIR analysis, potassium bromide (KBr) discs were prepared by mixing 1.5 mg dried and ground biochar samples with 200 mg dried IR grade KBr using an agate pestle and mortar. The homogeneous powder was then placed between stainless steel discs, pressure ( $\sim 10$  tonnes for  $\sim 1$  minute) applied by hydraulic press (Specac) and the resulting disc transferred into the FTIR spectrometer (Perkin Elmer spectrum 65) for analysis. Analysis conditions were: scanned wavenumber range 4000-400  $\text{cm}^{-1}$ ; scanning increment 2  $\text{cm}^{-1}$ ; number of scans set to 12; and background correction achieved using a blank KBr disc (i.e. no sample).

The soils used for the Cd immobilisation trial were from field 10 (Table 1, collected on the original sampling trip) and field 14 (collected from near the field 14/15 margin on a subsequent collection trip, total Cd 61.5 mg/kg, referred to henceforth as field 14b). These soils were selected because they were in the mid to high Cd contamination range and thus were representative of soils from this area that most warranted remediation trials. Finely ground (powderised) biochars were added to the soils at rates of 0, 1, 5, 10, 15 and 20% w/w, and after mixing and equilibration in 0.01 M  $\text{MgCl}_2$  solution for 2 days the isotopically exchangeable Cd was determined using the  $^{111}\text{Cd}$  method as described above (with E values calculated according to soil mass). Biochar only samples (i.e. no soil) were also assessed for isotopically exchangeable Cd and were found to have negligible amounts ( $\leq 0.1$  mg/kg).

Statistical evaluation of data was conducted via correlation, regression and analysis of variance (ANOVA) using Minitab17 following appropriate data suitability tests.

### **3. Results & Discussion**

#### 3.1 Extractable, isotopically exchangeable and rice grain Cd comparisons

The various abiotic measures of exchangeable (available) Cd employed produced comparable results for soils with total Cd  $<20$  mg/kg but were more varied for soils with greater contamination levels (Figure 2). Isotopically exchangeable Cd correlated well with Tessier 1 extracts across all soils ( $r = 0.904$ ,  $p = 0.002$ ), whereas BCR 1 and Tessier 2 extracts were far greater than E values for soils with high Cd. This may be related to both BCR 1 and Tessier 2 extracts accessing Cd bound to carbonate phases in the higher Cd soils that are not



isotopically labile. When isotopically exchangeable Cd was measured in soils previously subjected to the Tessier 1 extraction (Table 2), i.e. to more closely examine the relationship between Cd pools accessed by the two methods, in most cases the E values were substantially lower than for the equivalent soils not subjected to a prior extraction. The reduction was  $\geq 50\%$  for most soils, indicating that the Tessier 1 and isotopically exchangeable methods did access similar pools of soil Cd. The reductions were not 100%, however, indicating that the pools accessed did vary to a degree and/or that the Tessier 1 extraction process exposed a portion of the Cd that was previously not isotopically exchangeable. Interestingly, it was soil from field 14 that showed the lowest reduction in E value following Tessier 1 treatment (37%), and it was this soil that also stood out when rice grain concentrations were compared with the abiotic measurements of available Cd (see below).

Regression analysis revealed that rice grain Cd was associated with, and so could be predicted by, all abiotic measures of available Cd as well as total Cd (Table 3). Considering rice grain and all predictors across all fields, the regression coefficient was greatest for BCR 1 and lowest for E value. However, when rice grain Cd was back-predicted from the derived regression equations it was clear that E values were in fact the best predictor of rice grain Cd for several of the soils (e.g. F4, F18; Figure 3). All of the predictors grossly overestimated rice grain Cd for soils from field 14, which was unexpected considering that this soil is comparable to the others in terms of key parameters such as pH and clay content. This set of results may suggest that it is not the Cd availability estimates that have failed for soil from field 14 but rather that some other process has restricted Cd assimilation here, a process possibly linked to site management. As noted previously (Kosolsaksakul et al., 2014), field 14 remains wetter for longer than the other soils because of its position in the cascade irrigation system at the site and therefore an oxidation-reduction related process may be restricting the assimilation of Cd. This would fit with the Mn-Cd uptake competition theory discussed by Chaney and others (Chaney et al., 2016; Fulda et al., 2013) in which dissolved  $\text{Mn}^{2+}$  in soil solution of wet soils strongly inhibits Cd uptake but upon soil drying is re-oxidized to  $\text{MnO}_2$  and no longer competes with Cd for uptake by the roots. If this were the case, any abiotic measurement of available Cd in such soils may have to be interpreted with this point in mind.

Table 2. Isotopically exchangeable Cd ( $^{111}\text{Cd}$  E value, mg/kg, mean  $\pm$  standard error) in soils and in soils previously extracted with 1M  $\text{MgCl}_2$  at pH 7 (Tessier step 1 reagent)

| Soil | E value         | E value post Tess 1 extraction | Reduction (%) post Tess 1 |
|------|-----------------|--------------------------------|---------------------------|
| F1   | 1.93 $\pm$ 0.01 | 1.04 $\pm$ 0.01                | 46.1                      |
| F7   | 0.75 $\pm$ 0.01 | 0.25 $\pm$ 0.01                | 66.7                      |
| F4   | 6.92 $\pm$ 0.07 | 3.08 $\pm$ 0.07                | 55.5                      |
| F14  | 14.5 $\pm$ 0.16 | 9.12 $\pm$ 0.01                | 37.1                      |
| F10  | 19.0 $\pm$ 0.14 | 9.6 $\pm$ 0.41                 | 49.5                      |
| F18  | 18.0 $\pm$ 0.62 | 7.89 $\pm$ 0.34                | 56.2                      |

Table 3. Regression equations and coefficients for rice grain Cd (mg/kg) vs predictors total Cd (*aqua-regia* 3:1 HCl:HNO<sub>3</sub> digestion, BCR1 (0.11 M CH<sub>3</sub>COOH extraction at pH 2.85), Tessier 1 (1M MgCl<sub>2</sub> extraction at pH 7), Tessier 2 (1M CH<sub>3</sub>COONa extraction at pH 4.5), and <sup>111</sup>Cd E value

| Equation                             | Model p value and regression coefficient |
|--------------------------------------|--|
| 0.041 Total Cd (mg/kg) - 0.184       | p<0.001; R <sup>2</sup> = 86.4%          |
| 0.048 BCR1 Cd (mg/kg) - 0.029        | p<0.001; R <sup>2</sup> = 91.0%          |
| 0.262 Tessier 1 Cd (mg/kg) - 1.65    | p<0.001; R <sup>2</sup> = 89.5%*         |
| 0.057 Tessier 2 Cd (mg/kg) - 0.010   | p= 0.001; R <sup>2</sup> = 86.2%*        |
| 0.047 Tessier 1+2 Cd (mg/kg) - 0.310 | p= 0.001; R <sup>2</sup> = 87.15%*       |
| 0.166 E value Cd (mg/kg) - 0.312     | p= 0.001; R <sup>2</sup> = 66.5%         |

\* Regression derived from 4 fields only (Tessier extractions not performed on F1 or F7).

### 3.2 Cd immobilisation by biochar

Rice husk and miscanthus biochar CECs were 8.12±0.01 and 3.6±0.54 meq/100 g, respectively, which is comparable with CECs reported elsewhere for biochars produced from wheat-straw (0.5-5.1 meq/100 g) and peanut shell (0.3-7.2 meq/100 g) (Gai et al., 2014). FTIR analysis (Figure 4) revealed the two biochars to have similar surface functional groups, with features including non-H bonded OH groups (~3500 cm<sup>-1</sup>), carbonyl C=O (e.g. 1697 cm<sup>-1</sup>), alcohol/phenol O-H bend (e.g. 1378 cm<sup>-1</sup>), C-O groups (e.g. 1106 cm<sup>-1</sup>) and aryl C-H groups (e.g. 873 cm<sup>-1</sup>), reflecting the observations made by Jindo et al. (2014) for rice husk and rice straw biochars. However, as shown in Figure 4, there were some discernible differences in surface functional groups between the rice husk and miscanthus biochars; the rice husk biochar had more prominent bands at lower wavelengths and this is attributed to a greater silica content on the grounds that silica is a major component in the chemical structure of rice materials and that silica is known to give strong peaks in this region (Jindo et al. 2014). Moreover, it has been found that rice husk and rice straw biochars have more silica compared to biochars made from other (i.e. woody) source materials (Jindo et al. 2014).

Isotopically exchangeable Cd measurements in amended and non-amended soils revealed statistically significant (ANOVA, with Tukey pairwise comparisons) reductions in E values for field 14b soils at rice biochar application rates of 5% and above (Figure 5). When amended with miscanthus biochar, the same soil only showed significant reductions at application rates of 15% and above (Figure 5). Contrastingly, neither biochar was able to significantly reduce isotopically exchangeable Cd in field 10 soils at any application rate tested (Figure 5). The precise reason for this failure is unclear but it may be linked to the higher organic matter content of field 10 soil (i.e. 4.75% vs 1.25%), which may have enabled indigenous soluble soil organic matter to maintain Cd mobility despite the sorbing capacity of the biochar. Considering that recent work has demonstrated how higher pyrolysis temperatures produce biochars with greater metal sorption capacity (Rodríguez-Vila *et al.* 2017), it may be that employing biochars produced at 550°C (which would have greater sorption capacity than those used in the present study) could overcome this theorised mobilisation effect. Hypotheses aside, the results appear comparable with those of Ippolito *et al.* (2017) who observed varying degrees of success in reducing extractable Cd in mine contaminated soils treated with pine and tamarisk biochars. They found 0.01M CaCl<sub>2</sub> extractable Cd was only modestly reduced in some soils treated with tamarisk biochar at application rates of 10% w/w (i.e. from ~27 to 18 mg/kg and from ~10 to 6 mg/kg for two tested soils respectively) but was more substantially reduced by pine biochar applications (i.e. down to ~5 and 3 mg/kg, respectively, for the same two soils). Houben *et al.* (2013), on the other hand, saw substantial (i.e. 50%) decreases in 0.01M CaCl<sub>2</sub> extractable

Cd in contaminated soils treated with miscanthus biochar at 10% application rates. However, those reductions were largely attributed to the increase in pH brought about by biochar additions (from an acidic ~5.7 to a more neutral 6.7), which would not have occurred to the same extent in the soils tested in the current study because of their much higher natural pH values (~7.7). Therefore, biochar properties and site specific circumstances need to be considered when remediation efforts involving biochar are considered.

Although the results of the current study support the concept, in principle at least, that biochar applications can restrict Cd availability in some soils, the degree of any restriction achieved and its ecological and human health relevance in terms of successful site amelioration needs to be evaluated. If one considers the isotopically exchangeable Cd content of the biochar amended soils tested here and uses them to predict (using regression equations in Table 3) the rice grain Cd that would arise in a crop, it becomes apparent that even for soil 14b (which showed statistically significant reduction in E value following biochar applications) the amount of Cd in the resulting rice would still be an order of magnitude above the safe limit of 0.4 mg/kg (FAO-WHO, 2006). Moreover, this would still be the case at the highest biochar application rate tested (i.e. 20% w/w). Therefore, even at the impractical and likely uneconomical rate of biochar application of 20% by mass (which would equate to ~240 t/ha considering the top 10 cm of soil and a soil bulk density of 1.2 g/cm<sup>3</sup>), the biochars tested here would not achieve the desired level of site amelioration for these soils. This is in contrast to findings reported by Bian and co-workers (Bian et al., 2013) where field applications of wheat biochar to rice paddy soils in China (pH 5.0-6.3; total Cd 0.2-21.8 mg/kg) successfully reduced rice grain Cd to near or below the 0.4 mg/kg health limit in 4 out of 5 soils treated at 20 and 40 t/ha. Again, this emphasises the need to evaluate, select and even deliberately engineer, the properties of biochars produced from different feedstocks and to consider the implications of important site specific conditions such as pH and water (flood irrigation) management.

#### **4. Conclusions**

For the Cd contaminated soils investigated, step 1 of the BCR scheme and step 1 of the Tessier scheme showed the highest R<sup>2</sup> values in regressions with rice Cd (91% and 90%, respectively), but all predictors examined were strongly linked to rice Cd ( $p < 0.001$ ) and could be used for prediction purposes. However, all predictors over-estimated grain Cd by a factor of 2.5 to 5.7 for soil from field 14, suggesting that rice grain Cd had been restricted here by the differing flooding regime and subsequent changes to redox conditions. The precise mechanisms involved should be further investigated to determine whether this phenomenon is explained by the Mn-Cd uptake competition theory. E values and Tessier step 1 extractions were closely related, indicating that these measurements access similar pools of soil Cd.

Biochar addition did reduce the isotopically exchangeable Cd in one soil (at 5% rice husk and 15% miscanthus biochar addition rates, respectively), but made no difference in the second soil. Moreover, based on E-value – rice grain Cd relationships established here, the reductions in isotopically exchangeable Cd observed would not be sufficient to predict reductions in rice grain Cd below health limit thresholds. This indicates that while biochars can reduce Cd mobility, further work is needed to customise biochar properties to suit specific soil and contaminant situations if they are to be used successfully for remediation of metal contaminated soils.

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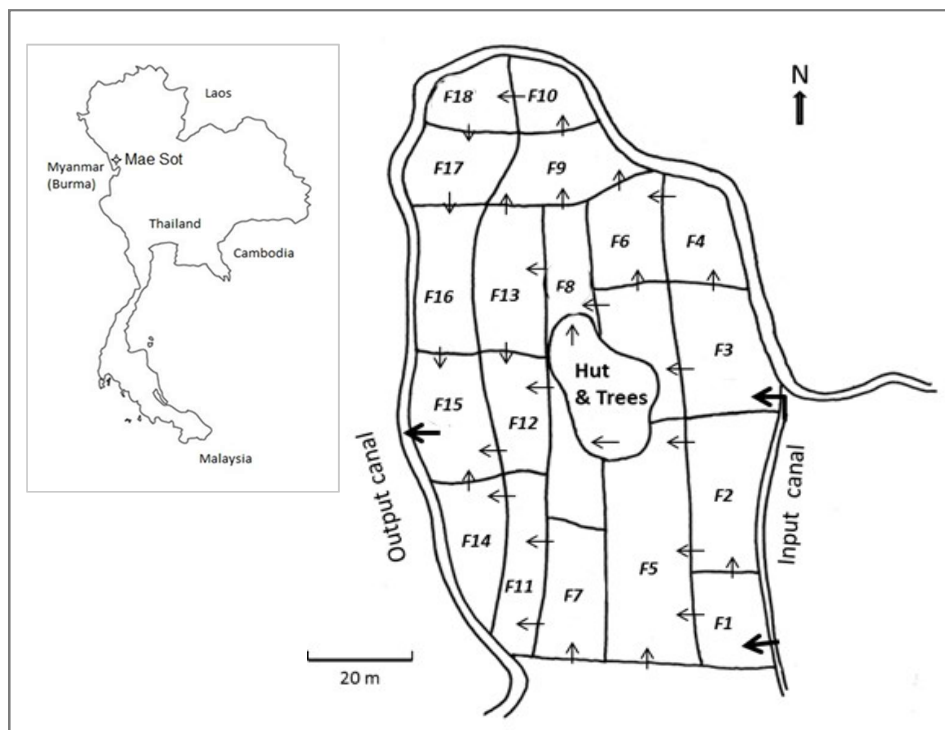


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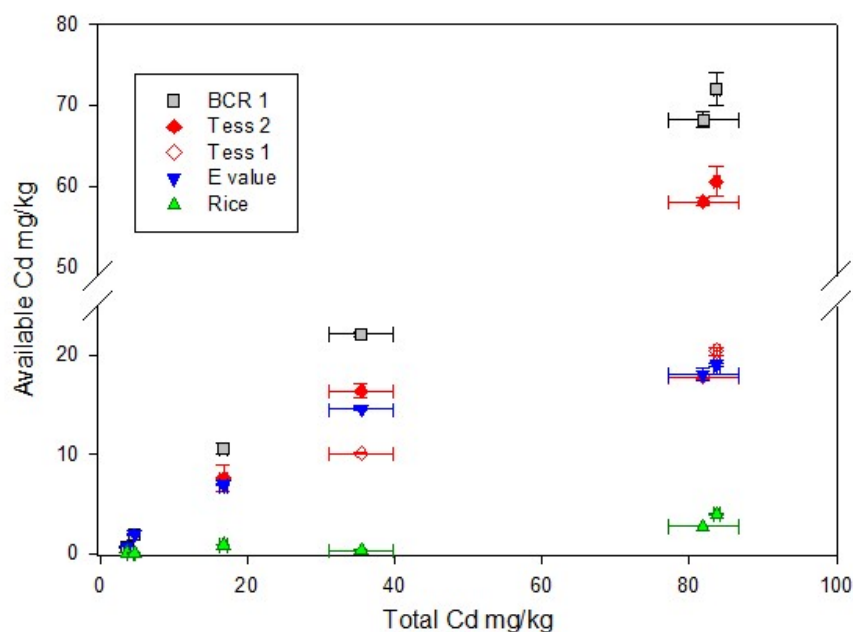
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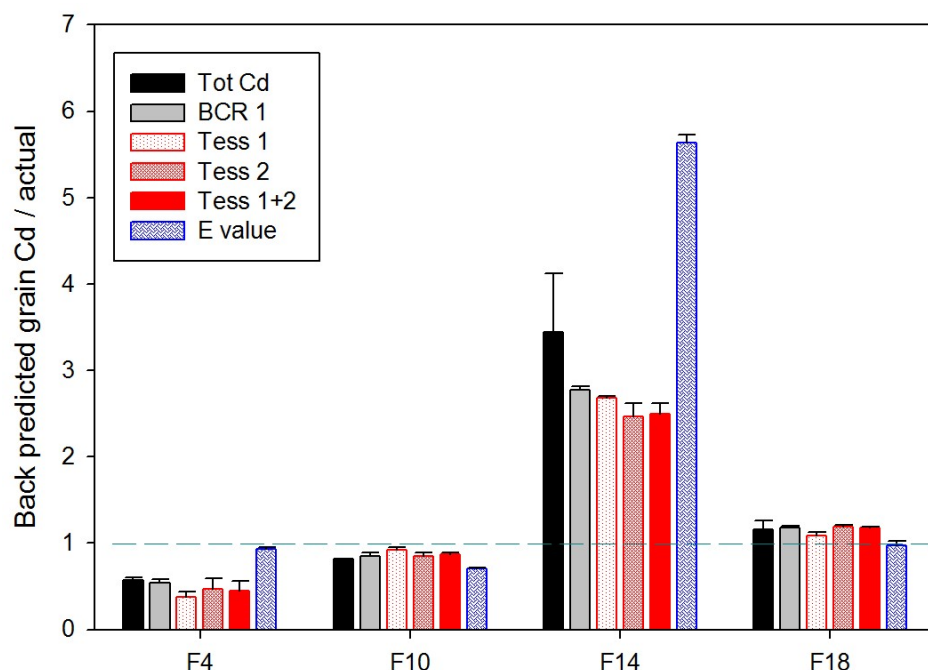
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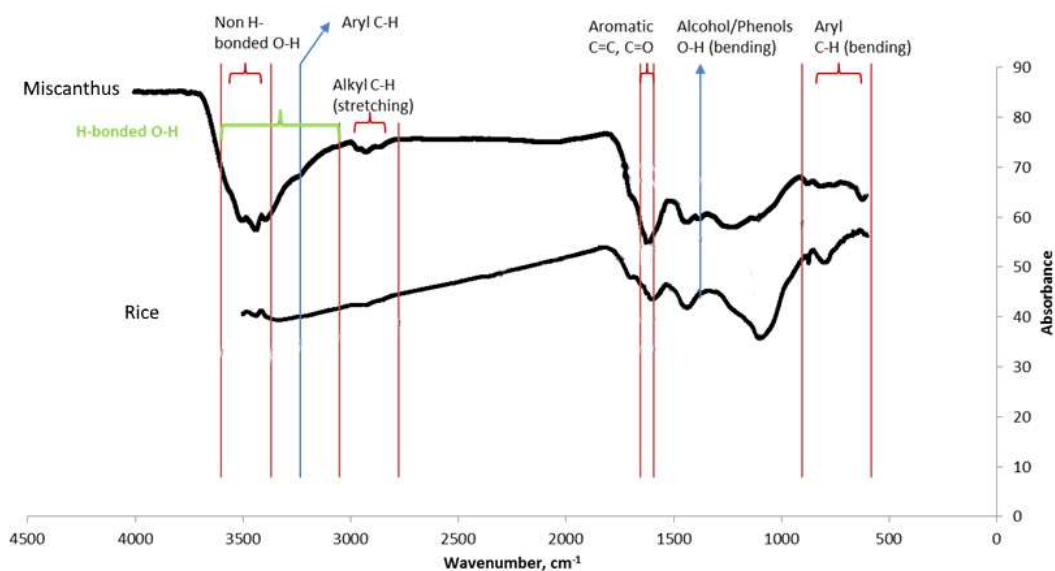
**Figure 1.** Sketch map indicating orientation and water flow direction for the set of rice paddy fields examined (inset map indicates location of the Mae Sot area within Thailand) (adapted from Kosolsaksakul et al. 2014).



**Figure 2.** Comparison of rice grain Cd and various abiotic measures of available soil Cd: step 1 of the BCR extraction scheme (BCR1 = 0.11 M  $\text{CH}_3\text{COOH}$  at pH 2.85), steps 1 and 2 of the Tessier extraction scheme (Tess 1, Tess 2 = 1M  $\text{MgCl}_2$  at pH 7 followed by 1M  $\text{CH}_3\text{COONa}$  at pH 4.5) and isotopically exchangeable Cd ( $^{111}\text{Cd}$  E value). Soils presented according to total soil Cd (note Tessier scheme not performed on soils from Field 1 or 7).

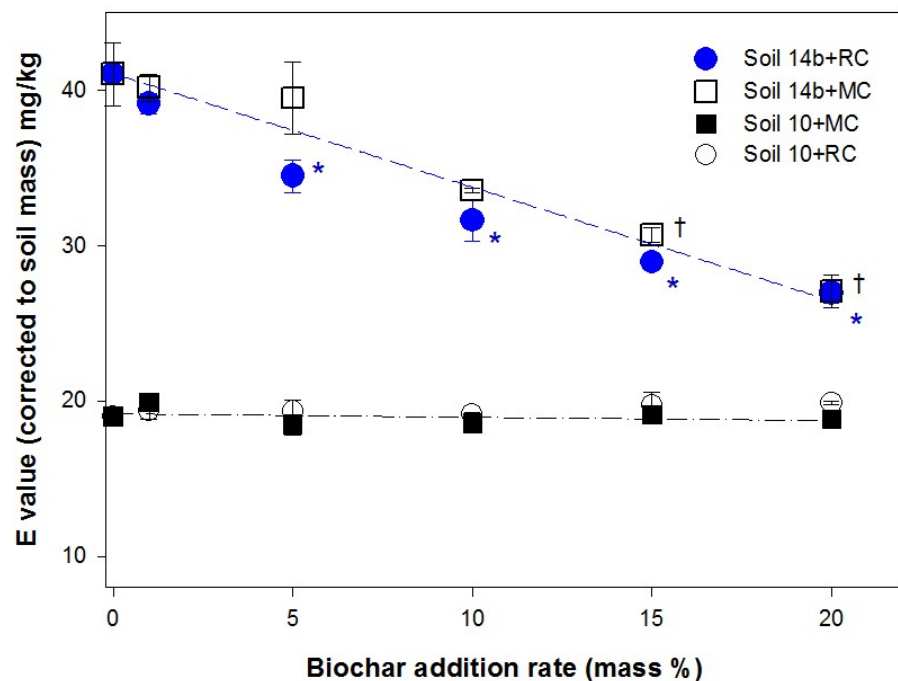


**Figure 3.** Rice grain Cd back-predicted from regression equations in Table 3 divided by measured (actual) values. BCR1 = 0.11 M  $\text{CH}_3\text{COOH}$  extraction at pH 2.85, Tess 1 = 1M  $\text{MgCl}_2$  extraction at pH 7, Tess 2 = 1M  $\text{CH}_3\text{COONa}$  extraction at pH 4.5, E value = isotopically exchangeable Cd ( $^{111}\text{Cd}$ ). Error bars indicate standard deviations. The horizontal dashed line represents a perfect (1:1 predicted:measured) value. Fields 1 and 7 not shown as Tessier extractions were not performed on them.



**Figure 4.** Fourier-transform infrared (FTIR) spectra of the rice husk and miscanthus biochars.





**Figure 5.** Isotopically exchangeable Cd (E value, mg/kg) for field 10 and field 14b soils amended with varying rates of rice (RC) and miscanthus (MC) biochar. Error bars indicate standard errors. Asterisks (\*) indicate values significantly lower than control (0% biochar) for rice biochar treatments while daggers (†) indicate same for miscanthus treatments.

## GRAPHICAL ABSTRACT

