PAPER

View Article Online
View Journal | View Issue

Assessing the effect of phosphate and silicate on Cd bioavailability in soil using an *Escherichia coli* *cadAp::luc*-based whole-cell sensor

Cite this: *Environ. Sci.: Processes Impacts*, 2014, 16, 890

Qi-Hui Hou,^{†a} An-Zhou Ma,^{†a} Ye Li,^b Xu-Liang Zhuang,^a Zhi-Hui Bai,^a Xin-Ke Zhang^c and Guo-Qiang Zhuang^{*a}

An *Escherichia coli* *cadAp::luc*-based whole-cell sensor was constructed to measure cadmium (Cd) bioavailability and assess the immobilizing efficiency of phosphate and silicate on Cd. In previous induction experiments, a linear response ($R^2 = 0.97$, $P < 0.01$) from 0.1 to 5 $\mu\text{mol L}^{-1}$ of Cd was detected by this sensor after a 2 h incubation. The sensor was then used to estimate Cd bioavailability in soils spiked with different amounts of dipotassium phosphate (DKP, K_2HPO_4) or sodium silicate (SS, $\text{Na}_2\text{SiO}_3 \cdot 9\text{H}_2\text{O}$). The total Cd in soil–water extracts (TSWE) was determined with ICP-MS, and the bioavailable Cd in soil–water extracts (BSWE) and bioavailable Cd in soil–water suspensions (BSWS) were measured by the *E. coli* *cadAp::luc*-based whole-cell sensor. Final results showed that spiked SS (Si : Cd = 2 : 1, mol mol^{-1}) reduced the different forms of Cd (TSWE, BSWE and BSWS) from 56.47 mg kg^{-1} , 42.11 mg kg^{-1} , and 206.72 mg kg^{-1} to 16.63 mg kg^{-1} , 15.90 mg kg^{-1} , and 67.57 mg kg^{-1} , respectively. In other words, SS had 25.68%, 19.5%, and 9.54% better immobilizing efficiency, respectively, compared with DKP. All the results supported SS was more efficient than DKP at immobilizing Cd in soil, and higher soil pH and higher solubility of the immobilizing agents may have been the major factor affecting immobilizing efficiency. In addition, the total and bioavailable Cd in soil–water extracts was only 16.13–35.41% of the sensor contact assay-determined Cd (BSWS), which indicated that the whole-cell sensor-based contact assay was more practical in assessing the risk of Cd in soil after immobilization since it would not overrate the immobilizing capacity of the agents.

Received 9th November 2013
Accepted 17th December 2013

DOI: 10.1039/c3em00598d

rsc.li/process-impacts

Environmental impact

Environmental contamination by cadmium (Cd) is a world-wide problem. Dipotassium phosphate (DKP, K_2HPO_4) and sodium silicate (SS, $\text{Na}_2\text{SiO}_3 \cdot 9\text{H}_2\text{O}$) are effective and traditional immobilizing agents for metals in soil due to their effectiveness to reduce the mobility of metals. However, a direct comparison of their immobilizing efficiencies for Cd in soil has not been carried out before, more importantly, the mobility of metals did not represent their actual bioavailability and toxicity. Therefore an *Escherichia coli*-based whole cell sensor for detecting the bioavailability of Cd was constructed, and the immobilizing efficiency of DKP and SS for Cd in soil was then compared with this whole-cell sensor.

Introduction

Cadmium (Cd) is a highly toxic trace element that naturally exists primarily as cadmium oxide, cadmium chloride, and cadmium sulfate. Extensive industrial and agricultural activities such as mining, refinement, agricultural fertilizing, irrigation of sewage sludge, and pesticide use have led to serious Cd contamination. Cd pollution can severely damage soil

ecological factors, agricultural products, water, and animal health. In addition, Cd can enter the human body *via* food, touch, and air and remain there for long periods of time, leading to acute toxicity and chronic carcinogenesis in various human organs.¹ At present, Cd ranks sixth on the list of toxic substances from the Agency for Toxic Substances and Disease Registry. The safety threshold for Cd in agricultural soil in the European Union is 3 mg kg^{-1} .² In China, the first class quality of Cd in soil was less than 0.20 mg kg^{-1} (GB15618-1995).

As with other heavy metals, the remediation technologies frequently used to address Cd in polluted soil include physical transfer, plant absorption, microbial remediation, and chemical immobilization. These strategies aim to either reduce total heavy metal or bioavailable heavy metal in contaminated soil. Amongst these technologies, chemical immobilization is an

^aResearch Center for Eco-Environmental Sciences, Chinese Academy of Sciences, Beijing 100085, China

^bLibrary of ShanDong Agricultural University, Taian 271018, China

^cCollege of Biological Sciences and Biotechnology, Beijing Forestry University, Beijing 100083, China

[†] Qi-Hui Hou and An-Zhou Ma contributed equally to this study.

economical and readily available method of reducing the mobility and bioavailability of heavy metals in soil. Immobilizing reagents convert toxic heavy metals into stable states through surface adsorption, precipitation, complex formation, or ion exchange, thereby lessening their harm to the environment.^{3–5}

It has been confirmed that phosphate is a kind of effective chemical agent to immobilize heavy metals in soil. For instance, studies have shown that phosphate can reduce 89–96% of leachable Cd,⁶ 72–100% of water soluble lead (Pb),⁷ and 63–87% of leachable copper (Cu) in soils.⁸ A comparison of the immobilizing efficiency of seven different phosphate materials found that dipotassium phosphate (DKP, K_2HPO_4) was the most efficient reagent for reducing the concentration of plant-available Cd in soil; this efficiency was thought to occur *via* a pH-induced increase in the soil and phosphorous adsorption.⁹ Silicate can also decrease available water soluble Cu, zinc (Zn), Cd, and nickel (Ni),¹⁰ increase the yield of grain, and decrease Cd and Cu concentrations in grain.¹¹ The immobilizing efficiencies of phosphate and silicate in soil can vary due to different chemical characteristics and immobilization mechanisms. However, a direct comparison of their immobilizing efficiencies for Cd in soil has not yet been conducted.

Methods for assessing the immobilizing efficiency for metal in soil include physical X-ray diffraction, scanning electron microscopy, trichlorophenol, sequential extraction, botanic accumulation test, *etc.*^{5–9} Each of these methods have their own advantage on the basis of different assessing point of view. However, these physical and chemical analyses cannot represent the actual bioavailability of metal,^{12,13} and the botanic accumulation test can be too time-consuming. Compared with the above methods, whole-cell sensors are a cheaper, faster, and more convenient and accurate tool that, more importantly, can reflect the actual bioavailability of metal.^{12,13} Recently, numerous whole-cell sensors have been used to detect the bioavailability of Cd, As, Hg, Pb, and Zn in soil or water^{12,14–17} as well as assess the effect of liming, cyclonic ash, compost or phosphate rock on bioavailable Ni,¹⁸ Zn¹ and Pb.¹⁹ We constructed an *Escherichia coli* *cadAp::luc*-based whole-cell sensor to improve the ease and authenticity of assessing chemical immobilization. Using the whole-cell sensor, the immobilizing efficiencies of DKP and sodium silicate (SS, $Na_2SiO_3 \cdot 9H_2O$) for spiked Cd in soil were compared and analyzed.

Experimental

Reagents and enzymes

High-fidelity DNA polymerase (Vent), restriction endonucleases, and T4 ligase were obtained from NEB (USA). Analytical-reagent grade DKP and SS were obtained from Sinopharm Chemical Reagent Co., Ltd. $CdSO_4$ was purchased from the National Research Center for Certified Reference Material, China.

Soil sampling and characterization

Unmodified soil samples were collected from a wheat field in the Fangshan District, a main wheat production area in Peking,

China. The soil was air-dried and passed through a 2 mm sieve before analysis. Soil samples were digested for 12 h at 120 °C by aqua regia and then by $HClO_4$ until white or gray-white (120 °C) to measure total Cd analysis using inductively coupled plasma mass spectrometry (ICP-MS, Plasma Quad 3, UK). Soil and distilled water were mixed at ratio of 1/2.5 ($g\ mL^{-1}$), and soil pH was then measured using the METTLER TOLEDO FE20-FiveEasy™ pH meter (USA). Total carbon (C) and nitrogen (N) and the cation-exchange capacity (CEC) of the soil samples were determined by the Institute of Agricultural Resources and Regional Planning at the Chinese Academy of Agricultural Sciences. Soil characteristics are shown in Table 1.

Soil remediation

Soil with a high concentration of Cd was prepared to ensure that the sensor was effectively detecting Cd. First, 4 mL $CdSO_4$ (2 mmol L^{-1}) was added to 4 g of preprocessed soil sample for a concentration of approximately 450 $mg\ kg^{-1}$ Cd in a 50 mL centrifuge tube (Corning, USA). DKP or SS solution was then added to the soil samples at ratios of 1 : 2, 1 : 1, and 2 : 1 (DKP or SS:Cd, mol mol^{-1}). Soil without the addition of DKP or SS was used as a negative control. The treated soil samples were then homogenized and air dried for two months. Three independent experiments were conducted for each condition.

Strains and plasmids

The bacterial strains and plasmids used in this study are shown in Table 2.

Construction of the *E. coli* DH5 α -based sensor

The BAC/PAC DNA isolation system (Omega Bio-Tec, USA) was used to isolate the *E. coli*-based sensor of plasmid pI258 from *Staphylococcus aureus*. The isolated plasmid was purified by the Omega Bio-Tec Cycle Pure Kit. Gene segments containing the *cad* promoter and the downstream regulatory gene *cadC* were amplified from the pI258 plasmid using polymerase chain reaction (PCR) with the following primers: P_{cadF} , 5'-CCCGA GCTCGTGTATTTTAAATAAATTATTTTAC-3' and P_{cadR} , 5'-AAGT CTCGAGCCCTTTCAGACATTGACCTTC-3'.

ACCTTC-3'

The resulting PCR product was also purified by Omega Bio-Tec Cycle Pure Kit, and these gene segments were then inserted into the plasmid pUC18luc (Fig. 1). *SacI* and *XhoI* restriction enzyme cleavage sites (*italics*) were added to the primers. A new sensor plasmid (Fig. 1) was created by digesting the PCR product with *SacI* and *XhoI* and then inserting it into pUC18luc cleaved by the same enzymes. The recombinant plasmid *pcadCluc* was then transformed into *E. coli* DH5 α *via* heat shock at 42 °C to assemble the whole-cell sensor for Cd bioavailability detection.

Sensor cell culture and luminescence measurements

Standard induction assays were conducted as follows. First, a single colony of *E. coli* DH5 α harboring the *pcadCluc* plasmid was cultured for 16 h shaking (200 rpm, 37 °C) in 50 mL Luria–Bertani

Table 1 Soil chemical properties

Total Cd (mg kg ⁻¹)		pH (water)	Total C (g kg ⁻¹)	Total N (g kg ⁻¹)	CEC (cmol (+) kg ⁻¹)
No addition	Addition				
0.09 ± 0.00 ^a	450.09 ± 0.00	7.70 ± 0.03	20.35 ± 0.43	1.25 ± 0.08	12.82 ± 0.01

^a Average value of three replicates ± standard deviation.

(LB) medium containing 100 µg mL⁻¹ ampicillin. Next, 400 µL of culture was pipetted into 20 mL fresh LB (1 : 50, V/V) with 100 µg mL⁻¹ ampicillin and incubated with shaking (200 rpm) at 37 °C until *OD*₆₀₀ reached 0.6–0.8. The cell culture was then diluted to *OD*₆₀₀ at 0.2 with fresh LB medium.

To induce cellular luminescence, 50 µL of various Cd concentrations in MilliQ water were pipetted onto 96-well cell culture clusters (Costar, USA). An equal volume of diluted bacterial cultures was then added, and the mixed solution was incubated using the Thermo Shaker Incubator (Thermo, Finland) at 30 °C (200 rpm). After incubation, sensor cells were lysed using the E1500 luciferase assay system (Promega, USA), and cellular luminescence was detected using the Glomax-Multi microplate multimode reader (Promega, USA), according to manufacturer's instructions. At least three independent experiments were conducted for each condition. The limit of detection (LOD) of the sensor was determined by the statistically significant change ($P < 0.05$) of luminescence compared with the background value. *E. coli* DH5α containing plasmid pUC18NLR, which constitutively expresses the reporter gene *luc*, was used as a positive control.

Detection of Cd in soil

The total Cd in soil–water extracts (TSWE) was detected by ICP-MS while the bioavailable Cd in soil–water extracts (BSWE) and suspensions (BSWS) were detected by the whole-cell sensor. Soil–water extracts were prepared by mixing 1 g air-dried soil and 9 mL deionized water in a 50 mL centrifuge tube (Corning, USA). The suspensions were shaken for 24 h at 25 °C and then centrifuged at 16 000 g for 5 min.²⁰ Next, 50 µL of diluted bacterial culture was added at equal volumes to the soil–water extracts or suspensions. The soil–water suspensions were diluted by a factor of 100 or 1000 with water, and the respective extracts were diluted 1 : 10 with water.

To avoid the influence of impurities or particles on luminescence emission, the mixture of soil–water suspension and

sensor cells was diluted until the correction factor (CF) was 1.²⁰ Cd bioavailability was then determined by linear regression of the standard Cd solution luminescence from three independent experiments. *E. coli* DH5α containing plasmid pUC18NLR was used as a positive control to eliminate the interference of impurities or particles with the sensor. Bioavailable Cd was expressed as a percent of the total Cd in soil. At least three independent experiments were conducted for each condition.

Results and discussion

Construction of pcdCluc as a whole-cell sensor

The response element of pUC18luc is comprised of the T7 promoter, downstream reporter gene *luc*, and T7 termination. In prokaryotes, the 5' Shine–Dalgarno sequence is critical for translational efficiency through its base-pairing with the 3' region of 16S ribosomal RNA.^{21,22} Thus, to ensure efficient and high levels of *luc* translation, the Shine–Dalgarno sequence (AGGAG) of the T7 phage g10 gene was inserted upstream of *luc*. The induced luciferase expression showed detectable luminescence that responded to the addition of IPTG in *E. coli* BL21 (DE3) containing pUC18luc (results not showed).

The *cad* operon of pI258 allows *S. aureus* to survive in heavy metal environments, such as those containing Cd and Pb ions. In this system, *cadA* and *cadC* are the primary gene elements. CadA is responsible for metal resistance by efflux while CadC negatively regulates *cadA* translation.²³ Here, the 572-bp regulatory gene *cadC* and the upstream promoter sequence of pI258 replaced the T7 promoter of pUC18luc, resulting in a 4756-bp plasmid pcdCluc.

pcdCluc induction with increasing induction time and Cd dose

The response time of sensor to effector largely depends on the reporter gene employed. Studies have shown that *luc* is both relatively fast and highly sensitive.^{24,25} A comparison of

Table 2 Bacterial strains and plasmids

Strains/plasmids	Genotype/phenotype	Source
<i>E. coli</i> DH5α	<i>supE44ΔlacU169</i> (Φ80 <i>lacZΔM15</i>) <i>hsdR17 recA1 endA1 gyrA96 thi-1relA1</i>	Tiangen Biotech, China
<i>E. coli</i> BL21 (DE3)	<i>hsdS gal (λctts857 ind1 Sam7 nin5 lac UV-5-7 gene1)</i>	Tiangen Biotech, China
<i>Staphylococcus aureus</i> (containing plasmid pI258)	Amp ^r	ATCC
pUC18luc	Amp ^r	This laboratory
pcdCluc	Amp ^r	This study
pUC18NLR	Amp ^r	This study

Fig. 1 Plasmid construction of pcadCluc from the plasmid frame pUC18luc.

induction time between *luc*, the bioluminescence gene *lux-CDABE*, the fluorescent protein gene *gfp*, and *dsRed* found that *luc* required the shortest incubation time (30 min) to produce enough luminescence to mercury (Hg) and arsenic. The *gfp* and *dsRed* reported required 2 and 8 h, respectively. The shorter induction time of *luc* can be explained by the shorter time it needs to fold after translation.²⁵

The time-dependent luminescence of pcadCluc in the presence of Cd ($2 \mu\text{mol L}^{-1}$) was determined by incubating the sensor at increasing intervals from 0.5 to 8 h. The kinetic profile of the sensor showed that the sensor produced an obvious response after only 0.5 h of induction. Luminescence intensity increased as induction time increased. Furthermore, the rate of luminescence increase during the first 2 h was faster than that after 2 h (Fig. 2). Thus, 2 h was used as the routine induction time in the following experiments.

To investigate the dose-response regularity of the sensor to Cd, the sensor was incubated with increasing concentrations of Cd (Fig. 3). Cd concentrations $<5 \mu\text{mol L}^{-1}$ could be detected by the sensor while Cd concentrations $>5 \mu\text{mol L}^{-1}$ resulted in an obvious decrease in luminescence from both the sensor and the control strain with pUC18NLR. This may be the result of higher

Cd concentrations exceeding the toxicity resistance of the bacterial cells. In this study, the statistically significant ($P < 0.05$) LOD of the sensor to Cd was $0.1 \mu\text{mol L}^{-1}$. The LOD of this *luc*-based sensor is lower than that of a previously constructed *luxAB*-fused whole-cell sensor ($0.5 \mu\text{mol L}^{-1}$)²⁶ but higher than that of a similarly constructed *luc*-based sensor ($>0.3 \text{ nmol L}^{-1}$).²⁷ Other factors besides the reporter gene can also affect the LOD of the sensor, including the host strain, the host strain concentration, and culture medium.

A calibration curve was established using linear regression for Cd concentrations from 0.1 to $5 \mu\text{mol L}^{-1}$. The regression formula $y = 2.39 \times 10^6 x + 1.59 \times 10^6$ ($R^2 = 0.97$, $P < 0.01$), where x represents Cd concentration and y represents luminescence, was used to calculate Cd concentration in the following experiments.

The effects of immobilizing reagents on Cd bioavailability in soil-water extracts

DKP and SS decreased the total and bioavailable Cd in soil-water extracts compared to controls (Fig. 4). The metal in soil pore water represents the most instantaneously bioavailable due to its free mobility and is considered the most environmentally toxic and risky metal fraction.^{28–30} Higher

Fig. 2 Kinetics of the relative luminescence unit of the whole-cell sensor harboring pcadCluc over time.

Fig. 3 Dose-dependent induction of the whole-cell sensor harboring pcadCluc and the control strain harboring pUC18NLR.

concentrations of DKP and SS (2 : 1, 1 : 1, and 1 : 2, P or S : Cd, mol mol⁻¹) had a higher capacity for lowering total Cd concentration in soil–water extracts after 60 days. At their highest concentrations, DKP and SS significantly reduced Cd water solubility (from 56.47 to 31.13 mg kg⁻¹ and 16.63 mg kg⁻¹, a reduction of 44.87% and 70.55%, respectively)

compared to controls. At all three concentrations, soil treated with DKP had higher Cd water solubility than that treated by SS. Specifically, the total Cd in soil–water extracts of DKP-treated soil were 1.50, 1.70, and 1.87 times higher than that in SS soil at concentrations of 2 : 1, 1 : 1, and 1 : 2 (P or Si : Cd, mol mol⁻¹), respectively. Therefore, SS appeared to be more effective at reducing Cd mobility in soil.

The total metal found in soil pore water does not reflect its actual bioavailability since significant amounts of metal chelators may also be present.^{31,32} For example, EDTA, CTDA, or Na₂S in aqueous reduced the bioavailable Cd and Pb due to their complexation to Cd and Pb.^{33,34} Thus, the pcadCluc sensor was also used to test the actual Cd bioavailability in soil pore water. At concentrations of 2 : 1 (P or Si : Cd, mol mol⁻¹), the concentration of bioavailable Cd in soil–water extracts decreased from 42.11 mg kg⁻¹ to 36.60 mg kg⁻¹ and 30.84 mg kg⁻¹ after 60 days with DKP and SS, respectively. At DKP and SS concentrations of 1 : 1 (P or Si : Cd, mol mol⁻¹), the residual bioavailable Cd in soil–water extracts decreased to 32.98 mg kg⁻¹ and 20.98 mg kg⁻¹, respectively. When the concentrations of DKP and SS were highest at 1 : 2 (P or Si : Cd, mol mol⁻¹), bioavailable Cd in soil–water extracts decreased to 21.13 mg kg⁻¹ and 10.90 mg kg⁻¹ (49.82% and 74.12% reductions), respectively, compared to controls. When the immobilizing capacities of DKP and SS were compared, we found that SS had an increased immobilizing efficiency of 26.61%, 28.5%, and 24.29% for all concentrations (2 : 1, 1 : 1, and 1 : 2), respectively, compared to DKP. Thus, SS appeared to be the better reagent for decrease Cd bioavailability in soil pore water.

Various mechanisms contribute to the immobilizing ability of phosphate and silicate to metal, including surface complexation, precipitation, and pH-mediated adsorption.^{3–5} In addition, soil pH, the solubility of immobilizing agents, dissolved organic carbon (DOC), and other competitive metal ions in soils are major factors that can affect these mechanisms.^{6,35,36} In this study, SS was better than DKP for immobilizing water-soluble Cd in soil. Variations in pH could have played a significant role in enhancing the immobilizing efficiency of SS. After immobilization, there was a pH increase of 0.30–0.55 in the SS-treated soil. In contrast, the pH in DKP-treated soil decreased by 0.13–0.19 (Fig. 5), which was consistent with previous findings.^{7,11,37,38} The increase in pH observed after the SS treatment may have increased charge exchange between positive soil particle charges and metals in soil pore water.^{39,40} This would contribute to the decrease in metal bioavailability in soil–water extracts. Soil pH could also affect the production of other metal complex precipitates. For instance, an increase in pH could facilitate the generation of CdCO₃ or Cd(OH)₂ precipitates, which are difficult to dissolve or are adsorbed tightly by soil.^{41,42}

Moreover, the difference of immobilizing efficiency could be attributed to the increased solubility of SS compared to DKP because SS was a crystal consisting of Na₂SiO₃ and 9H₂O, which could lead to a greater dissolution rate and consequently immobilization of Cd in soil. In other words, there may be more Si–Cd precipitation compared with P–Cd precipitation. Theoretically, the higher release of SS in soil was an original factor to increase the pH of soil due to its alkalinity.

Fig. 4 Total Cd in soil–water extracts (TSWE), bioavailable Cd in soil–water extracts (BSWE), and bioavailable Cd in soil–water suspensions (BSWS) after DKP or SS addition at different levels (a) P or Si : Cd = 2 : 1 (mol mol⁻¹), (b) P or Si : Cd = 1 : 1 (mol mol⁻¹) and (c) P or Si : Cd = 1 : 2 (mol mol⁻¹).

Fig. 5 Variations in soil pH after immobilizing by DKP or SS.

Fig. 6 Percentage of total Cd in soil-water extracts (TSWE) or bioavailable Cd in soil-water extracts (BSWE) to bioavailable Cd in soil-water suspensions (BSWS) after DKP or SS addition at different levels (a) P or Si : Cd = 1 : 2 (mol mol⁻¹), (b) P or Si : Cd = 1 : 1 (mol mol⁻¹) and (c) P or Si : Cd = 2 : 1 (mol mol⁻¹).

The effect of immobilizing reagents on bioavailable Cd in soil-water suspensions

Although metal bioavailability in soil-water extracts is a significant proportion of total metal in soil, it does not represent the total amount of bioavailable metal. Studies have shown that when metal bioavailability in soil-water suspensions is detected directly by a sensor, a higher amount of bioavailable Cd, Hg, and Pb is obtained.^{20,43} This resulted from the metals bound to the soil particles being absorbed by the sensor cells in soil-water suspensions. Consequently, this is also a potentially bioavailable fraction of metal if microbial and plant cells come in contact with these soil particle-bound metals in the environment.

Using diluted soil-water suspensions (1 : 100 dilution, CF = 1), we found that the bioavailable Cd in soil-water suspensions was significantly higher (about 3 to 5-fold higher) than in corresponding soil-water extracts. This indicated the

presence of soil particle-bound Cd that was bioavailable to the sensor cells. Similar to our findings above, higher concentrations of DKP and SS gradually enhanced their immobilizing efficiencies for Cd in soil-water suspensions. At DKP and SS concentrations of 1 : 2 (P or Si : Cd, mol mol⁻¹), the bioavailable Cd decreased by 119.43 mg kg⁻¹ and 139.15 mg kg⁻¹ (57.53% and 67.31% reductions), respectively, in soil-water suspensions compared to controls (206.72 ± 8.94 mg kg⁻¹). SS was also more efficient at decreasing Cd bioavailability in soil-water suspensions (Fig. 4) compared to DKP. SS had a higher immobilization capacity of 11.5%, 19.08%, and 9.54% at concentrations of 2 : 1, 1 : 1, and 1 : 2 (P or Si : Cd, mol mol⁻¹), respectively. Except for pH and solubility, the other possible reason that led to the difference of immobilizing efficiencies of DKP and SS in this direct contact assay was that DKP-bound with Cd was used as the phosphate source by the *E. coli* sensor cells.^{44,45} However, the better immobilizing efficiency was not as stark as those for the total and bioavailable Cd in soil-water extracts. This may be due to the uptake of the Cd adsorbed on the soil surface by the sensor cells, however the specific mechanisms at play in the increased bioavailability of Cd in soil-water suspensions have to be further elucidated. In short, the immobilizing efficiencies of DKP and SS differ based on their immobilization mechanisms. Here, SS was more efficient at immobilizing Cd.

Finally, the differentiation of Cd detected by the above three modes was conducted, and results showed that the total and bioavailable Cd in soil-water extracts was respectively only 24.62–35.41% and 16.13–24.2% (Fig. 6) of the sensor contact assay-determined Cd (BSWS), which indicated that the whole-cell sensor-based contact assay was more practical in assessing the risk of Cd in soil after immobilization since it would not overrate the immobilizing capacity of the agents.

Conclusion

Whole-cell sensors are an effective tool for assessing metal bioavailability in soil. Although they cannot replace traditional chemical analyses, they can complement these chemical analytical methods to quantify the actual bioavailable fraction of metal. Several chemical agents have been employed to immobilize Cd in water or soil, however a direct comparison of immobilizing efficiencies between SS and DKP for Cd in soil has not yet been conducted. What is more meaningful is that a microbial biosensor was applied to assess the immobilizing efficiencies of SS and DKP for Cd in soil.

In this study, an *E. coli*-based whole-cell sensor was successfully constructed to detect Cd bioavailability. The p_{cad}Cluc plasmid was used as a negative regulatory system where the CadC repressor from *S. aureus* controlled expression of the reporter gene *luc*. Induction experiments showed that the sensor produced luminescence in response to Cd after an induction of 0.5 h. Increasing Cd concentrations from 0.1 to 5 µmol L⁻¹ also resulted in a corresponding linear increase in luminescence ($R^2 = 0.97$, $P < 0.01$). The sensor was then used to measure Cd bioavailability in soil after DKP and SS immobilization.

SS could immobilize more Cd than DKP in all soil samples (soil-water extracts and suspensions), and the immobilization

efficiencies of both SS and DKP increased as their concentrations increased. At the highest concentration of 1 : 2 (P or Si : Cd, mol mol⁻¹), SS decreased the total and bioavailable Cd in soil–water extracts and the bioavailable Cd in soil–water suspensions by 70.55%, 74.12%, and 67.31%, respectively. The immobilization efficiency of SS was 25.68%, 24.29%, and 9.54% higher, respectively, than that of DKP. The variations of soil pH and solubility of the immobilizing agents may be the major factors contributing to this difference in immobilization efficiency. In addition, the total and bioavailable Cd in soil–water extracts was respectively only 24.62–35.41% and 16.13–24.2% of the sensor contact assay-determined Cd (BSWS). Therefore it indicated that the whole-cell sensor-based contact assay was more practical in assessing the risk of Cd in soil after immobilization since it would not overrate the immobilizing capacity of the agents.

In summary, SS was more efficient than DKP at immobilizing Cd in soil, and the conduct assay of *E. coli cadAp::luc*-based whole-cell sensor could feasibly assess Cd bioavailability in soil.

Acknowledgements

This work was supported by the National Key Technology R&D Program (2012BAJ24B01). We are also grateful to the anonymous reviewers for their helpful comments on the manuscript.

Notes and references

- M. Nachtegaal, M. A. Marcus, J. E. Sonke, J. Vangronsveld, K. J. T. Livi, D. van Der Lelie and D. L. Sparks, *Geochim. Cosmochim. Acta*, 2005, **69**, 4649–4664.
- P. C. Nagajyoti, K. D. Lee and T. V. M. Sreekanth, *Environ. Chem. Lett.*, 2010, **8**, 199–216.
- J. Kumpiene, A. Lagerkvist and C. Maurice, *Waste Management*, 2008, **28**, 215–225.
- R. X. Cao, L. Q. Ma, M. Chen, S. P. Singh and W. G. Harris, *Environ. Pollut.*, 2003, **122**, 19–28.
- P. Theodoratos, N. Papassiopi and A. Xenidis, *J. Hazard. Mater.*, 2002, **94**, 135–146.
- U. Thawornchaisit and C. Polprasert, *J. Hazard. Mater.*, 2009, **165**, 1109–1113.
- X. Cao, A. Wahbi, L. Ma, B. Li and Y. Yang, *J. Hazard. Mater.*, 2009, **164**, 555–564.
- R. Liu and D. Zhao, *Chemosphere*, 2007, **68**, 1867–1876.
- C. O. Hong, D. Y. Chung, K. Lee do and P. J. Kim, *Arch. Environ. Contam. Toxicol.*, 2010, **58**, 268–274.
- A. Usman, Y. Kuz'yakov and K. Stahr, *J. Soils Sediments*, 2005, **5**, 245–252.
- P. Li, X. Wang, T. Zhang, D. Zhou and Y. He, *J. Environ. Sci.*, 2008, **20**, 449–455.
- V. H. Liao, M. T. Chien, Y. Y. Tseng and K. L. Ou, *Environ. Pollut.*, 2006, **142**, 17–23.
- S. Magrisso, Y. Erel and S. Belkin, *Microb. Biotechnol.*, 2008, **1**, 320–330.
- K. Yoshida, K. Inoue, Y. Takahashi, S. Ueda, K. Isoda, K. Yagi and I. Maeda, *Appl. Environ. Microbiol.*, 2008, **74**, 6730–6738.
- A. Rantala, M. Utriainen, N. Kaushik, M. Virta, A. L. Välimaa and M. Karp, *Anal. Bioanal. Chem.*, 2011, **400**, 1041–1049.
- S. Magrisso, S. Belkin and Y. Erel, *Water, Air, Soil Pollut.*, 2009, **202**, 315–323.
- P. Liu, Q. Huang and W. Chen, *Environ. Pollut.*, 2012, **164**, 66–72.
- J. L. Everhart, D. McNear Jr, E. Peltier, D. van der Lelie, R. L. Chaney and D. L. Sparks, *Sci. Total Environ.*, 2006, **367**, 732–744.
- W. Geebelen, D. C. Adriano, D. van der Lelie, M. Mench, R. Carleer, H. Clijsters and J. Vangronsveld, *Plant Soil*, 2003, **249**, 217–288.
- A. Ivask, M. Francois, A. Kahru, H. C. Dubourguier, M. Virta and F. Douay, *Chemosphere*, 2004, **55**, 147–156.
- S. C. Makrides, *Microbiol. Rev.*, 1996, **60**, 512–538.
- K. Lee, C. A. Holland-Staley and P. R. Cunningham, *RNA*, 1996, **2**, 1270–1285.
- G. Endo and S. Silver, *J. Bacteriol.*, 1995, **177**, 4437–4441.
- P. Billard and M. S. DuBow, *Clin. Biochem.*, 1998, **31**, 1–14.
- K. Haktila, M. Maksimow, M. Karp and M. Virta, *Anal. Biochem.*, 2002, **301**, 235–242.
- P. Corbisier, G. Ji, G. Nuyts, M. Mergeay and S. Silver, *FEMS Microbiol. Lett.*, 1993, **110**, 231–238.
- S. Tauriainen, M. Karp, W. Chang and M. Virta, *Biosens. Bioelectron.*, 1998, **13**, 931–938.
- K. E. Giller, E. Witter and S. P. McGrath, *Soil Biol. Biochem.*, 2009, **41**, 2031–2037.
- W. J. G. M. Peijnenburg and T. Jager, *Ecotoxicol. Environ. Saf.*, 2003, **56**, 63–77.
- I. Worms, D. F. Simon, C. S. Hassler and K. J. Wilkinson, *Biochimie*, 2006, **88**, 1721–1731.
- R. Turpeinen, J. Salminen and T. Kairesalo, *Environ. Sci. Technol.*, 2000, **34**, 5152–5156.
- K. K. Brandt, A. Petersen, P. E. Holm and O. Nybroe, *FEMS Microbiol. Ecol.*, 2006, **56**, 281–291.
- K. Riether, M. A. Dollard and P. Billard, *Appl. Microbiol. Biotechnol.*, 2001, **57**, 712–716.
- S. M. Tauriainen, M. P. J. Virta and M. T. Karp, *Water Res.*, 2000, **34**, 2661–2666.
- J. Matusik, T. Bajda and M. Manecki, *J. Hazard. Mater.*, 2008, **152**, 1332–1339.
- F. Lang and M. Kaupenjohann, *Eur. J. Soil Sci.*, 2003, **54**, 139–147.
- F. Madrid, E. Díaz-Barrientos, M. C. Florido and L. Madrid, *Water, Air, Soil Pollut.*, 2008, **192**, 117–125.
- X. L. Zhao and S. Masaihiiko, *J. Environ. Sci.*, 2007, **19**, 343–347.
- M. Elzahabi and R. N. Yong, *Eng. Geol.*, 2001, **60**, 61–68.
- C. Appel and L. Ma, *J. Environ. Qual.*, 2002, **31**, 581–589.
- M. Gräfe, M. Nachtegaal and D. L. Sparks, *Environ. Sci. Technol.*, 2004, **38**, 6561–6570.
- S. A. Hasan, Q. Fariduddin, B. Ali, S. Hayat and A. Ahmad, *J. Environ. Biol.*, 2009, **30**, 165–174.
- A. Ivask, M. Virta and A. Kahru, *Soil Biol. Biochem.*, 2002, **34**, 1439–1447.
- H. Rosenberg, R. G. Gerdes and K. Chegwiddden, *J. Bacteriol.*, 1977, **131**, 505–511.
- G. R. Willsky and M. H. Malamy, *J. Bacteriol.*, 1980, **144**, 156–165.