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Full Length Article

Using Passivation Materials to Immobilize Soil Copper in a Rape-Rice Rotation System

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Abstract

Copper (Cu) contamination in agricultural soils is a serious concern and imposing health risks to individuals through Cu soil-food chain transfer. Hence, soil Cu immobilization is essential to reduce its bioavailability in the soil system. This two year (2016–2018) field experiment was conducted to investigate the impacts of nano-hydroxyapatite, quicklime, biochar, bio-organic fertilizer, and mesoporous ceramic functional nanomaterials amendments on Cu bioavailability and its uptake by rape and rice in an acidic soil (pH=5.04). Compared to control plots, passivation materials (PMs) application significantly ($P \leq 0.05$) improved soil pH and organic matter contents by 0.39–12.3% and 3.08–41.3%, respectively. Among them, quicklime was the best to increase soil pH while biochar had the greatest effect on increasing soil organic matter. Moreover, these materials reduced the soil available Cu (DTPA-extractable Cu) and Cu removal by rice and rape crops. After two years restoration, Cu contents in rape and brown-rice seeds ranged between 6.32 to 8.50 and 6.56 to 8.47 mg kg^{-1}, respectively, lower than the *National Standard of Pollutants in Food of China* (GB 15199-1994, 10 mg kg^{-1}). Nonetheless, these materials had slight effect on growth, yield and related traits of both crops. The results showed that the crop yields, plant height, dry weight (DW), 1000-grain weight with PMs were slightly influenced. In conclusion, this two-year field study indicated that the rape-rice rotation combined with PMs application (quicklime and mesoporous ceramic functional nanomaterials in particular) can reduce Cu uptake by rice and rape crops, and therefore, improve their safety. From the perspective of food safety, rape is recommended for Cu-contaminated acidic soil because it had low Cu uptake ability compared with rice.

Keywords: Copper contamination; Soil Cu immobilization; Soil available Cu; Acidic soil; Passivation materials; Rape-rice rotation

Introduction

Soil pollution of heavy metals is one of the major environmental problems in the world (Koptsik 2014). In China, recent rapid development of industry and economy has been a major cause of heavy metals' pollution in agricultural soils. Currently, 13.9% of Chinese grain production is affected by heavy metals due to the intensive human activities such as mining, smelting, industry, sewage irrigation, urban development, and heavy fertilization (Zhang *et al*. 2015; Wang *et al*. 2019).

Because of its wide application and significant output, copper (Cu) has become one of the most common heavy metals in agricultural soils (Seiler and Berendonk 2012). The absence of Cu may cause physiological dysfunction, but its excessive intake causes toxicity in human body (Mclaughlin *et al.* 1999; Mackie *et al*. 2012; Gul *et al*. 2018). Pawar *et al*. (2016) showed that high concentration of Cu (>5 mg L^{-1}) in human body is associated with health problems such as renal failure and liver diseases. Goldhaber's reported that dietary Cu intake of about 200 mg kg^{-1} body weight can cause human death (Goldhaber 2003).

The content of Cu in plants generally ranged from 2 to 50 mg kg^{-1} dry weight (Burkhead *et al*. 2009). Increased concentration of Cu in plant tissues (> 20 mg kg^{-1} DW) may induce the production of reactive oxygen species, causing toxicity. For instance, Yan *et al.* (2006) showed that Cu stress significantly inhibited the occurrence and growth of tillers in rice (*Oryza sativa* L.), resulting in yield reduction. In 2014, the *National Soil Pollution Investigation Bulletin* issued by the Ministry of Environmental Protection of China and the Ministry of Land and Resources of China reported that Cu content in 2.1% of national soils exceeded the standard (50 mg kg^{-1}) (Ran *et al*. 2019). In 2015, 3.01% of Chinese farmland soils were Cu polluted, suggesting the importance of soil Cu contamination in China (Zhang *et al*. 2015).

Rice and rape (*Brassica napus* L.) are both very

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important and widely cultivated crops in southern China. In addition, paddy-dryland rotations are popular agricultural production systems in China and other Asian countries (Ran *et al.* 2019), covering an estimated area of 26.7 million ha in Asia. Because of the suitable climate and people's dietary preferences, rape is not only an oil crop, but also a regional ornamental crop in China (Ran *et al.* 2019). However, intensive agricultural management and industrial activities have resulted in over Cu accumulation in Chinese paddy-dryland rotation soils. Irrigation with contaminated river water (Cu mining and smelting), over application of agrochemicals (*e.g.*, fertilizers and pesticides) and atmospheric deposition are the main causes of soil Cu contamination in Tongling mining area (Koptsik 2014; Zhou *et al*. 2019).

Recently, *in situ* passivation materials (PMs) have been receiving major attention for heavy metals’ restoration and remediation in soils (Ok *et al.* 2011; Mahar *et al.* 2015; Zeng *et al.* 2018). For instance, Bade *et al.* (2012) showed that lime had a significant effect on soil Cu fixation in Janghang, Chungnam, South Korea. In a five years field experiment, Cui *et al.* (2016) reported that available Cu ($CaCl_2$ and $MgCl_2$ extractable Cu) significantly decreased after apatite application in Cu-contaminated acid Guixi farmland soils, East China. In Jiangxi Province, East China, application of various passivators (*e.g.*, limestone, calcium magnesium phosphate, calcium silicate, pig manure, and peat) decreased and increased rice Cu removal and yield, respectively (Li *et al.* 2008). Nevertheless, previous studies have mainly focused on indoor simulation or potted plant conditions to explore the impact of different PMs on restoration of soil Cu, while field trials remained rarely investigated.

Tongling, Anhui Province, East China, is one of the eight major non-ferrous metal industrial bases in China. Tailings, slag, and acid wastewater from smelting, and production of Cu, gold, silver, pyrite, and other polymetallic deposits are the major sources of heavy metals’ pollution in Tongling mining area farmland soils (Duruibe *et al.* 2007; Ye *et al.* 2012; Zhan *et al*. 2012). Hence, restoration of Cu contaminated farmland soils in this area using various PMs is essential, particularly in agricultural soils and crops such as rice and rape. Therefore, this study was conducted with the hypothesis that different PMs application can increase soil pH and organic matter (SOM) coupled with decrease in Cu bioavailability and removal by rice and rape crops and will improve their growth and yield. Moreover, the results of this study will provide basis to farmers and managers to remediate Cu-contaminated soils, improving soil health together with crop productivity and safety.

Materials and Methods

Site and soil

This field trial was conducted from November 2016 to October 2018 in a Cu-contaminated soil in Tongling mining area, Anhui Province, East China (30°56 '39 ''N, 117°59' 16'' E). The study area has a subtropical humid monsoon climate with mean annual temperature and precipitation of 17.6°C and 1300 mm, respectively. In this region, rice-rape rotation is the dominant cultivation scheme. Soils in Tongling mining area are moderately Cu polluted with mean Cu contents of 236 mg kg^{-1}, approximately four times higher than the second-level National Soil Environmental Standard (50 mg kg^{-1}). This acid soil is clay loam in texture with pH 5.04, 23.9 mg kg^{-1} organic matter, 1.38 mg kg^{-1} total N, 236 mg kg^{-1} total Cu, 84.2 mg kg^{-1} available Cu. Available N, P, K content were 112, 50 and 219 mg kg^{-1}, respectively.

Passivation materials for soil amendment

The PMs applied in this study were nano hydroxyapatite ($Ca_{10}(PO_4)_6(OH)_2 \geq 96\%$) with Ca/P of 1.67 and particle size of 60 nm; quicklime ($CaO \geq 95\%$) with strong water absorption; biochar made from peanut shell by pyrolysis and carbonization at 350–500°C; The biological humic acid bacteria agent with a specific surface of 8.9 m^2 g^{-1} and organic carbon of more than 460 g kg^{-1}; bio-organic fertilizer composed of chicken manure and wheat bran. The proportions of N, P_2O_5, and K_2O in bio-organic fertilizer were 2:1:1. It also contained effective bacteria such as *Bacillus subtilis*, and its organic matter content was higher than 20%. Mesoporous ceramic functional nanomaterials, mainly composed of SiO_2 and silicate, have a maximum specific surface area of 180 m^2 g^{-1}. The basic properties of the materials are given in Table 1.

Experimental design

This field experiment consisted of six treatments including: no passivator (CK); nano hydroxyapatite (NHA: 3330 kg ha^{-1} per season); quicklime (QL: 3330 kg ha^{-1} per season); bio-organic fertilizer (BOF: 1000 kg ha^{-1}) of biological humic acid bacteria agent (BHAB) for rape cultivation in the first season and 8330 kg ha^{-1} of BOF per other three seasons; biochar (1000 kg ha^{-1}) in the first season of rape cultivation and 8330 kg ha^{-1} per other three seasons; mesoporous ceramic functional nanomaterials (MCFN: 6670 kg ha^{-1} per season). Experiment was laid out following randomized complete block design and individual treatments had three replicates with net plot size of 5 m × 6 m for each plot (Fig. 1). The plots were separated with plastic film covering the field ridge (0.3 m high and 0.4 m wide). The PMs in each plot was applied uniformly by hand, with a uniformity of 75–95%.

Crops and fertilizers

Rape and rice, the most widely cultivated crops in Tongling mining area, were planted in this study. The rice seed (Zhendao 18) and rape seed (Fengyou 737) varieties were purchased from Hefei Fengle Seed Industry Co., Ltd. in Anhui Province, East China, and Jiaxing Academy of Agricultural Sciences in Zhejiang Province, East China, respectively. The top soil (0–20 cm) was turned over with a hoe, and the soil was thoroughly mixed two times. Seeds were planted after one week.

The fertilizers applied in this study were potash-magnesium phosphate fertilizer (with a mass fraction of 15% for N, P_2O_5 and K_2O) (Tongling Hongxing Chemical Plant, Anhui Province) and urea (total nitrogen ≥ 46.4%) (Tongling Chemical Plant, Anhui Province). Potash-magnesium phosphate was applied as the base fertilizer (375 kg ha^{-1}), topdressing with urea (150 kg ha^{-1}). Both fertilizers were applied according to the local high-yielding cultivation techniques. In addition, the field management for planting, fertilizing, and weeding in each plot was consistent with the local farmers.

Sample collection and analysis

At the maturity stage of crops, each plot was sampled along five diagonal points. Five plants were randomly selected from each plot; the plant height was measured with a ruler. At the same point, the aboveground part of the crops and the corresponding rhizosphere soil samples were collected. The samples were stored in the plastic bags and transferred to the laboratory. After removing rocks and plant roots, the soils were air-dried and passed through 1 mm and 0.149 mm, respectively. The crop samples were separated into stalks, husks, and seeds, washed thoroughly, oven-dried at 70°C to constant weight, measure the DW, powdered and then sieved through a 0.149 mm nylon sieve for further analysis. Data regarding yield and related traits of rape and rice crops were recorded following Ijaz *et al*. (2018) and Jabran *et al*. (2015), respectively.

Soil pH was determined with a glass electrode pH meter (Sartorius PB 10, Shanghai) using a soil: water ratio of 1:2.5. Soil available Cu was extracted by DTPA-TEA-$CaCl_2$ (HJ 804-2016) (10 g: 20 mL soil: solution ratio) for 2 h at 22°C in an end to end shaker (180 RPM). Then after, the supernatant was centrifuged (4000 RPM) for 10 min and filtered through a 20 μm filter paper. The SOM, total nitrogen, and available contents of nutrients (N, P and K) were measured according to Bao (Bao 2000).

To measure plant content of Cu, plant tissues were digested by HNO_3-$HClO_4$ (3:1, v:v) (GB 5009.13-2017) (0.5 g: 10 mL HNO_3 and 0.5 mL $HClO_4$ dried plant weight: solution ratio) in a 50 mL clean beaker at 120°C for 0.5 h and 180°C for 3 h. Before digestion, nitric acid and perchloric acid were added to the sample. Afterwards, the samples were covered by surface dishes and left overnight for pre-digestion at room temperature (25°C). The concentration of Cu was determined by a Flame Atomic Absorption Spectrometry (FAAS) (ZEEnit-700P, JENA, Germany). The reagents used in this study were all of excellent grade purity. All the glassware used for analysis of heavy metals were washed and used after soaking with 20% HNO_3. Blanks and standard references for both plant (GSB-26) and soil samples (ASA-10) were used for quality control during sample analysis.

Data processing and statistics

All the experiments in this study were carried out in triplicates. The mean and standard error values were calculated using I.B.M. S.P.S.S. 22.0 (S.P.S.S. Institute, USA). The significant differences among the treatments were evaluated by one-way analysis of variance (ANOVA) and Duncan's multiple range test was used to compare treatments means at $P \leq 0.05$. Figures were plotted by Origin 2017 (Origin Lab., USA).

Results

Soil pH and organic matter

Application of PMs had significant effect on soil pH and SOM after rape and rice harvest in both years of study (Table 2). Application of QL as PMs significantly increased pH compared with all treatments including control after rape and rice harvest during both years of study. Moreover, maximum SOM contents were recorded after rice and rape harvest where BHAB/BC was applied as PMs in both years of study (Table 2). In rape season, the content of SOM in BC treatment was 30 mg kg^{-1}, 34.8% higher than that in CK (22.3 mg kg^{-1}). Under NHA, MCFN, and BOF treatments, SOM content in rape season increased by 4.64, 4.11 and 3.92%, respectively. In rice season, SOM treated with biochar and BOF increased by 23 and 11.6%, respectively, compared with control. SOM under NHA and QL treatments increased by 1.64 and 1.74%, respectively, but SOM treated with MCFN decreased by 2.41% compared with CK (Table 2).

Soil Cu availability

In this two-year study, the application of PMs had a significant impact on soil Cu availability after crop harvest (Fig. 2). All the passivators used significantly reduced soil available Cu compared with control after rice harvest during both years of study. Moreover, BOF had the worst inhibition effect on soil available Cu (Fig. 2). Overall, continuous application of PMs under rape-rice rotation reduced soil available Cu to less than 50 mg kg^{-1}. Compared with control, PMs (except BHAB) significantly ($P < 0.05$) reduced soil available Cu by 22–39% in rape season (2017). However, the available Cu in BOF treated soil was only reduced by 7.26% (Fig. 2). Likewise, PMs significantly reduced soil available Cu in rice season (2017) by 23.4–34.9%. In this season, QL had the highest impact on soil Cu reduction to 34.2 mg kg^{-1}, whereas MCFN and NHA ranked second with reduction of soil Cu availability to 36 and 38.3 mg kg^{-1}, respectively. Compared to CK, significant reduction was also seen in soil Cu availability by 21.8–29% in 2^{nd} rice season. Comparatively, QL, MCFN, and NHA significantly reduced soil available Cu by 29, 27.6 and 26.7%,

Table 1: Basic properties of applied passivation materials

PMs	pH	Cu (mg kg^{-1})	Producer
Nano hydroxyapatite	8.27	2.58	Nanjing Epry Nanomaterials Co., Ltd.
Quicklime	12.9	/	Zhejiang Guxian Road Green Fiber Co., Ltd.
Biochar	9.91	4.37	Henan Shangqiu Sanli New Energy Co., Ltd.
Bio-organic fertilize	8.17	5.8	Beijing Century Arms Biotechnology Co., Ltd.
Mesoporous ceramic functional nanomaterials	10.7	17.6	Wuhu Gefeng Technology Materials Co., Ltd.

"/" indicate cannot be detected

Table 2: Effect of passivation materials on soil pH and organic matter after rape and rice harvest

Treatments	Rape				Rice			
	pH		Organic matter (mg kg^{-1})		pH		Organic matter (mg kg^{-1})	
	2016	2017	2016	2017	2017	2018	2017	2018
CK	5.28 ± 0.00c	5.14 ± 0.15c	23 ± 0.02b	22.6 ± 0.13b	6.75 ± 0.13abc	5.97 ± 0.05c	22.3 ± 0.88d	22.9 ± 0.29bc
NHA	5.81 ± 0.06b	6 ± 0.21b	25.2 ± 0.74ab	21.5 ± 0.44b	6.82 ± 0.01ab	6.06 ± 0.04c	23.3 ± 0.33cd	22.6 ± 0.89c
QL	6.27 ± 0.05a	6.75 ± 0.14a	22.7 ± 0.84b	20.5 ± 0.98b	7.01 ± 0.08a	7.21 ± 0.06a	23.5 ± 0.13bc	22.4 ± 0.53c
BHAB/BC	5.28 ± 0.03c	5.34 ± 0.04c	26.8 ± 1.44a	33.3 ± 1.37a	6.53 ± 0.09c	5.93 ± 0.21c	25.4 ± 0.36a	30.1 ± 1.07a
BOF	5.22 ± 0.02c	5.07 ± 0.11c	23.8 ± 0.54ab	22.6 ± 0.39b	6.68 ± 0.10bc	5.91 ± 0.04c	24.6 ± 0.06ab	25.9 ± 1.67b
MCFN	5.67 ± 0.09b	5.14 ± 0.15c	25.2 ± 1.16ab	21.3 ± 0.14b	6.98 ± 0.02a	6.81 ± 0.11b	23 ± 0.43cd	21.1 ± 0.68c

Means with different letters, within a column for each year and each trait, differ significantly from each other at *P* 0.05 according to Duncan's Multiple Range test
Here BHAB/BC indicates the fourth treatment in rape season with BHAB application in 2016 and BC application in the next three seasons; CK: no passivator; NHA: nano hydroxyapatite; QL: quicklime; BHAB: biological humic acid bacteria; BC: biochar; BOF: bio-organic fertilizer; MCFN: mesoporous ceramic functional

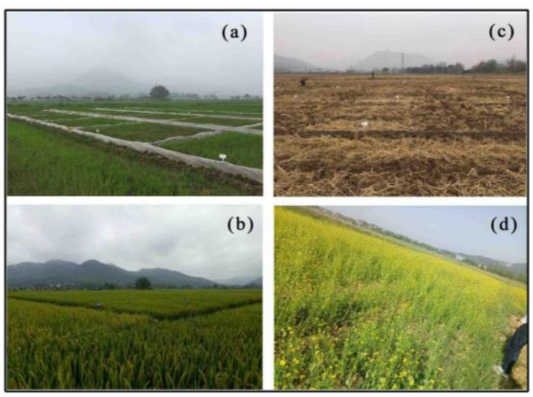

Fig. 1: Rape-rice rotation field plot: (**a**) Rice planting plot (**b**) Rice maturity stage (**c**) Rape planting plot (**d**) Rape flowering stage

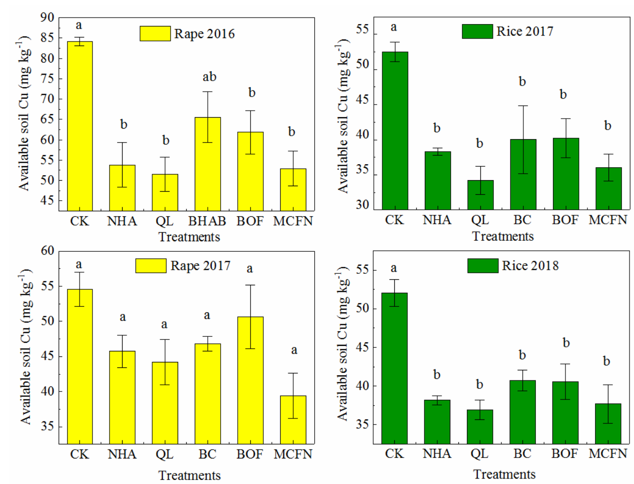

Fig. 2: Effect of passivation materials on soil available Cu in rape and rice fields
CK: no passivator; NHA: nano hydroxyapatite; QL: quicklime; BHAB: biological humic acid bacteria; BOF: bio-organic fertilizer; MCFN: mesoporous ceramic functional nanomaterials

respectively, in this season. BC and BOF had similar impacts on soil available Cu with a reduction rate of about 22% (Fig. 2).

Plant Cu removal

Application of PMs had significant effect on Cu uptake by stems or straw, hulls, and seeds after rape and rice harvest in both years of study (Table 3). Application of QL as PM significantly decreased plant Cu compared with all treatments including control in rape and rice plant parts during both years of study. Copper enrichment in the above-ground organs of rape was ordered as: hulls>seeds>stems. Different from rape, Cu uptake by above-ground rice tissues followed the order: hulls>straw>seeds. Compared with CK, PMs (except BOF in 2017) significantly ($P < 0.05$) inhibited rape grains and seeds' uptake of Cu by 19.4–36.7% and decreased in rice Cu uptake by 8.2–31.7% (Table 3).

In 2016, compared with CK, the content of Cu in NHA and QL treated rape grains decreased by 46%, followed by BHAB and MCFN decreasing Cu content by 45.1 and 42.7%, respectively. Under BOF treatment, Cu content of rape seeds decreased to 10.1 mg kg^{-1}, 44% lower than CK. In 2017, Cu contents in MCFN and QL treated rapeseeds decreased to 6.3 and 6.51 mg kg^{-1}, respectively, 29.4 and 27.3% lower than the corresponding seasonal CK, respectively. In this season, rape seeds' uptake of Cu in NHA, BC and BOF treated soils were 7.4, 7.7, and 8.5 mg kg^{-1}, respectively.

In 2017, Cu content of rice seeds treated with QL decreased to 7.75 mg kg^{-1} ($P < 0.05$), 22.2% less than CK, followed by biochar, MCFN, BOF, and NHA decreasing Cu content by 18.2, 13.7, 12.4 and 8.2%, respectively. In 2018,

Table 3: Effects of passivation materials on Cu uptake by different tissues of rape and rice

Treatments	Rape						Rice					
	Stems (mg kg^{-1})		Hulls (mg kg^{-1})		Seeds (mg kg^{-1})		Straw (mg kg^{-1})		Hulls (mg kg^{-1})		Brown rice (mg kg^{-1})	
	2016	2017	2016	2017	2016	2017	2017	2018	2017	2018	2017	2018
CK	4.23 ± 0.00c	4.74 ± 0.31a	16.3 ± 0.42bc	18.5 ± 0.31a	15.3 ± 2.27a	8.95 ± 0.02a	10.7 ±0.02a	9.86±0.64ab	11.6 ± 0.75bc	16.5±0.79a	9.96±0.83aba	9.61 ± 0.27a
NHA	3.88 ± 0.28c	2.98 ± 0.10b	11.6 ± 1.16d	11.2 ± 0.51c	8.23 ± 0.16b	7.40 ± 0.37cd	11.7 ±1.78a	8.26±0.20ab	16.4 ± 0.52a	12.9±0.79b	9.14 ± 0.36ab	7.02±0.35bc
QL	6.53 ± 0.54ab	3.01 ± 0.25b	16.8 ± 0.18b	12.6 ± 0.24c	8.23 ± 0b	6.51 ± 0.37de	12.2 ±0.53a	9.38±0.71ab	14.9 ± 1.75ab	11.4±1.06b	7.75 ± 0.32b	6.56±0.50bc
BHAB/BC	4.77 ± 0.26c	3.36 ± 0.36ab	13.4 ± 0.31cd	16.1 ± 1.18b	8.37 ± 0.24b	7.70 ± 0.05bc	12.0 ±0.75a	8.10±0.79b	12.5±1.42abc	13.5±0.09b	8.15 ± 0.06ab	7.93±0.51bc
BOF	7.59 ± 0.23a	3.57 ± 0.38ab	21.2 ± 1.22a	11.4 ± 0.21c	10.1 ± 0.36b	8.50 ± 0.15ab	11.7 ±0.96a	9.16±0.81ab	9.84 ± 0.75c	13.2±1.35b	8.73 ± 0.51ab	8.47±0.81ab
MCFN	6.35 ± 0.32b	3.39 ± 0.33ab	14.5 ± 0.9bcd	7.83 ± 0.41d	8.74 ± 0.23b	6.32 ± 0.34e	9.50 ±0.62a	10.2±0.58a	12.8±1.50abc	12.8±1.04b	8.60 ± 0.32ab	6.95±0.44bc

Means with different letters, within a column for each year and each trait, differ significantly from each other at *P* 0.05 according to Duncan's Multiple Range test
Here BHAB/BC indicates the fourth treatment in rape season with BHAB application in 2016 and BC application in the next three seasons; CK: no passivator; NHA: nano hydroxyapatite; QL: quicklime; BHAB: biological humic acid bacteria; BC: biochar; BOF: bio-organic fertilizer; MCFN: mesoporous ceramic functional

Table 4: Effects of passivation materials on growth and yield of rape and rice

Treatments	Rape						Rice					
	Plant height (cm)		DW (g $plant^{-1}$)		Yield (kg ha^{-2})		Plant height (cm)		1000-grain weight (g)		Yield (kg ha^{-2})	
	2016	2017	2016	2017	2016	2017	2017	2018	2017	2018	2017	2018
CK	150 ± 2.60b	146 ± 3.93^{ns}	70.3 ± 8.83ab	61.4 ± 4.97c	583 ± 0.0^{ns}	617 ± 16.7^{ns}	101 ± 0.8^{ns}	74.8 ± 0.9c	26 ± 0.56a	22.5 ± 0.80ab	9410 ± 372ab	8860 ± 315^{ns}
NHA	178 ± 11.0a	166 ± 15.8	79.3 ± 1.36ab	79.3 ± 0.71ab	627 ± 0.63	628 ± 22.2	105 ± 2.7	75.8 ± 0.8bc	26.1 ± 1.00a	23 ± 0.49ab	9350 ± 255ab	9070 ± 194
QL	162 ± 3.71ab	164 ± 7.32	72.4 ± 11.5ab	71.5 ± 0.02bc	627 ± 0.13	639 ± 24.2	106 ± 3.1	77.5 ± 0.7abc	22.8 ± 0.77b	21.6 ± 0.37b	9578 ± 241a	8900 ± 154
BHAB/BC	161 ± 5.46ab	153 ± 4.82	93 ± 19.6ab	71 ± 6.19bc	417 ± 0.25	650 ± 16.7	105 ± 1.5	83.9 ± 1.6a	26.3 ± 0.49a	23.5 ± 0.90ab	9550 ± 364a	9260 ± 354
BOF	149 ± 6.38b	150 ± 2.94	63.9 ± 3.33b	79.2 ± 0.67ab	583 ± 0.25	622 ± 11.1	105 ± 2.6	77.5 ± 3.1abc	23.6 ± 0.90b	22.7 ± 0.51ab	8440 ± 111b	8960 ± 202
MCFN	162 ± 6.34ab	171 ± 4.16	104 ± 6.44a	82.9 ± 3.72a	543 ± 0.38	650 ± 9.62	105 ± 2.7	81.9 ± 3.3ab	26.1 ± 0.33a	24 ± 0.73a	9510 ± 422a	9460 ± 286

Means with different letters, within a column for each year and each trait, differ significantly from each other at *P* 0.05 according to Duncan's Multiple Range test
Here BHAB/BC indicates the fourth treatment in rape season with BHAB application in 2016 and BC application in the next three seasons; CK: no passivator; NHA: nano hydroxyapatite; QL: quicklime; BHAB: biological humic acid bacteria; BC: biochar; BOF: bio-organic fertilizer; MCFN: mesoporous ceramic functional

Cu uptake by rice seeds was lower than that in 2017 meeting the *National Standard of Pollutants in Food of China* (GB 15199-1994, 10 mg kg^{-1}). Especially, Cu content of rice seeds treated with QL was 6.56 mg kg^{-1}, 31.7% lower than CK. While, Cu content of rice seeds treated with NHA, biochar, BOF, and MCFN decreased by 27, 17.4, 11.8 and 27.7% respectively, compared with control (Table 3).

Growth and yield of plants

Application of PMs had non-significant effect on yield of rape in both yeas and plant height in 2nd year of study while had significant effect on plant dry matter in both years and plant height during 1st year of study (Table 4). Likewise, PMs had non-significant effect on plant height and rice yield during 1st and 2nd year of study, respectively while had significant effect on 1000-grain yield in both years of study (Table 4). In short, biochar promoted the growth of both rice and rape, NHA and QL had stronger effects on rape yield, and MCFN had better effect on rice growth. Nonetheless, BOF application slightly impaired the growth and yield of rape and rice crops (Table 4).

Discussion

Application of PMs such as QL, MCFN, NHA and BC significantly increased the soil pH, while biochar and BOF increased SOM, which may lead to reduced Cu availability in soil and lesser intake by rape and rice crops (Fig. 2 and Tables 2–3). The increase of pH is beneficial to the decrease of Cu activity, while the increase of organic matter is beneficial to increase chelating adsorption of Cu ions.

Significant increases ($P < 0.05$) in pH values following the application of QL might be due to the strong alkaline substances of QL. NHA mainly produces a large amount of OH^{-1} through hydrolysis, so as to reduce the soil acidity. MCFN is a mixture of alkaline clay minerals, which neutralizes soil acidity and produces OH^{-1} by hydrolysis of SiO_3^{2-} and significantly improves pH compared with CK. (Chen *et al*. 1997; Cui *et al*. 2013; Bian *et al*. 2014; Chen *et al*. 2018). Biochar contains basic substances like carbonate and oxide, which can improve soil pH (Bian *et al*. 2014; Hussain *et al*. 2017). Nevertheless, BHAB had no significant effect on soil pH, which may be related to its weak alkalinity (Ren *et al*. 2016). In terms of SOM, this study showed that biocahr and BOF are more beneficial to increase SOM content than other PMs, which is consistent with the previous reports (Ma *et al*. 2015). The special traits of biochar such as high surface charge density, large surface area, and internal porosity enable this material to adsorb organic molecules and related nutrients, thereby reducing nutrient losses and increasing nutrient storage (Laird *et al*. 2010). This study indicated that biochar can promote rice and rape production stably, which may be attributed to its capability for carbon sequestration, fertilization, yield increase, and immobilization of heavy metals (Bian *et al*. 2014). BOF is rich in organic matter, so it can further improve the SOM after application (Bian *et al*. 2014; Pérez-Esteban *et al*. 2019).

An increase in soil pH following the application of PMs reduced soil Cu bioavailability and plant Cu removal. This was indicated by the significant negative correlations ($P < 0.05$) between soil pH and Cu bioavailability and Cu contents in edible parts of rape and rice. Similar results were reported by Zhang *et al*. (2010). Hence, immobilization of soil Cu as well as inhibiting Cu uptake by crops can increase the crop yield and safety (Bian *et al*. 2014). In this study, soil

Cu bioavailability decreased under MCFN treatment which, in turn, might be due to its large specific surface area resulting in Cu precipitation (Tong *et al*. 2011). Furthermore, NHA was an effective treatment for Cu fixation due to the following reasons: on one hand, specific sites on the surface of NHA rapidly complexes soil Cu; on the other hand, Ca ions on the surface of NHA can also adsorb soil Cu (Corami *et al*. 2007; Liu *et al*. 2018). The formation of $Cu_2(PO_4)OH(s)$ following the dissolution of NHA in the soil can also cause Cu precipitation (Oliva *et al*. 2011).

In addition to the alkalinity of the PMs, the high specific surface area of the nanomaterials has the effect of improving the soil quality and inhibiting Cu on the aggregate structure of contaminated soil and the complexation and antagonism of soil colloid (Koptsik 2014). Likewise, MCFN, NHA and biochar exhibited a high affinity to immobilize soil Cu due to its large specific surface area and active functional groups (*e.g*., -COOH and-OH) (Park *et al*. 2011; Xue *et al*. 2019). In addition, BC can decrease soil Cu availability by changing soil microbial community composition and redox potential, reducing Cu uptake by crops (Bian *et al*. 2014; Jones *et al*. 2016).

The PMs in this study can enhance the pH of soil and inhibit the activity of Cu. For example, QL, MCFN and NHA can also improve soil texture and structure, but they have no significant effect on soil fertility, so they make little contribution to crops yield (Table 4). Naturally, biochar was superior promoting the yields of rice and rape, which might be due to its high contents of nutrients, and capability for carbon sequestration and Cu immobilization (Wei *et al*. 2016; Hussain *et al*. 2017).

The different capabilities of various PMs to remediate Cu contaminated soils were further justified through the analysis of FTIR spectrum (Fig. 3). Comparatively, FTIR spectrum of QL amended soils induced a notable variation to change the band intensity from 1640 to 1650 cm^{-1} and 1040 to 1030 cm^{-1}, respectively, which might be the main mechanism for Cu complexation with functional groups. There are two significant peaks in BC amended soil: 1650 and 1040 cm^{-1}, representing the increase in the vibration of C=C, C=O, and olefins associated with organic matter. The shifts of these bands might account for application of biochar into soil which, in turn, could increase surface complexation and precipitation of Cu (Bashir *et al*. 2018; Mayans *et al*. 2019).

Conclusion

Continuous application of PMs significantly reduced soil available Cu by 50% to less than 50 mg kg^{-1} which is lower than the limit of Screening Value of Soil Pollution Risk for Agricultural soils (GB 15618-2018, pH < 5.5). Moreover, application of QL, MCFN, BC and NHA as PMs reduced the Cu contents in edible parts of rape and rice during both years of study. Compared with rice, rape is recommended for Cu-contaminated acidic soil because it contains lesser

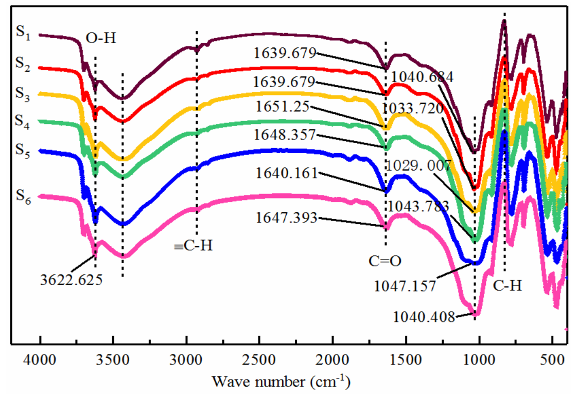

Fig. 3: FTIR analysis of Cu immobilization mechanism showed: (S_1) control soil, (S_2) nano hydroxyapatite amended soil, (S_3) quicklime amended soil, (S_4) biochar amended soil, (S_5) bio-organic fertilize amended soil and (S_6) mesoporous ceramic functional nanomaterials amended soil

contents of Cu in its edible parts. Quicklime by increasing soil pH; and biochar by increasing soil pH and organic matter meet the requirements of reducing the risk of soil Cu pollution in Cu-contaminated soil remediation.

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Author Contributions

H.H. designed the study, put forward key opinions on the revision of the paper and giving a great help in data analysis; Z.X. carried out the whole laboratory work, participated in data analysis and drafted the manuscript; Y.K.K. interpreted the results and helped to draft and revise the manuscript; N.L. and L.Z. carried out the statistical analyses and helped drafting the manuscript; Y.Z. and M.W. helped with data collection, analysis, and interpretation. All authors gave final approval for publication.

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