

**Effects of goethite on the fractions of Cu, Cd, Pb, P and soil enzyme activity with
hydroxyapatite in a heavy metal-contaminated soil**

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Text S1. Simplified bioaccessibility extraction test (SBET) procedure

Briefly, a gastric solution was prepared by dissolving 1.25 g of pepsin, 0.5 g sodium citrate, 0.5 g sodium malate, 500 μL acetic acid and 420 μL lactic acid in 1 L Milli-Q water with the pH adjusted to 2.5 using 12 mol L^{-1} HCl. About 0.4 g of soil sample was extracted using 40 mL of the gastric solution (solid:fluid = 1:100) in a 100 mL polypropylene centrifuge tube with shaking (37 °C) for 1 h. The pH of the extraction solution was measured every 30 min using a pH meter and, if needed, adjusted using 12 mol L^{-1} HCl or saturated NaHCO_3 solution to maintain a constant pH (1.5). After 1 h, the extractants were centrifuged (2810 \times g for 10 min), and 20 mL supernatant was directly taken from each tube and filtered through 0.45 μm filters (Whatman 1) as a stomach phase. They were stored at 4°C and analyzed within 48 h.

Text S2. Soil Cu, Cd and Pb sequential extraction procedure

A sequential extraction procedure modified from the method of Tessier et al. (1979) was described as follows:

(1). Exchangeable fraction: 16 mL magnesium chloride ($1 \text{ mol L}^{-1} \text{ MgCl}_2$, pH=7) was added in a 100-mL polypropylene centrifuge tube containing $2 \pm 0.0001 \text{ g}$ of soil sieved through a 0.15-mm mesh. The tube was shaken for 2 h at $25 \pm 1 \text{ }^\circ\text{C}$ on an end-over mechanical shaker operating at 120 rpm. After equilibration, the suspension was centrifuged at 4,000 rpm for 10 min. The supernatant was decanted and filtered through a 0.45- μm filter and transferred into a polyethylene container and stored at $4 \text{ }^\circ\text{C}$ until analysis.

(2). Bound to carbonates: The residue from (1) was shaken with 16 mL mol L^{-1} sodium acetate (CH_3COONa), with pH adjusted to 5.0 with acetic acid (CH_3COOH). The tube was shaken for 3 h at $25 \pm 1 \text{ }^\circ\text{C}$, and the extraction procedure was the same as in (1).

(3). Bound to Fe–Mn oxides: The residue from (2) was shaken with 40 mL hydroxylamine hydrochloride (0.04 mol L^{-1} , $\text{NH}_2\text{OH}\cdot\text{HCl}$) in 25 % (v/v) CH_3COOH . The tube was heated to $96 \pm 3 \text{ }^\circ\text{C}$ in a water bath for 6 h, and the extraction procedure was the same as in (1).

(4). Bound to organic matter fraction: 6 mL nitric acid (0.02 mol L^{-1} , HNO_3) and 10 mL hydrogen peroxide (30 % H_2O_2 , acidified to pH 2 with HNO_3) were added into the residue from (3), and the mixture was heated to $85 \pm 2 \text{ }^\circ\text{C}$ for 2 h in a water bath. Then, 6 mL H_2O_2 (30 %, acidified to pH 2 with HNO_3) was added, and the sample was heated again to $85 \pm 2 \text{ }^\circ\text{C}$ for 3 h. After cooling, 10 mL ammonium acetate (3.2 mol L^{-1} , $\text{CH}_3\text{COONH}_4$) in 20 % (v/v) HNO_3 was added at $25 \pm 1 \text{ }^\circ\text{C}$ for 30 min. The extraction procedure was the same as in (1).

(5). Residual fraction: The difference between the total and non-residual fractions was regarded as the residual fraction. Each fraction of the certified reference material was analyzed in triplicate. The residual fraction of the certified reference material was analyzed by digestion with HNO_3 –HF– HClO_4 (5:10:5 mL). The sum of concentrations of Cu and Cd in different fractions was compared with the concentrations obtained by total digestion. The accuracy ranged between 83 % and 116 %. Therefore, the difference between the total and non-residual fractions was called the residual fraction.

Text S3. Soil P sequential extraction procedure

A sequential extraction method for phosphorus fractions modified from the method of Tiessen and Moir (1993): (1) labile resin-P, 0.5 g air-dried soil was extracted with deionized water and one anion-exchange resin strip; (2) labile inorganic $\text{NaHCO}_3\text{-P}$ ($\text{NaHCO}_3\text{-Pi}$) and organic $\text{NaHCO}_3\text{-P}$ ($\text{NaHCO}_3\text{-Po}$) were extracted with $0.5 \text{ mol L}^{-1} \text{ NaHCO}_3$ at $\text{pH} = 8.5$; (3) moderately labile inorganic NaOH-P (NaOH-Pi) and organic NaOH-P (NaOH-Po) were extracted with NaOH (0.1 mol L^{-1}); (4) stable HCl-P was extracted with HCl (1 mol L^{-1}); (5) stable residual P was measured after the remaining soil digested with $\text{H}_2\text{SO}_4/\text{H}_2\text{O}_2$.

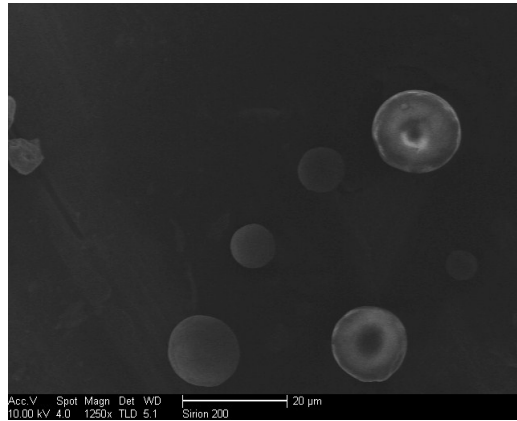


Fig. S1. The TEM imaging of hydroxyapatite (HAP)

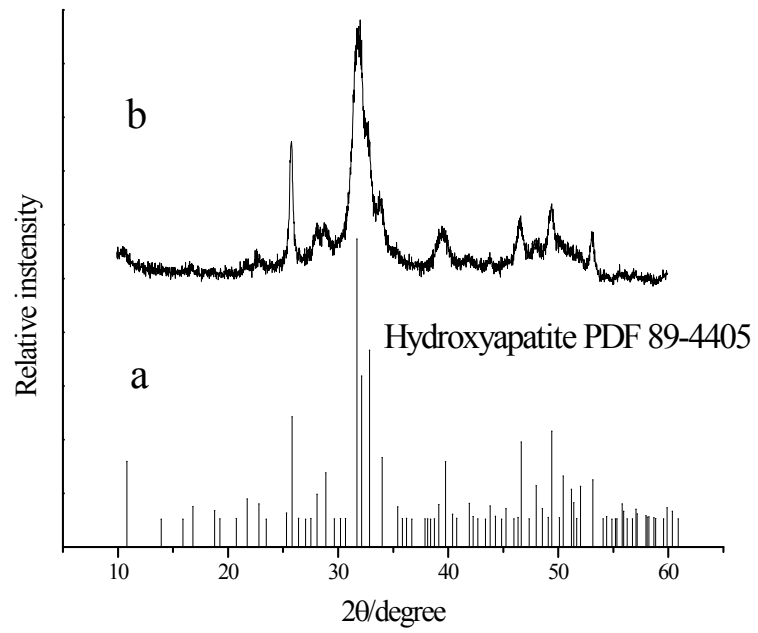


Fig. S2. Powder X-ray diffraction patterns of hydroxyapatite.

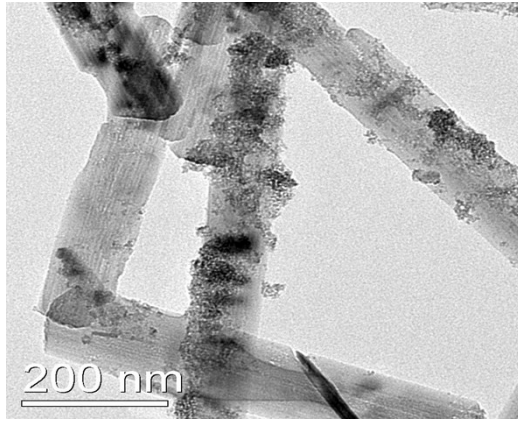


Fig. S3. The TEM imaging of goethite

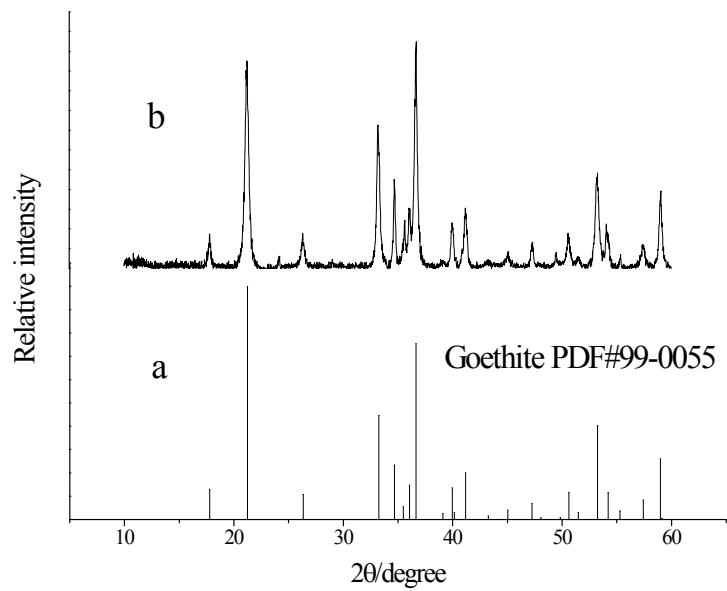


Fig. S4. Powder X-ray diffraction patterns of goethite.

Fig. S5. Effects of HAP and goethite applications on percentage of Cu (A), Cd (B), and Pb (C). CK=untreated soil, LG=0.5% goethite plus soil, HG=1% goethite plus soil, HAP=1% HAP plus soil, LGH=0.5% goethite and 1% HAP plus soil, HGH=1% goethite and 1% HAP plus soil. Means (n = 3) followed by different letters above the columns indicated significant difference at the $P < 0.05$. Error bars are standard error of the mean.

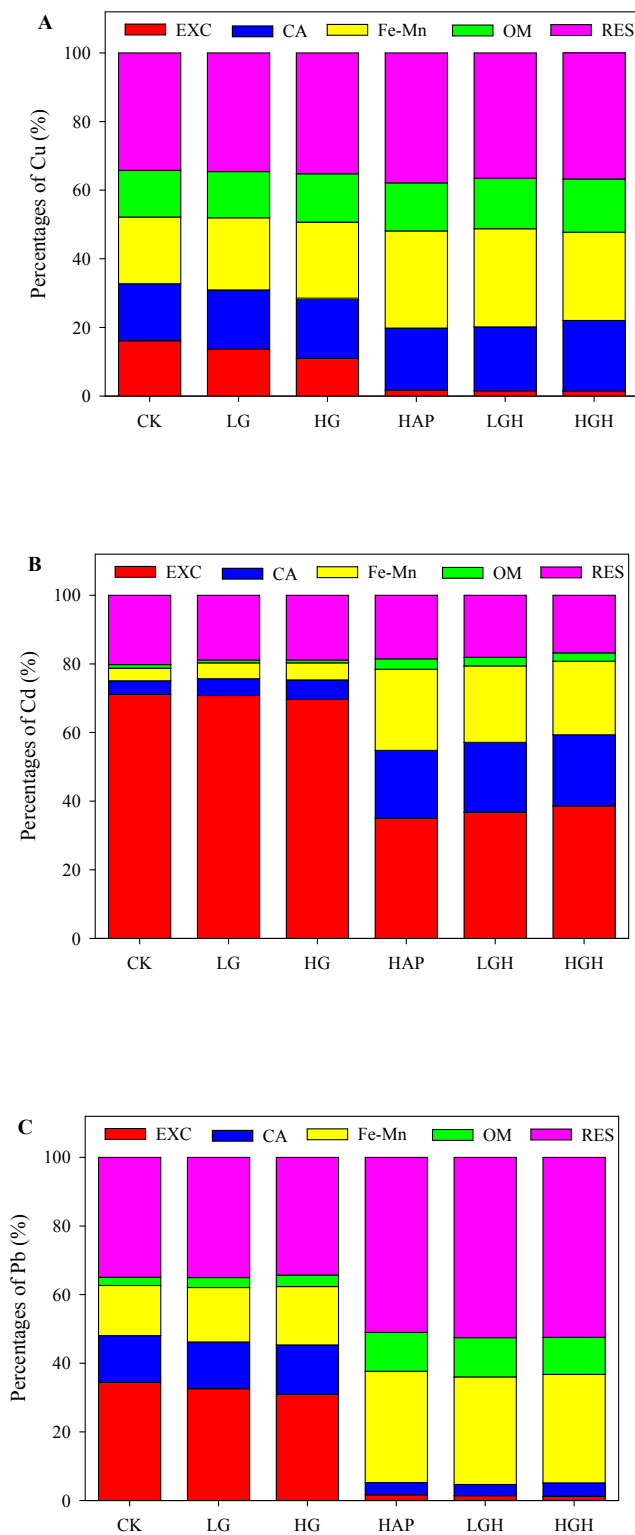


Fig. S6. Effects of HAP and goethite applications on the concentrations of bioaccessible Cu, Cd, and Pb. CK=untreated soil, LG=0.5% goethite plus soil, HG=1% goethite plus soil, HAP=1% HAP plus soil, LGH=0.5% goethite and 1% HAP plus soil, HGH=1% goethite and 1% HAP plus soil. Means (n = 3) followed by different letters above the columns indicated significant difference at the $P < 0.05$ Error bars are standard error of the mean.

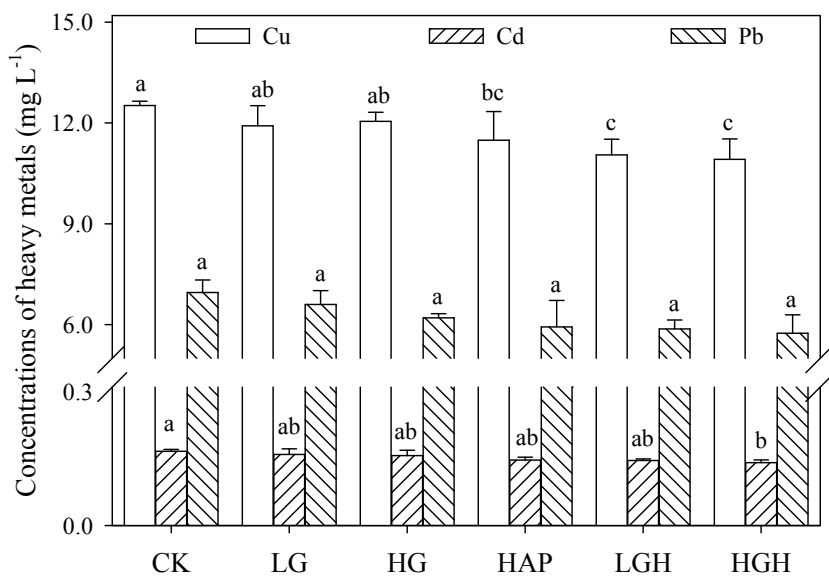


Table S1. Correlation coefficients between various extractable heavy metal concentrations and microbial activities (n=5).

	pH	Catalase	Urease	Phosphatase	Ca-Cu	Ca-Cd	Ca-Pb	EXC-Cu	EXC-Cd	EXC-Pb	Resin-P	NaHCO ₃ -Pi
pH	1											
Catalase	0.965**	1										
Urease	0.966**	0.947**	1									
Phosphatase	0.997**	0.973**	0.969**	1								
Ca-Cu	-0.981**	-0.946**	-0.948**	-0.978**	1							
Ca-Cd	-0.969**	-0.969**	-0.954**	-0.973**	0.968**	1						
Ca-Pb	-0.990**	-0.940**	-0.952**	-0.984**	0.993**	0.954**	1					
EXC-Cu	-0.995**	-0.966**	-0.965**	-0.993**	0.989**	0.984**	0.990**	1				
EXC-Cd	-0.971**	-0.963**	-0.978**	-0.946**	0.951**	0.994**	0.937**	0.974**	1			
EXC-Pb	-0.974**	-0.971**	-0.957**	-0.978**	0.969**	0.999**	0.958**	0.987**	0.996**	1		
Resin-P	0.905**	0.880**	0.911**	0.904**	-0.909**	-0.964**	-0.891**	-0.930**	-0.953**	-0.963**	1	
NaHCO ₃ -Pi	0.643*	0.634*	0.650*	0.648*	-0.654*	-0.760**	-0.618*	-0.678*	-0.761**	-0.760**	0.876**	1

Ca-Cu, CaCl₂-extractable Cu; Ca-Cd, CaCl₂-extractable Cd; Ca-Pb, CaCl₂-extractable Pb; EXC-Cu, exchangeable fraction of Cu; EXC-Cd, exchangeable fraction of Cd; EXC-Pb, exchangeable fraction of Pb;

* Significant at $P < 0.05$.

** Significant at $P < 0.01$.