The Immobilization of Soil Cadmium by the Combined Amendment of Bacteria and Hydroxyapatite

Xiaoxi Zeng, Hong Xu, Jijie Lu, Qimin Chen, Wen Li, Ling Wu, Jianxin Tang & Liang Ma

Soil is the main sink for metals and acts as a barrier to prevent their entry into the food chain. However, human activities such as mining and metal smelting have gradually transferred many toxic metals from the earth’s crust to the environment, resulting in the spread and contamination of heavy metals. In recent decades, heavy metal pollution has become a serious problem worldwide. In China, it was reported that 13.33% of all soil samples collected from 6.3 million square kilometres of land were polluted with high levels of metals. Cadmium pollution has been detected in all agricultural areas. Cadmium is chemically stable and does not undergo chemical or microbial degradation. Moreover, cadmium is not an essential element for living organisms. Generally, it is regarded as the most toxic among heavy metals. Cadmium in the soil harms plant cells by altering metabolic pathways, damaging chloroplasts and mitochondria, and causing oxidative damage to lipids and proteins. Cadmium also interferes with the uptake and transport of essential elements, e.g., P and K. Excessive Cd can reduce iron uptake by plants, affecting their photosynthesis. Cadmium can also accumulate in plants and animals and thus enters the human body through the food chain, threatening health.

Various physical, chemical and biological techniques have been used to remediate heavy metal-contaminated soils. In situ immobilization is generally considered a feasible technique to remediate metal-contaminated soils due to its cost effectiveness and ease of operation. Many researchers have reported on the use of various amendments to immobilize Cd in polluted soils, including phosphates, clay minerals, calcareous materials, and so on. However, the long-term use of a single chemical amendment can alter soil properties, resulting in soil alkalization, soil hardening, microbiologic ecological disturbances, and so on. Thus, identifying combinations of chemical and biological materials to compensate for these shortcomings has been a popular approach with respect to the immobilization of soil metals.

Microorganisms are living materials with various properties. Some bacteria and fungi have been investigated to remediate contaminated soils and water. The hybrid bio-nanocomposite (ANHP) can be recognized as a...
promising soil amendment candidate for effective remediation on the soils. In our previous work, the bacterium Cupriavidus sp. ZSK was isolated from heavy metal-contaminated soils. It was indicated that this strain is highly tolerant to five heavy metals (Cd, Cu, Zn, Cr, and Pb) and is a potential microorganism to adsorb cadmium ions. In this work, the effects of strain Cupriavidus sp. ZSK and HAP on DTPA-extractable cadmium were studied. The potential application of the combined amendment of this strain and HAP was investigated for the immobilization of soil cadmium.

**Materials and Methods**

**Flask experiments.** The flask experiments consisted of three parts. The first part involved strain growth in the presence of HAP. HAP was added to LB media in flasks at five different ratios (0, 1%, 2%, 3%, and 4%). The flasks were sterilized and inoculated with Cupriavidus sp. ZSK in accordance with normal procedures, after which the bacteria were cultured in a shaker at 180 rpm at 30 °C. According to the growth curve of the strain in previous work, the cells were collected at an interval of 3 h during the exponential and stationary phases, after which plate colony counting was implemented. Each treatment involved three replications, for a total of 15 flasks.

Diethylene-triamine-pentaacetic acid (DTPA)-extractable metal is well known as an index of available metals in soils. To investigate the effect of hydroxyapatite on the pH and the DTPA-extractable Cd in the soil, a second experiment was carried out. HAP was added to soils at five different ratios (0, 1%, 2%, 3%, and 4%) and mixed well. The mixtures were then loaded in flasks, and deionized water was added at a soil: water ratio of 2:1 (w/v). Each treatment was conducted in triplicate, for a total of 15 flasks. The flasks were shaken for 2 h to fully soak the soil and then were incubated at room temperature. The soils were sampled at an interval of 2 d and were divided into three portions to analyse the pH and cadmium concentration. The pH was tested by a pH meter PHSJ-4F (Shanghai Thunder magnetic equipment factory), and the cadmium was determined by ICP-MS.

In the third part, the combination of the strain and HAP was evaluated, which was denoted as SH. Specifically, the effects of SH on the pH and DTPA-extractable Cd of soil were studied. There were flasks with soil as described above, and they were established into four groups: Group A was the control flask experiment without HAP and the strain; group B was the flask experiment with 3% HAP; group C was the flask experiment with the strain (10^8 cells/mL); and group D was the flask experiment with the SH. Each group was replicated three times, for a total of 12 flasks.

The immobilization efficiency was calculated by the following formula:

\[
\text{Immobilization efficiency (\%) } = \frac{(a - b)}{a} \times 100\%
\]

where a (mg/kg) is the DTPA-extractable Cd of the control group (A) and b (mg/kg) is the DTPA-extractable Cd of the groups with SH (B/C/D).

**Pot experiments.** Ramie, dandelion, and daisy are common plant species in polluted areas near the Zhuzhou smelter. Pots 15 cm in height with a top diameter of 23.5 cm and a bottom diameter of 16 cm were used in the experiments. A total of 1.5 kg of cadmium-polluted soil was added to each pot. The seedlings of the plants from unpolluted soils were transplanted into pots. Each species included two groups: one was the control group without SH, and the other was the treatment group with SH, including 3% HAP (w/w) and 10^8 cells/g of the Cupriavidus sp. ZSK strain. The pots were arranged randomly under natural light. The plants were watered when needed and harvested after 45 d. Each group was replicated three times.

Cadmium determinations. Cadmium was extracted by DTPA solution at a soil: water ratio of 1:5 (w/v). The samples were then shaken at 180 rpm for 2 h on a thermostatic oscillator at 25 °C. The suspensions were centrifuged at 8000 rpm for 30 min and then filtered with a 0.45-μm membrane filter. The soil pH was determined in 1:2.5 soil: water suspensions after shacking over 0.5 h with a combination pH electrode. The soil samples should be digested in a solution consisting of HF: HClO₄: HCl: HNO₃ at a ratio of 1:1.6:2:4. The concentration of Cd in the extracts was tested by ICP-MS.
The potted plants were harvested after 45 d. First, the plants were collected, shaken to remove the soil particles and then weighed by an electronic scale. They were then thoroughly washed with tap water followed by demineralized water. The plant samples were ground to powder after naturally air drying to a constant weight. The soil samples were digested in a solution containing HNO₃:HClO₄ at a ratio of 6:1. The concentrations of Cd in the extracts were tested by ICP-MS.

**Statistical analysis.** The data were subjected to one-way ANOVA using SPSS 17.0 software. At least three independent replicates of each sample and determination were tested, and mean values and respective standard deviations were calculated.

**Results and Discussion**

**Soils, strains, and HAp.** The cadmium-contaminated soil (pH of 6.84, TOC pf 0.952%, total N of 1.73 g/kg, total P of 1.16 g/kg, cation exchange capacity of 0.039 cmol/kg; 37.8% sand, 38.7% silt, 23.4% clay) was collected from gardens near the Zhuzhou Smelter in Hunan Province, China (113°4′23.46″E, 27°51′54.3″N)¹⁸. It is classified as Ferralsols, according to the World Reference Base for soil resources (WRB)¹⁹. The soil was cleared of plant debris and stones, air dried at room temperature, and then ground to a particle size 2 mm for the experiments. The total cadmium was 13.82 mg kg⁻¹, and the DTPA-extractable Cd was 5.73 mg/kg. *Cupriavidus* sp. ZSK was isolated in our previous work. The bacterium was cultured and observed by optical microscopy to test its activity. HAp was supplied by Shanghai Hualan Chemical Technology Company. HAp is a ultra-fine powder with micron-nanometer dimensions (Fig. 2(a)). The Ca₁₀(PO₄)₆(OH)₂ was ≥99.5%, and the heavy metals were ≤1 ppm; the pH was 8.10.

**The effects of HAp on the bacterial strain.** The bacterial strain *Cupriavidus* sp. ZSK was cultured in LB media. Crystal violet staining was used to confirm its morphology. Figure 1(b) show that the strain is a rod-shaped bacterium, which means these bacteria were not contaminated with other bacteria. Microorganisms are often affected by the addition of soil amendments; for example, sepiolite and sodium alginate can increase soil microbial biomass and diversity²⁰,²¹, nano-silver is highly cytotoxic and is genetically toxic to nitrifying bacteria²², and soil bacterial diversity varies under the addition of HAp²³. Therefore, according to the growth curve in previous works, the effect of HAp on the strain growth was investigated. Figure 3 shows that without HAp, the peak growth was 8.85 x 10⁸ cells. In flasks with 1%, 2%, and 3% HAp, the peaks were similar to those of the control. With 4% HAp, the growth peak was 8.68 x 10⁸ cells, which was slightly lower than that of the control. In all groups, the peaks occurred at 24h, suggesting that 1–3% HAp has little effect on the growth of the strain.

**The effects of HAp on the pH and DTPA-extracted Cd.** The time course of the soil pH inoculated with HAp is shown in Fig. 4. In the control experiment without HAp, the pH of the soil was relatively stable, ranging from 6.02 to 6.05. In the other groups with HAp, the soil pH increased obviously. With 1, 2, 3, and 4% HAp, the soil pH increased from 6.02 to 6.49, 6.63, 6.82, and 7.04, respectively. In all groups, the pH reached stabilized on the 8th day. The DTPA-extracted Cd in the soils is presented in Fig. 5. In the control soils, the cadmium concentration changed slightly, ranging from 5.70 to 5.87 mg/kg. With increasing HAp, the DTPA-extracted Cd decreased gradually. In the flasks with 1, 2, 3, and 4% HAp, the DTPA-extracted Cd decreased by 24.62, 29.53, 35.10, and 37.89%, respectively.

At high pH, mobile cadmium might become immobile. However, an excessively high pH can alter soil properties, which influence soil ecology and plant growth. In general, the increase in pH should be better at values more greater than 1. For the above reason, the HAp concentration of 3% was selected for the following experiments.

**The effects of SH on the pH and DTPA-extractable Cd in flask experiments.** As shown in Table 1, the effects of SH on the pH and DTPA-extractable Cd were investigated. The soil pH of group A (without SH) was 6.02. Compared with that of the control group (A), the pH of groups B (with HAp) and D (with SH) obviously...
increased, reaching 6.75 and 6.77, respectively. In group C (with the strain), the pH was 6.05, which had slightly increased. These results suggested that the strain has no notable effect on the pH and that the effect of the SH on soil pH is dependent on HAP. The statistical analysis showed that the pH of groups B and D were significantly different from the pH of the control group (A) \((p < 0.05)\), while that of group C was not \((p = 0.91)\).
Table 1. The combined effects of the bacterial strain and HAP on soil and cadmium. Group A represents the control flask experiment without HAP and the strain; group B represents the flask experiment with 3% HAP; group C represents the flask experiment with the strain (10⁸ cells/mL); and group D represents the flask experiment with the SH. Each group was replicated three times. According to ANOVA (Tukey’s test), there is no significant difference in the value of the same letter within a single column at p < 0.05 (Significant at 95% confidence interval, F: statistics, df: degrees of freedom).

| Groups | pH (F = 168.14; df = 3) | DTPA-extractable Cd (mg/kg; F = 55.53; df = 3) | Immobilization efficiency (%) |
|--------|------------------------|---------------------------------------------|-------------------------------|
| A      | 6.02 ± 0.04b           | 5.85 ± 0.43a                                | —                             |
| B      | 6.75 ± 0.05a           | 3.92 ± 0.25b                                | 33.0%                         |
| C      | 6.05 ± 0.08b           | 4.76 ± 0.32b                                | 18.6%                         |
| D      | 6.77 ± 0.03a           | 2.45 ± 0.31c                                | 58.2%                         |

The effects of SH on the biomass and cadmium accumulation of the plants. The potted plants were harvested. The biomass values are shown in Fig. 6. All of the biomass values of the plants treated with SH were much greater than those of the control plants. The biomass of dandelion and daisy increased slightly in the treatments with SH addition compared to the control treatment (p > 0.05). In ramie, the biomass significantly increased in the treatments with SH addition compared to the control treatment (p = 0.01), increasing by 28.3%. This suggests that HAP not only is harmless but also somewhat benefits the growth of the plants. However, compared with that in the non-SH groups, the cadmium accumulation in all plants in the other groups decreased (Fig. 7). In the dandelion group, cadmium accumulation decreased from 6.82 mg/kg to 3.34 mg/kg. This decrease is the maximum among the three plant species, which reached 51.0%. The cadmium accumulation decreased by 44.9% and 38.7% in the ramie and daisy groups, respectively. The statistical analysis showed that the cadmium accumulation in dandelion and ramie significantly decreased (p < 0.05) but that the accumulation in the daisy did not (p = 0.14). This means that SH can significantly reduce the bioavailable cadmium and induce plants to absorb cadmium from the soil.

Immobilization is considered a potentially reliable, cost-effective technique for the reclamation of metal-contaminated soils. At present, some chemical amendments have been investigated and applied for the immobilization of heavy metals. Phosphates and their derived materials are considered effective amendments for contaminated soils. HAP is a typical inorganic chemical amendment that immobilized heavy metals. Generally, the primary mechanisms of HAP for metal immobilization are adsorption, precipitation, cation exchange, and surface complexation, and involved processes as the follows:

$$\text{Ca}_{10}(PO_4)_6(OH)_2 + 14\text{H}^+ \rightarrow 10\text{Ca}^{2+} + 6\text{H}_2\text{PO}_4^- + 2\text{OH}^-$$  (2)
Moreover, in this work, the cadmium contaminated soil is from Zhuzhou city nearby the Xiangjiang River, in southern China. The soil is slight acid, marked as ferralsol. In the area, weaker atmospheric dispersion, higher humidity and more sunlight favor the formation of acid rain. Acid rain could cause soil acidifying and damages the soil construction. Introduction of the alkaline matters in soils can weaken soils acidity. Thus, HAP can not only immobilize metals but also benefiting the soils stabilizations.

It is well known that HAP can increase the pH to form stable metal-phosphate precipitates in heavy metal-contaminated soils. With a high specific surface area and reactivity, nano-hydroxyapatites (n-HAPs) are more effective than bulk HAP particles for immobilizing metals. Considering that n-HAP particles tend to self-agglomerate during the process of application, the micro-nanometer HAP was used in this work. In this study, Figs. 4 and 5 show that the pH obviously increased with increasing HAP in the soils, and the DTPA-extracted Cd decreased. The micro-nanometer HAP can increase the soil pH to reduce the availability of heavy metals. This result is consistent with some reports.

Some microorganisms also can immobilize heavy metal ions. reported that the *Rhizobium pusense* KG 2 can immobilize Cd$^{2+}$ from soil. studied that hybrid bio-nanocomposites of fungal hyphae and nano-hydroxyapatites immobilize Cd and Pb in contaminated soils suggested it can be recognized as a promising soil amendment candidate. In our previous study, the bacteria strain *Cupriavidus* sp. ZSK can tolerant to and adsorb heavy metals. When the bacterial strain *Cupriavidus* sp. ZSK was introduced with HAP in the flask experiments, the soil pH did not change obviously, but the DTPA-extractable Cd decreased from 3.35 mg/kg to 2.45 mg/kg. This suggests that the efficiency of immobilizing cadmium by the combined amendment is obviously better than that by the strain or HAP. The adsorption ability of the *Cupriavidus* sp. strain contributes to the stabilization of cadmium ions in the soil. Compared with that in other similar studies, the efficiency of immobilization by SH is moderate to high.

The pot experiments suggest that SH obviously decreases the cadmium accumulation in the three plant species, which means that the bioavailable cadmium decreased in the soil. Moreover, SH is benefit to plant growth. Thus, the combination of the *Cupriavidus* sp. ZSK strain and HAP is considered a potential amendment for the immobilization of soil Cd. The detail relationship and interactive mechanism of the strain and HAP need further investigate.

Conclusions

Micro-nanometer HAP caused the soil pH to increase, and DTPA-extractable cadmium decreased significantly. The efficiency of immobilizing cadmium by the combined amendment of the *Cupriavidus* sp. ZSK strain and HAP was obviously higher than that by the strain or HAP alone. The combined amendment somewhat benefits plant growth and can significantly decrease cadmium accumulation in plants. Thus, the combination of the bacterial strain *Cupriavidus* sp. ZSK and HAP represents a potential method to decrease available cadmium in the soil and cadmium accumulation in plants.

Received: 30 August 2019; Accepted: 13 January 2020;
Published online: 10 February 2020

References

1. Yasir, H. et al. An explanation of soil amendments to reduce cadmium phytoavailability and transfer to food chain. *Sci. Total. Environ.* **660**, 80–96 (2019).
2. Sinha, S., Mishra, R. K., Sinan, G. & Mallick, S. Comparative evaluation of metal phytoremediation potential of trees, grasses, and flowering plants from Tannery-wastewater-contaminated soil in relation with physicochemical properties. *J. Soil. Contamination.* **22**, 958–983 (2013).
3. Maria, J. M. S., Salvador, M. L. & Lucia, B. M. M. Importance of the oral arsenic bioaccessibility factor for characterizing the risk associated with soil ingestion in a mining-influenced zone. *J. Env. Manage.* 116, 10–17 (2013).
4. Jacquiod, S. et al. Long-term industrial metal contamination unexpectedly shaped diversity and activity response of sediment microbiome. *J. Hazard. Mater.* 344, 299–307 (2017).
5. Lee, P. K., Jo, H. Y., Paik, S. W. & Chi, S. J. Metal contamination and solid phase partitioning of metals in the stream and bottom sediments in a reservoir receiving mine drainage. *Appl. Geochem.* 28, 80–90 (2013).
6. Hamid, Y. et al. Comparative efficacy of organic and inorganic amendments for cadmium and lead immobilization in contaminated soil under rice/wheat cropping system. *Chemosphere.* 214, 259–268 (2019).
7. Guan, M. Y., Zhang, H. H. & Pan, W. Sulfide alleviates cadmium toxicity in Arabidopsis plants by altering the chemical form and the subcellular distribution of cadmium. *Sci. Total. Environ.* 627, 663–670 (2018).
8. Clemens, S. Toxic metal accumulation, responses to exposure and mechanisms of tolerance in plants. *Biochimie.* 88, 1707–1719 (2006).
9. Bertoli, A. C. et al. Lycopersicon esculentum submitted to Cd-stressful conditions in nutrition solution: nutrient contents and translocation. *Ecotoxicol. Env. Saf.* 86, 176–181 (2012).
10. Mombo, S. et al. Management of human health risk in the context of kitchen gardens polluted by lead and cadmium near a lead recycling company. *J. Soils Sediments.* 16, 1069–1077 (2016).
11. He, M., Shi, H., Zhao, X., Yu, Y. & Qu, R. Immobilization of Pb and Cd in contaminated soil using nano-crystallite hydroxyapatite. *Procedia. Sci.* 18, 657–665 (2013).
12. Niu, L. L., Yang, F. X., Xu, C., Yang, H. Y. & Liu, W. P. Status of metal accumulation in farmland soils across China: from distribution to risk assessment. *Env. Pollut.* 176, 55–62 (2013).
13. Yang, Z. H. et al. Combination of microbial oxidation and biogenic schwertmannite immobilization: a potential remediation for highly arsenic-contaminated soil. *Chemosphere.* 181, 1–8 (2017).
14. Cui, L. et al. Biochar amendment greatly reduces rice Cd uptake in a contaminated paddy soil: a two-year field experiment. *Bioresources.* 6, 2605–2618 (2011).
15. Boldetsadik, D. et al. Effects of biochar and alkaline amendments on cadmium immobilization, selected nutrient and cadmium concentrations of lettuce (lactuca sativa) in two contrasting soils. *SpringerPlus.* 5, 397 (2016).
16. Zeng, X. et al. Characterization of strain cupriavidus sp. zk2, and its biosorption of heavy metal ions. *J. Biobased Mater. Bio.* 11, 154–158 (2017).
17. Yang, Z. H. et al. Simultaneous immobilization of cadmium and lead in contaminated soils by hybrid bio-nanocomposites of fungal hyphae and nano-hydroxyapatites. *Env. Sci. Pollut. Res.* 25, 11970–11980 (2018).
18. Wang, Z. X. et al. A land use-based spatial analysis method for human health risk assessment of heavy metals in soil and its application in Zhuzhou City, Hunan Province, China. *J. Cent. South. Univ.* 23, 1915–1923 (2016).
19. IUSS, World Reference Base for Soil Resources 2006. EEA, Rome (2006).
20. Huang, D. L. et al. Immobilization of Cd in river sediments by sodium alginate modified nanoscale zero-valent iron: Impact on enzyme activities and microbial community diversity. *Water Res.* 106, 15–25 (2016).
21. Sun, R. B. et al. Fungal community composition in soils subjected to long-term chemical fertilization is most influenced by the type of organic matter. *Env. Microbiol.* 18, 5137–5150 (2016).
22. Samaraweera, A. D. et al. Effect of silver nano-particles on soil microbial growth, activity and community diversity in a sandy loam soil. *Env. Pollut.* 220, 504–513 (2017).
23. Cui, H. B. et al. Effect of different grain sizes of hydroxyapatite on soil heavy metal bioavailability and microbial community composition. *Agric. Ecosyst. Environ.* 267, 165–173 (2018).
24. Liu, R. Q. & Zhao, D. Y. Synthesis and characterization of a new stabilized of apatite nanoparticles and applying the particles to in situ Pb immobilization in a fire-range soil. *Chemosphile.* 91, 594–601 (2013).
25. Liu, H. & Guo, X. Hydroxyapatite reduces potential Cadmium risk by amendment of sludge compost to turf-grass grown soil in a consecutive two-year study. *Sci. Total. Environ.* 661, 48–54 (2019).
26. Rehman, M. Z. et al. Effect of limestone, lignite and biochar applied alone and combined on cadmium uptake in wheat and rice under rotation in an efluent irrigated field. *Env. Pollut.* 227, 560–568 (2017).
27. Mahar, A. et al. (Im)mobilization of soil heavy metals using CaO, FA, sulfur, and Na2S: a 1-year incubation study. *Int. J. Env. Sci. Technol.* 15, 607–620 (2018).
28. Xiao, R. et al. Lime and phosphate amendment can significantly reduce uptake of Cd and Pb by field-grown rice. *Sustainability.* 9, 430 (2017).
29. Wang, X. et al. Exploratory of immobilization remediation of hydroxyapatite (HAP) on lead–contaminated soils. *Env. Sci. Pollut. Res.* 26, 26674–26684 (2019).
30. Chen, S. et al. In Situ Stabilization of Toxic Metals in Polluted Soils Using Different Soil Amendments: Mechanisms and Environmental Implication [M]/Twenty Years of Research and Development on Soil Pollution and Remediation in China. Springer, Singapore. 563–572 (2018).
31. Liang, S. X., Ding, L., Chen, Q. S. & Liu, W. Immobilization Mechanism of Nano-Hydroxyapatite on Lead in the Ryegrass Rhizosphere Soil under Root Confinement. *Bull. Env. Contam. Toxicol.* 103, 330–335 (2019).
32. Wang, W. & Wang, T. On the origin and the trend of acid precipitation in China. *Water, Air, Soil. Pollution.* 85, 2295–2300 (1995).
33. Ma, J., Wang, F., Huang, Z. & Wang, H. Simultaneous removal of 2,4-dichlorophenol and Cd from soils by electrokinetic remediation combined with activated bamboo charcoal. *J. Hazard. Mater.* 176, 715–720 (2010).
34. Antoniades, V. & Golia, E. E. Sorption of Cu and Zn in low organic matter-soils as influenced by soil properties and by the degree of soil weathering. *Chemosphere.* 138, 364–369 (2015).
35. Xu, L. et al. Changes in the heavy metal distributions in whole soil and aggregates affected by the application of alkaline materials and phytore-mediation. *RSC Adv.* 7, 41033–41042 (2017).
36. Zhang, W. H., Sun, R. B., Xu, L., Liang, J. N. & Zhou, J. Assessment of bacterial communities in Cu-contaminated soil immobilized by a one-time application of micro-/nanohydroxyapatite and phytoremediation for 3 years. *Chemosphere.* 2, 49 (2019).
37. Li, Y. et al. The potential of cadmium ion-immobilized Rhizobium pusense KG 2 to prevent soybean root from absorbing cadmium in cadmium-contaminated soil. *J. Appl. Microbiol.* 126, 919–930 (2019).
38. Hong, L., Ou, J. Y., Wang, X. D., Yan, Z. G. & Zhou, Y. Y. Immobilization of soil cadmium using combined amendments of illite/smectite clay with bone char. *Env. Sci. Pollut. Res.* 25, 20723–20731 (2018).
39. Xu, Y. Z., Fang, Z. Q. & Tsang, E. P. In situ immobilization of cadmium in soil by stabilized biochar-supported iron phosphate nanoparticles. *Env. Sci. Pollut. Res.* 23, 19164–19172 (2016).
40. Khan, M. A., Khan, S. & Khan, A. Soil contamination with cadmium, consequences and remediation using organic amendments. *Sci. Total. Environ.* 601-602, 1591–1605 (2017).
41. Wen, Li. et al. Isolation, genetic identification and degradation characteristics of COD-degrading bacterial strain in slaughter wastewater. *Saudi. J. Biol. Sci.* 25, 1800–1805 (2018).
Acknowledgements
This research was funded by the National Key Technology R&D Program (2015BAD05B02 and 2018YFE0110200), the National Natural Science Foundation of Hunan Province (2018JJ2090), the National Natural Science Foundation of China (51774128 and 51774129), the Key Program of the Hunan Provincial Department of Science and Technology (2016NK2096) and the Social Development and Livelihood Program of Zhuzhou City of China (2018 and 2019).

Author contributions
X.X.Z., W.L. and L.M. conceived and designed the experiments; H.X., Q.M.C., J.J.L, and L.W. performed the experiments; X.X.Z. and L.W. analyzed the data; J.X.T., X.Z. and W.L. performed the statistical analyses; and X.X.Z., H.X., L.M. and W.L. wrote the paper.

Competing interests
The authors declare no competing interests.

Additional information

Correspondence and requests for materials should be addressed to W.L. or L.M.

Reprints and permissions information is available at www.nature.com/reprints.

Publisher's note
Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Open Access This article is licensed under a Creative Commons Attribution 4.0 International License, which permits use, sharing, adaptation, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons license, and indicate if changes were made. The images or other third party material in this article are included in the article’s Creative Commons license, unless indicated otherwise in a credit line to the material. If material is not included in the article’s Creative Commons license and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder. To view a copy of this license, visit http://creativecommons.org/licenses/by/4.0/.

© The Author(s) 2020