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RESEARCH ARTICLE

Accumulation, availability, and uptake of heavy metals in a red soil after 22-year fertilization and cropping

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Abstract Fertilization is important to increase crop yields, but long-term application of fertilizers probably aggravated the risk of heavy metals in acidic soils. In this study, the effect of 22-year fertilization and cropping on accumulation, availability, and uptake of heavy metals in red soil was investigated. The results showed that pig manure promoted significantly cadmium (Cd) accumulation (average 1.1 mg kg^{-1}), nearly three times higher than national soil standards and, thus, increased metal availability. But the enrichment of heavy metals decreased remarkably by 50.5 % under manure fertilization, compared with CK (control without fertilization). On the contrary, chemical fertilizers increased greatly lead (Pb) availability and Cd activity; in particular, exceeding 85 % of soil Cd became available to plant under N (nitrogen) treatment during 9–16 years of fertilization, which correspondingly increased their enrichment by 29.5 %. Long-term application of chemical fertilizers caused soil acidification and manure fertilization led to the increase in soil pH, soil organic matter (SOM), and available phosphorus (Olsen P), which influenced strongly metal behavior in red soil, and their effect had extended to deeper soil layer (20~40 cm). It is advisable to increase application of manure alone with low content of heavy metals or in combination with chemical fertilizers to acidic soils in order to reduce toxic metal risk.

Keywords Long-term fertilization · Manure · Heavy metal · Red soil · Uptake

Introduction

Application of inorganic and organic fertilizers has long been regarded as one of the most important measures to increase crop yields. A 10-year continued increase in grain production in China has been closely linked to great fertilizer consumption (Bi et al. 2013). Global food demand is expected to double by 2050 (Godfray et al. 2010), suggesting that fertilizers will still have an important role in promoting grain production.

Fertilizers especially animal manures generally contain a concentration of most heavy metals such as cadmium (Cd), copper (Cu), lead (Pb), zinc (Zn), and chromium (Cr); therefore, their long-term and frequent application could result in metal accumulation in soils (Hejcman et al. 2009; Duan et al. 2012; Fan et al. 2013; Zhao et al. 2006, 2014) and, consequently, in toxicity hazard to plant and animals or in entry of toxic metals into the human food chain (Westfall et al. 2005). In fact, entry of soil metals into the food chain depends not only on metal amount but also soil properties and rate of uptake by plants. Organic fertilizers might reduce metal availability due to promoting remarkably soil organic matter (SOM) and phosphorus (P) that immobilized metals by complexation or precipitation (Bolan et al. 2003; Karlsson et al. 2006); inorganic fertilizers could also reduce the bioavailability of metals via producing binding sits or promoting the normal metabolism of plants or changing forms of metals (Singh et al. 2010). As a result, application of inorganic and organic fertilizer has been tried as a technique for remediating metal-contaminated cropland soils (Xu et al. 2014). However, overuse of chemical fertilizers contributes substantially to regional soil acidification (Debreczeni and Kismányoky 2005; Guo

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et al. 2010), causing the increase in mobility and availability of heavy metals in soils. Obviously, the impact of long-term fertilization on metal availability and potential risk is extremely complex, where it maybe is not as serious as it might appear or it probably is very serious and is overlooked. Yet, heavy metal contamination caused by long-term fertilization has not been paid enough attention.

Red soils, corresponding to Typic Hapludults in the soil taxonomy (Soil Survey Staff 2010), are widely distributed in subtropical regions of China and are its main acidic cropland soils, where the bioavailability and uptake of heavy metals are usually higher (Westfall et al. 2005). Thus, more attention should be focused on long-term fertilization influencing the accumulation, availability, and uptake of heavy metals in these acidic soils. In this study, we investigated the behaviors of five metals (Cu, Zn, Pb, Cr, and Cd) in red soil after 22-year fertilization and cropping in order to 1) elucidate the long-term effect of manure and chemical fertilizers on metal risk in acidic soil and 2) present a proposal of rational fertilization for sustainable production in acidic croplands.

Materials and methods

Study site and experimental design

The long-term field experiment was initiated in 1990 at Red Soil Agro-Ecological Experimental Station of Chinese Academy of Agricultural Sciences, Qiyang County, Hunan Province, China (26° 45′ N, 111° 52′ E). Average annual temperature is 18.0 °C, and annual precipitation is 1250 mm. There is a frost-free period of about 300 days and the cumulative temperature (≥10 °C) is 5600 °C. The soil derived from quaternary red clay is referred to as red soil, and its initial properties before experiment are shown in Table 1.

Six treatments with two replicates were selected in this study, including N (nitrogen), NP (nitrogen + phosphorus), NPK (nitrogen + phosphorus + potassium), M (manure), NPKM (nitrogen + phosphorus + potassium + manure), and CK (control without fertilization) (Table 2). Each plot had an area of 196 m^2, and winter wheat (*Triticum aestivum* L.)-summer maize (*Zea mays* L.) rotation was designed. For each year, winter wheat (Xiangmai 11) was sown in early November and harvested in mid-May in the following year; summer maize (Yedan 13) was sown between the wheat strips in early April and harvested in lately July. No irrigation water was applied during the growing season, but other field managements followed the same practices as the local farms, e.g., herbicides and pesticides were applied as needed. For all the treatments except CK, the total amount of nitrogen applied was the same (300 kg N ha^{-1} $year^{-1}$), with 100 % urea-N for chemical fertilizer treatments (N, NP, and NPK), and 70 and 100 % manure-N for NPKM and M treatments, respectively. The properties of pig manure (dry matter) were listed as follows: pH 8.75, ash alkalinity (82.7 cmol kg^{-1}), organic C

Table 1 Initial chemical properties of red soil (0~20 cm)

Item	Content
pH (H_2O)	5.7
Organic matter (SOM) (g kg^{-1})	13.6
Total nitrogen (g kg^{-1})	1.07
Available nitrogen (mg kg^{-1})	79
Total phosphorous (g kg^{-1})	0.45
Olsen phosphorous (mg kg^{-1})	13.9
Total potassium (g kg^{-1})	13.7
Available potassium (mg kg^{-1})	104
Cation exchange capacity (CEC) (cmol kg^{-1})	10.32
Grain size distribution (%)[a]	
Sand	3.7
Silt	34.9
Clay	61.4
Total metal (mg kg^{-1})	
Cu	31.75
Zn	115.07
Pb	41.83
Cr	115.76
Cd	0.099
0.1 M HCl-extractable metal (mg kg^{-1})	
Cu	2.43
Zn	5.27
Pb	6.82
Cr	5.29
Cd	0.025

[a] Sand (2–0.05 mm), silt (0.05–0.002 mm), and clay (<0.002 mm)

Table 2 The annual fertilization rates (kg ha^{-1})

Treatment	Wheat				Maize			
	N	P_2O_5	K_2O	Manure[a]	N	P_2O_5	K_2O	Manure
CK	0	0	0	0	0	0	0	0
N	90	0	0	0	210	0	0	0
NP	90	36	0	0	210	84	0	0
NPK	90	36	36	0	210	84	84	0
M	0	0	0	18,000	0	0	0	42,000
NPKM	27	36	36	12,510	63	84	84	29,200

CK unfertilized control, *N* nitrogen, *NP* nitrogen + phosphorus, *NPK* nitrogen + phosphorus + potassium, *M* manure, *NPKM* nitrogen + phosphorus + potassium + manure

[a] Manure is fresh pig manure with water content of 76.3 %. Nitrogen (urea, 46 % N), phosphorus (calcium superphosphate, 12 % P_2O_5), potassium (potassium chloride, 60 % K_2O), and pig manure are applied as basal fertilizers

(388.0 g kg^{-1}), N (21.0 g kg^{-1}), P (16.0 g kg^{-1}), K (16.5 g kg^{-1}), Na (5.7 g kg^{-1}), Ca (32.6 g kg^{-1}), and Mg (15.0 g kg^{-1}) (Cai et al. 2015). Urea (46 % N), calcium superphosphate (12 % P_2O_5), potassium chloride (60 % K_2O), and fresh pig manure (76.3 % water) are applied, respectively. Their application rate for wheat and maize is listed in Table 2, respectively, and all fertilizers were applied as basal fertilizers. That is, 30 and 70 % of total N were applied for planting wheat and maize, respectively, where both chemical fertilizers and manure were applied by banding at a depth of 10 cm, followed by sowing of each crop and then covering with surface soil.

Soil and plant sampling

Every year, soil samples were collected after maize harvest. Five random cores were taken from the 0~20 cm (additional 20~40 cm in 2012) soil layer in each plot for each treatment. Soils were air-dried and ground to pass through a 1-mm nylon sieve for the analysis of soil pH; SOM; available soil N, P, K; and trace metal (Cu, Zn, Pb, Cr, and Cd). Part of each soil sample (<1 mm) was ground to pass through a 0.15-mm nylon sieve for the analysis of total soil trace metals. For analysis of soil total/available metal contents, only those soil samples collected in 1990, 1996, 1999, 2002, 2006, 2009, and 2012, respectively, were used.

In 2012, the grain and straw of wheat and maize, respectively, in each plot for each treatment were collected and dried in an oven at 70 °C for 2 days and ground through a 0.25-mm nylon sieve for the analysis of trace metals.

Chemical analysis

Soil pH was determined in a soil/water ratio of 1:2.5 (*V*:*V*); SOM was measured by potassium dichromate oxidation titration; available N, P, and K were determined by extracting with 1 M NaOH, 0.5 M $NaHCO_3$, and 1 M NH_4OAC, respectively (ISS 1978).

Available metals were extracted by 0.1 M HCl procedure developed for acidic soils (Page et al. 1982) where after 2 h-continuous shaking at 25 °C, soil solution was centrifuged and filtered through a 0.45-μm membrane. Total amounts of metals in soils were determined by a tri-acid digestion method (HNO_3-$HClO_4$-HF) (Shuman 1985). The contents of metals in plants and pig manure were determined by HNO_3 digestion (Wu et al. 1997). Finally, the concentrations of Cu, Zn, Pb, Cr, and Cd in the digestion solutions and extracts were measured using atomic absorption spectroscopy (AAS Zeenit 700).

In addition, national standard substances (GBW07406 and GBW10035) were used as quality control. Analytic reagents and ultrapure water were used in the laboratory analysis.

Statistical analysis

Comparison of means (*t* test) was done using MedCalc 14.10 (MedCalc Software bvba, Belgium); multiple linear stepwise regression analysis was conducted using IBM SPSS 20.0 (IBM, USA); correlation analysis and graphics and associated curve-fitting were conducted with SigmaPlot 12.0 (Systat Software Inc., USA).

Results

Effect of long-term fertilization on the accumulation of heavy metals in red soil

Long-term (22 years) fertilization did not affect the accumulation of Pb and Cr in red soil; application of chemical fertilizers also did not cause the accumulation of Cu, Zn, and Cd in the soil; however, application of pig manure (M and NPKM treatments) resulted in significant accumulation of Cd (average 1.1 mg kg^{-1}) and Cu (average 81.5 mg kg^{-1}) in the acid soil, above the national soil standards (0.3 and 50 mg kg^{-1}, respectively) (SEPAC 2006) (Table 3). In particular, Cd accumulation, nearly three times higher than allowable concentration in soils, would become the most serious environmental issues in red soil, which need to be taken into account. Furthermore, there was also significant accumulation of Cd and Cu in subsurface layer (20~40 cm) after 22-year manure fertilization (Table 3), indicating that manure had the greatest potential for Cd and Cu accumulation in red soil.

The relationship analysis between metal accumulation and pig manure input (Fig. 1) showed that compared with Cu, Cd accumulation in red soil depended more on manure input due to higher relationship (R^2=0.8032, p<0.0001), suggesting that Cd concentration in pig manure applied to the acidic soil should be controlled priorly.

Effect of long-term fertilization on the availability and relative activity of heavy metals in red soil

Here, the use of 0.1 M HCl extraction is thought to reflect bioavailability of metals (Page et al. 1982; Qian et al. 1996; Meers et al. 2007), and relative activity (RA) is also regarded as an index of available metals (Wang et al. 2008a). RA is calculated as follows:

$$\mathrm{RA} = \frac{[\text{0.1 M HCl–extractalbe metal}]}{[\text{soil total metal}]} \times 100\% \qquad (1)$$

It is presented in Table 4 that compared with CK, 22-year manure fertilization significantly increased availability of Cu, Zn, Cr, and Cd (both 0.1 M HCl-extractability and RA), where maximum increase occurred for Cr exceeding 20-fold,

Table 3 Total amount (mg kg^{-1}) of heavy metals in red soil after 22-year fertilization and cropping

Soil layer (cm)	Treatment	Cu	Zn	Pb	Cr	Cd
0~20	CK	32.35±0.28a[a]	93.14±1.75ab	30.03±1.33ab	98.39±9.22a	0.248±0.039b
	N	32.84±1.74a	95.05±0.95ab	30.58±2.52ab	95.73±6.36a	0.067±0.011a
	NP	32.27±1.03a	98.51±0.51ab	30.55±2.31ab	103.44±20.89a	0.105±0.018a
	NPK	32.14±2.42a	92.71±2.35ab	35.61±7.69bc	105.84±7.31a	0.097±0.010a
	M	79.12±3.81c	148.07±2.05d	35.33±3.62bc	96.42±0.50a	1.031±0.084c
	NPKM	83.93±9.80c	158.01±6.36e	42.50±2.71c	103.20±2.72a	1.168±0.142d
20~40	CK	33.41±0.70a	90.58±3.27a	31.51±5.03ab	98.74±15.05a	0.108±0.029a
	N	31.58±1.71a	92.42±2.87ab	25.54±2.53a	92.73±11.78a	0.048±0.003a
	NP	29.55±0.52a	98.15±4.44ab	26.18±2.89a	91.39±4.84a	0.101±0.019a
	NPK	30.54±2.35a	98.93±4.24b	28.27±7.61ab	106.55±6.33a	0.073±0.023a
	M	43.62±0.49b	112.76±2.24c	39.57±2.07c	106.65±3.27a	0.239±0.015b
	NPKM	45.99±9.94b	113.11±11.86c	30.53±4.25ab	104.03±5.09a	0.273±0.025b
Before experiment[b]		31.75±2.46	115.07±9.00	41.83±3.86	115.76±8.92	0.099±0.013
Limit concentration[c]		50	200	80	150	0.3

[a] Mean value±standard deviation, and the same letter in the same column means no significant difference among different treatments (p>0.05)

[b] Metal content in red soil (0~20 cm)

[c] SEPAC (2006)

followed by Cu, with about 14-fold (from 1.95 to 28.45 mg kg^{-1}) and 5-fold (from 6.0 to 34.9 %) increase in 0.1 M HCl-extractable Cu and RA_{Cu}, respectively. On the contrary, application of chemical fertilizers (N, NP, and NPK treatments) significantly increased Pb availability (average 1-fold higher than that in CK). For RA_{Cd}, on the one hand, higher values were found in all treatments (Table 4), mainly ascribed to lower soil pH; on the other hand, there were obviously different changes with fertilization year between chemical and organic fertilizer applications (Fig. 2), where very high values (>85 %) were kept during 12–16 years of application of chemical fertilizers; whereas a continuous increase (up to 60 %) was observed during 22-year manure fertilization.

Additionally, Table 4 also shows that like their effect on metal accumulation mentioned above (Table 3), long-term organic and chemical fertilizer applications have enhanced metal availability in deeper soil layers.

Uptake of heavy metals by wheat and maize in red soil after 22-year fertilization and cropping

Metal uptake by crops was no longer considered in N treatment (pH<4.1) because they could not be grown there after 2006. It was found from Fig. 3 that compared with CK, with a few exceptions, although there was no significant difference in metal uptake by crops among treatments, applied manure reduced the uptake of Pb and Cr by about 26.1 % (from 2.69 to

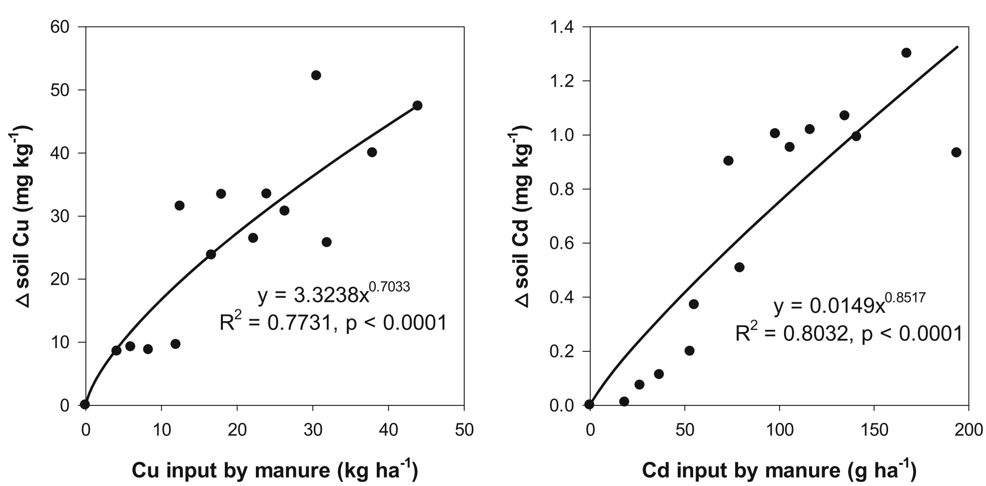

Fig. 1 The relationships between accumulation of Cu and Cd and their inputs from pig manure application. The symbol (Δ) means the difference in metal content between after and before manure fertilization

Table 4 0.1 M HCl-extractable metals (mg kg^{-1}) and their relative activity (%) in red soil after 22-year fertilization and cropping

Soil layer (cm)	Treatment	Cu	Zn	Pb	Cr	Cd
0~20	CK	1.95±0.03a (6.04)[a]	3.09±0.69a (3.32)	6.74±0.56b (22.44)	0.12±0.04a (0.13)	0.097±0.008b (39.02)
	N	1.95±0.12a (5.94)	1.41±0.24a (1.49)	13.07±0.48d (42.74)	0.44±0.15ab (0.46)	0.036±0.005a (53.42)
	NP	2.04±0.16a (6.32)	2.77±0.10a (2.81)	11.31±0.95c (37.02)	1.16±0.21de (1.13)	0.036±0.011a (34.64)
	NPK	1.63±0.14a (5.07)	2.78±0.24a (2.99)	11.09±0.44c (31.14)	1.12±0.23cde (1.05)	0.036±0.004a (37.16)
	M	28.73±0.06d (36.32)	33.09±0.10c (22.35)	4.79±0.23a (13.55)	2.68±0.12f (2.78)	0.636±0.058d (61.70)
	NPKM	28.17±3.89d (33.56)	34.11±6.99c (21.59)	6.01±0.13ab (14.15)	3.25±0.51f (3.15)	0.699±0.009e (59.85)
20~40	CK	1.16±0.36a (3.46)	1.43±0.30a (1.58)	5.13±1.31a (16.28)	0.23±0.13a (0.23)	0.024±0.013a (22.52)
	N	1.80±0.23a (5.69)	1.79±0.11a (1.93)	9.87±1.24c (38.64)	0.57±0.11abc (0.62)	0.013±0.002a (27.12)
	NP	1.29±0.14a (4.38)	3.21±0.75a (3.27)	6.08±0.35ab (23.21)	0.44±0.12ab (0.48)	0.023±0.008a (22.65)
	NPK	1.15±0.50a (3.76)	2.51±0.39a (2.54)	7.06±2.04b (24.97)	0.71±0.37abcd (0.67)	0.011±0.004a (15.36)
	M	8.79±0.12b (20.15)	12.49±0.17b (11.08)	4.88±0.40a (12.34)	0.98±0.01bcd (0.92)	0.186±0.003c (77.55)
	NPKM	12.75±5.71c (27.72)	17.00±9.96b (15.03)	4.76±0.89a (15.61)	1.67±0.82e (1.60)	0.192±0.002c (70.23)
Initial value[b]		2.43±0.95 (7.66)	5.27±2.32 (4.58)	6.82±1.22 (16.31)	5.29±1.56 (4.57)	0.025±0.006 (25.21)

[a] The data with no brackets stand for 0.1 M HCl-extractable metal concentration (mean value±standard deviation); the data within brackets stand for relative activity of metals. The same letter in the same column means no significant difference among different treatments (p>0.05)

[b] 0.1 M HCl-extractable metal concentration in red soil (0~20 cm)

1.99 mg kg^{-1}) and 20.3 % (from 0.39 to 0.31 mg kg^{-1}), respectively; whereas applied chemical fertilizers (e.g., NP treatment) increased their uptake by 6.4 % from 2.69 to 2.88 mg kg^{-1} and 20.6 % from 0.39 to 0.49 mg kg^{-1}, respectively. Special attention should be focused on Cd uptake in manure treatments (e.g., NPKM) because significantly higher Cd concentration (0.07~0.69 mg kg^{-1}) was observed (average 1-fold higher than that in CK); especially its content in wheat grain (0.244 mg kg^{-1}) was about 1- and 2-fold more than that in CK (0.16 mg kg^{-1}) and national hygienic standards (0.1 mg kg^{-1}) (MOHC 2005), respectively.

The total amount of metal removed by crops was presented in Table 5, where more metal was removed under manure application especially for NPKM treatment, due to its higher biomass, with about 3-, 4-, 5-, 8-, and 10-fold higher uptake of Pb, Cr, Zn, Cu, and Cd, respectively, than those in CK. These results again suggested that we should pay more attention to Cd uptake and animal manure application at long-term scale in acidic soil.

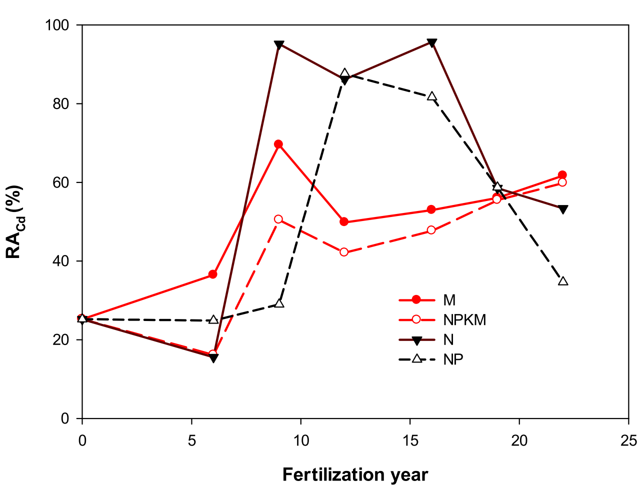

Fig. 2 The changes of relative activity of Cd (RA_{Cd}) with fertilization year

Enrichment coefficient (EC) could be used to evaluate accumulating capacity of plant to heavy metals (Zu et al. 2004; Sagiroglu et al. 2006; Galinha et al. 2010), and EC is calculated as follows:

$$EC = \frac{\text{heavy metal concentration in plant above ground}}{\text{heavy metal concentration in soil}} \quad (2)$$

Table 6 exhibited the EC values of five heavy metals in wheat and maize, with the order of average EC: Cd (0.87) >>Zn (0.30)>Cu (0.09)>Pb (0.07)>Cr (0.004). And multiple comparisons showed significant difference (p<0.01) in EC values between Cd and other metals. In addition, the EC was wheat (0.33)>maize (0.21) (p<0.05), indicating that wheat had more accumulating ability to heavy metals. There is considerable concern about Cd enrichment in crops planted in acidic soil in view of ECs higher than 1 and even 3 in some cases (e.g., NP and NPK treatment), which exceeded hyperaccumulating criterion (Sagiroglu et al. 2006).

It is also presented in Table 6 that after long-term application of chemical fertilizers the average of EC (0.39) increased by 29.5 %, as compared with CK (0.28); but it reduced remarkably after long-term manure fertilization by 50.5 % from 0.28 to 0.14 (60.5 % for Cd, 42.6 % for Pb, 45.2 % for Cu, 21.2 % for Cr, and 33.6 % for Zn). So, manure had the greatest inhibitory effect on Cd enrichment by wheat and maize.

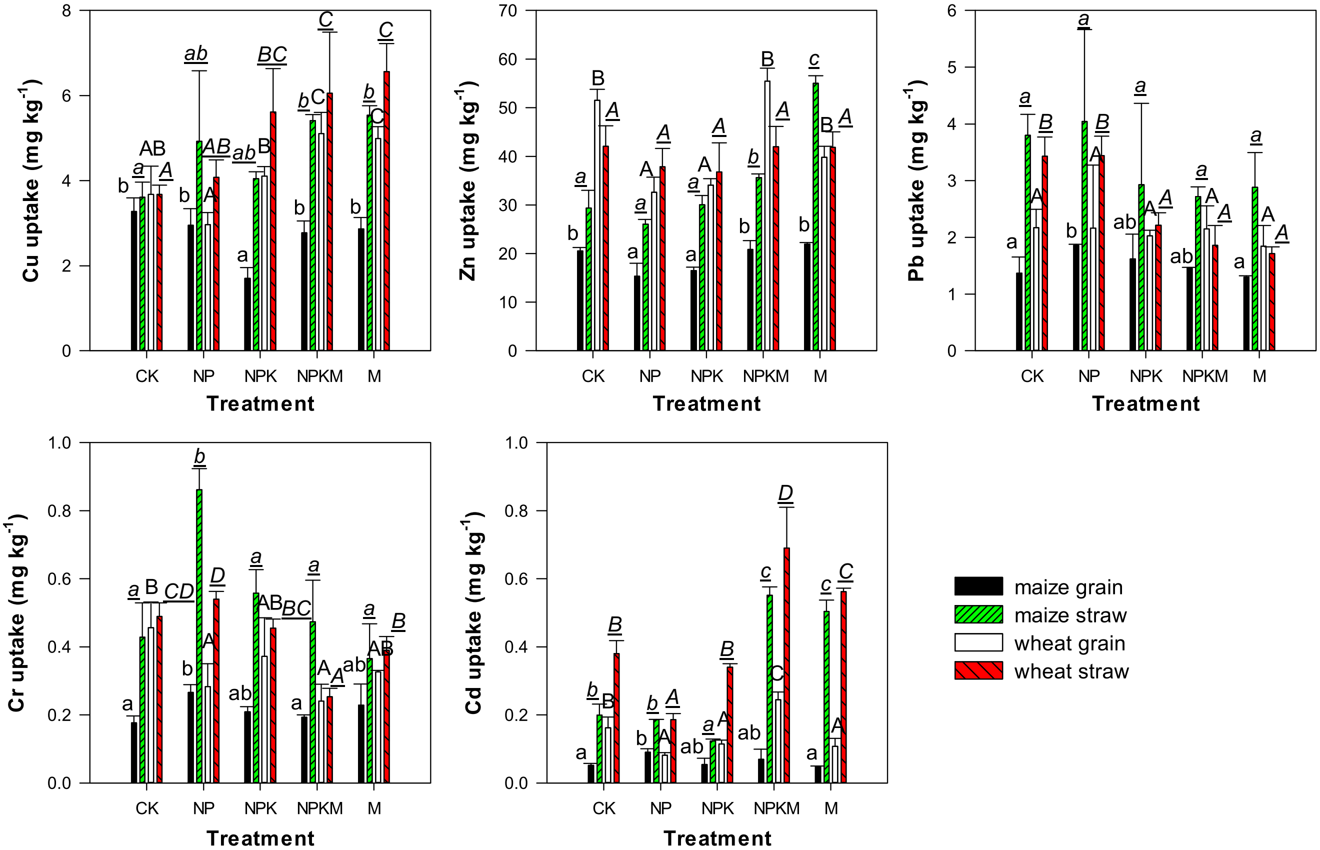

Fig. 3 Uptake of heavy metals in red soil by wheat and maize after 22-year fertilization and cropping. The *same letter* in different treatments means no significant difference (p>0.05)

Discussion

Factors controlling metal availability in fertilized red soil

Usually higher inputs of metals would result in their higher accumulation and thus higher availability in soil (Fan et al. 2011). For example, in our study, high Cd content (0.62 mg kg^{-1}) in pig manure was observed, causing serious soil Cd contamination after 22-year manure fertilization, where both total and 0.1 M HCl-extractable Cd were 10 to 25 times higher than they were before beginning the experiment (Tables 3 and 4).

In fact metal solid-solution equilibrium is controlled by key soil properties (SOM, pH, etc.), besides soil phases (SOM,

Table 5 The amount of metal removal per year by maize and wheat (g ha^{-1}) after 22-year fertilization and cropping

Treatment	Maize					Wheat					Total				
	Cu	Zn	Pb	Cr	Cd	Cu	Zn	Pb	Cr	Cd	Cu	Zn	Pb	Cr	Cd
CK	3.91	30.12	3.55	0.41	0.18	4.38	53.53	3.63	0.57	0.37	8.29	83.65	7.18	0.98	0.55
NP	14.14	74.41	10.69	2.06	0.50	9.87	95.88	8.03	1.21	0.40	24.01 (2.90)[a]	170.29 (2.04)	18.72 (2.61)	3.27 (3.35)	0.90 (1.63)
NPK	14.98	122.34	11.96	1.99	0.46	16.13	113.31	6.80	1.35	0.83	31.11 (3.75)	235.65 (2.82)	18.76 (2.61)	3.34 (3.42)	1.29 (2.34)
NPKM	40.71	281.13	20.77	3.30	3.06	30.48	246.89	10.39	1.32	2.88	71.19 (8.58)	528.02 (6.31)	31.16 (4.34)	4.62 (4.74)	5.94 (10.71)
M	31.30	286.28	15.56	2.22	2.03	28.23	192.40	8.20	1.72	1.92	59.53 (7.18)	478.68 (5.72)	23.76 (3.31)	3.94 (4.03)	3.95 (7.14)

Biomass (straw + grain) was the average value of cropping years (1991–2012) for each treatment

[a] The data within brackets stand for metal uptake values in different fertilization for multiples of that obtained from non-fertilization treatment (CK)

Table 6 Enrichment coefficient (EC) of heavy metals in wheat and maize after 22-year fertilization and cropping

Metal	Plant	CK	NP	NPK	NPKM	M
Cu	Maize grain	0.101	0.091	0.053	0.033	0.036
	Maize straw	0.112	0.153	0.126	0.065	0.070
	Wheat grain	0.114	0.092	0.128	0.061	0.063
	Wheat straw	0.114	0.126	0.175	0.072	0.083
Zn	Maize grain	0.220	0.156	0.178	0.132	0.148
	Maize straw	0.315	0.265	0.324	0.226	0.372
	Wheat grain	0.553	0.331	0.368	0.351	0.269
	Wheat straw	0.452	0.384	0.397	0.266	0.283
Pb	Maize grain	0.046	0.061	0.045	0.034	0.037
	Maize straw	0.126	0.132	0.082	0.064	0.082
	Wheat grain	0.072	0.071	0.057	0.051	0.052
	Wheat straw	0.114	0.113	0.062	0.044	0.048
Cr	Maize grain	0.002	0.003	0.002	0.002	0.002
	Maize straw	0.004	0.008	0.005	0.005	0.004
	Wheat grain	0.005	0.003	0.004	0.002	0.003
	Wheat straw	0.005	0.005	0.004	0.002	0.004
Cd	Maize grain	0.207	0.859	0.558	0.059	0.047
	Maize straw	0.802	1.776	1.271	0.472	0.488
	Wheat grain	0.649	0.774	1.182	0.209	0.104
	Wheat straw	1.529	1.775	3.519	0.591	0.545

oxides, clay) and soluble ligands (dissolved organic carbon, phosphate, carbonate, etc.) (Young 2013). During 22-year application of manure and chemical fertilizers and cropping, the remarkable changes of red soil properties occurred, where soil pH continuously decreased in those treatments of chemical fertilizers whereas SOM and Olsen P continuously increased in those treatments of manure fertilization (Fig. 4). Similar findings were reported by some researchers (Brown et al. 2003; Wei et al. 2006; Zhang et al. 2009). The total amount of N applied was the same (300 kg N ha^{-1} $year^{-1}$) for all the fertilization treatments, meaning that 21.4 kmol H^+ ha^{-1} $year^{-1}$ would be produced from nitrification of urea-N (assumed urea-N fully oxidized to NO_3-N) or 8.8 to 12.7 kmol H^+ ha^{-1} $year^{-1}$ in M (100 % manure-N) and NPKM (70 % manure-N), respectively (Cai et al. 2015). However, pig manure contained higher content of ash alkalinity (82.7 cmol kg^{-1} dry matter), equals to about 9.6 and 13.8 kmol OH^- ha^{-1} $year^{-1}$ in NPKM and M, respectively (Cai et al. 2015). Thus, as 70 % or more total N source, continuous manure application can fully prevent or reverse red soil acidification process. Additionally, higher amount of P and C (16.0 and 388.0 g kg^{-1} dry matter, respectively) in pig manure would result in higher content of soil Olsen P and SOM in manure fertilization treatments (M and NPKM). Stepwise regression analysis (Table 7) showed a significantly dependent association of metal availability with fertilization-derived soil property (SOM, Olsen P, pH, and total metal amount), where except Pb 0.1 M HCl-extractable metals were positively correlated with those soil factors, which was ascribed to long-term manure fertilization. However, a significant negative correlation between available Pb and soil pH suggested that the availability of Pb was mainly influenced by application of chemical fertilizers. Further stepwise regression analysis showed that significant negative correlation occurred between RA of Pb and Cd and soil pH (standardized coefficient of −0.691 and −0.449, respectively), indicating soil acidification caused by chemical fertilizers would promote their mobility and activity, as mentioned in Table 4. Among heavy metals, Cd is the most sensitive to soil pH (Singh and Myhr 1998; Blake and Goulding 2002), so that at pH<4.5 (N, NP, and NPK treatments) almost all soil Cd (>85 %) became available to plant for a longer period of time (Fig. 2). At the same time, available Cd was also the most correlated to its total content in soils (Table 7), supporting that its availability or activity (0.1 M HCl-extractability and RA) after long-term manure application became more and more evident as time progressed (Table 4 and Fig. 2).

Additionally, Table 4 showed that RA of Cu and Zn significantly increased in those treatments of manure fertilization. About 900 data for long-term fertilization in China also evidenced that manure fertilization increased markedly RA of Cu and Zn (unpublished work). It means that manure promoted SOM, Olsen P, and even soil pH, thus increased the activation of Cu and Zn by complexation (Wang et al. 2008a; Fan et al. 2012) or that Cu and Zn mainly existed in the available form (0.1 M HCl-extractability) in fresh pig manure. In short, long-term fertilization changed soil basic properties such as SOM, pH, and Olsen P that controlled strongly metal availability.

Cd accumulation and risk in red soil after long-term fertilization

Cd pollution has become the most serious environmental problem in the acidic red soil with pH of 5.7 (Table 1), for higher soil Cd activity caused much higher uptake by plants, with Cd content in wheat grain (0.16 mg kg^{-1}) under 22-year continued cropping without fertilization (CK) above the maximum level allowed in grains (MOHC 2005) (Fig. 3). Pollution of Cd in China's acidic agricultural soils and thus grain Cd exceeding hygienic standards were commonly observed (Wang et al. 2008b), and Cd activity was controlled mostly by soil pH (Singh and Myhr 1998; Blake and Goulding 2002), with higher activity at lower pH, as well as total Cd content. Our study also showed that at pH<4.5 (chemical fertilizer treatments) and manure treatments exceeding 50 % of soil Cd became available (Table 4 and Fig. 2). So, it is very necessary to focus on Cd accumulation in red soil and its entry into food chain.

Long-term application of chemical fertilizers increased the risk of Cd due to soil acidification (Fig. 4), with higher RA

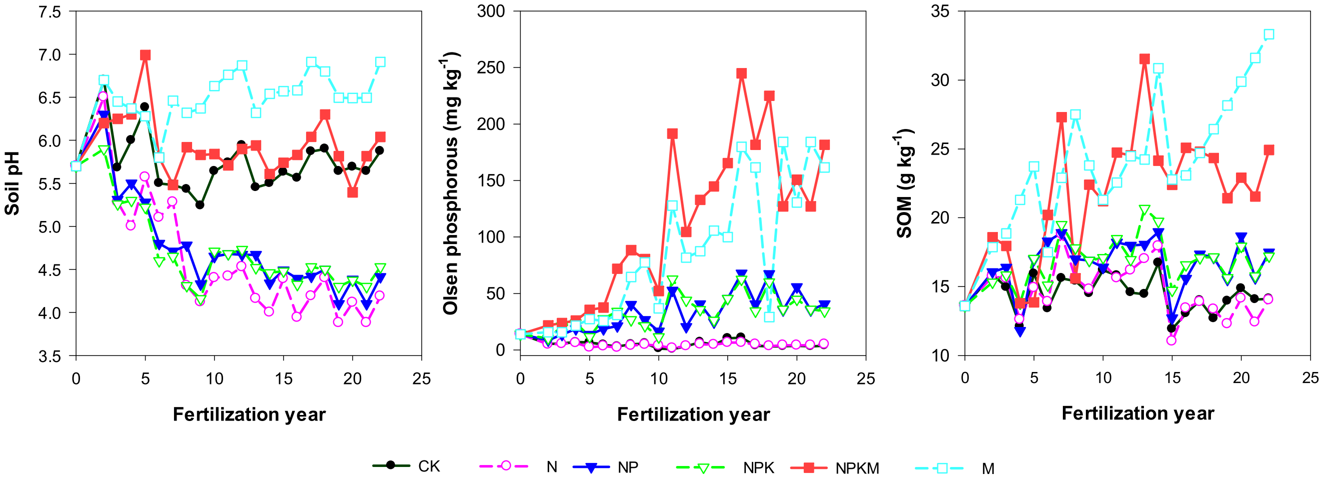

Fig. 4 Dynamic changes of soil pH, Olsen phosphorous, and SOM of red soil during long-term fertilization and cropping

and EC of Cd (Fig. 2 and Tables 4 and 6). Overuse of urea or other nitrogen fertilizers caused soil acidification, which is now becoming more widely recognized (Debreczeni and Kismányoky 2005; Guo et al. 2010; Tong and Xu 2012; Cai et al. 2014). Thus, reducing unnecessary N fertilization by balancing nutrient management for the red soil is the first step in maintaining its health. Long-term application of pig manure resulted in higher Cd accumulation and availability in red soil, as given in Fig. 1 and Tables 3 and 4. Li et al. (2009) also showed 17 years of pig manure fertilization caused Cd contents in red soil and rice exceeding national standards. In China's agricultural soils, livestock manures accounted for approximately 55 % of the total Cd inputs (Luo et al. 2009); and metal concentrations in animal feed and manure were positively correlated (Wang et al. 2013). So, it is time to reduce the concentration of toxic metals especially Cd in animal manures by limiting their content in animal feeds or feed additives. In a word, in strong acidic soils such as red soil, it is critical to reduce Cd risk by carefully selecting and applying fertilizers. In particular, more attention should be paid to manure fertilization, for which had a unique role in acidic soils. For example, under long-term manure fertilization pH, SOM, and Olsen P of red soil increased and uptake and enrichment of metals decreased (Figs. 3 and 4 and Table 6). That is, manure could improve soil quality and reduce the risk of transfer of soil-borne toxic metals in acidic soils into the food chain, so advocating increased application of manure alone or in combination with chemical fertilizers would be a viable strategy in these soils. But be sure to pay attention to the controlling the content of toxic metals especially Cd in animal manures.

Remediation strategies of heavy metal-contaminated acidic soils based on long-term experimental results

Our study showed that red soil would be contaminated by Cd, Cu, and Pb after long-term fertilization and cropping, and manure input and soil acidification are the main cause of pollution in this area. These toxic metals could be richened in the edible parts of the crops exceeding food hygiene standards, thus resulting in their entry into food chain and ultimately threatening human health. So, the contaminated soils must be managed and remediated effectively.

Because high-quality agricultural soil resources are insufficient to supply food for its enormous population, many crops are still planted in the contaminated soils in China. An issue that needs to be considered is how to

Table 7 Stepwise regression analysis between available metal (Y) and total metal (X1), soil pH (X2), Olsen phosphorous (X3), and SOM (X4) (n=37)

Y	R^2	X1		X2		X3		X4	
		Coefficient[a]	p	Coefficient	p	Coefficient	p	Coefficient	p
Cu	0.949	0.491	0.000	0.184	0.001	0.403	0.000		
Zn	0.918			0.260	0.000	0.482	0.000	0.349	0.001
Pb	0.529			−0.727	0.000				
Cr	0.307							0.554	0.000
Cd	0.932	0.966	0.000						

[a] These are standardized coefficients, indicating in one model the more the value the greater its effect on dependent variable

reduce the metal transfer from soil to plant. Firstly, those crops or crop cultivars with lower enrichment coefficients and higher tolerance to heavy metals were selected to plant in contaminated red soil. For example, not wheat but maize and pea, not indica and hybrid rice but japonica should be preferred. Secondly, promotion of soil pH was a critical to reduce metal activity in acidic soil, so long-term manure application and/or mixed application of lime materials would be very effective in high metal contaminated soils. Thirdly, adequate fertilization was a good strategy to remediate lightly contaminated soils by heavy metals, including the type and rate of organic and inorganic fertilizers, which had been summarized in our monographs (Xu et al. 2014; Zhou et al. 2015). Fourthly, for heavy contaminated soils, maybe some stabilized/immobilized amendments of heavy metals such as clays, red muds, phosphates, biochars, fly ash, etc., would be used during crop growth (Kumpiene et al. 2008), or maybe crop production should be given up and all kinds of chemical, physiochemical, and biological remediation technologies should be used to remove or immobilize pollutants.

Conclusions

A 22-year fertilization and cropping indicated that manure resulted in metal accumulation in red soil, mainly Cd, followed by Cu, and thus in the increase of metal availability except Pb, but it caused the decrease in uptake (mainly Pb and Cr) and enrichment (mainly Cd) of heavy metals by wheat and maize. On the contrary, chemical fertilizers promoted the availability of Pb and relative activity of Cd, and thus increased the uptake of Pb and enrichment of Cd. The effect of long-term fertilization on metal accumulation and availability extended to soil subsurface (20~40 cm), and generally chemical fertilizers would aggravate the risk of uptake and enrichment of heavy metals due to the increased soil acidity; manure fertilization could reduce their risk in red soil due to the increase in soil pH, SOM, and Olsen P. In acidic soils, Cd has the highest risk and special attention should be paid to it, so Cd content in manure and feed should be controlled and the maintenance of soil weak acidification would be necessary. There is no doubt that increased application of manure alone with low content of heavy metals or in combination with chemical fertilizers is important to securing acidic soils for sustainable agriculture.

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