Detection of bioavailable cadmium, lead, and arsenic in polluted soil by tailored multiple *Escherichia coli* whole-cell sensor set

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Abstract Microbial whole-cell sensor has been widely used to assess bioavailability and risk of toxic elements, but their environmental use is still limited due to the presence of other interfering pollutants and the nonspecific binding in cells, which leads to inaccurate results. Here, we proposed a strategy combining *Escherichia coli* sensor set with binary regression models for the specific detection of bioavailable cadmium (Cd), lead (Pb), and arsenic (As) in a co-polluted environment. Initial tests suggested that the sensor set respectively termed pcdCluc, pzntRluc, and parsRluc could be classified into two groups according to their specific response to Cd, Pb, and As: group 1 (pcdCluc and pzntRluc) induced by a Cd-Pb mix and group 2 (parsRluc) induced by a Cd-As mix. Based on the variance in responses of each sensor to mixtures of target elements, three binary linear equations for two sensor groups were set up to calculate the individual concentrations in the

mixture solutions. This method was then used to quantify the bioavailable Cd, Pb, and As in soils from a co-polluted mining region and to compare the results with other methods. Results showed that the conventional single target sensor method overestimated the bioavailability of each element, while sensor set was credible for accurate bioavailable Cd, Pb, and As quantification and comparable with the results from inductively coupled plasma mass spectrometry (ICP-MS) analysis. Our method can potentially be extended to cover the specific detection of other bioavailable toxic elements in different environmental settings.

Keywords Heavy metals · Bioavailability · Specificity · Whole-cell sensor set · Cross-mixed induction · Binary linear regression

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Introduction

Cadmium (Cd), lead (Pb), and arsenic (As) contamination are attracting continuing concern in many parts of the world due to their high content in agricultural products, high toxicity, and accumulation in the human body. Chemical analysis based on a series of analytical instrumentations such as Atomic Absorption Spectroscopy (AAS) and inductively coupled plasma mass spectrometry (ICP-MS) can give accurate and sensitive determination of the total metal content in the environment. However, due to the sorption and complexation of trace elements in soil, only a fraction of the total content is available for uptake to biota. The associated environmental risk of hazardous elements in polluted environments might therefore be more accurately determined from their bioavailable fraction. Bioavailable metal can be defined as the metal fraction available for crossing an organism's cell membrane [1], so it not only evaluates soluble metals but also those in other chemical

forms which can cross the cell membrane. Chemical-assessing methods such as single chemical extraction or sequential extraction procedures (SEPs) are frequently employed to quantify the bioavailable metals. However, these calculated bioavailable fractions do not correlate well with actual bioavailability because these were obtained using non-biological protocols. For instance, bioavailable Cd extracted by weak acid in polluted field soils was dozens of times higher than that determined using a microbial whole-cell sensor and the amount of Pb in sequential leaches was obviously different with microbial whole-cell sensor-determined Pb [2, 3].

Whole-organism assay is an effective approach to assess actual biological response, bioavailability, and toxicity of metals in soil. Numerous microbial whole-cell sensors have been developed to measure the soil bioavailability of toxic elements such as Cd, Pb, As, Hg, and Cr [4, 5]. However, almost all the developed whole-cell sensors are nonspecific to a certain metal since the regulatory proteins, such as CadC, ZntR, and ArsR, could concurrently recognize several different metals [6, 7]. Nonspecific microbial sensors that respond to multiple metals in a certain environment will tend to overestimate the bioavailability of target metals and environmental risks in a co-polluted environment.

In order to realize the specific and accurate detection of bioavailable Cd, Pb, and As in a co-polluted environment, a multiple *Escherichia coli* whole-cell sensor set and binary linear equations were built in this study to eliminate the interference between Cd, Pb, and As by a series of experiment procedures such as specificity analysis, sensor grouping, and mixture induction. The test of soil samples showed that this method was more feasible for detecting bioavailable metals compared to using single whole-cell sensors in a co-polluted environment, which tend to overestimate the levels.

Experimental

Materials and methods

Vent DNA polymerase, restriction endonucleases, and T4 ligase were purchased from NEB (USA). CdSO₄, PdSO₄, and NaAsO₂ were purchased from the National Research Center for Certified Reference Material, China. Oligo primer (see Electronic Supplementary Material (ESM) Table S1) synthesis and sequencing were performed by Invitrogen (China).

E. coli whole-cell Cd, Pb, and As sensors

The parsRluc (see ESM Fig. S1a) and pcadCluc (ESM Fig. S1b) sensors were constructed in the previous research by our laboratory [8, 9]. The pzntRluc sensor (ESM Fig. S1c) was constructed by this study as follows: The Pznt promoter was amplified using the primers Pznt-F/Pznt-R and linked to

the upstream of *luc* in pUC18luc [8], which was then digested using *Sac*I and *Xho*I. Next, the primers PnptII-F/PnptII-R, Pznt-R-F/Pznt-R-R, and PrrNB-F/PrrNB-R were used to construct constitutively expressed *zntR* (NC_007779) fragment from *E. coli* DH5 α (Biomed, China). The restriction enzyme sites at the ends of the *zntR* fragment were then changed from *Bam*HI and *Eco*RI to *Sac*I using the P1 and P2 primers, and the resulting fragment was reversely linked to pUC18luc.

Soil samples

Soil samples were obtained from the Shuikoushan mining area in Hengyang City, Hunan Province, China. Detailed information about the soil samples is shown in ESM Table S2.

Specificity analysis of sensors to Cd, Pb, and As

Standard induction assays [8] for the pcadCluc, pzntRluc, and parsRluc sensors were performed to determine their specificity to Cd, Pb, and As. Relative luminescence units (RLUs) were measured using the GloMax-Multi microplate multi-mode reader (Promega, USA). The induction experiment of sensor cells (OD₆₀₀=0.6–0.8) by standard metal solution and RLU measure respectively took 2 h and 30 min.

Construction of binary linear equation groups

*Cd*²⁺-*Pb*²⁺ and *Cd*²⁺-*As*³⁺ induction experiments

Standard Cd²⁺ and Pb²⁺ solutions of equal volumes (25 μ L) and concentrations (0, 0.4, 0.5, 1, 1.5, 2, 4, 6, 8, and 20 μ M) were cross-mixed and then used to induce pcadCluc and pzntRluc in the 96-well plates. Similarly, standard of As³⁺ (0, 0.4, 0.5, 1, 1.5, 2, 4, 6, 8, and 20 μ M) and Cd²⁺ (0, 20, 22, 24, 28, 32, 40, 48, 56, and 80 μ M) solutions of equal volumes (25 μ L) were cross-mixed and used to induce parsRluc. Three-dimensional diagrams of RLU variation with mixtures of the toxic elements were generated using Origin 8.0. Three replicate experiments were conducted for each experimental condition.

Linear response analysis of sensors to mixed metals

In order to better describe the analysis procedure, mixed metals (Cd²⁺-Pb²⁺ or Cd²⁺-As³⁺) were set as metal A and metal B. Then, *k* was defined as the slope of RLU at different initial metal A and increasing metal B. The relationship between RLU and the concentrations of mixture metals was expressed as variational *k* at different initial metal A. If the increase of *k* was linear at a certain concentration range of metal A, this range was set as the range in which metal A could induce a linear increase of RLU at the presence of metal B. All the six linear ranges were also determined by this way.

At last, binary linear equations were then generated using MATLAB R2013a according to the results of mixture induction experiments and the linear response ranges.

Detection of the bioavailable concentration of Cd, Pb, and As in soil

The digestion of total Cd, Pb, and As was carried out using aqua regia ($\text{HCl}/\text{HNO}_3=3:1$ in v/v) and HClO_4 . Total As concentration of digested solution was detected by ICP-MS (Plasma Quad 3; UK). Soil water extracts were prepared according to the pre-existing method [10]. Serial dilutions of the soil water extracts were then performed to ensure that their total metal concentrations were within the range of detection of the single sensor and sensor groups. A pUC18NLR plasmid in *E. coli* DH5 α , which constitutively expresses *luc* under the *nptII* promoter, was used as a positive control to detect possible spurious toxicity in the samples which may significantly interfere ($P>0.05$) with the analysis.

Results and discussion

Specificities of sensor regulators to Cd, Pb, and As

Specificity analysis showed that the RLU of both pcdCluc and pzntRluc sensors increased with Cd^{2+} and Pb^{2+} concentration but not significantly increased with As^{3+} concentration (Fig. 1a, b). For the parsRluc sensor, the RLU just significantly increased with both Cd^{2+} and As^{3+} concentration, although the RLU increase induced by Cd^{2+} was not as prominent as that induced by As^{3+} (Fig. 1c). These results indicated that, when the individual pcdCluc, pzntRluc, or parsRluc sensors were used with mixed Cd-Pb-As solution, Cd and Pb may mutually interfere their respective measured results and Cd may affect the measured result of As. These findings are consistent with those of previous studies, which also observed nonspecific binding of metals such as Cd and Pb to CadC; Pb, Cd, Hg, and Zn to ZntR; and As and Sb to ArsR [6, 7].

The detection limit was determined using statistically significant changes ($P<0.05$) in RLU compared with background RLU [11]. The detection limit of both pcdCluc and pzntRluc sensors for Cd and Pb was lower than 0.1 and 0.05 μM , respectively. The detection limit of parsRluc sensor for Cd and As was 5.0 μM and lower than 0.1 μM , respectively. These results suggested that, when a non-target metal concentration was higher than the detection limit of sensor for target metal determination, overestimation would occur when a single cell sensor was used.

Fig. 1 Dose-response curves of pcdCluc (a), pzntRluc (b), and parsRluc (c) sensors to Cd, Pb, and As. Error bars mean the standard deviations of the three individual results

Sensor set responses to mixtures of toxic elements

Although interferences by the different elements occur, the response strength of each sensor to the same mixture solution is different. This means that the interferences can be distinguished by applying a set of sensors in combination with statistical analysis treatment.

According to the results of specificity analysis, the sensor set was divided into two groups: group 1 comprised of pcdCluc and pzntRluc sensors in order to distinguish the interference of Cd and Pb, and group 2 comprised of parsRluc sensor, associated with group 1, to detect the specific bioavailable As. Three-dimensional mesh plots (ESM Fig. S2) showed that a mixture of 0.1 to 2.0 μM Cd-Pb significantly induced pcdCluc and resulted in a RLU that was much higher than that induced by either Cd or Pb alone. Similarly, 0.1 to 2.0 μM Cd-Pb, 5.0 to 10.0 μM Cd, and 0.1 to 2.0 μM As

mixtures noticeably induced pzntRluc and parsRluc, respectively. The concentration ranges of mixed Cd-Pb or As-Cd that could linearly increase the RLU of each sensor were confirmed using parameter k (Table 1).

Mathematical treatment to determine specific concentration

For eliminating the interferences between Cd, Pb, and As to sensors, binary linear regression (Fig. 2) and

equations (Eqs. 1, 2, 3) were established by MATLAB R2013a on the basis of grouping and concentration ranges of mixed metals that could linearly increase the RLU of sensor group. For sensor group 1, the overlapping concentration ranges (0.1–1.0 μM) of mixed Cd and Pb were determined according to their concentration ranges, linearly increasing the RLU of each sensor (Table 1).

Sensor group 1:

$$\begin{cases} \text{RLU}_{\text{pcadCluc}} = 33.01\text{E}5 \cdot c_{\text{Cd}} + 49.72\text{E}5 \cdot c_{\text{Pb}} + 22.42\text{E}5 & R^2 = 0.99 & (c_{\text{Pb}}: 0.1\text{--}1.0 \mu\text{M}) & (1) \\ & & (c_{\text{Cd}}: 0.1\text{--}1.0 \mu\text{M}) \\ \text{RLU}_{\text{pzntRluc}} = 26.99\text{E}5 \cdot c_{\text{Cd}} + 11.96\text{E}5 \cdot c_{\text{Pb}} + 5.94\text{E}5 & R^2 = 0.97 & (c_{\text{Pb}}: 0.1\text{--}1.0 \mu\text{M}) & (2) \\ & & (c_{\text{Cd}}: 0.1\text{--}1.0 \mu\text{M}) \end{cases}$$

Sensor group 2:

Pb detection by pzntRluc:

$$\begin{aligned} \text{RLU}_{\text{parsRluc}} &= 79.57\text{E}5 \cdot c_{\text{As}} + 8.85\text{E}5 \cdot c_{\text{Cd}} - 9.84\text{E}5; R^2 \\ &= 0.97 \quad (c_{\text{As}} = 0.1\text{--}1.0 \mu\text{M}; c_{\text{Cd}} = 5.0\text{--}10.0 \mu\text{M}) \end{aligned} \quad (3)$$

$$\begin{aligned} \text{RLU}_{\text{pzntRluc}} &= 7.54\text{E}5 \cdot c_{\text{Pb}} + 4.68\text{E}5; R^2 \\ &= 0.97 \quad (\text{Pb} = 0.1\text{--}1.0 \mu\text{M}) \end{aligned} \quad (5)$$

As detection by parsRluc:

$$\begin{aligned} \text{RLU}_{\text{parsRluc}} &= 4.2\text{E}6 \cdot c_{\text{As}} + 3.5\text{E}6; R^2 \\ &= 0.98 \quad (\text{As} = 0.1\text{--}1.0 \mu\text{M}) \end{aligned} \quad (6)$$

The significance test of equations was illustrated in ESM Text S1. For comparisons, regressions for single sensors to measure bioavailable concentrations of Cd, Pb, and As in individual standard solutions are also presented in Eqs. 4, 5, and 6.

Cd detection by pcadCluc:

$$\begin{aligned} \text{RLU}_{\text{pcadCluc}} &= 2.7\text{E}6 \cdot c_{\text{Cd}} + 2.12\text{E}6; \\ R^2 &= 0.99 \quad (\text{Cd} = 0.1\text{--}1.0 \mu\text{M}) \end{aligned} \quad (4)$$

Bioavailable concentrations of Cd, Pb, and As in soil samples

Among the 24 soil samples, only water-soluble Cd and Pb in P1–P7 were 0.2–15.8 mg/kg (0.2–15.6 μM) and 0.3–23.2 mg/kg (1.6–12.4 μM), respectively, and water-soluble

Table 1 Mixed metal concentration ranges for linear sensor response (k =the slope of RLU at different initial metal A and increasing metal B)

	Sensor	Metal A	Metal B	Linear equation of k variation (y) with increasing metal A (x)	R^2	Concentration range of metal A (x) (μM)	Detection limit (μM)
Sensor group 1	pcadCluc	Pb	Cd	$y = 7.94\text{E}5x + 3.48\text{E}6$	0.86**	0.1–1.0	Pb=0.1–1.0
		Cd	Pb	$y = 9.63\text{E}5x + 4.77\text{E}6$	0.98**	0.1–1.0	Cd=0.1–1.0
	pzntRluc	Cd	Pb	$y = 9.78\text{E}5x + 8.82\text{E}5$	0.97**	0.1–1.0	
		Pb	Cd	$y = 7.51\text{E}5x + 2.76\text{E}6$	0.95**	0.1–1.5	
Sensor group 2	parsRluc	As	Cd	$y = 1.48\text{E}5x + 3.87\text{E}6$	0.95**	0.1–1.0	As=0.1–1.0
		Cd	As	$y = 1.48\text{E}5x + 1.40\text{E}6$	0.90**	5.0–10.0	Cd=5.0–10.0

** $P < 0.01$

As in P2 and P5 was 0.7–1.2 mg/kg (0.1–1.7 μM), which were respectively higher than the detection limit of p_{cad}Cluc, p_{znt}Rluc, and p_{ars}Rluc sensors. Therefore, the bioavailability

of Cd and Pb in samples P1–P7 and that of As and Cd in sample P2 and P5 could be evaluated respectively by sensor groups 1 and 2 since the total metal concentrations of soil water extractions would be in the independent variable ranges of binary linear regression equations after dilution. In addition, the total content of Cd, Pb, and As in the selected samples ranged from 13.2 to 68.4 mg/kg, from 216.6 to 8868.1 mg/kg, and from 268.9 to 442.2 mg/kg, respectively, which were respectively 16 to 85-fold, 2 to 110-fold, and 3 to 9-fold higher

Fig. 2 Binary linear regressions of p_{cad}Cluc (a) and p_{znt}Rluc (b) sensors in group 1 to Cd-Pb mixtures and of p_{ars}Rluc (c) sensor in group 2 to As-Cd mixtures

Fig. 3 Cd (a), Pb (b), and As (c) concentrations in soil samples P1–P7. Error bars mean the standard deviations of the three individual results

than the quality standard of agricultural land in China (GB15618-2008). Hence, all the seven soil samples were seriously polluted by Cd, Pb, and As as judged using the quality standard.

When the conventional single whole-cell sensor was used to measure these environmental samples, with the exception of Cd in P5, the bioavailable concentrations of Cd, Pb, and As were higher than their corresponding total water-soluble concentrations (Fig. 3) due to the presence of multiple interferents and the sensors' non-specificity. Statistically, the bioavailable concentrations of Cd, Pb, and As were respectively 1.45–3.34-fold, 6.61–27.67-fold, and 1.6–2.02-fold of their corresponding total water-soluble concentrations. Results also showed that the effect of sample impurity in soil water extractions on bioluminescence emission of sensor could be negligible (ESM Table S3). Obviously, because of the nonspecific response of core regulator protein, the results, especially the bioavailable Pb, were interfered and overrated by other metals. The interference strength can also be demonstrated by the coefficient ratio in Eqs. 1–3, in which it ($\text{coefficient}_{c_{pb}}/\text{coefficient}_{c_{cd}}$, $\text{coefficient}_{c_{cd}}/\text{coefficient}_{c_{pb}}$, $\text{coefficient}_{c_{cd}}/\text{coefficient}_{c_{As}}$) was 1.51, 2.26, and 0.11, respectively. Therefore, the strength order of suffered interference in the three single sensors was $RLU_{pzntRluc} > RLU_{pcadCluc} > RLU_{parsRluc}$.

In contrast, the Cd bioavailable concentration determined using group 1 ($pcadCluc$ and $pzntRluc$) was 29.6–69.8 % (mean 41.3 %) of that determined using $pcadCluc$ alone, and the Pb bioavailable concentration determined using group 1 ($pcadCluc$ and $pzntRluc$) was 8.4–16.0 % (mean 11.2 %) of that determined using $pzntRluc$ alone. Similarly, the As bioavailable concentration determined using group 2 ($parsRluc$) in P2 and P5 was 60.9 and 52.9 % (mean 56.9 %), respectively, of that determined using $parsRluc$ alone (Fig. 3). These results indicated that, with the single sensor, the Pb bioavailability was significantly overestimated by the Cd interference, whereas the Cd and As bioavailability was partly overestimated by the interference of Pb and Cd. However, with the sensor set, the specific determination of bioavailable Cd, Pb, and As could be realized and this can provide a significant step towards accurately assessing the actual bioavailable concentration of each toxic element in a co-polluted environment.

The bioavailable concentrations of Cd, Pb, and As determined using the sensor groups were lower than their corresponding total water-soluble concentrations, and these results also supported that the bioavailable concentration of Cd, Pb, and As determined with the sensor set was effective and dependable. In detail, the fractions of Cd, Pb, and As that are bioavailable, according to the sensor groups, were 59.1–90.7, 38.2–78.1, and 77.1–85.8 %, respectively, of their total water-soluble contents for P1 to P7. These findings are not unexpected since they can be explained by the presence of metal chelator in the soil water extraction, such as dissolved organic

matter and other metal chelators, which could sorb or bind heavy metals but non-bioavailable, thereby decreasing their bioavailability [12–14].

Conclusion

Based on the conventional whole-cell sensor technique, we developed a novel method that could effectively distinguish and eliminate the interference of multiple elements (Cd, Pb, and As) with tailored microbial whole-cell sensor set, and then applied this for the specific and accurate detection of bioavailable Cd, Pb, and As. The test results that indicated actual bioavailable Cd, Pb, and As calculated using a sensor set were 41.3, 11.2, and 56.9 %, respectively, of the value calculated using a single sensor. In addition, the results based on a sensor set were comparable with the results from ICP-MS analysis. This method could be a significant improvement for the rapid assessment of the actual bioavailability and risk of toxic element in co-polluted environments. The exhaustive arithmetical process we used can also be adapted to measure other heavy metals such as Hg, Zn, and Cu, in complex soil and water samples.

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Conflict of interest The authors declare that they have no competing interests.

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