

Fractionation of copper and cadmium and their binding with soil organic matter in a contaminated soil amended with organic materials

Ibrahim Mohamed · Bocar Ahamadou · Ming Li ·
Changxiu Gong · Peng Cai · Wei Liang ·
Qiaoyun Huang

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Abstract

Purpose The contamination of agricultural soils by heavy metals is a worldwide problem. Organic amendments can be used for the immobilization and binding of heavy metal ions in soils by complexation, adsorption, and precipitation. A field trial was carried out to evaluate the influence of some low-cost organic materials such as rice straw (RS), green manure (GM), and pig manure (PM) on the distribution of Cu and Cd and the retention of these metals by organic matter fractions in heavy metal-polluted soils.

Materials and methods The experiment was conducted in Miaoyunao Village, Daye County, Hubei province, China. PM, GM (peanut plants), and RS were obtained from a farm close to the village. Sixteen treatments with three replicates were designed. Soil chemical properties such as soil pH, electrical conductivity (EC), organic matter (OM), and available P were measured by standard methods. Soluble/exchangeable, organic-bound, inorganic precipitates and residual Cu and Cd in the soil were sequentially extracted and analyzed. The amounts of Cu and Cd bound with soil particulate organic matter (POM) fractions and humic substances were also determined.

Results and discussion The addition of organic amendments declined significantly the concentrations of soluble/exchangeable Cu and Cd, but increased the amounts of these metals in organic-bound and inorganic precipitate forms in the soil. RS was more effective than GM and PM in diminishing the solubility of Cu and Cd. The largest retention for Cu and Cd by humic substances and POM was noticed in RS treatments, whereas the lowest was found in PM treatments. Humic substances showed higher potential in the fixation of Cu and Cd than POM fractions. The conversion of soluble/exchangeable Cu and Cd to other insoluble forms after the application of organic amendments may be ascribed to the increases of soil OM, pH, EC, and available P contents. The highest binding of Cu and Cd with POM fractions and humic substances after the incorporation of RS mainly resulted from the greatest increase of soil OM contents.

Conclusions RS, GM, and PM can be employed as good and cheap substances for the immobilization of Cu and Cd in heavy metal-polluted soils. RS was the best amendment in decreasing the solubility of Cu and Cd, and also in enhancing the retention of these metals by humic substances and POM fractions in the soil. Future studies should focus on the influence of these organic amendments or their mixtures on the phytotoxicity of Cu and Cd for different plants in heavy metal-contaminated soils.

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I. Mohamed · B. Ahamadou · M. Li · C. Gong · P. Cai ·
W. Liang · Q. Huang (✉)
State Key Laboratory of Agricultural Microbiology,
Key Laboratory of Subtropical Agriculture
and Environment of Ministry of Agriculture,
Huazhong Agricultural University,
Wuhan 430070, People's Republic of China
e-mail: qyhuang@mail.hzau.edu.cn

Q. Huang
State Key Laboratory of Agricultural Microbiology,
Huazhong Agricultural University,
Wuhan 430070, People's Republic of China

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1 Introduction

Pollution of agricultural soils with heavy metals such as copper (Cu) and cadmium (Cd) is still one of the most serious concerns for the functionality of ecosystems in the world. This

contamination with Cu and Cd is mainly caused by mining and smelting activities (Cang et al. 2004; Quartacci et al. 2006) and application of industrial effluents as water source for irrigation of crop plants (Rattan et al. 2005; Abbas et al. 2007). Accumulation of Cu and Cd in the soils has toxic effects on microorganisms, plants, and ultimately on animals and human health via the food chain (Rulkens et al. 1998). Several physical, chemical, phyto, and engineering techniques have been used for the remediation of heavy metal-polluted soils (Mench et al. 1994; Shuman 1999). The chemical stabilization methods were considered to be the most cost-effective ways to immobilize heavy metals in the soils (Chen et al. 2000). The influence of organic substances on the availability of the heavy metals depends on the nature of these metals, soil types, and the organic matter properties, particularly the degree of humification (Walker et al. 2004). Organic matter has a vital role in controlling the mobility of heavy metals in soils. It may decrease the available concentrations of heavy metals in soils by precipitation, adsorption, or complexation processes (Bernal et al. 2007).

A number of studies have been carried out to examine the remediating effects of different organic wastes on heavy metal-polluted soils over the past years. For example, the addition of mushroom compost to a clay loam soil in Ankara, Turkey decreased the concentration of available Cu from 2.20 to 1.90 mg kg⁻¹ and Cd from 0.057 to 0.005 mg kg⁻¹ (Karaca 2004). Incorporation of pig manure to a paddy soil in China reduced the concentrations of available Cu and Cd by 76.1% and 25.7%, respectively (Li et al. 2008). In a calcareous soil in Murcia, Spain, the amount of available Cu was slightly reduced from 11.3 to 10.4 mg kg⁻¹ after the addition of cattle manure (Walker et al. 2003). Application of chicken manure compost in a Ferralsol, China decreased the concentration of soluble/exchangeable Cd by 71.8–95.7%, but increased the values of inorganic precipitated Cd by 0.6–1.5 times and organic-bound Cd by 0.9–7.8 times (Liu et al. 2009).

Particulate organic matter (POM) is the light fraction of soil organic matter and is mainly composed of identifiable animal and plant residues, root fragments, fungal hyphae, spores, and fecal pellets (Gregorich et al. 2006). The addition of vine-shoot, oak bark compost, and urban compost enhanced the binding of Cu, Pb, and Zn with POM in sandy loamy, France (Balabane et al. 1999). The fixation of Cu with POM increased by 73% due to the incorporation of 100 m³ ha⁻¹ conifer compost as compared with the control in clay-loamy soil, France (Sebastia et al. 2008). Humic substances constitute important fractions of soil organic matter, and their binding capacity affects the fate of metal cations and plays an important role in their mobility (Stevenson 1994). Humic acids (HA), fulvic acids (FA), and humin are the main components of humic substances, and they are different in the molecular weights, elemental, and functional group contents. It was reported

that HA and FA extracted from an ombrotrophic peat bog have essential roles in the retention of Cu, Cd, and Pb (Gondar et al., 2006a, b). The retention percentage of Hg²⁺ with HA and FA separated from the black soil in China was 50.9% and 82.0%, respectively (Zhang et al. 2009).

In view of the severe situation of soil pollution, searching for the lower cost and more effective approaches to immobilize heavy metals is still the most important tasks for soil and environmental scientists. Also, to our knowledge, there are no systematic published studies on the binding of metal ions with humic substances due to the addition of organic amendments under the field conditions. The aims of this work were: (1) to estimate the efficiency of pig manure, green manure, and rice straw application, through field trial, at various rates on the immobilization of Cu and Cd; (2) to examine in-depth the distribution and binding of Cu and Cd among different soil organic matter fractions in a heavy metal-contaminated soil in Hubei province, China.

2 Materials and methods

2.1 Site description and field experiment

A field trial was conducted for a period of 3 months (August–November 2008) in Miaoyunao Village, Daye County (30°6' N and 114°58' E) which is located in the south of Huangshi City, Hubei province, China. The site is characterized with a subtropical continental climate. The average annual temperature is 17°C, while the mean annual precipitation is 1,400 mm. The soil is contaminated with Cu and Cd due to mining activities. Sixteen treatments with triplicates were organized in a randomized complete block. The size of each plot was 3 m². Pig manure, green manure (peanut plants), and rice straw were obtained from a farm close to the village. Before the incorporation of organic amendments in the soil, pig manure was passed through a 2-mm sieve, while the rice straw and green manure were cut to small pieces (1–2 cm). The treatments of our study are listed in Table 1.

2.2 Analyses of soil and organic amendments

Soil samples were collected from the surface layer (0–15 cm) two times, at the beginning of the experiment and 3 months after the addition of the organic materials. Soil pH and electrical conductivity (EC) were measured using soil/water ratio (*w/v*) of 1:2 (Thomas 1996). The pH and EC of pig manure (PM) were determined in a PM/water ratio (*w/v*) of 1:10. Soil particle size distribution was evaluated by the international pipette method (Klute 1986). Soil organic matter content was estimated according to the method of Nelson and Sommers (1982). The organic matter content of the organic substances was obtained by ashing duplicate

Table 1 Treatments of the field experiment

Treatments	Amounts (t ha ⁻¹)	Treatments	Amounts (t ha ⁻¹)	Treatments	Amounts (t ha ⁻¹)
CK	0	GM1	5.2	PM1	2.2
RS1	5.2	GM2	7.7	PM2	3.3
RS2	7.7	GM3	10.3	PM3	4.4
RS3	10.3	GM4	15.4	PM4	6.5
RS4	15.4	GM5	23.2	PM5	9.8
RS5	23.2				

samples of each batch in muffle furnace at 540°C for 6 h. The change in the dry weight of these organic wastes before and after ashing was used to calculate the OM content. Soil and organic amendment samples were digested by a mixture of H₂SO₄ and HClO₄ (4:1 ratio), and the concentrations of total Cu and Cd were analyzed by atomic absorption spectrophotometer (Varian AA240FS). Total N and P concentrations in the digested solution were determined by FIA-star 5000 analyzer. Soil available phosphorus was extracted with 0.5 M NaHCO₃ (Hess 1972) and measured also by FIA-star 5000 analyzer. The amounts of cellulose, hemicellulose, and lignin were measured using the procedure of Goering and van Soest (1970). Some pertinent characteristics of the soil and organic amendments are shown in Table 2.

2.3 Sequential extraction of Cu and Cd in soil

Chemical fractionations of Cu and Cd in soils collected after the field trial were investigated using the scheme of Sposito et al. (1982). Four fractions were obtained: (1) soluble/exchangeable fraction, (2) organic-bound fraction, (3) inorganic precipitates, and (4) residual fraction. A brief summary of the procedure is presented in Table 3. The concentrations

of Cu and Cd for each fraction were determined by atomic absorption spectrophotometer (Varian AA240FS).

2.4 Analysis of Cu and Cd bound with POM

POM was separated from soil samples as described by Balescient (1996). One hundred grams of soil samples (<2 mm) was placed into a 500-ml flask and mixed with 250 ml deionized water in the presence of 20 glass beads (6 mm). The samples were shaken for 16 h at 200 rpm and 25°C. The POM was isolated by flotation in water and wet-sieved to 200 and 50 µm. The two POM fractions were dried at 55°C. The total Cu and Cd content in the POM fractions were measured by atomic absorption spectrometry (Varian AA240FS) after the digestion with H₂SO₄ and HClO₄ (4:1 ratio).

2.5 Determination of Cu and Cd linked with soil humic substances

The humic substances (humic acid, fulvic acid, and humin) were obtained from soil samples according to the procedure of Schnitzer (1982). Briefly, 100 g of soil sample (<2 mm) was weighed and mixed with 1 L of 0.1 M Na₄P₂O₇ + NaOH

Table 2 Some chemical properties of soil and organic amendments used in the experiment

Properties	Soil	Rice straw	Green manure	Pig manure
Moisture (%)	3.51	4.70	67.02	40.38
EC (dS m ⁻¹)	0.08	–	–	4.00
pH	6.03	–	–	8.11
Total N (g kg ⁻¹)	1.01	7.60	22.10	28.90
Total P (g kg ⁻¹)	0.52	2.90	13.00	23.70
Organic matter (g kg ⁻¹)	30.14	796.7	587.0	456.8
Total Cu (mg kg ⁻¹)	289.9	6.13	24.34	35.71
Total Cd (mg kg ⁻¹)	4.55	0.08	0.84	2.57
Cellulose (g kg ⁻¹)	–	375.4	268.6	105.3
Hemicellulose (g kg ⁻¹)	–	184.8	133.7	70.4
Lignin (g kg ⁻¹)	–	139.5	124.3	58.9
Sand (g kg ⁻¹)	402.2	–	–	–
Silt (g kg ⁻¹)	388.1	–	–	–
Clay (g kg ⁻¹)	209.7	–	–	–
Texture	Loam	–	–	–

Table 3 Summary of the sequential extraction steps of Cu and Cd fractionation

Steps	Fraction	Reagent	Soil/solution ratio (<i>w/v</i>)	Experiment conditions
1	Soluble/exchangeable	0.5 mol l ⁻¹ KNO ₃ , H ₂ O (3 times)	1:10	Shake 16 h at 20°C
2	Organic-bound	0.5 mol l ⁻¹ NaOH	1:10	Shake 16 h at 20°C
3	Inorganic precipitates	0.05 mol l ⁻¹ Na ₂ -EDTA	1:10	Shake 16 h at 20°C
4	Residual	4 mol l ⁻¹ HNO ₃	1:10	Shake 16 h at 80°C

(pH 10). The mixture was shaken for 24 h at 200 rpm and 25°C. The solution was separated from the residue (humins) by centrifugation at 3,800 rpm for 30 min. The supernatant was divided into HA and FA fractions by acidifying the extract to pH 1 using 6 M HCl. HA was isolated from FA by centrifugation for 30 min at 3,800 rpm and 25°C. HA and FA were collected and freeze-dried, while the humin fraction was dried at 50°C. The concentrations of Cu and Cd in each fraction were detected in the digested solution by atomic absorption spectrometry (Varian AA240FS).

2.6 Statistical analysis

Data were analyzed using M-STATC statistical package, and the differences between the treatment means were evaluated using the least significant difference test.

3 Results

3.1 Effect of organic amendments on soil chemical properties

The influences of the organic amendments on soil chemical properties are shown in Table 4. The values of soil pH, EC,

OM, and available P (AP) were markedly increased due to the addition of organic amendments as compared to the control. These increments became more obvious with raising amendment application rates. Soil pH values ranged from 6.04 in CK to 7.48 in PM5. The lowest value of soil EC was noticed in CK (0.12 dS cm⁻¹), whereas the highest was recorded in PM5 (1.68 dS cm⁻¹). The content of soil OM enhanced from 29.8 mg kg⁻¹ for CK to 46.2 mg kg⁻¹ for soils treated with RS5. The amounts of soil AP varied from 20.1 mg kg⁻¹ in CK to 50.6 mg kg⁻¹ in PM5.

3.2 Effect of organic amendments on Cu and Cd distribution in soil

As shown in Fig. 1, the application of PM, GM, and RS in the soil significantly reduced the concentrations of soluble/exchangeable Cu and Cd as compared to the control. The decrease of soluble/exchangeable Cu and Cd was considerable with increasing rates of applied amendments. The amounts of soluble/exchangeable Cu lowered from 8.97 mg kg⁻¹ in CK to 5.35, 4.15, and 2.46 mg kg⁻¹ in PM1, GM1, and RS1, respectively. In PM5, GM5, and RS5, the amounts of soluble/exchangeable Cu declined to 1.37, 0.64, and 0.28 mg kg⁻¹, respectively. The values of soluble/exchangeable Cd remarkably reduced from 1.00 mg kg⁻¹

Table 4 Influences of organic amendments at different rates on soil chemical properties

Treatments	pH	EC (dS cm ⁻¹)	Organic matter (g kg ⁻¹)	Available P (mg kg ⁻¹)
CK	6.04 i	0.12 h	29.8 i	20.1 l
RS1	6.12 h	0.28 gh	36.7 fgh	22.2 kl
RS2	6.24 gh	0.40 fgh	39.1 def	24.0 k
RS3	6.36 ghi	0.54 efg	41.5 bcd	27.2 j
RS4	6.53 fgh	0.68 def	43.6 ab	30.5 hi
RS5	6.61 ef	0.83 de	46.2 a	34.9 fg
GM1	6.41 ghi	0.36 fgh	34.0 gh	27.3 j
GM2	6.53 fgh	0.55 efg	36.0 fgh	29.2 ij
GM3	6.65 ef	0.77 de	38.3 efg	32.7 gh
GM4	6.78 de	0.98 cd	41.0 bcd	37.0 ef
GM5	6.91 cd	1.19 bc	42.8 bc	41.2 cd
PM1	6.81 de	0.80 de	31.6 h	35.8 f
PM2	6.97 cd	1.02 cd	34.0 gh	38.9 de
PM3	7.13 ab	1.23 bc	36.0 fgh	42.0 c
PM4	7.29 b	1.45 ab	38.1 efg	45.3 b
PM5	7.48 a	1.68 a	40.2 cde	50.6 a

All the values are means of three replicates. Different letters in the same column are significantly different at $P < 0.05$

RS rice straw, GM green manure, PM pig manure

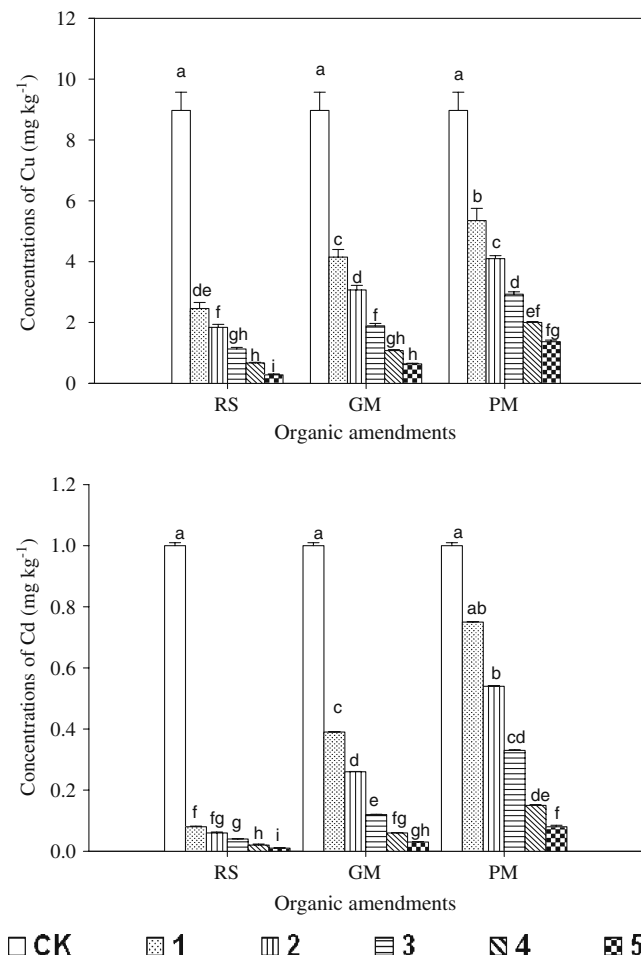


Fig. 1 Concentrations of soluble / exchangeable Cu and Cd influenced by different organic amendments

in CK to 0.08–0.01 mg kg⁻¹ for soil treated with RS, 0.39–0.03 mg kg⁻¹ due to the addition of GM, and 0.75–0.08 mg kg⁻¹ after the incorporation of PM.

The data in Fig. 2 demonstrated that organic amendments significantly increased the concentrations of organic-bound Cu and Cd in the soil. The amounts of organic-bound Cu conspicuously increased by 7.5–17.1%, 12.3–23.7%, and 23.6–33.2% due to the application of PM, GM, and RS. The addition of PM1, GM1, and RS1 caused increases in the amounts of organic-bound Cd by 20.0%, 43.6%, and 77.4%, respectively. At PM5, GM5, and RS5, the concentrations of organic-bound Cd enlarged by 108.4%, 136.9%, and 164.8%, accordingly.

The amounts of inorganic precipitate Cu and Cd in the soil treated with different organic substances are shown Fig. 3. At the lowest rates of RS, GM, and PM, the quantities of inorganic precipitate Cu enhanced by 5.6%, 7.3%, and 12.8%, respectively. The values of inorganic precipitate Cu slightly raised by 10.4%, 12.3%, and 21.2% at the highest rates of RS, GM, and PM,

respectively. The supply of RS, GM, and PM treatments obviously increased the amounts of inorganic precipitate Cd by 13.5–100.6%, 69.2–122.7%, and 84.5–152.9%, respectively.

The concentrations of soil residual Cu and Cd are illustrated in Fig. 4. In general, the addition of RS, GM, and PM caused noticeable changes in the amounts of soil residual Cu and Cd as compared with CK. There are no significant differences noticed with increasing rates of the applied amendments. The highest concentrations of residual Cu and Cd were recorded in PM treatments, whereas the lowest values were found in RS treatments.

3.3 Influence of organic amendments on Cu and Cd bound by POM and humic substances

Table 5 shows that the incorporation of RS, GM, and PM enlarged significantly the values of Cu and Cd in POM fractions as compared to CK. The greatest amounts of Cu and Cd in POM fractions were recorded in RS treatments, whereas the lowest were in PM treatments. The contents of

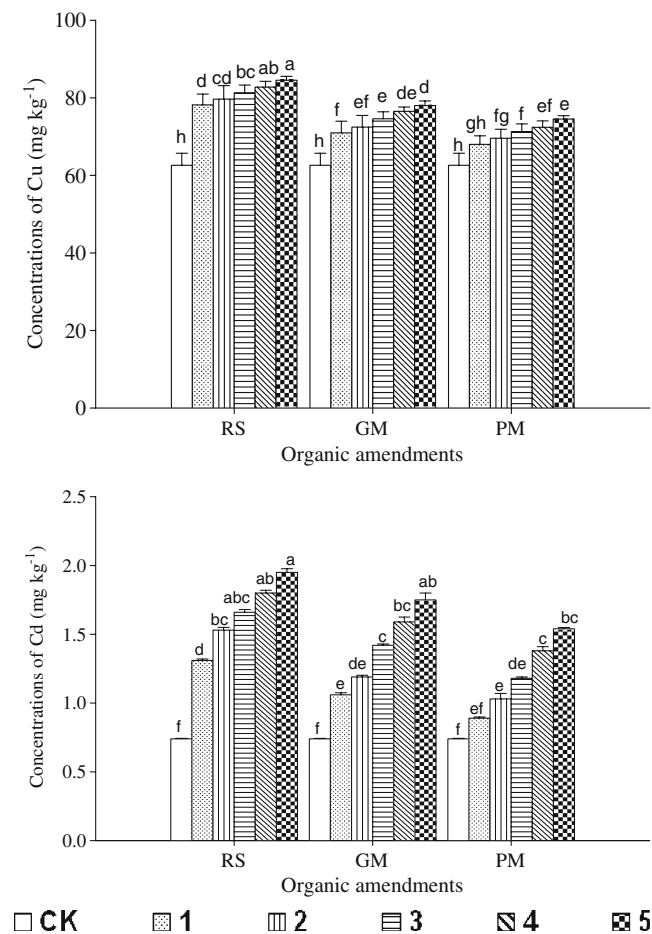


Fig. 2 Concentrations of organic-bound Cu and Cd influenced by different organic amendments

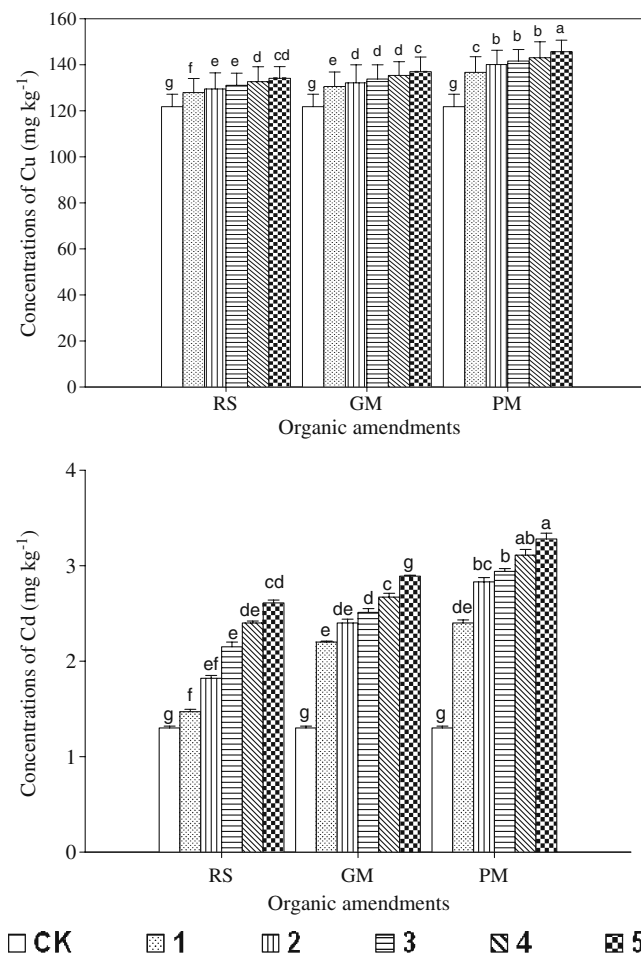


Fig. 3 Concentrations of inorganic precipitated Cu and Cd influenced by different organic amendments

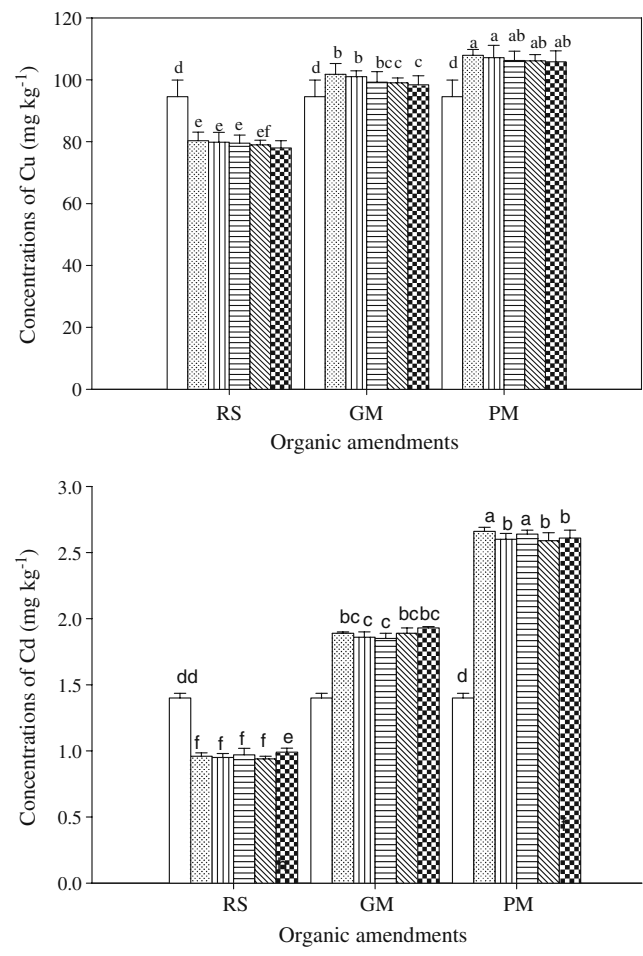


Fig. 4 Concentrations of residual Cu and Cd influenced by different organic amendments

Cu and Cd in POM fractions increased with decreasing the POM size. The quantities of Cu in the larger fraction of POM (2,000–200 μm) increased from 0.87 mg kg⁻¹ in CK to 1.35–4.52, 1.70–6.00, and 2.41–7.12 mg kg⁻¹ for PM, GM, and RS treatments, respectively. In CK treatment, Cd concentration in the larger fraction of POM was 0.01 mg kg⁻¹, and it was enhanced to 0.04, 0.06, and 0.10 mg kg⁻¹ for soil treated with PM1, GM1, and RS1, while at PM5, GM5, and RS5, the amounts of Cd increased to 0.23, 0.35, and 0.47 mg kg⁻¹, respectively. In the smaller fraction of POM (200–50 μm), the values of Cu ranged from 1.80 mg kg⁻¹ in CK to 2.40–6.02 mg kg⁻¹ as results of PM additions, 3.07–7.39 mg kg⁻¹ in GM-amended soils, and 4.05–10.9 mg kg⁻¹ for soils treated with RS. The application of RS, GM, and PM raised the contents of Cd in the smaller fraction of POM from 0.05 mg kg⁻¹ in CK to 0.18–0.71, 0.13–0.60, and to 0.11–0.52 mg kg⁻¹, respectively.

The addition of RS, GM, and PM in soil caused significant increases in the amounts of Cu and Cd bound

with humic substances as compared to CK (Table 6). The greatest amounts of Cu and Cd were recorded in HA, whereas the lowest were found in HU. The effects of RS treatments on Cu and Cd concentrations in humic substances fractions were more obvious than GM and PM treatments. In HA, the values of Cu enhanced from 25.4 mg kg⁻¹ in CK to 28.9, 30.5, and 32.3 mg kg⁻¹ in PM1, GM1, and RS1, while it enhanced to 34.0, 36.0, and 38.5 mg kg⁻¹ for soil amended with PM5, GM5, and RS5, correspondingly. The concentration of Cd in HA was 0.33 mg kg⁻¹ at CK and increased to 0.42, 0.58, and 0.70 due to the application of PM1, GM1, and RS1, respectively. At PM5, GM5, and RS5, the values of Cd increased to 0.90, 1.03, and 1.48 mg kg⁻¹, accordingly. The addition of PM, GM, and RS treatments caused increases in the amounts of Cu in fulvic acid ranging from 20.4 mg kg⁻¹ in CK to 21.6–26.7, 23.4–28.0, and 24.5–30.0 mg kg⁻¹, respectively. The values of Cd in FA varied from 0.22 mg kg⁻¹ for CK to 0.28, 0.40, and 0.59 mg kg⁻¹ for

Table 5 Amounts of Cu and Cd (mg kg⁻¹ soil) bound with POM fractions influenced by organic amendments at different rates on soil chemical properties

Treatments	Cu-POM (2,000–200 μm)	Cu-POM (200–50 μm)	Cd-POM (2,000–200 μm)	Cd-POM (200–50 μm)
CK	0.87 i	1.80 i	0.01 f	0.05 g
RS1	2.41 fgh	4.05 fg	0.10 de	0.18 ef
RS2	3.81 def	5.92 de	0.18 cd	0.26 de
RS3	4.63 cde	7.11 bc	0.26 bc	0.39 cd
RS4	5.75 ab	9.16 b	0.33 ab	0.62 ab
RS5	7.12 a	10.9 a	0.47 a	0.71 a
GM1	1.70 gh	3.07 ghi	0.06 ef	0.13 f
GM2	2.68 fgh	4.12 fg	0.09 de	0.22 de
GM3	3.80 def	5.20 ef	0.15 cd	0.36 cd
GM4	4.91 bc	6.45 cd	0.26 bc	0.49 bc
GM5	6.00 ab	7.39 bc	0.35 ab	0.60 ab
PM1	1.35 hi	2.40 hi	0.04 ef	0.11 f
PM2	2.10 gh	3.40 gh	0.06 ef	0.17 ef
PM3	3.00 efg	4.33 fg	0.10 de	0.25 de
PM4	3.89 def	5.23 ef	0.16 cd	0.38 cd
PM5	4.52 cde	6.02 de	0.23 bc	0.52 bc

All the values are means of three replicates. Different letters in the same column are significantly different at *P*<0.05

RS rice straw, GM green manure, PM pig manure, POM particulate organic matter

soil ameliorated with PM1, GM1, and RS1 and to 0.80, 0.91, and 1.16 mg kg⁻¹ at PM5, GM5, and RS5, respectively. The lowest concentration of Cu in HU was noticed in CK (12.1 mg kg⁻¹), whereas the highest was found in RS5 (20.8 mg kg⁻¹). The amounts of Cd in HU was 0.11 mg kg⁻¹ in CK and, it increased after the incorporation of PM, GM, and RS to 0.15–0.60, 0.21–0.69, and 0.41–0.90 mg kg⁻¹, respectively.

4 Discussion

Our results showed that the application of RS, GM, and PM reduced the contents of soluble/exchangeable Cu and Cd, but increased the amounts of organic-bound and inorganic precipitates Cu and Cd in the soil. These findings suggested that the soluble/exchangeable Cu and Cd may be converted to organic-bound, inorganic precipitates, and residual. The

Table 6 Impact of organic amendments on the binding of Cu and Cd (mg kg⁻¹ soil) with humic substances

Treatments	Cu-HA	Cd-HA	Cu-FA	Cd-FA	Cu-HU	Cd-HU
CK	25.4 g	0.33 j	20.4 h	0.22 g	12.1 j	0.11 h
RS1	32.3 de	0.70 efg	24.5 efg	0.59 def	15.5 gh	0.41 ef
RS2	34.4 cd	0.81 def	25.9 def	0.66 de	16.8 ef	0.53 cd
RS3	35.5 bc	0.94 bcd	27.2 bcd	0.79 bc	18.2 cd	0.66 b
RS4	36.8 ab	1.33 b	28.7 ab	0.94 ab	19.4 ab	0.80 ab
RS5	38.5 a	1.48 a	30.0 a	1.16 a	20.8 a	0.90 a
GM1	30.3 efg	0.58 gh	23.4 fgh	0.40 fg	14.1 hi	0.21 gh
GM2	31.1 def	0.66 fgh	24.5 efg	0.51 efg	15.2 gh	0.32 fg
GM3	33.0 de	0.78 def	25.2 ef	0.64 de	16.1 fg	0.46 de
GM4	34.2 cd	0.90 bcd	26.6 cde	0.78 bc	17.4 de	0.55 cd
GM5	36.0 bc	1.03 b	28.0 bc	0.91 ab	18.8 bc	0.69 b
PM1	28.9 fg	0.42 i	21.6 gh	0.28 g	13.2 i	0.15 h
PM2	30.1 efg	0.50 hi	22.8 fgh	0.35 fg	14.5 hi	0.23 gh
PM3	31.3 def	0.64 fgh	24.0 efg	0.47 efg	15.5 gh	0.38 fg
PM4	32.5 de	0.78 def	25.2 ef	0.69 de	16.8 ef	0.47 de
PM5	34.0 cd	0.90 bcd	26.7 cde	0.80 bc	18.0 cd	0.60 bc

All the values are means of three replicates. Different letters in the same column are significantly different at *P*<0.05

RS rice straw, GM green manure, PM pig manure, HA humic acid, FA fulvic acid, HU humin

transformation of soluble/exchangeable Cu and Cd to insoluble fractions can be explained by the increase of soil OM content, pH, EC, and available P as results of RS, GM, and PM addition in the polluted soil. It was noticed by Wang et al. (2009) that the increases of soil pH, EC, OM contents, and total P were the most important factors affecting the distribution of Cu, Cd, Pb, and Zn fractions in soils near a Pb/Zn smelter, China. RS treatments were more effective than GM and PM treatments in decreasing the concentrations of soluble/exchangeable Cu and Cd and in increasing the values of organic-bound Cu and Cd. This could be related to the higher amounts of OM in soil treated with RS than with GM and PM (see Table 4). The increment of soil OM content encouraged the formation of stable organometallic complexes which could diminish the solubility of the metal ions in the soil (Udom et al. 2004). The greatest enhancements of soil pH, EC, and AP due to the addition of PM (see Table 4) were assumed to be reasons for the highest values of inorganic precipitates Cu and Cd in the soil. It was reported that the increases of soil pH from 5.95 to 7.38 and total P from 0.44 to 1.68 mg kg⁻¹ increased the concentrations of inorganic precipitates Cd by 0.6–1.5 times after the incorporation of chicken manure compost in a Ferralsol, China (Liu et al. 2009).

As compared with CK, the addition of RS, GM, and PM markedly increased the fixation of Cu and Cd with different fractions of POM. The greater efficiency of RS treatments than that of GM and PM treatments in the retention of Cu and Cd with POM fractions may be explained by the larger amounts of some organic compounds such as cellulose, hemicellulose, and lignin in RS in comparison to GM and PM (see Table 2). These compounds contain several phenol groups which may affect strongly the binding of Cu and Cd (Rocha et al. 2009). Another possible reason is the higher enhancements of OM content in soils amended with RS than with GM and PM (see Table 4). A study by Sebastia et al. (2008) showed that the increment of OM content from 5.5 to 34.7 g kg⁻¹ after the addition of conifer compost (100 m³ ha⁻¹) enhanced the amount of Cu bound with POM fractions from 5.5 to 9.5 mg kg⁻¹ in a clay-loamy soil, France. The enrichment of Cu and Cd in the smaller fraction of POM was higher than in the coarser fraction, which may be due to the larger surface area and greater number of active sites for the smaller POM fraction. Similar results were also obtained by Labanowski et al. (2007) who found that the amounts of Cu and Cd bound with the smaller fraction of POM (200–50 μm) increased by 2.4 and 3.0 times, respectively, as compared with the larger fraction (2,000–200 μm) in a sandy-loamy soil, France.

In the current investigation, the status of HA and FA clearly improved due to the addition of RS, GM, and PM as compared with CK (Fig. 5). Possibly, the largest presence of total OM in RS (see Table 2) can be responsible for the

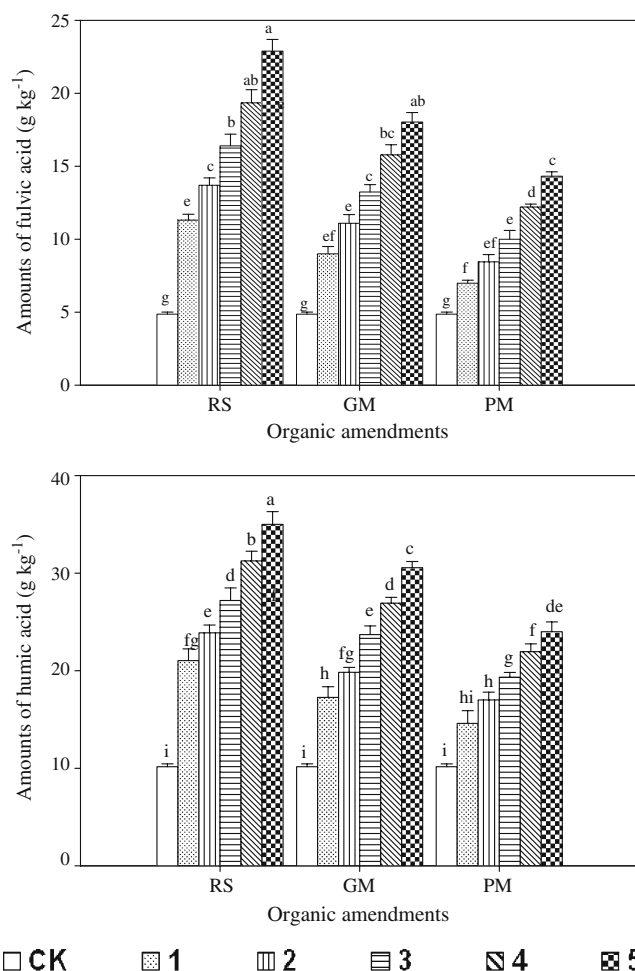


Fig. 5 Amounts of fulvic acid and humic acid in soils treated different organic amendments

highest quantities of HA and FA in RS-amended soil. In comparison with CK, the incorporation of RS, GM, and PM into the contaminated soil increased significantly the retention of Cu and Cd by humic substances. The ability of humic substances to bind heavy metal ions can be attributed to their high content of oxygen-containing functional groups, including carboxyl, phenol, hydroxyl, enol, and carbonyl structures of various types (Stevenson 1994). The increases of soil OM contents and pH values via the addition of RS, GM, and PM were important in governing the fixation of Cu and Cd by HA, FA, and HU fractions. In a previous study, Gondar et al. (2006a, b) found that the variation of pH from 4.5 to 6.5 increased the binding constant of Cu from 4.70 to 5.40 and from 4.20 to 4.9 for HA and FA, respectively, extracted from the upper horizon of an ombrotrophic peat bog. The highest retention of Cu and Cd with HA, FA, and HU in soil treated with RS probably resulted from the greatest increases of OM in this soil (see Table 4). In all treatments, the larger presence of HA in the soil as compared with FA (see Fig. 5) may have

contributed to the greater efficiency of HA in the complexation of Cu and Cd. It was noticed that the amounts of Cu bound with FA were 1.0, 0.8, and 1.7 mg kg⁻¹, while for HA, it increased to 3.3, 4.0, and 5.2 mg kg⁻¹ in a calcareous soil (0–20 cm), Mexico, irrigated with wastewater for 5, 46, and 89 years, respectively (Reyes-Solís et al. 2009). However, the concentrations of Cu and Cd bound with FA enhanced by 1.3 and 6.8 times than those with HA isolated from the surface layer of an Andosol (0–16 cm), Romania (Donisa et al. 2003). Our results showed that the smallest enrichment of Cu and Cd were noticed in HU fraction in all treatments. This may be explained by the lowest surface area and functional group contents of HU fraction.

5 Conclusions

The incorporation of pig manure, green manure, and rice straw were effective in reducing the solubility of Cu and Cd especially at the highest applied rates. Also, both pig manure, green manure, and rice straw can be used as good and inexpensive substances for the immobilization of Cu and Cd in heavy metal-polluted soils. Among different organic materials, rice straw was the most efficient material in diminishing the extractability of Cu and Cd in the contaminated soils. The addition of organic amendments strongly affected the retention and the distribution of Cu and Cd by humic substances and POM. The highest binding of Cu and Cd with soil organic matter fractions were recorded in rice straw treatments. The retention of Cu and Cd by POM fractions depended on their size. The greatest ability in the fixation of Cu and Cd was observed in the smaller size fraction of POM. In all treatments, humic acid recorded higher capacity in the binding of Cu and Cd, higher than fulvic acid, humin, and POM fractions in the contaminated soil. Accordingly, future long-term investigations should be carried out in order to provide the impacts of these organic wastes or different mixtures of them on the immobilization of Cu and Cd and their phytotoxicity for crops grown on heavy metal-contaminated soils.

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