Supplementary Material Cover Sheet

pH-dependent transport of neonicotinoid pesticides in saturated soil: Single and combined functions of rhamnolipid and biochar colloids

Lixia Li [1], Tao Jia [1], Zhichong Qi [2], Usman Farooq [2], Yanbin Ma [2, 3], Fanyan Yang [2, 3], Minghui Lv [1, *],Taotao Lu [4, **]

[1] Shandong Provincial Geo-mineral Engineering Exploration Institute, Shandong Provincial Bureau of Geology & Mineral Resources, Shandong Engineering Research Center for Environmental Protection and Remediation on Groundwater, Key Laboratory of Geological Disaster Risk Prevention and Control，Emergency Management Department of Shandong Province, Jinan 250353, China

[2] Henan Joint International Research Laboratory of Environmental Pollution Control Materials, College of Chemistry and Molecular Sciences, Henan University, Kaifeng 475004, China

[3] Ningxia Fundamental Geological Survey Institute (Geological and Mineral Resources Center Laboratory of Ningxia), Geological Bureau of Ningxia Hui Autonomous Region, Yinchuan 750021, China

[4] College of Hydraulic Science and Engineering, Yangzhou University, Yangzhou, 225009, China

Manuscript prepared for *Environmental Science: Nano*

[*]Corresponding author: Minghui Lv (lmhyoxiang@163.com);

[**]Corresponding author: Taotao Lu (Taotao.Lu@yzu.edu.cn).

Number of pages: 21

Number of tables: 6

Number of figures: 6

S1. Particle size distribution (PSD) of soil

In this work, the particle size distribution (PSD) of soil grains was measured by the sieve-pipette method (SPM). The SPM is based on the Stokes' Law [S1]. Soil was dispersed using an ultrasonic vibrator (ca. 20 g soil and ca. 60 mL water in a beaker; sonicated for 2 min). The soil fraction was collected by passing the soil-water suspension through a 53-μm sieve. A 50 mL sample of suspension (< 53 μm) was collected to measure silt plus clay. The suspension was allowed to settle for 8 h in a temperature-controlled room (20 °C), and a sample was collected with a 50-mL pipette from a depth of 10 cm for determination of clay [S1]. Clay (< 2 μm), silt (2–53 μm), and sand (53–2000 μm) contents were calculated as the percentage (%) of recovered sample mass based on the USDA soil texture classification system.

S2. Determination of the CEC of soil

The cation exchange capacity (CEC) of soil was measured by following the ammonium acetate method [S2]. In brief, the saturation of the exchange sites by ammonium is carried out by percolating a 1 mol/L ammonium acetate solution (75 mL) through a test portion of 2.5 g of soil. The excess reagent is eliminated with several rinsings with ethanol (75 mL). After drying in air, the solid phase is agitated in 50 mL of a 1 mol/L solution of sodium chloride. The exchanged ammonium is measured by spectrocolorimetry, which permits the measurement of CEC (cmol/kg).

S3. Determination of the neonicotinoid pesticide concentrations in the effluent

The neonicotinoid pesticide concentrations in the effluent in each sample were measured after solvent extraction [S3, S4]. Specifically, 3 mL of each sample was taken into 10 mL centrifuge tubes with 0.5 mL 0.25 M NaH_2PO_4 and 0.5 mL acetonitrile. Then, the vials were shaken at 25 °C in the dark for 2 h. Afterward, the samples were sonicated for 45 min to free the neonicotinoid pesticides associated with biochar colloids and immediately filtered through 0.1 μm pore size polytetrafluoroethylene membrane for analysis. The concentration of acetamiprid and nitenpyram was analyzed directly by using a Waters high-performance liquid chromatography system (HPLC, e2695, Waters Alliance) equipped with a symmetry reversed-phase C18 column (4.6 × 150 mm) using a UV/visible detector at a wavelength of 245 nm and 270 nm, respectively.

S4. Effects of rhamnolipid on the adsorption of neonicotinoid pesticides onto biochar colloids

To investigate the roles of rhamnolipid in the binding affinities between neonicotinoid pesticides and biochar colloids, the adsorption characteristics of neonicotinoid pesticides onto biochar colloids were determined with or without 20 mg/L rhamnolipid under different pH conditions based on a previously developed technique [S5]. First, a series of dialysis bags (500 Da), each containing ~3 mL of electrolyte solution (1 mM NaCl at different pH values), was put in 40 mL amber vials containing various concentrations of neonicotinoid pesticides (1 mg/L) and 30 mg/L biochar colloids. The pH values of the experimental solution were adjusted using 0.1 M HCl or NaOH accordingly. The vials were then tumbled for 12 h in an orbital shaker at room temperature. Subsequently, the neonicotinoid pesticide concentrations inside the bags were measured by HPLC, as mentioned in Section S3. The adsorbed neonicotinoid pesticides onto colloids (q (mg/g)) were calculated according to the mass balance. Considering the loss of neonicotinoid pesticides during operational processes other than sorption (e.g., binding to dialysis bag), the control experiments without biochar colloids were also carried out. The calculation of the distribution coefficients (K_d) of neonicotinoid pesticides between biochar colloids and the aqueous phase was performed using the equation $K_d = q/C_e$. The K_d values were expressed in terms of L/g. All experiments were performed in triplicate.

S5. Adsorption studies of rhamnolipid onto soil grains

Adsorption experiments studied the adsorption capacity of rhamnolipid on soil grains under different conditions. First, about 1 g of soil was weighed and transferred to a 20 milliliter amber glass bottle with 20 mL of solution containing 1 mM of NaCl and amounts of surfactants (20 mg/L). The vials were then balanced by horizontal shaking for 12 hours (the duration was equivalent to the transport experiment). In addition, the vials were centrifuged at 8000 rpm for 20 min, and the supernatant was extracted.

The concentration of rhamnolipid was determined by the chromogenic method. Rhamnolipid is a kind of glycolipid. The phenol-sulfuric acid method is the most commonly used quantitative method [S6]. That is, 2.00 mL supernatant was poured into the colorimetric tube, each of which was added with 0.05 mL 80% phenol reagent and 5 mL concentrated sulfuric acid. After full oscillation, the solution was boiled in water at 25-30℃ for 10-20 min and then cooled to room temperature. To quantify rhamnolipid, the absorbance of each concentration was measured at a wavelength of 480 nm by an ultraviolet spectrophotometer and recorded. All absorbance measurements were made using an ultraviolet-visible spectrophotometer (TU-1810PC, Purkinje General, Beijing, China).

The adsorbed surfactants were then determined by the difference between the initial and final surfactant concentrations in the aqueous phase. All experiments were run in triplicate.

S6. Adsorption of rhamnolipid onto biochar colloids

Adsorption experiments were also conducted to determine the adsorbed amount of rhamnolipid onto biochar colloids under different solution chemistry conditions. The initial concentration of surfactant in the 20-mL amber glass vial was 20 mg/L, and the initial concentration of colloids was 30 mg/L. The vials were mixed, then left on an orbital shaker operated at room temperature for 12 h. The liquid and solid phases were separated by centrifugation at 8000 rpm for 30 min, and then the supernatants were filtered through a 0.45 μm filtering membrane. The concentrations of rhamnolipid in supernatants were determined based on the method mentioned in Section S5. The adsorbed rhamnolipid was then determined by the difference between the initial and final phosphate concentrations in the aqueous phase. All experiments were run in triplicate.

Table S1. Elemental compositions of biochars

Biochars	Bulk element composition				Ash (wt. %)	(O+N)/C [a]	H/C [a]
	C (wt. %)	O (wt. %)	N (wt. %)	H (wt. %)			
BC	68.61	23.60	0.98	4.54	2.22	0.271	0.794

[a] Bulk atomic ratio, O/C: atomic ratio of oxygen to carbon, H/C: atomic ratio of hydrogen to carbon.

Table S2. Assignments of FTIR spectrum [S7, S8].

Wavenumber	Assignments
3311~3494 cm^{-1}	H-bonded hydroxyl groups or amino groups
2854~2931 cm^{-1}, 1370~1458 cm^{-1}	aliphatic C–H
1500~1620 cm^{-1}	aromatic C=C
1700~1750 cm^{-1}	C=O of carboxyl, aldehyde, ketone, and ester groups
1103~1205 cm^{-1}	C–O or C-N
1040 cm^{-1}, 800 cm^{-1}	Si–O–Si
745~881 cm^{-1}	aromatic C–H or C=C-H

Table S3. Adsorption amount of rhamnolipid onto soil grains under different solution chemistry conditions. Error bars represent standard deviations from replicate experiments (n=3)

Electrolyte solution	pH	Rhamnolipid concentration	*q* (mg-rhamnolipid /kg-soil)
1 mM NaCl	5.0	20 mg/L	35.2 ± 0.5
1 mM NaCl	7.0	20 mg/L	31.3 ± 0.8
1 mM NaCl	9.0	20 mg/L	26.7 ± 1.3

Table S4. Summary of zeta potential of soil grains under different solution chemistry conditions.

No.	Background solution	pH	ζ-potential (mV)
1	1 mM NaCl	5.0	-27.9 ± 0.7
2	1 mM NaCl + 20 mg/L rhamnolipid	5.0	-35.7 ± 0.5
3	1 mM NaCl	7.0	-31.6 ± 0.3
4	1 mM NaCl + 20 mg/L rhamnolipid	7.0	-35.2 ± 1.2
5	1 mM NaCl	9.0	-36.9 ± 0.2
6	1 mM NaCl + 20 mg/L rhamnolipid	9.0	-38.7 ± 1.1

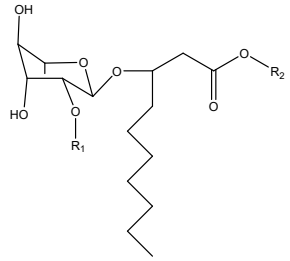
Table S5. Summary of zeta potential and D_h values of biochar colloids (30 mg/L) under different solution chemistry conditions.

No.	Materials [a]	Background solution	pH	ζ-potential (mV)	D_h (nm)
1	biochar colloids	1 mM NaCl	5.0	-15.7 ± 0.5	378.2 ± 12.3
2	biochar colloids	1 mM NaCl + 20 mg/L rhamnolipid	5.0	-20.1 ± 1.2	337.5 ± 9.6
3	biochar colloids	1 mM NaCl	7.0	-22.2 ± 0.7	325.6 ± 8.6
4	biochar colloids	1 mM NaCl + 20 mg/L rhamnolipid	7.0	-25.6 ± 1.1	300.7 ± 12.9
5	biochar colloids	1 mM NaCl	9.0	-24.0 ± 0.2	278.0 ± 7.5
6	biochar colloids	1 mM NaCl + 20 mg/L rhamnolipid	9.0	-26.3 ± 0.9	251.9 ± 6.7

Table S6. Adsorption amount of rhamnolipid onto biochars under different solution chemistry conditions. Error bars represent standard deviations from replicate experiments (n=3)

Biochar colloid concentration	Electrolyte solution	pH	Rhamnolipid concentration	q (mg-rhamnolipid /g-biochar)
30 mg/L	1 mM NaCl	5.0	20 mg/L	112.5 ± 2.3
30 mg/L	1 mM NaCl	7.0	20 mg/L	83.7 ± 5.2
30 mg/L	1 mM NaCl	9.0	20 mg/L	69.5 ± 1.8

(a)



(b)

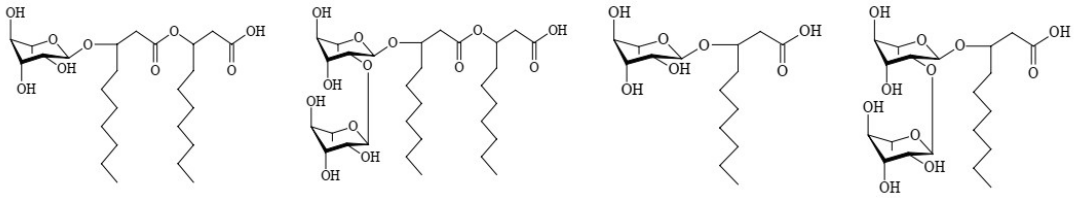


Fig. S1. Chemical structure of rhamnolipid with general formula [S9]; and (b) four common rhamnolipid structures. Typically, RL-1($RhC_{10}C_{10}$) and RL-2($Rh_2C_{10}C_{10}$) were considered to be the main rhamnolipidic congeners, having variable relative proportions in mixture (RL respents rhamnolipid) [S10].

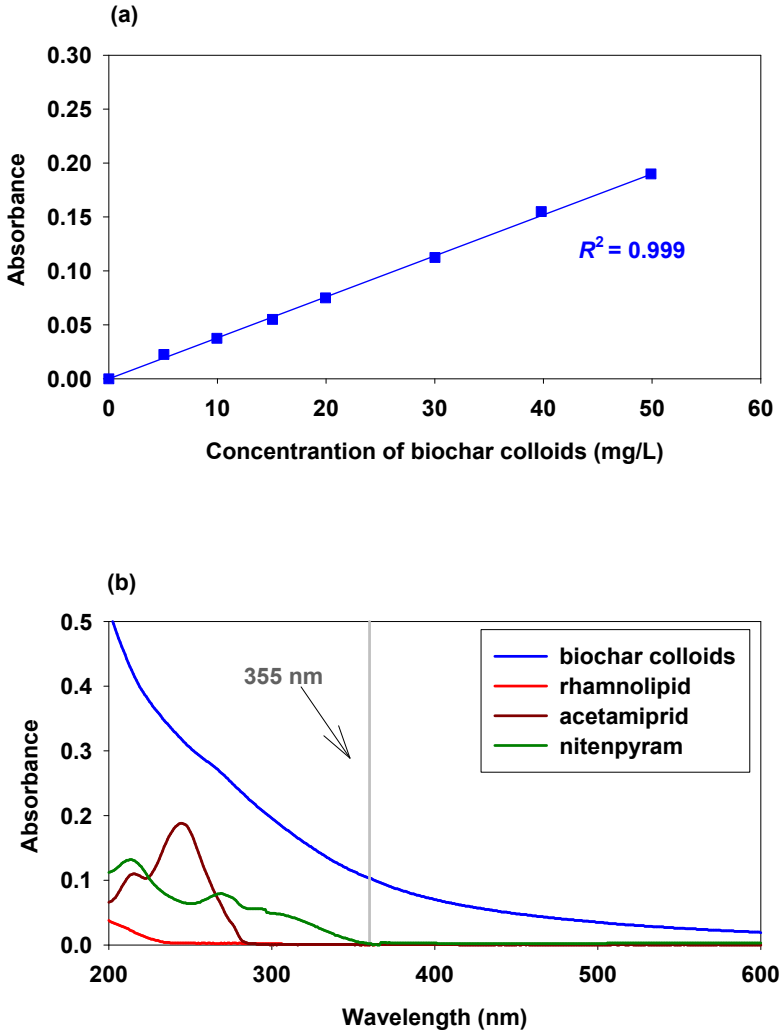


Fig. S2. (a) Calibration curve as absorbance vs. concentrations of biochar colloids in suspension (absorbance at the wavelength of 350 nm); and (b) UV/Vis spectra of biochar colloids (30 mg/L) and surfactants (30 mg/L) dispersed in DI water.

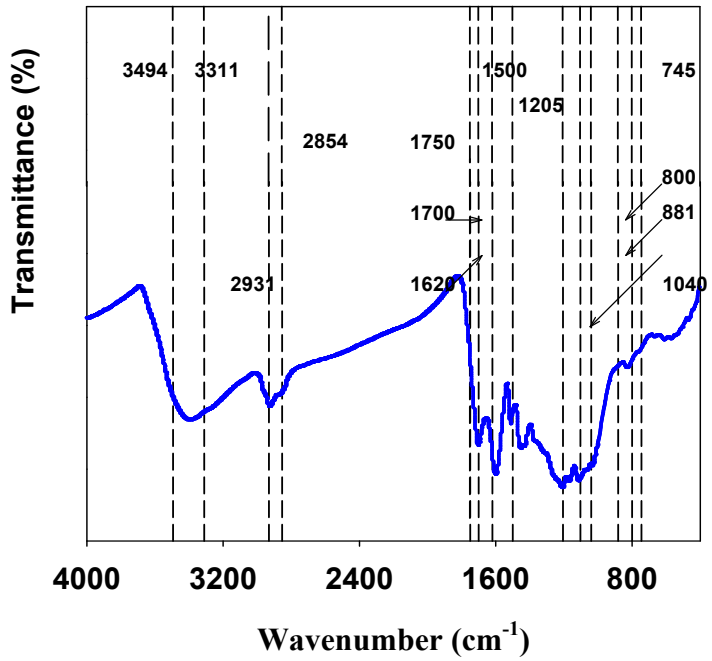


Fig. S3. Fourier transform infrared spectra of biochars (the assignments of functional groups were shown in Table S2).

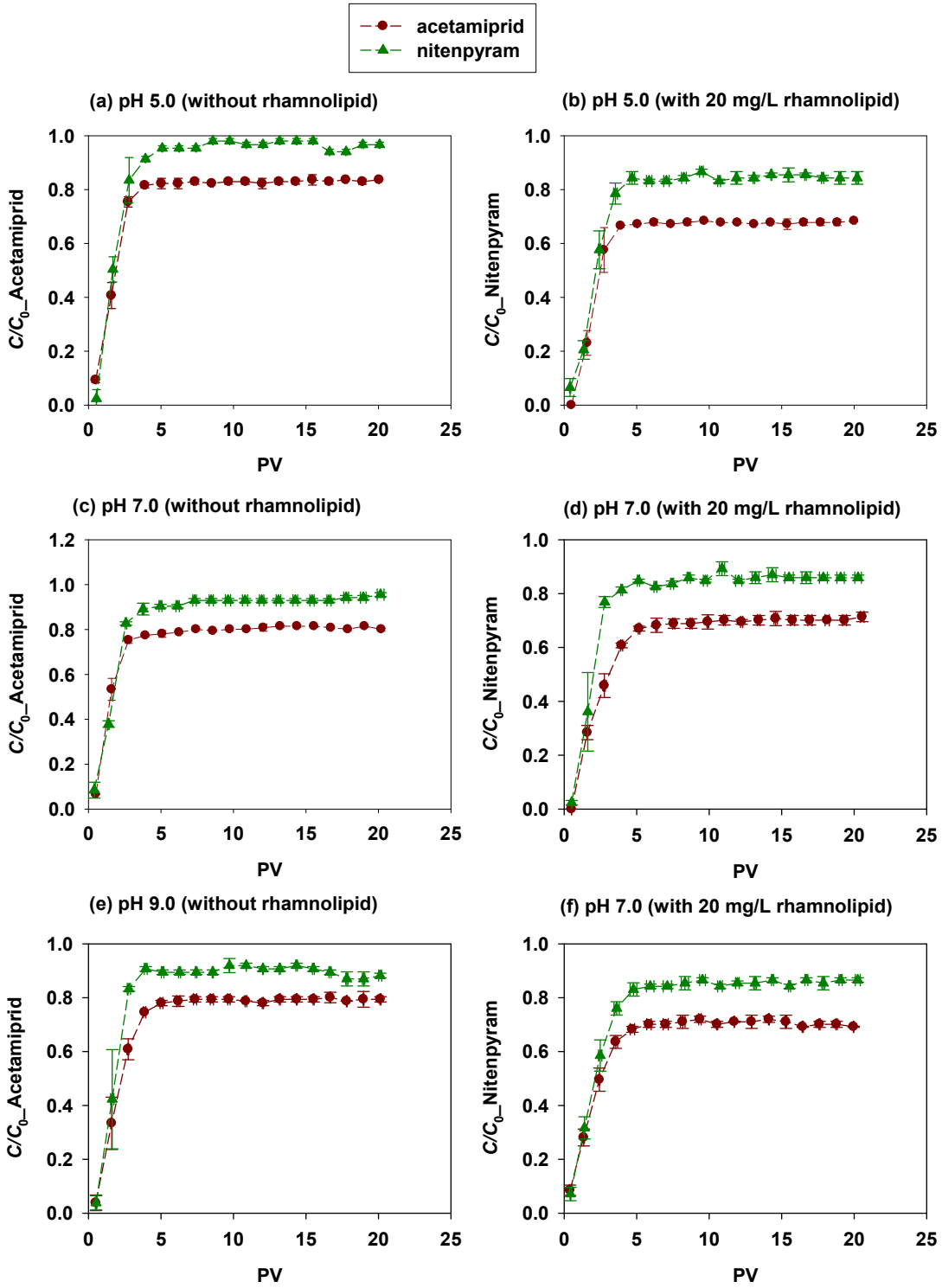


Fig. S4. Transport of neonicotinoid pesticides in saturated soil columns under different pH conditions (at a fixed ionic strength): (a), (c), and (e) in the absence of rhamnolipid (columns 1, 3, 5, 7, 9, and 11, Table 2); (d)–(f) in the presence of rhamnolipid (columns 2, 4, 6, 8, 10, and 12, Table 2).

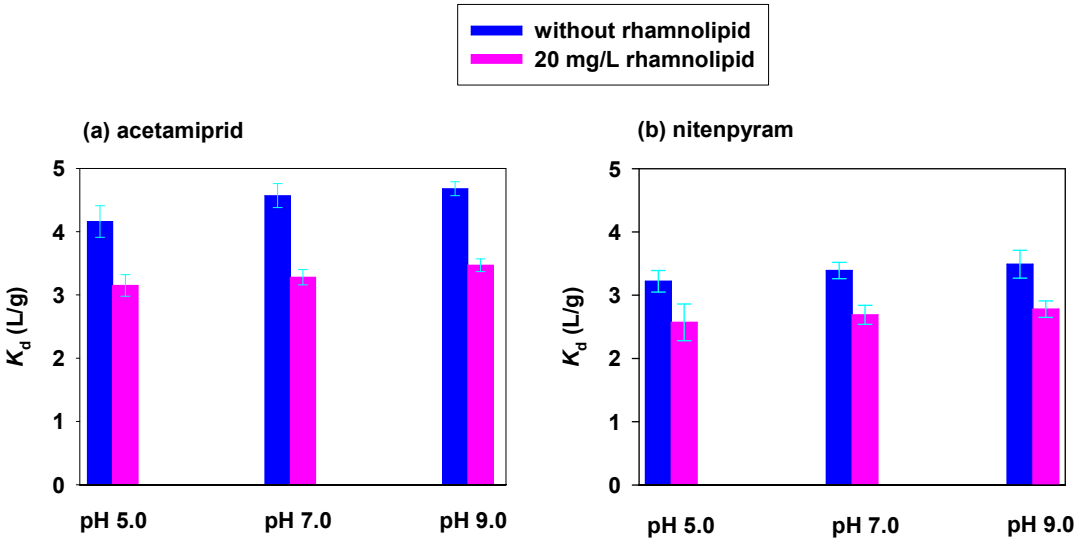


Fig. S5. Effect of rhamnolipid on the adsorption coefficients (K_d) of (a) acetamiprid and (b) nitenpyram (1 mg/L) to biochar colloids (30 mg/L) at different pH conditions.

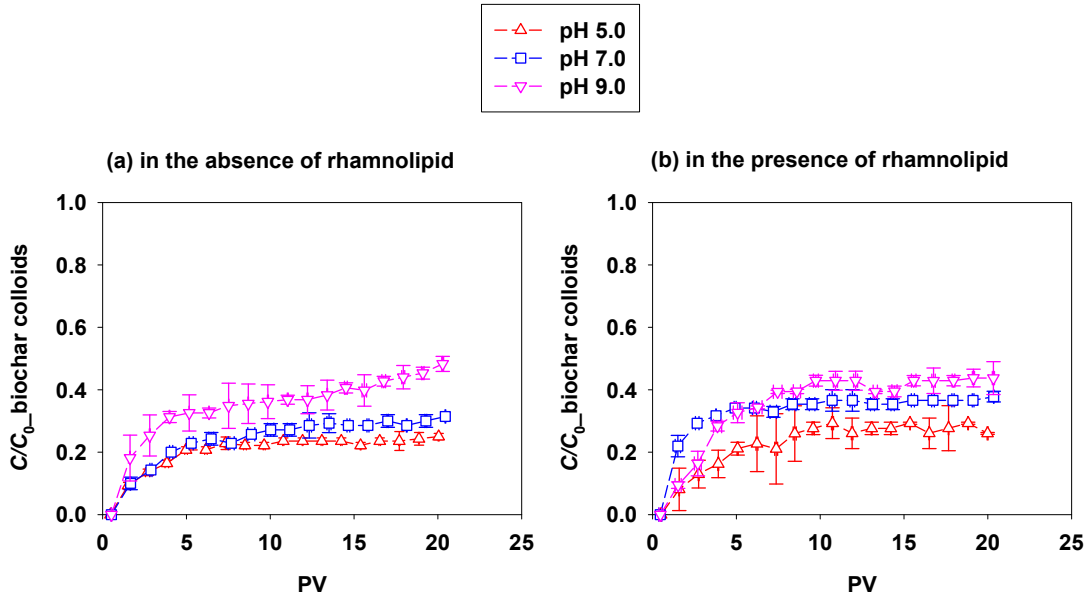


Fig. S6. Comparative transport of biochar colloids in saturated soil columns under different pH conditions: (a) in the absence rhamnolipid and (b) in the presence of rhamnolipid.

References

S1. G.W. Gee and D. Or, Particle size analysis. In Dane J, Topp C (eds.) Methods of Sand Analysis, *Sand Sci. Soc. Am. Madison.*, 2006, pp. 255–293.

S2. M. Pansu and J. Gautheyrou, Handbook of Soil Analysis. Mineralogical, Organic and Inorganic Methods. Springer, Berlin 2006.

S3. S. Irace-Guigand and J.J. Aaron, The role of organic colloids in herbicide transfer to rivers: a quantitative study of triazine and phenylurea interactions with colloids, *Anal. Bioanal. Chem.*, 2003, **376**, 431–435.

S4. K. Sun, S. Dong, Y. Sun, B. Gao, W. Du, H. Xu and J. Wu, Graphene oxide-facilitated transport of levofloxacin and ciprofloxacin in saturated and unsaturated porous media, *J. Hazard. Mater.*, 2018, **348**, 92–99.

S5. S. Kamrani, M. Rezaei, M. Kord and M. Baalousha, Co-transport and remobilization of Cu and Pb in quartz column by carbon dots, *Sci. Total Environ.*, 2018, **626**, 995–1004.

S6. J. Jiang, M. Jin, X. Li, Q. Meng, J. Niu, and X.W. Long, Recent progress and trends in the analysis and identification of rhamnolipids. *Appl. Microbiol. Biot.*, 2020, **104**, 8171–8186.

S7. W. Chen, R. Wei, L. Yang, Y. Yang, G. Li and J. Ni, Characteristics of wood-derived biochars produced at different temperatures before and after deashing: Their different potential advantages in environmental applications, *Sci. Total Environ.*, 2019, **651**, 2762–2771.

S8. M. Keiluweit, P.S. Nico, M.G. Johnson and M. Kleber, Dynamic molecular structure of plant biomass-derived black carbon (biochar), *Environ. Sci. Technol.* 2010, **44**, 1247–1253.

S9. T.T. Nguyen and D.A. Sabatini, Characterization and emulsification properties of rhamnolipid and sophorolipid biosurfactants and their applications. *Int. J. Mol. Sciences*, 2011, **12**, 1232–1244.

S10. Y.P. Guo, Y.Y. Hu, R.R. Gu and H. Lin, Characterization and micellization of rhamnolipidic fractions and crude extracts produced by Pseudomonas aeruginosa mutant MIG-N146. *J. Colloid Interface Sci.* 2009, **331**, 356–363.