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Title: Magnet bioreporter device for ecological toxicity assessment on heavy metal contamination of coal cinder sites

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Keywords: whole-cell bioreporter; magnetic nanoparticles (MNPs); magnet bioreporter device; heavy metal; toxicity

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Abstract: A novel magnet bioreporter device was developed in this research for soil toxicity assessment, via magnetic nanoparticles functionalized whole-cell bioreporters. The whole-cell bioreporter ADPWH_recA kept response capability to DNA damage after magnetic nanoparticles (MNPs) functionalization, and could be harvested from soil samples by permanent magnet to reduce the soil particle disturbance. Compared to conventional treatments applying bioreporter directly in soil-water mixture (SW-M treatment) or supernatant (SW-S treatment), MNPs functionalized bioreporter via the magnet device (MFB) treatment achieved high sensitivity to evaluate the toxicity and bioavailability of chromium contamination in soils from 10 mg/kg to 5,000 mg/kg soil dry weight. The MNPs functionalized bioreporter also achieved high reproducibility with pH value from 5.0 to 9.0, salinity from 0% to 5% and temperature from 20°C to 40°C. A case study was carried out on the ecological toxicity assessment of heavy metal contamination at the coal cinder site via the magnet bioreporter device. The heavy metal toxicity declined with the increasing distance to the coal cinder point, and a significant accumulation of heavy metal toxicity was observed along the vertical distribution. No direct link was found between the pollution load index (PLI) and heavy metal toxicity, and the results suggested the bioreporter test monitored the toxicity of heavy metals in soils and was an important approach for ecological risk assessment. Magnet bioreporter device also offered the high throughput biological measurement and was feasible for in situ monitoring.

Cover letter

To:
Editor of Sensor and Actuators B: Chemical

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Dear Editor

I would like to submit this manuscript, entitled "*Magnet bioreporter device for ecological toxicity assessment on heavy metal contamination of coal cinder sites*", for the consideration in *Sensor and Actuators B: Chemical*. The heavy metals leaching from coal cinder have severely contaminated soils in China and its toxicity was hard to be evaluated on the field by traditional chemical analysis. This paper published a novel concept of magnetic bioreporter device to improve the sensitivity and reliability of whole-cell bioreporter in soil measurement. Functionalized with the magnetic nanoparticles, the whole-cell bioreporter could sense the toxicity of soils in situ and subsequently isolated by magnetic field for bioluminescent signal detection. The magnetic bioreporter device was further applied for the toxicity assessment on a coal cinder sites in China. The results illustrated the horizontal/vertical toxicity distribution and its correlation with pollution load index. This novel magnetic device benefitted the biosensor application on the field, particularly contributing to the soil measurement, with high feasibility in practical environmental monitoring.

No conflict of interest exits in the submission of this manuscript, and the manuscript has approved by all authors for publication. The authors would like to declare that the work described is original research that has not been published previously, and is not under consideration for publication elsewhere, in whole or in part.

Thanks for your consideration. If you have any questions, please feel free to contact with me.

Yours sincerely

Dr Dayi Zhang

Reviewer #1: The paper describes magnetic bioreporter device for ecological toxicity assessment on heavy metal contamination of coal cinder sites. However, before it could be publish, some points need to be addressed as follows:

3. Results and discussions

No discussion on optimization parameter of magnetic whole-cell bioreceptor based, such as pH, temperature, medium etc. Please give comment on this issue.

Answer:

Thanks for the comments and the author has carried out more research work on the impacts of pH and temperature. All these data are included in the reproducibility part.

3.1. Higher sensitivity ...

Please give a mechanism of whole-cell bioreceptor toward heavy metals.

In this subtitle, it could be better if the analytical characteristic is summarized in Table, in term of linear range, sensitivity, LOD, reproducibility etc.

What was the life time of this magnetic bioreporter cells?

Answer:

The mechanisms of whole-cell bioreporter's response to heavy metal are the SOS response activated by the DNA damage. The author has added more description and discussion on this issue. The additional table (Table 1) is added to summarize the main features of the magnetic bioreporter.

3.2. Heavy metal ...

The heavy metal profile in Table 1 needs to added with information regarding the instrumentation used for the determination of heavy metal listed.

Answer:

The analytical instrument for Hg is DMA-80 Hg analyzer (Milestone S.r.L., Italy) and the other elements were analysed by ICP-MS (X series II, Thermo Fischer Scientific, USA). The information has been listed with detailed detection procedure in the

materials and methods section. The author has added in the note of the table for better description in accordance with the comments.

Other comment

It could be better if the performance of the proposed magnetic bioreporter cells could be compared with other toxicity assessment on heavy metals.

Answer:

Thanks for this good comments and the performance of ADPWH_recA has been tested and compared to other toxicity assessment in our previous research (Y.Z. Song, G.H. Li, S.F. Thornton, I.P. Thompson, S.A. Banwart, D.N. Lerner, et al., Optimization of Bacterial Whole Cell Bioreporters for Toxicity Assay of Environmental Samples, *Environ Sci Technol*, 43(2009) 7931-7938). Sharing the same mechanisms of SOS response, the author believes the magnetic bioreporter has the same performance comparing to other toxicity assay. The authors have added some more discussions in the revised manuscript.

Reviewer #2: This manuscript presented a magnet bioreporter device for soil toxicity assessment by magnetic nanoparticles functionalized whole-cell bioreporters. The whole-cell bioreporters with magnetic nanoparticles functionalization could be seperated from soil samples to reduce the soil particle interference. Although this work is innovate, major revision should be performed before this manuscript can be accepted for publication.

1. Why did the author choose the whole-cell Acinetobacter ADP1_recA reporter for toxicity assessment? And why did the author choose chromium as the target analyte? Did the reporter represent any specificity to chromium? Did other heavy metals have genotoxic effects on the reporter?

Answer:

Various whole-cell bioreporter can be applied for toxicity test, like *E. coli*, but they are not suitable for soil detection mainly due to two reasons as induction condition and response stability. First of all, the induction condition for *E. coli* is 37 degree,

which is much higher than natural temperature in soils. *Acinetobacter* can survive and response to carcinogens from 15 degree to 37 degree, allowing the toxicity assessment at the real soil temperature. Secondly, the reporter gene in *E. coli* is on the plasmid, requiring antibiotics to maintain the plasmid copies and responsive sensitivity. The reporter gene in *Acinetobacter* is on the chromosome, which is single copy and stable with no need of antibiotic cultivation. From these reasons, the author suggests that *Acinetobacter* is suitable candidate as bioreporter strain for environmental monitoring and assessment.

From the chemical analysis of heavy metal profiles in the contaminated soils, chromium is the major contaminants and the highest pollutant level compared to other heavy metals. For this reason, chromium is chosen as the targeting analyte to investigate the biological response and the feasibility that the magnetic biosensor device can be applied for heavy metal contamination evaluation. The description was added in the materials and methods section.

From our previous work, ADPWH_recA responds to various carcinogens, including mitomycin C, UV light, ethidium bromide and H_2O_2 (Y.Z. Song, G.H. Li, S.F. Thornton, I.P. Thompson, S.A. Banwart, D.N. Lerner, et al., Optimization of Bacterial Whole Cell Bioreporters for Toxicity Assay of Environmental Samples, *Environ Sci Technol*, 43(2009) 7931-7938). From the SOS response mechanisms, it is not a specific bioreporter to sense targeting chemical molecules, but responding to the generic SOS activation of *recA* gene. It is obvious that other heavy metals might also cause the bioluminescent response, which is undergoing. Current work shows that ADPWH_recA only represents positive response to Zn and Be. Further work is still undergoing and the outcome might be discussed in future publication. The author thanks reviewers' comments and added more discussions in the revised manuscript addressing the details shown above.

2. When detecting real soil samples by the whole-cell bioreporter, no extra preprocessing produres were introduced. Is it possible that some substances in samples affect the bioreporter during the incubation and further interfere the toxicity evaluation? How did you solve the interference problem?

Answer:

From our previous work on toxicity assessment on soils (Y.Z. Song, G.H. Li, S.F. Thornton, I.P. Thompson, S.A. Banwart, D.N. Lerner, et al., Optimization of Bacterial Whole Cell Bioreporters for Toxicity Assay of Environmental Samples, *Environ Sci Technol*, 43(2009) 7931-7938; B. Jiang, D. Zhu, Y. Song, D. Zhang, Z. Liu, X. Zhang, et al., Use of a whole-cell bioreporter, Acinetobacter baylyi, to estimate the genotoxicity and bioavailability of chromium(VI)-contaminated soils, Biotechnol Lett, 37(2015) 343-348), some substances (organic matters, other heavy metals, etc.) in soils significantly affected the bioavailability of heavy metals and they are part of the soil meso-environment determining the ecological risks of heavy metal contamination. As for other environmental factors, like pH, temperature and salinity, are further discussed with new experiment in the revised version. From the data, all these factors will not influence the bioreporter response within the soil properties. The author thinks that the interference will not affect the toxicity assessment by whole-cell bioreporter.

3. How did the reporter attach to the magnetic nanoparticles? Is the magnetic nanoparticles coupled with any chemical groups?

Answer:

The magnetic nanoparticles were attached on the membrane of whole-cell bioreporter by electrostatic attraction between Fe-OO- and amino-groups. More discussions are made in the revised version.

4. In figure 4, the ordinate is the "bioluminescence response ratio", and in figure 5, the ordinate is the "relative bioluminescence response ratio". In the text, the two words were also used alternatively. Did these two words have different meanings or not?

Answer:

Thanks for the comments and the author misrepresented the phrase. It should be “relative bioluminescence response ratio” (as defined in the Materials section) throughout the manuscript. The author has corrected all the mistakes.

5. Why the MFB present positive bioluminescent response to the low chromium concentration in soil and negative response to high chromium concentration? Why are the simulation curves parabolic shaped? And how could the toxicity of the contaminated soils be predicted by the curves? According to the curve, even under the treatment to two samples in different chromium concentration, the reporter may present same bioluminescent response. The author should explain it in detail.

Answer:

Chromium has both cytoxicity and genotoxicity on living microorganisms. At lower concentration, the cytoxicity is not obvious and would not affect bacterial growth, and the positive relationship between chromium amount and bioluminescent response was therefore observed. At higher concentration (>100 mg/kg dry soil weight in this study), the cytoxicity of chromium became dominant and significantly affected cell growth and activity. The bacterial growth curve was significantly supressed by the high chromium concentration. The author has added the growth curve against different concentration of chromium (Fig. S1). The results showed that no growth change was observed when the chromium concentration was less than 100 mg/kg and this is the range for positive relationship between bioluminescent response and chromium contamination. At higher level, the cell growth and activity was inhibited, causing the dramatic loss of bioluminescent signal. The synergetic effects of cytoxicity and genotoxicity were then the main reason causing the parabolic curve.

To determine the real toxicity, both relative bioluminescent response ratio and growth curve were considered. If the growth was not affected, the bioluminescence data belonged to the positive relationship range, and it was on the opposite when the growth was inhibited. The author has added more detailed discussion in the revised version.

6. What is the detection limit and linear range of the magnet bioreporter device?

Answer:

From our previous publication (Jiang, B., Zhu, D., Song, Y., Zhang, D., Liu, Z., Zhang, X., Huang, W.E. and Li, G.*, (2015) Use of a whole-cell bioreporter, Acinetobacter baylyi, to estimate the genotoxicity and bioavailability of chromium(VI)-contaminated soils, Biotechnology Letters, 37(2), 343-348), the limit of detection is 130 mg/kg soil and the linear range is 130-5200 mg/kg (ultrasonic treatment). Without pretreatment, the response of ADPWH_recA to chromium contaminated soils is too weak to be quantified, similar as our research results. The test of this magnet bioreporter work demonstrated a higher sensitivity that the limit of detection is 1 mg/kg soil and the linear range is 1-100 mg/kg. Compared with the bioreporter growth data, the detection range of the magnetic device can achieve 1-5000 mg/kg. The author has added the discussion and summarized the limited of detection and other key parameters in Table 1.

7. In figure 4, why did the response ratio not fit well with the model prediction of 10% chromium bioavailability when chromium concentration was below 100 mg/kg soil dry weight?

Answer:

The bioavailability of chromium changes with the contamination level. With lower chromium in the soils, different bioavailability will be observed due to different adsorption by soil particles. The author thought it is the main reason causing the unexpected bioluminescent response ratio when chromium concentration was <100 mg/kg soil. Some further discussion was addressed on this issue in the revised version.

8. In figure 5, the authors concluded that" the toxicity of heavy metals declined with the increasing distance to the coal cinder point" because "Except for 0 m point, the bioluminescence response ratio dropped from 1.47 (10 m) to 1.10 (150 m) in the surface soil, 1.34 (10 m) to 1.16 (150 m) in the middle soil, and 1.26 (0 m) to 0.58 (150 m) in the bottom soil." However, did lower bioluminescence response ratio really mean lower toxicity? Please combine the results obtained from figure 4 to explain the correlations between toxicity and bioluminescence response ratio.

Answer:

Thanks for the comments. As discussed for the bioreporter growth curve, whether the growth is inhibited can be the indicator to determine the toxicity range of soil samples. From Fig. S4, all the bioreporter growth curve behaved similar without any inhibition, and the results indicated that no strong cytoxicity was found in the heavy metal contaminated soils. Located in the linear relationship range, higher relative bioluminescent response ratio represented higher ecological toxicity. The author has revised the discussion in the new manuscript.

9. What about the reproducibility of magnetic nanoparticles functionalized whole-cell bioreporters?

Answer:

Thanks for the comments and it is the authors' mistake missing the description for the reproducibility test. In our experiment, three biological replicates were carried out for each test, and five individual tests were repeated for the same sample sets to evaluate the reproducibility. The data and error bar on each graph were the mean and standard error of five individual tests. The author has corrected the sentence in the materials and methods section.

10. Since the soil ecological risks were not associated with neither the load of PC1 nor PC2, the circles in figure 6 is needless and should be deleted.

Answer:

Thanks for the comments and the author has moved Figure 6 to Supplementary Material as supporting information for the manuscript.

Reviewer #3: This paper reported a magnet biosensor for soil toxicity monitoring. It was stated that this biosensor "offered the high throughput biological measurement and was feasible for in situ monitoring".
This reviewer would like to suggest some revisions below.
1. Replicates and errors. No related data were given in this draft. The authors

should perform some efforts focusing on replicates and sensing errors.

Answer:

For all the tests, three biological replicates were carried out. All the data in the graph were the average of replicates with error bars. The information was in Section 2.2.

2. What was the repeatability of this biosensor. What was the testing error between biosensors of different lots?

Answer:

Thanks for the comments and it is the authors' mistake missing the description for the reproducibility test. Actually, three biological replicates were carried out for each sample, and five individual tests were repeated for the same sample sets to evaluate the reproducibility. The data and error bar on each graph were the mean and standard error of five individual tests. The author has corrected the sentence in the materials and methods section.

3. Did the authors considered the influence from other competing ions, pollutions and moisture on your biosensor?

Answer:

The author has added the influence of salinity on the bioreporter's performance and the results indicated <4% salinity did not affect its response ratio. During the bioluminescence measurement, the soils were saturated and the original moisture of soils will not affect the assessment results.

From the mechanisms of SOS response and ADPWH_recA bioreporter, it responded to all the DNA damage. Thus, any other carcinogen will also cause the positive response of bioreporter. However, the chromium was just chosen as the target analyte since it is the main heavy metal contamination at the sites. The MNPs functionalized bioreporter actually evaluate the synergetic toxicity of all the contaminants in the environmental samples. The author has added more discussion in the revised manuscript.

4. The authors should try more practical samples to testify the accuracy and practicability of this biosensor.

Answer:

Thanks for the comment. The author wanted to collect more samples in different direction and at different distance and depth, to further test the reproducibility and reliability of the magnetic biosensor by more sample testing. However, on the north and west to the cinder sites, nearly all the surface is the hard surface (within the main chemical production area) and no appropriate soils can be collected. The east and south soils were near to the road and the soils might be contaminated by the coal transportation activities. Furthermore, the local company did not allow us to take samples below 50 cm to protect the underground pipeline. From local investigation, we have tried out best to choose the most appropriate sampling line and sampled the maximal samples for the work, as illustrated in the figure.

More tests were carried out according to reviewers' suggestions, and the impacts of pH, salinity and temperature were comprehensively discussed in the revised version.

To further testify the accuracy and practicability, the authors have more samples collected from different cinder sites around China (about 70 samples from 5 companies), and further work will focus on the relationship between heavy metal profiles, biosensor results and microbial community, to deeper understand how the magnetic biosensor device can be applied to assess the toxicity and how the toxicity has affected indigenous microorganisms. In this paper, our main goal is to prove the feasibility of magnetic bioreporter device at one site, and it will be used as a technical tool for more samples to illustrate the ecological impacts of coal cinder in the next step.

Magnet bioreporter device for ecological toxicity assessment on heavy metal contamination of coal cinder sites

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Abstract

A novel magnet bioreporter device was developed in this research for soil toxicity assessment, via magnetic nanoparticles functionalized whole-cell bioreporters. The whole-cell bioreporter ADPWH_recA kept response capability to DNA damage after magnetic nanoparticles (MNPs) functionalization, and could be harvested from soil samples by permanent magnet to reduce the soil particle disturbance. Compared to conventional treatments applying bioreporter directly in soil-water mixture (SW-M treatment) or supernatant (SW-S treatment), MNPs functionalized bioreporter via the magnet device (MFB) treatment achieved high sensitivity to evaluate the toxicity and bioavailability of chromium contamination in soils from 10 mg/kg to 5,000 mg/kg soil dry weight. The MNPs functionalized bioreporter also achieved high reproducibility with pH value from 5.0 to 9.0, salinity from 0% to 5% and temperature from 20°C to 40°C. A case study was carried out on the ecological toxicity assessment of heavy metal contamination at the coal cinder site via the magnet bioreporter device. The heavy metal toxicity declined with the increasing distance to the coal cinder point, and a significant accumulation of heavy metal toxicity was observed along the vertical distribution. No direct link was found between the pollution load index (*PLI*) and heavy metal toxicity, and the results suggested the bioreporter test monitored the toxicity of heavy metals in soils and was an important approach for ecological risk assessment. Magnet bioreporter device also offered the high throughput biological measurement and was feasible for *in situ* monitoring.

Key words: whole-cell bioreporter, magnetic nanoparticles (MNPs), magnet bioreporter device, heavy metal, toxicity

1. Introduction

Heavy metals are the key anthropogenic environmental contaminants, mainly caused by industrial activities [1, 2]. All around the world and particularly in China, numerous heavy metal contaminated sited are found due to the improper disposal of various chemical wastes [3], including coal cinders [4], and the key pollutants include chromium, mercury, arsenic, lead, cadmium, manganese, cobalt, copper, nickel and zinc. They have high mobility through the leachate and further contaminate the biospheric soils [5, 6], with respective carcinogenic, teratogenic and mutagenic effects [7]. The high level of heavy metal in soils threatens the ecological system [8], poses potential risks to human health [9] and draws attention on early warning for potential cancer induction [10]. Due to the complex composition and synergetic effects in soils, traditional chemical and physical analysis only provides the amount information [3], but the toxicity and bioavailability of heavy metal contamination from coal cinder are hard to be evaluated.

Recently, whole-cell bioreporter has become initiative and legislative tool for environmental monitoring, with capability to sense the bioavailability and toxicity of contaminated water and soil samples [11]. With genetically engineered bacteria, yeast, fungi, or animal cells, the biological signals of whole-cell bioreporter are initiated by phenotypic color (*lacZ*), fluorescent (*gfp*/*yfp*) or bioluminescent (*luc*/*lux*) genes [12-14]. It offers highly sensitive, rapidly analytic, easy operation and cost-effective feasibility for *in situ* pollutants assessment [15]. Some whole-cell bioreporter specifically senses the heavy metal molecules [16, 17] or their cytotoxicity/genotoxicity [18].

Though the application of whole-cell bioreporter in water sample is successful, it suffers from the heterogeneous features of soils [19]. Exposed to whole-cell bioreporter, the soil particles will absorb the bioluminescent signal (*lux* or *luc*) or give strong fluorescent interference (*gfp*). Some recent work has assessed the bioavailability and toxicity of copper [20], cobalt and nickel [21] via direct exposing the whole-cell bioreporter to the soils [22], but the biological sensitivity and specificity are significantly reduced. Some pre-treatments, like water extraction or ultrasonication, are therefore applied to transfer contaminants into aqueous phase for biological analysis [23]. Particularly for heavy metal, the aqueous extraction has been used for whole-cell bioreporters to sense the bioavailability of chromium [24],

mercury [16], lead and cadmium [25, 26] in soils. Nevertheless, the main drawback is the neglect of the real occurrence of pollutants in the porous soil [17]. Technically, a new type of bioreporter device is required to sense the soil contaminants *in situ* and effectively separate the living reporter cells from the soil particles for biological signal detection. Magnetic nanoparticles (MNPs) functionalization offers the feasibility of magnetic remote control and is biocompatible for whole cell bioreporter [27]. Its equipping and portability for *in situ* monitoring is still under development and required further research.

In this work, a novel magnet bioreporter device was developed and optimized for effective monitoring and assessment of coal cinder contaminated soils. With whole-cell *Acinetobacter* ADP1_recA reporter [28], the magnet device effectively reduced the impacts of soil particles and improved the sensitivity and reproducibility, comparing to the direct exposure of bioreporters to the soils. The MNPs functionalized bioreporter was able to evaluate the ecological toxicity of heavy metal contamination, via the high throughput and easy operation magnet device. This work showed the feasibility and potential of *in situ* environmental risk assessment via whole-cell bioreporter for the coal contaminated sites.

2. Material and methods

2.1 Bioreporter strain and incubation

In this research, the *Acinetobacter baylyi* ADPWH_recA whole-cell bioreporter was introduced for environmental ecological toxicity evaluation [28, 29]. Compared to other plasmid based or *Escherichia coli* hosted toxicity bioreporter, the reporter gene was located on the chromosomal with high stability and *Acinetobacter* was soil bacterium to tolerate the ambient soil environment and achieve high sensitivity. After cultivation in Luria-Bertani (LB) medium overnight at 30°C, the 10.0 mL ADPWH_recA cells were harvested by 3,000 rpm centrifugation for 10 minutes. The bioreporter pellets were further washed by deionized water and resuspended in 10 mL deionized water for magnetic nanoparticles functionalization or 10 mL fresh MMS medium for toxicity measurement. The 1.0 litre MMS medium contained 1.0 g $(NH_4)SO_4$, 2.5 g KH_2PO_4, 0.1 g $MgSO_4{\cdot}7H_2O$, 0.005 g $FeSO_4{\cdot}7H_2O$, 0.25 g nitrilotriacetic acid (NTA), 0.55 g NaOH, 3.24 g sodium succinate (20 mM) and 1 ml Bauchop and Elsden solution.

2.2 Direct toxicity measurement on soil samples

The direct toxicity measurement of soil samples were conducted for the supernatant of soil/water mixtures (SW-S) and the bulk soil/water mixtures (SW-M) respectively. For SW-S treatment, the 100 mg soil sample was suspended in 1 mL deionized water and homologized by 150 rpm shaking for 10 min. The 20 μL supernatant and 180 μL bioreporter suspensions were added into each well of a black clear-bottom 96-well microplate. For SW-M treatment, the 1.0 mL bioreporter suspensions were added into 100 mg soil sample, and the mixture was directly transferred into the microplate. The incubation and induction was conducted at 30°C for 4 hours, in the Spectra M5 Plate Reader (Molecular Devices, USA). Three biological replicates were carried out for each sample and the bioluminescent signal was measured every 10 minutes with 5 seconds shaking before each reading.

2.3 Magnetic nanoparticles synthesis and functionalization

All the chemicals in this study were analytical grade and purchased from Sigma Aldrich (UK) without specific statement. Magnetic nanoparticles synthesis followed chemical deposition method [27]. The 12.5 mL NaOH (1.5 M) was added dropwisely into the mixture of 1.0 mL $FeCl_3$ (2 M in 2 M HCl) and 0.5 mL $FeCl_2$ (1 M in 2 M HCl) with 40k Hz ultrasonic homologization, until the appearance of dark iron oxide precipitates. The precipitates were further separated by permanent magnet and washed with deionized water until the supernatant reached pH=7.0.

For bioreporter functionalization, 10 mL bioreporter suspensions (in deionized water) were mixed with 1 mL MNPs suspension, incubated at room temperature for 10 minutes with 150 rpm shaking. The MNPs-bioreporter was subsequently harvested by a permanent magnet and washed twice by deionized water. The magnetized bioreporter was finally suspended in 10 mL MMS medium for soil toxicity assessment via the magnet bioreporter device.

2.4 Magnet bioreporter device and operation

The magnet bioreporter device contained the magnet probe assay and plastic cover for bioreporter strain transfer, as illustrated in Fig. 1a. The magnet probe assay was assembled by 96 magnet probes (1 cm length and 3 mm id), fixed on the plastic base and patterned (20.66 mm distance between each magnet probe) for the high throughput bioreporter measurement on the 96-well microplate. For MNPs

functionalized bioreporter via the magnet device (MFB treatment), the 1.0 mL bioreporter suspension was mixed with 100 mg soil samples and transferred into each well of the 96-well microplate (Fig. 1 b-1). For the determination of the best cultivation time before magnetic separation, the bioreporter cells were magnetically harvest at 0, 15, 30, 45, 60, 75, 90, 105 and 120 min. The recovered MNPs functionalized bioreporter was counted by plate count and the bioluminescent response was also measured.

After incubation at 30°C for 1 hour as the optimal cultivation condition, the magnet probe assay (with plastic cover) was emerged into the reaction system for 30 seconds (Fig. 1 b-2). The bioreporter cells were then separated from the soil suspension and attached on the plastic cover by magnetic field. The magnet device was transferred and emerged in another 96-well microplate, supplemented with 200 μL fresh MMS medium (Fig. 1 b-3). Removing the magnet probe, the plastic cover and microplate was incubated at 30°C for 5 minutes with 150 rpm shaking (Fig. 1 b-4). The bioreporter cells were resuspended in the fresh MMS medium and the bioluminescence was further measured on the Spectra M5 Plate Reader (Molecular Devices, USA). The detection and data analysis followed the same instruction for the direct toxicity measurement on soil samples.

For reproducibility test, MNPs-bioreporter was applied to sense the toxicity of 100 mg/kg chromium contaminated soils in the medium with different pH values and salt contents. The pH value in the induction medium was adjusted by 1.0 M HCl or 1.0 M NaOH solution as 4.0, 5.0, 6.0, 7.0, 8.0, 9.0 and 10.0. The series of salty medium was prepared by adding NaCl into the MMS medium with the final concentration of 1%, 2%, 3%, 4%, 5% and 10%. As for the temperature influence, the temperature during induction period was controlled by the Spectra M5 Plate Reader at 10°C, 15°C, 20°C, 30°C, 37°C, 40°C and 45°C. To evaluate the life-time of MNPs-bioreporters, the bioreporter suspension was stored at 4°C and taken out for direct toxicity measurement without any pre-treatment.

2.5 Toxicity model for heavy metal contaminated soils

For the calibration of heavy metal toxicity in soils, chromium was chosen at the target analyte since it was the main heavy metal contamination at the coal cinder site. The artificial chromium contaminated soils were prepared by mixing 1.0 g

uncontaminated soil with 1 mL potassium bichromate solution with the concentration of 0, 0.1, 0.2, 0.5, 1, 2, 5, 10, 20, 50, 100, 200, 500, 1000, 2000 and 5,000 mg/L. The soil slurry was air dried in the laminar hood. The chromium contamination in soils was 0, 0.1, 0.2, 0.5, 1, 2, 5, 10, 20, 50, 100, 200, 500, 1000, 2000 and 5,000 mg/kg soil dry weight, respectively.

The genotoxicity of hexavalent chromium in soil was identified as several mechanistic processes, including mutagenic effects on DNA and direct inductive immunological responses [30]. Such genotoxic effects consequently caused the accumulation of single stranded DNA (ssDNA) and the activation of SOS response for damaged DNA repair. The SOS response (bioluminescent response of ADPWH_recA) is the function of LexA-like SOS repressor (*LSR*, cell^{-1}) and can be simulated by the previous gene expression model [31, 32], as shown in Equation (1).

$$SOS_{r,s} = 1 + \left(\frac{k_{dSLR}}{2\cdot(1+k_{ssDNA})} \cdot [LSR]\right) \cdot \frac{[Cr]}{(K_{mLSR}\cdot K_{Cr}\cdot k_{ssDNA}\cdot k_{Cr})^{-1}+[Cr]} \quad (1)$$

Here, $SOS_{r,s}$ (SOS response ratio) is dependent on the hexavalent chromium contamination level in soils ($[Cr]$, mg/kg). K_{Cr} is the isotherm equilibrium of chromium-DNA adduct (DNA phosphodiester backbone with chromium) and k_{Cr} represents ssDNA generation constant from the chromium-DNA adduct (L/(cell·g) chromium). k_{dSLR} represents the equilibrium coefficient of *LSR* dimer ($dSLR$, cell^{-1}) and monomer ($mLSR$, cell^{-1}) and k_{ssDNA} represents the cleavage reaction constant of LSR dimer. K_{mLSR} is the dynamic gene expression (SOS response) level activated by *LSR* monomer.

2.6 Sites description

A total of 16 soil samples were taken from the methanol plant of Yulin Energy and Chemical Industry, Yanzhou Coal Corporation, China (Fig. 2). The site (698,000 m^2) was located in Yulin Shaanxi Province (N38°34’41.9’’, E109°55’50.4’’), in the junction of Maowusu Sandy Land and the Loess Plateau. The annual coal consumption was 31,200 tonnes and the soils have been seriously contaminated by the coal with high heavy metal content. The sampling sites were designed along the leeward direction of the cinder heap, with the distance of 0, 10, 50, 80 and 150 m. The uncontaminated soil sample was collected in the living area of the plant, 500 m away from the heap. At each point, the soils were sampled at different depth of 0-20 cm

(surface soil), 20-35 cm (middle soil) and 35-50 cm (bottom soil) to evaluate the toxicity profiles caused by the trace metal transportation.

2.7 Chemical analysis

Before chemical analysis, all of soil samples were seized by 200 mesh. Mercury was determined by DMA-80 Hg analyzer (Milestone S.r.L., Italy). For other trace elements, the samples were digested in an UltraCLAVE microwave high pressure reactor (Milestone S.r.L., Italy), containing 330 mL distilled H_2O, 30 mL 30% H_2O_2 and 2 mL 98% H_2SO_4 as the digestion solution [33]. With 50 bars initial nitrogen pressure, the microwave digestion program was listed in Table S1. Further digestion for 50 mg soil sample was conducted in 5 mL 40% HF, 2 mL 65% HNO_3, and 1 mL 30% H_2O_2 [34]. The inductively coupled plasma mass spectrometry (ICP-MS, X series II, Thermo Fischer Scientific, USA) was used for the determination of the trace elements in a pulse counting mode (three points per peak). In this study, the multi-element standards (Inorganic Ventures, CCS-1, CCS-4, CCS-5, and CCS-6) were referenced for the calibration of trace element concentrations. As and Se were determined by ICP-MS with collision cell technology (CCT) due to their volatility [35]. Polyfluoroalkoxy volumetric flasks were used without drying on electric hot plate to avoid As/Se volatile loss. With the 1 μg/L tuning solution, the torch position and ion lenses were optimized before real sample measurement. The optimal parameters of the ICP-CCT-MS and calibration curves of As/Se were listed in Table S2 and S3.

2.8 Data analysis

The bioluminescence response was calculated by averaging the bioluminescent signal from the 7 time points between 180 and 240 minutes for each well. The relative bioluminescence response ratio was the specific value of the bioluminescence response of contaminated soil samples to that of the uncontaminated soils. The heavy metal profiles in soil samples were statistically analysed by SPSS software (Version 15.0 for Windows) via Principal Component Analysis (PCA). The equality and normality of data were tested by Brown-Forsythe and Shapiro-Wilk test respectively, and the null hypothesis was rejected for $p<0.05$.

Contamination factor (*CF*) is defined as the ratio of the heavy metal concentration in the sample soil to the baseline concentration in background soil, as shown in Equation

(2) [36]. Pollution load index (*PLI*) is determined as the n^{th} root of the *n* CF in Equation (3) [36]. The *CF* and *PLI* are empirical indices to evaluate the level of heavy metal contamination, and the higher values indicate heavier contamination of individual and multiple heavy metals respectively.

$$CF = \frac{[Heavy\ metal\ in\ sample\ soils]}{[Heavy\ metal\ in\ background\ soils]} \quad (2)$$

$$PLI = (CF_1 \times CF_2 \times \cdots \times CF_n)^{1/n} \quad (3)$$

3. Results and discussions

3.1 Higher sensitivity and reproducibility of magnet bioreporter device

The MNPs functionalized bioreporter could be magnetic remote controlled for effectively separation from the soil particles. The MNPs were biocompatible, and the viability and bioluminescent signal of whole-cell bioreporter remained over 99% comparing to the native bioreporter cells [27]. With the strong electrostatic attraction between the negative iron oxide (Fe-OO^-) and positive amino-groups (-NH_3^+) on bacterial membrane, the separation effectiveness by magnetic field was above 99.6% and the synthesized MNPs had neither cytotoxicity nor genotoxicity on bacterial bioreporter cells [37]. MNPs functionalized whole-cell bioreporter therefore had the feasibility to sense the toxicity of soil samples *in situ* and subsequently isolated for bioluminescent signal measurement.

Due to the cell division, the MNPs functionalized bioreporter gradually lost their magnetic capacity [38]. Though longer incubation with soil samples could improve the chemical uptake by bioreporter cells for higher responsive ratio, the less recovery rate consequently resulted in lower bioluminescent signal and lower sensitivity. Fig. 3 illustrated that, within 45 minutes incubation, over 90% living bioreporter cells were isolated from the soil/water mixture based on plate count. The results fitted with previous study that about 12% free bioreporter cells were observed after 120 min cultivation in rich medium [27]. As for the bioluminescence and relative response ratios, the bioluminescent signals were stable from 3600 RLU to 3800 RLU when the incubation time was less than 75 minutes, and the response ratio ranged from 1.90 to 2.00. The results suggested a highly reliable responsive period between 30 and 70 minutes. The optimal incubation time for sufficient bioreporter cell recovery and high

response sensitivity was identified as 60 minutes, and it was further applied in the following work on calibration curve and real soil sample assessment.

The summarized features of the magnetic ADPWH_recA whole-cell bioreporter were listed in Table 1 from the reproducibility test. After 1 hour pre-incubation of the MNPs functionalized bioreporter, the cells were captured by permanent magnet and resuspended in fresh medium without soil disturbance for another 4 hours. As a soil bacterium, ADPWH_recA had strong tolerance to the environmental variations and maintained high reproducibility under different pH, salinity and temperature condition. The relative bioluminescent response ratio maintained stable (1.44 to 1.51) when pH value ranged from 5.0 to 9.0, dramatically dropping to 1.25 at pH=4.0 and 1.12 at pH=10.0 (Fig. 4a). The results were similar to previous research on the pH influence on *Acinetobacter baylyi* ADP1 that *Acinetobacter* based bioreporter could tolerate large pH variation [39]. Fig. 4b also illustrated the good responsive performance of MNPs functionalized ADPWH_recA at 20°C (relative bioluminescent response ratio=1.47), 30°C (relative bioluminescent response ratio=1.50) and 37°C (relative bioluminescent response ratio=1.49). The tiny reduction of bioluminescent response at 15°C and 40°C attributed to the less bacterial activities at inappropriate temperatures, and the response was very weak under even lower (10°C) or higher (45°C) temperature conditions. Salinity did not significantly affect the reproducibility of ADPWH_recA and the relative bioluminescent response ratios were above 1.45 when the salinity was no higher than 3%, but were gradually suppressed at higher salinity level (Fig. 4c). Therefore, the MNPs functionalized bioreporters had high reproducibility under the normal pH value, salinity and temperature conditions of natural soils and no specific pre-treatment was required for real soil sample assessment. High activity and responsive sensitivity of MNPs functionalized whole-cell bioreporters was also observed after 30 days storage at 4°C (Fig. 4d). Without any pre-treatment, the stored bioreporter cells could be directly applied for soil assessment and the relative bioluminescent response ratio was above 1.45 for chromium contaminated soils of 100 mg/kg soil dry weight. The life-time of MNPs functionalized bioreporter was the same to the original *Acinetobacter* based bioreporters [28, 40], indicating that MNPs functionalization had minimal impacts on the bacterial activities and was an appropriate approach to expand its application in soil contamination assessment.

From the calibration curve of soil/water supernatant (SW-S), soil/water mixture (SW-M) and MNPs functionalized bioreporter (MFB) (Fig. 5), magnet device had the highest responsive sensitivity and illustrated the chromium bioavailability in contaminated soils. In SW-S and SW-M treatments, ADPWH_recA bioreporter did not show any positive response to the chromium due to the strong light adsorption by soil particles. The negative bioluminescent response was observed when chromium concentration was above 100 mg/kg soil dry weight for both treatments. Significant positive response was only found in MFB treatment and the limit of detection was 1 mg/kg soil dry weight (Fig. 5 and Table 1). From 1 mg/kg to 100 mg/kg chromium contamination in dry soils, the relative bioluminescence response ratio showed a linear relationship to quantify the toxicity and bioavailability of chromium in soil samples, ranging from 1.05 to 1.60. Above 500 mg/kg soil dry weight, chromium predominantly behaved the cytoxicity effects and all the three treatments had similar inhibited bioluminescent signal. From the whole-cell bioreporter growth curve (Fig. S1), there was no significant growth difference when the chromium concentration was less than 500 mg/kg soil dry weight, in which range that the relative bioluminescent response ratio was positively correlated with chromium. It therefore explained the decreasing bioluminescent response ratio at higher chromium level that strong cytoxicity of chromium inhibited bioreporter growth and activities.

Given the model simulation of bioreporter's response to chromium with different bioavailability in aqueous phase in Fig. 5 [41], the results further revealed the bacteria-contaminant interaction within the porous soils and its impacts on bioreporter response. With lower chromium bioavailability, the calibration curve shifted towards higher chromium values. The SOS response coefficient ($K_{sLSR} \cdot K_{Cr} \cdot k_{ssDNA} \cdot k_{MO}$, 3.8) and genotoxicity coefficient ($\frac{k_{dsSLR}}{2 \cdot (1+k_{ssDNA})} \cdot [LSR]_{total}$, 1.724 L/mg) kept stable, similar to previous research [41]. Referring to the synergetic efficiency through the SOS repair process (including genotoxin DNA damage, ssDNA recognition and SOS box activation), the similar SOS response coefficients indicated the same SOS mechanism of bioreporter's responsive to chromium genotoxicity and cytotoxicity in the soils via the magnet bioreporter device [32]. Since the bioluminescent signal of ADPWH_recA was regulated by the SOS process, all the carcinogens causing DNA damage would activate its response, including mitomycin C, UV light, ethidium bromide and H_2O_2 [22]. The bioreporter therefore did not respond to a particular

heavy metal (like chromium), but evaluated the synergistic toxicity of all the carcinogens in environmental samples.

From the parabolic curve of MNPs functionalized bioreporter to hexavalent chromium, both the relative bioluminescent response ratio and growth curve (Fig. S1) were considered to evaluate the toxicity of unknown environmental samples. In absence of growth inhibition, the sample had low cytoxicity and its bioluminescent response belonged to the positive relationship range, oppositely in presence of growth inhibition. Only the MFB treatment had the positive bioluminescence response when chromium concentration was less than 200 mg/kg soil dry weight, and the response ratio fitted well with the model prediction of 10% chromium bioavailability when chromium concentration was above 100 mg/kg soil dry weight. At lower chromium contamination level, chromium bioavailability changed due to the complex adsorption effects of soil particles and the irregular bioluminescent response ratio represented the changing bioavailable fraction. Given heavy chromium contamination level (>500 mg/kg soil dry weight), MFB and SW-M treatments had similar responsive results, significantly higher than SW-S treatment. Since the whole-cell bioreporter only sensed the water soluble chromium in the supernatant of soil-water mixture in SW-S treatment, it measured the chromium toxicity in the unbound water phase. The dominant fraction of chromium existed in the bound water or was absorbed on the soil particles, and their carcinogenic effects was only assessable by the direct-contact bioreporter assay [24]. The results indicated magnetic functionalized bioreporter could effectively evaluate the real toxic effects of chromium in soils, by directly contacting soil particles and seeking for chromium in unbound/bound water or adsorbed on particle surface. The portable magnet bioreporter device provided the ready-to-use and nature-inspired technique for soil *in situ* measurement by optimizing the operation procedure and enhancing the bioluminescent signal [42].

3.2 Heavy metal contamination profiles in soils

The heavy metal profiles of the 16 investigated soil samples and the coal cinder were listed in Table 2. The chromium was 23 times enriched in the rough cinder (from 38.18 mg/kg to 920.82 mg/kg), followed by nickel (5.5 times enrichment). The enrichment of other heavy metals ranged from 1.8 to 3.7 times, except for zinc (no significant change) and mercury (loss due to volatile during the combustion process) [43]. Similar to previous research on coal combustion residues [44, 45], chromium

and nickel were mostly found concentrated in bottom ash or cinder as the dominant toxic heavy metal residues, due to their low volatility and high stability.

As for heavy metal contamination in soils, the investigated soils only showed significant contamination of chromium from 448.66 mg/kg to 802.77 mg/kg soil dry weight, with the *CF* values from 1.12 to 2.01 (398.94 mg/kg soil dry weight in background). The levels of the other heavy metals were similar to the background soils (p-value>0.05), as the *CF* value of beryllium ranged from 0.80 to 1.19, nickel from 0.40 to 0.95, copper from 0.50 to 1.34, zinc from 0.43 to 0.78, arsenic from 0.41 to 0.88, selenium from 0.33 to 1.37, cadmium from 0.50 to 0.96, lead from 0.83 to 1.40, uranium from 0.38 to 1.28 and mercury from 0.13 to 4.13. The results indicated that the key ecological toxicity was attributed to chromium, same as revealed by many previous research on mutagens in heavy metal contaminated soils [46], and its toxicity in soils could be effectively evaluated by various biological assays [47, 48]. The magnet bioreporter device in this study was therefore feasible to enhance the toxicity test sensitive by directly exposing MNPs-functionalized whole-cell bioreporter cells and diagnosing their bioluminescent response signal.

For soils nearer to the coal cinder point (0 m and 10 m), there was higher heavy metal contamination in the upper layer soil. The chromium contamination declined from 745.15 mg/kg (0-20 cm, 0 m) to 505.60 mg/kg (35-50 cm, 0 m), and from 802.77 mg/kg (0-20 cm, 10 m) to 525.79 mg/kg (35-50 cm, 10 m), respectively. Except for Be, Pb and Hg, all the other heavy metal elements (Ni, Cu, Zn, As, Se, Cd, and U) has the same vertical decreasing distribution. Comparing to the heavy metal composition in the cinders, chromium was also the key carcinogenic heavy metal in soils. Given the sequence of the exchangeable fractions of heavy metals as Cd > Zn > Cu > Ni > Pb > Cr [5], chromium was further concentrated in top soils with less mobility. The results further suggested that the main source of heavy metal contamination in soils was the leachates from the cinders and their residues were dependant on the transportation process in soils.

3.3 Ecological risk profiles of heavy metal contaminated soils

In the ecological toxicity evaluation by directly applying whole-cell bioreporters to the soil (SW-M, Fig. S2 in Supplementary Materials) and soil-water supernatant (SW-S, Fig. S3 in Supplementary Materials), ADPWH_recA only behaved negative

(relative bioluminescence response ratio < 1.0) or neutral signal (relative bioluminescence response ratio = 1.0) and was not suitable to quantify the toxicity impacts of heavy metal contamination *in situ*. Fig. 6 illustrated the ecological toxicity profiles of the soil samples by the magnetic bioreporter device (MFB treatment), and the toxicity of heavy metals declined with the increasing distance to the coal cinder point. From the whole-cell bioreporter growth curve for the soil samples (Fig. S4), all the heavy metal contaminated soils did not show inhibition effects on bacterial growth, indicating all the bioluminescent signals were within the linear response range and the relative bioluminescence response ratio had positive relationship with the ecological toxicity in soils. Except for 0 m point, the relative bioluminescence response ratio dropped from 1.47 (10 m) to 1.10 (150 m) in the surface soil, 1.34 (10 m) to 1.16 (150 m) in the middle soil, and 1.26 (0 m) to 0.58 (150 m) in the bottom soil. At the 0 m point, the low bioluminescence signal of surface soil was caused by the high cytotoxicity effects of chromium (745.15 mg/kg soil dry weigh) and the growth of ADPWH_recA bioreporter was inhibited. The soil sample at 0 m point was therefore characterized with the highest ecological risk.

From the toxicity vertical distribution, the ecological risks had a significant decline in deeper soils. Attributing to the heavy metals leachates from the coal cinders, the ecological risk distribution fitted well with chemical analysis and previous studies. The high ecological risk at the surface soils than bottom soils suggested the leakage and vertical transportation chromium in soils [49]. Comparing to the horizontal ecological risk distribution, the results further identified the main toxicity sources as the heavy metals from the coal cinders.

3.4 Correlation between soil heavy metal profiles and ecological risk

The Principle Component Analysis (PCA) illustrated the main factors causing the ecological risks in soil samples (Fig. S5a). More precisely, the principle component 1 (PC1) was the heavy metal contamination level, accounting for 60.5% of the total variance. At the sampling points nearer to the coal cinder site (0 m and 10 m), the surface and middle soils were heavily contaminated and therefore recognized as isolated square (red) and circle (blue) to the higher value of PC1-axis. For the rest soils, they gathered due to similar contamination level (*PLI*). PC1 was therefore derived from the external heavy metal sources, leaching from the coal cinder for the surface soil (0-20 cm) and heavy metal vertical transportation for middle soil (20-35

cm). The soil depth was the principle component 2 (PC2), contributing to 13.3% of the total variance). Heavy metals distribution and mobility were reported to depend on soil properties and depth [50], and their spatial distribution in different depths of soils also affected the mobility and bioavailability [51]. Nevertheless, the soil ecological risks (illustrated as the area of each symbol) were associated with neither the load of PC1 nor PC2, suggesting that they were complicatedly affected by both heavy metal profiles and soil features.

There was also no significant correlation between heavy metal pollution load index (*PLI*) and ecological risk (p-value>0.05) (Fig. S5b). Higher *PLI* indicated high heavy metal contamination level, but did not fit with the ecological risk distribution. Previous research had shown the positive correlation between heavy metal content and ecological toxicity at the contaminated sites with individual heavy metal pollutant, like chromium residues [24] or copper contaminated agricultural soils [52]. The ecological toxicity was only affected by the individual *EF* value and bioavailability in soil. At the coal cinder contaminated sites, we found the existence of multiple heavy metals and their synergic/antagonistic effects consequently resulted in complicated ecological toxicity [53]. Many evidences had revealed that the toxicity of individual or multiple heavy metals behaved antagonistic or additive effects, dependent on the composition and soil features, like organic matters or pH value [54, 55]. In this case, *PLI* was an empirical indicator evaluating the multiple heavy metal contamination level, but suffered from identifying and characterizing the interaction between various heavy metal molecules and their association with soil particles. From the mechanisms of ADPWH_recA to sense all the carcinogens activating SOS process, the response of whole-cell bioreporter effectively represented the synergic/antagonistic effects of multiple heavy metals. By directly exposing the living bioreporter cells to the contaminated samples *in situ*, the MNPs functionalized bioreporter had its feasibility as an important approach, supplementary to chemical analysis, in ecological risk assessment and environmental risk management.

4. Conclusion

This work developed a novel magnet bioreporter device for soil toxicity assessment, via magnetic nanoparticles functionalized whole-cell bioreporters. The living magnetic bioreporter cells could sense the carcinogenic chemicals in the soil and effectively separated from the soil-water slurry in the bioluminescence detection step

to avoid the disturbance of soil particles. Comparing to the conventional treatments directly applying bioreporter in soil-water mixture or supernatant, the magnet bioreporter device achieved high sensitivity and reproducibility under soil pH, salinity and temperature conditions. The dose-toxicity calibration curve revealed the impacts of chromium bioavailability on its ecological risk in soils, where strong genotoxicity was identified when chromium concentration was from 1 mg/kg to 500 mg/kg soil dry weight and the cytotoxic inhibition was found at chromium over 500 mg/kg soil dry weight. For the first time, the ecological toxicity of heavy metal contaminated soils was evaluated by the whole-cell bioreporter at the coal cinder site. Though the existence of heavy metal contamination contributed to the main ecological risks at the site, the pollution load index (*PLI*) had no significantly relationship with the ecological toxicity distribution. The synergic and antagonistic effects of soil multiple heavy metal contamination brought the challenges for environmental risk assessment by chemical analysis. The magnetic bioreporter device behaved as an alternative approach for the high throughput biological measurement and was feasible for *in situ* monitoring.

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Reference

[1] X.D. Li, O.W.H. Wai, Y.S. Li, B.J. Coles, M.H. Ramsey, I. Thornton, Heavy metal distribution in sediment profiles of the Pearl River estuary, South China, Appl Geochem, 15(2000) 567-81.

[2] K. Loska, D. Wiechula, I. Korus, Metal contamination of farming soils affected by industry, Environ Int, 30(2004) 159-65.

[3] S.R. Smith, A critical review of the bioavailability and impacts of heavy metals in municipal solid waste composts compared to sewage sludge, Environ Int, 35(2009) 142-56.

[4] H. Alizai, D. Preston, S.S. Meng, M. Kolkas, Heavy metal contamination in the coastal sediments of the Lower New York Bay, Northeastern Geology and Environmental Sciences, 25(2003) 226-31.

[5] P.J. He, Z. Xiao, L.M. Shao, J.Y. Yu, D.J. Lee, In situ distributions and characteristics of heavy metals in full-scale landfill layers, J Hazard Mater, 137(2006) 1385-94.

[6] Z. Dang, C.Q. Liu, M.J. Haigh, Mobility of heavy metals associated with the natural weathering of coal mine spoils, Environ Pollut, 118(2002) 419-26.

[7] M. Valko, H. Morris, M.T.D. Cronin, Metals, toxicity and oxidative stress, Curr Med Chem, 12(2005) 1161-208.

[8] K.E. Giller, E. Witter, S.P. McGrath, Toxicity of heavy metals to microorganisms and microbial processes in agricultural soils: A review, Soil Biol Biochem, 30(1998) 1389-414.

[9] L. Jarup, Hazards of heavy metal contamination, Br Med Bull, 68(2003) 167-82.

[10] M.L. Farre, R. Brix, D. Barcelo, Screening water for pollutants using biological techniques under European Union funding during the last 10 years, Trends in Analytical Chemistry, 24(2005) 532-45.

[11] S. Belkin, Microbial whole-cell sensing systems of environmental pollutants, Curr Opin Microbiol, 6(2003) 206-12.

[12] T.K. Van Dyk, E.J. DeRose, G.E. Gonye, LuxArray, a high-density, genomewide transcription analysis of *Escherichia coli* using bioluminescent reporter strains, J Bacteriol, 183(2001) 5496-505.

[13] E.A. Meighen, Genetics of bacterial bioluminescence, Annu Rev Genet, 28(1994) 117-39.

519 [14] J. Sanseverino, R.K. Gupta, A.C. Layton, S.S. Patterson, S.A. Ripp, L. Saidak, et
520 al., Use of Saccharomyces cerevisiae BLYES expressing bacterial bioluminescence
521 for rapid, sensitive detection of estrogenic compounds, Appl Environ Microbiol,
522 71(2005) 4455-60.
523 [15] S.F. D'Souza, Microbial Sensors, Biosens Bioelectron, 16(2001) 337-53.
524 [16] L.D. Rasmussen, S.J. Sorensen, R.R. Turner, T. Barkay, Application of a mer-lux
525 biosensor for estimating bioavailable mercury in soil, Soil Biol Biochem, 32(2000)
526 639-46.
527 [17] A. Ivaska, M. Virtab, A. Kahrua, Construction and use of specific luminescent
528 recombinant bacterial sensors for the assessment of bioavailable fraction of cadmium,
529 zinc, mercury and chromium in the soil, Soil Biol Biochem, 34(2002) 1439-47.
530 [18] S. Rodriguez-Mozaz, M.J. Lopez de Alda, D. Barcelo, Biosensors as useful tools
531 for environmental analysis and monitoring, Analytical and Bioanalytical Chemistry,
532 386(2006) 1025-41.
533 [19] J.R. van der Meer, S. Belkin, Where microbiology meets microengineering:
534 design and applications of reporter bacteria, Nat Rev Microbiol, 8(2010) 511-22.
535 [20] P. Corbisier, E. Thiry, L. Diels, Bacterial biosensors for the toxicity assessment
536 of solid wastes, Environmental Toxicology and Water Quality, 11(1996) 171-7.
537 [21] C. Tibazarwa, P. Corbisier, M. Mench, A. Bossus, P. Solda, M. Mergeay, et al.,
538 A microbial biosensor to predict bioavailable nickel and its transfer to plants, Environ
539 Pollut, 113(2001) 19-26.
540 [22] Y. Song, B. Jiang, S. Tian, H. Tang, Z. Liu, C. Li, et al., A whole-cell bioreporter
541 approach for the genotoxicity assessment of bioavailability of toxic compounds in
542 contaminated soil in China, Environ Pollut, 195C(2014) 178-84.
543 [23] V.H.C. Liao, M.T. Chien, Y.Y. Tseng, K.L. Ou, Assessment of heavy metal
544 bioavailability in contaminated sediments and soils using green fluorescent protein-
545 based bacterial biosensors, Environ Pollut, 142(2006) 17-23.
546 [24] B. Jiang, D. Zhu, Y. Song, D. Zhang, Z. Liu, X. Zhang, et al., Use of a whole-cell
547 bioreporter, *Acinetobacter baylyi*, to estimate the genotoxicity and bioavailability of
548 chromium(VI)-contaminated soils, Biotechnol Lett, 37(2015) 343-8.
549 [25] R. Turpeinen, J. Salminen, T. Kairesalo, Mobility and bioavailability of lead in
550 contaminated boreal forest soil, Environ Sci Technol, 34(2000) 5152-6.

[26] H. Fritze, J. Perikomaki, T. Petanen, T. Pennanen, M. Romantschuk, M. Karp, et al., A microcosms study on the effects of Cd containing wood ash on the coniferous humus fungal community and the Cd bioavailability, Soils Sediments, 1(2001) 146-50.
[27] D. Zhang, R.F. Fakhrullin, M. Özmen, H. Wang, J. Wang, V.N. Paunov, et al., Functionalization of whole-cell bacterial reporters with magnetic nanoparticles, Microbial Biotech, 4(2011) 89-97.
[28] Y.Z. Song, G.H. Li, S.F. Thornton, I.P. Thompson, S.A. Banwart, D.N. Lerner, et al., Optimization of Bacterial Whole Cell Bioreporters for Toxicity Assay of Environmental Samples, Environ Sci Technol, 43(2009) 7931-8.
[29] D. Zhang, A. Ding, S. Cui, C. Hu, S.F. Thornton, J. Dou, et al., Whole cell bioreporter application for rapid detection and evaluation of crude oil spill in seawater caused by Dalian oil tank explosion, Water Res, 47(2013) 1191-200.
[30] K.P. Nickens, S.R. Patierno, S. Ceryak, Chromium genotoxicity: A double-edged sword, Chem Biol Interact, 188(2010) 276-88.
[31] A.A. Al-Anizi, M.T. Hellyer, D. Zhang, Toxicity assessment and modeling of *Moringa oleifera* seeds in water purification by whole cell bioreporter, Water Res, 56(2014) 77-87.
[32] D. Zhang, Y. Zhao, Y. He, Y. Wang, Y. Zhao, Y. Zheng, et al., Characterization and Modeling of Transcriptional Cross-Regulation in *Acinetobacter baylyi* ADP1, Acs Synthetic Biology, 1(2012) 274-83.
[33] S. Dai, X. Wang, Y. Zhou, J.C. Hower, D. Li, W. Chen, et al., Chemical and mineralogical compositions of silicic, mafic, and alkali tonsteins in the late Permian coals from the Songzao Coalfield, Chongqing, Southwest China, Chem Geol, 282(2011) 29-44.
[34] S. Dai, X. Wang, V.V. Seredin, J.C. Hower, C.R. Ward, J.M.K. O'Keefe, et al., Petrology, mineralogy, and geochemistry of the Ge-rich coal from the Wulantuga Ge ore deposit, Inner Mongolia, China: New data and genetic implications, Int J Coal Geol, 90(2012) 72-99.
[35] X. Li, S. Dai, W. Zhang, T. Li, X. Zheng, W. Chen, Determination of As and Se in coal and coal combustion products using closed vessel microwave digestion and collision/reaction cell technology (CCT) of inductively coupled plasma mass spectrometry (ICP-MS), Int J Coal Geol, 124(2014) 1-4.

583 [36] M.A.H. Bhuiyan, L. Parvez, M.A. Islam, S.B. Dampare, S. Suzuki, Heavy metal
584 pollution of coal mine-affected agricultural soils in the northern part of Bangladesh, J
585 Hazard Mater, 173(2010) 384-92.
586 [37] C. Chen, D. Zhang, S.F. Thornton, M. Duan, Y. Luo, A. Ding, et al.,
587 Functionalization and immobilization of whole cell bioreporters for the detection of
588 environmental contamination, Environmental Engineering and Management Journal,
589 12(2013) 1417-22.
590 [38] D. Zhang, J.P. Berry, D. Zhu, Y. Wang, Y. Chen, B. Jiang, et al., Magnetic
591 nanoparticle-mediated isolation of functional bacteria in a complex microbial
592 community, ISME J, 9(2015) 603-14.
593 [39] C. Li, H. Wang, L. Zhou, Y. Zhang, F. Song, J. Zhang, Quantitative
594 measurement of pH influence on SalR regulated gene expression in Acinetobacter
595 baylyi ADP1, J Microbiol Methods, 79(2009) 8-12.
596 [40] D. Zhang, Y. He, Y. Wang, H. Wang, L. Wu, E. Aries, et al., Whole-cell
597 bacterial bioreporter for actively searching and sensing of alkanes and oil spills,
598 Microbial Biotech, 5(2012) 87-97.
599 [41] Y. Xu, J. Zhu, H. Li, Y. Du, D. Zhang, Removal and immobilization of
600 hexavalent chromium contaminated water by magnetic nanoparticles, Environ Sci
601 Technol, Under review.
602 [42] E. Michelini, L. Cevenini, M.M. Calabretta, S. Spinozzi, C. Camborata, A. Roda,
603 Field-deployable whole-cell bioluminescent biosensors: so near and yet so far, Anal
604 Bioanal Chem, 405(2013) 6155-63.
605 [43] R. Meij, L.H.J. Vredenbregt, H.T. Winkel, The fate and behavior of mercury in
606 coal-fired power plants, J Air Waste Manag Assoc, 52(2002) 912-7.
607 [44] F. Goodarzi, F.E. Huggins, H. Sanei, Assessment of elements, speciation of As,
608 Cr, Ni and emitted Hg for a Canadian power plant burning bituminous coal,
609 International Journal of Coal Geology, 74(2008) 1-12.
610 [45] F. Goodarzi, Assessment of elemental content of milled coal, combustion
611 residues, and stack emitted materials: Possible environmental effects for a Canadian
612 pulverized coal-fired power plant, International Journal of Coal Geology, 65(2006)
613 17-25.
614 [46] P.A. White, L.D. Claxton, Mutagens in contaminated soil: A review, Mutat Res-
615 Rev Mutat, 567(2004) 227-345.

[47] H.Q. Wang, Clastogenicity of chromium contaminated soil samples evaluated by Vicia root-micronucleus assay, Mutation Research-Fundamental and Molecular Mechanisms of Mutagenesis, 426(1999) 147-9.
[48] J.C. Codina, C. Pereztorrente, A. Perezgarcia, F.M. Cazorla, A. Devicente, Comparison of microbial tests for the detection of heavy metal genotoxicity, Archives of Environmental Contamination and Toxicology, 29(1995) 260-5.
[49] X. Ma, Z. Lu, J. Cheng, Ecological risk assessment of open coal mine area, Environ Monit Assess, 147(2008) 471-81.
[50] L. Hernandez, A. Probst, J.L. Probst, E. Ulrich, Heavy metal distribution in some French forest soils: Evidence for atmospheric contamination, Sci Total Environ, 312(2003) 195-219.
[51] K. Nemati, N.K. Abu Bakar, M.R. Abas, E. Sobhanzadeh, Speciation of heavy metals by modified BCR sequential extraction procedure in different depths of sediments from Sungai Buloh, Selangor, Malaysia, J Hazard Mater, 192(2011) 402-10.
[52] K.K. Brandt, P.E. Holm, O. Nybroe, Bioavailability and toxicity of soil particle-associated copper as determined by two bioluminescent *Pseudomonas fluorescens* biosensor strains, Environ Toxicol Chem, 25(2006) 1738-41.
[53] M. Holmstrup, A.M. Bindesbol, G.J. Oostingh, A. Duschl, V. Scheil, H.R. Kohler, et al., Interactions between effects of environmental chemicals and natural stressors: A review, Sci Total Environ, 408(2010) 3746-62.
[54] C.A.M. VanGestel, P.J. Hensbergen, Interaction of Cd and Zn toxicity for Folsomia candida Willem (Collembola:Isotomidae) in relation to bioavailability in soil, Environmental Toxicology and Chemistry, 16(1997) 1177-86.
[55] S. Preston, N. Coad, J. Townend, K. Killham, G.I. Paton, Biosensing the acute toxicity of metal interactions: Are they additive, synergistic, or antagonistic?, Environ Toxicol Chem, 19(2000) 775-80.

Figure caption

Fig. 1 Schematic instruction for magnet bioreporter device. (a-1) and (a-2) for magnetic probe assay and the 96-well microplate respectively. (b-1) The 1.0 mL MNPs functionalized bioreporter was mixed with soil samples and further incubated at 30°C for 1 hour; (b-2) separation from soil suspensions via magnetic probe. (b-3) Resuspension in fresh MMS medium; (b-4) incubation and bioluminescence measurement 30°C for 3 hours.

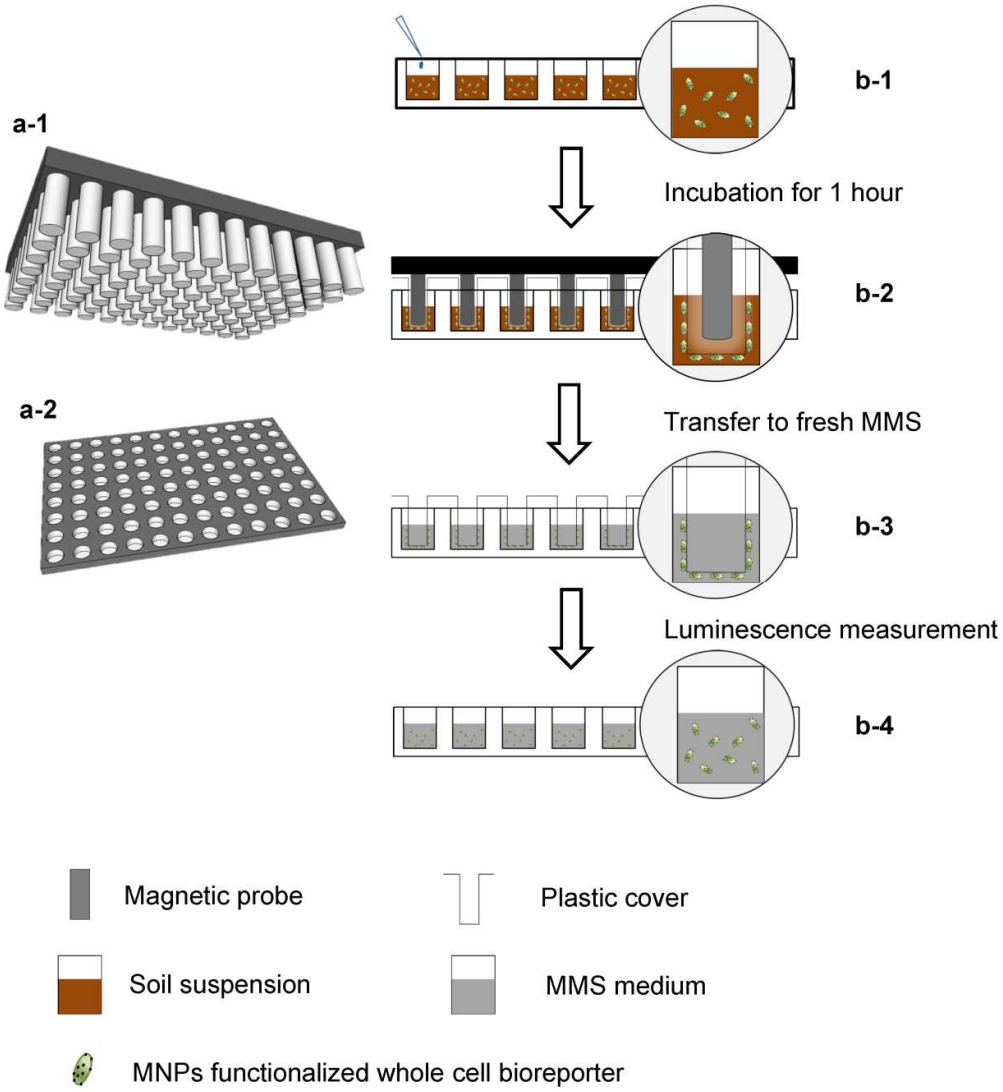
Fig. 2. Location of research area in Yulin and the sampling sites.

Fig. 3. The cell recovery rate (A) and bioluminescent response (B) of whole-cell bioreporter against the incubation time with soils. Over 90% of living bioreporter cells were successfully harvested from the soil/water mixture within 45 minutes incubation. The relative bioluminescence response ratio ranged between 1.90 and 2.00 when the incubation time was less than 75 minutes. The 60-minute incubation was identified as the optimal time for sufficient bioreporter cell recovery and high response sensitivity.

Fig. 4. The impacts of pH (a), temperature (b), salt (c) and storage time (d) on magnetic bioreporter's response to artificial chromium contaminated soils. The chromium concentration was 100 mg/kg soil dry weight.

Fig. 5. The calibration curve for toxicity assessment on artificial chromium contaminated soils. Grey circle refers to magnet bioreporter device (MFB); white diamond represents direct measurement of soil/water supernatant (SW-S); white circle is the direct measurement of soil/water mixture (SW-M). The black line represents the simulation of whole-cell bioreporter's response to chromium toxicity with 100% 100% bioavailability, and a significant bioluminescent response curve shift was found for 50% (red line), 30% (yellow line) and 10% (green line) chromium bioavailability respectively.

Fig. 6. Ecological toxicity assessment of heavy metal contaminated soils via magnetic bioreporter device.



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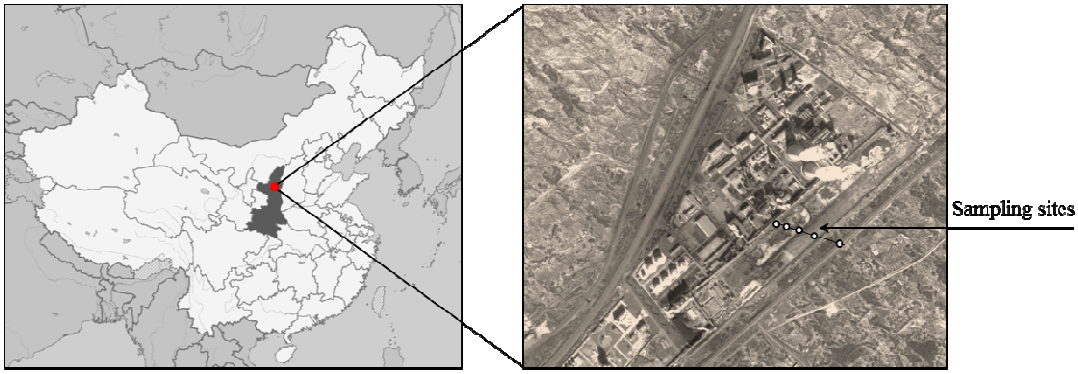

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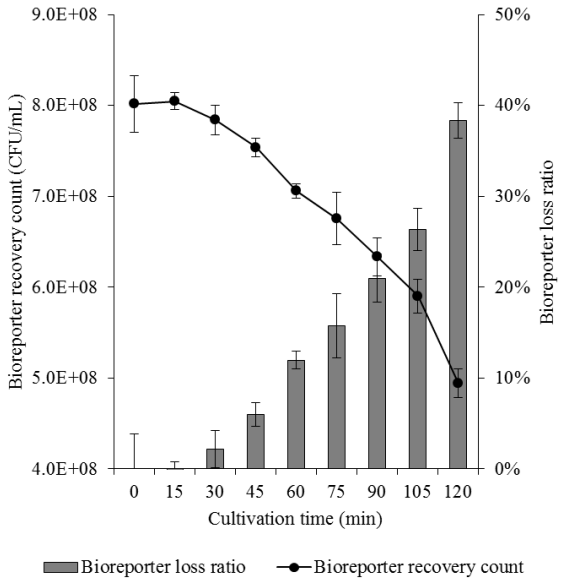
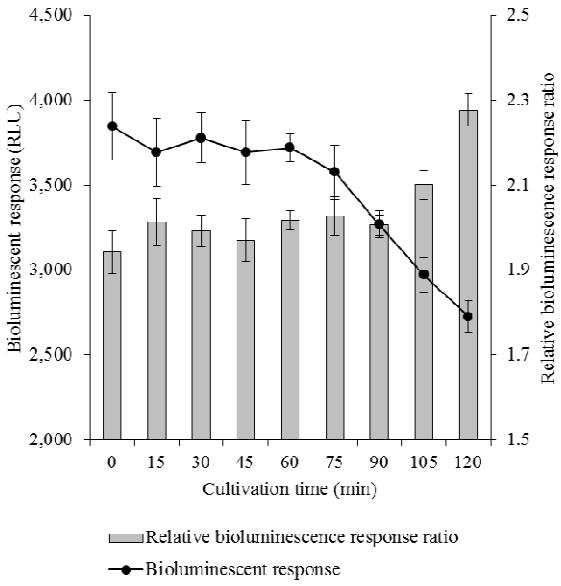


(a)



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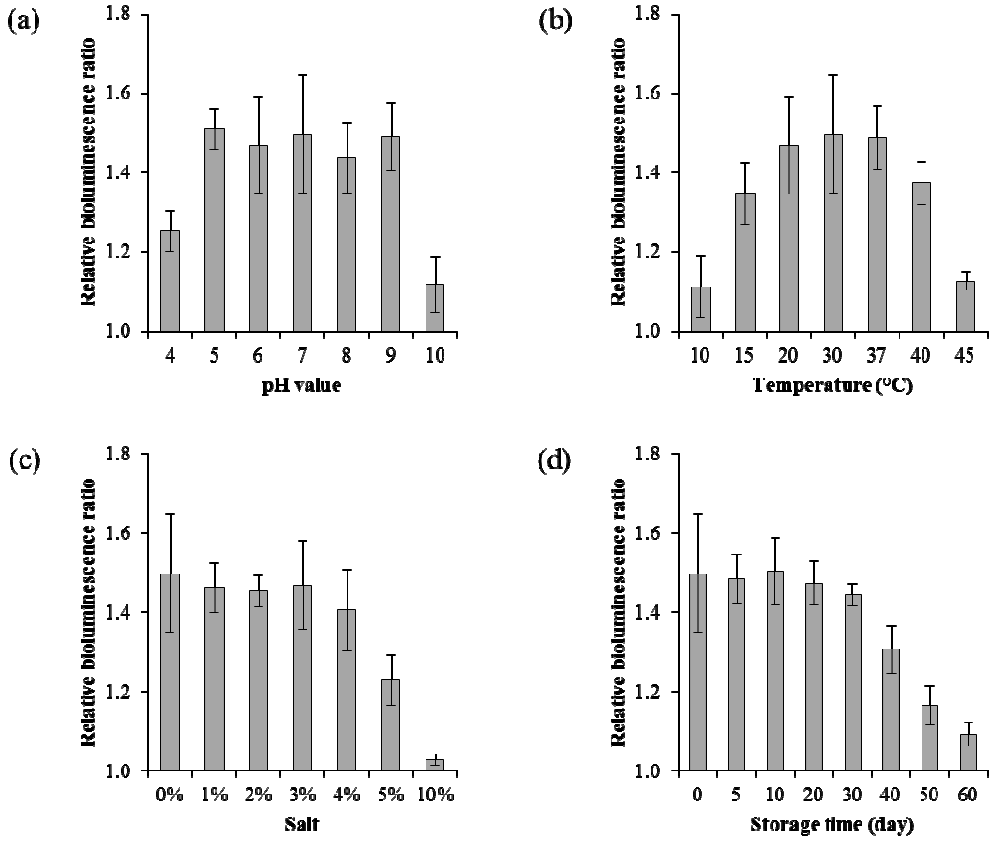

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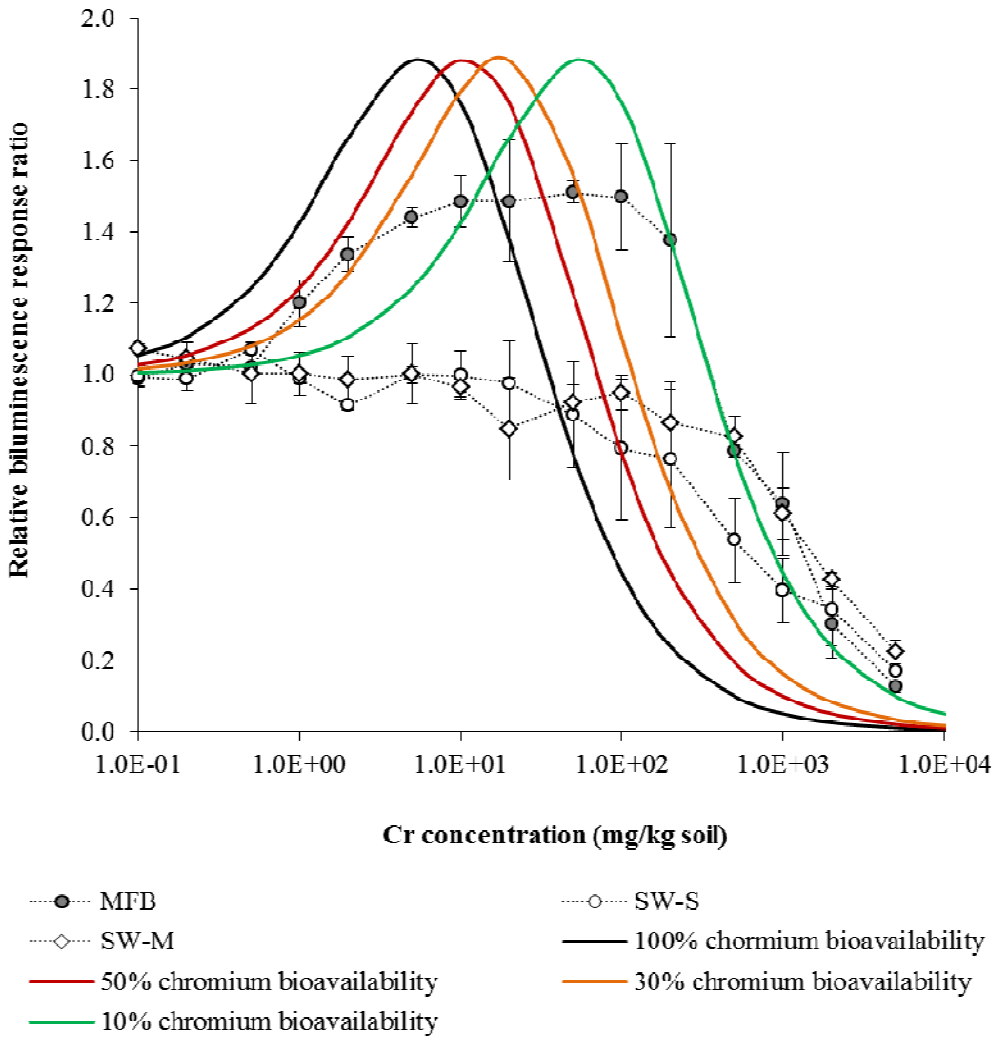

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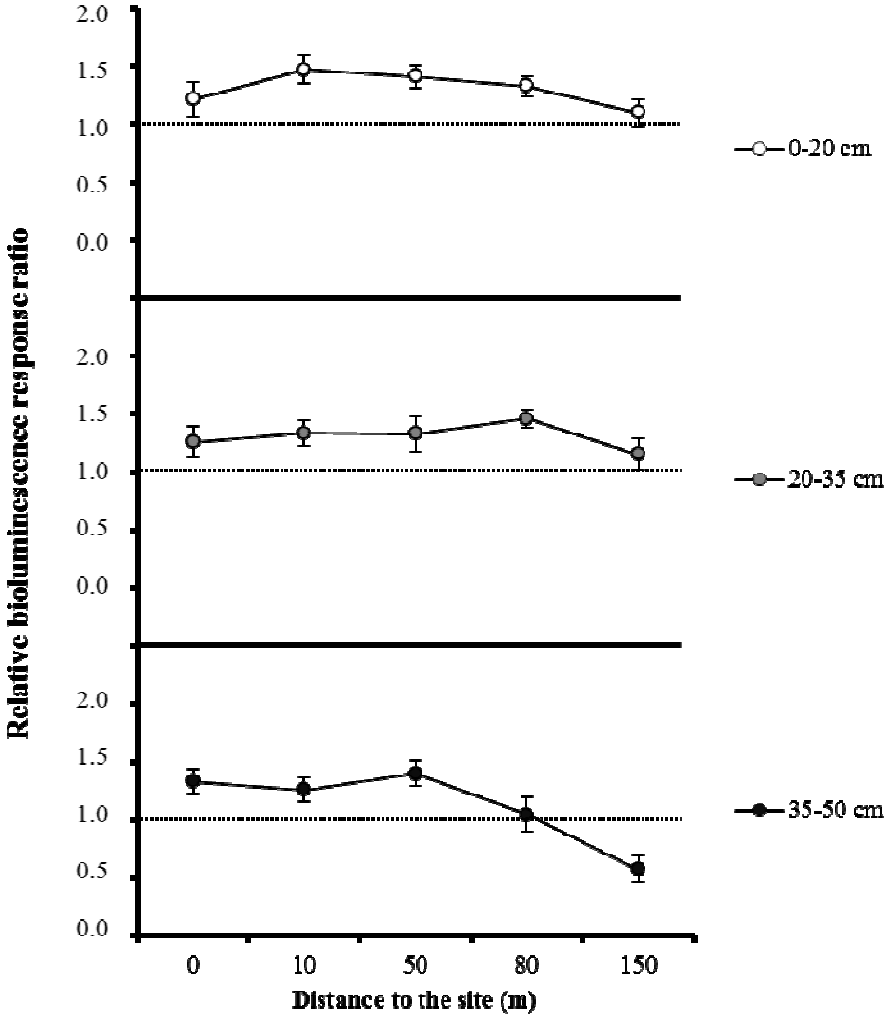

711

712 **Fig. 6.** Ecological toxicity assessment of heavy metal contaminated soils via magnetic
713 bioreporter device.

714

715

Table

Table 1. Analytical characteristics of magnetic ADPWH_recA whole-cell bioreporter.

Characteristics	Description
Limit of detection	1 mg/kg chromium(VI) in dry soil
Linear range	1-100 mg/kg chromium(VI) in dry soil
Pre-incubation time	1 hour
Detection time	4 hour
Reproducibility	High reproducible when pH value is from 4.0 to 9.0, salinity ranges from 0% to 3%, and temperature is from 20 °C to 37 °C
Storage time	30 days

Table 2. Heavy metal contamination in coal/cinder (mg/kg coal or cinder dry weight) and coal cinder contaminated sites (mg/kg soil dry weight).

Samples		Be	Cr	Ni	Cu	Zn	As	Se	Cd	Pb	U	Hg
Raw coal		0.27	38.18	4.93	6.62	8.55	2.26	0.23	0.05	3.43	0.48	0.006
Rough cinder		0.83	920.82	26.89	22.66	9.01	4.14	0.84	0.12	7.44	1.42	0.001
Background soil		1.33	398.94	30.99	16.22	37.91	6.36	0.87	0.26	21.62	1.20	0.008
0 m	**0-20 cm**	1.07	745.15	23.11	17.92	28.78	4.71	0.75	0.25	18.19	1.29	0.003
	20-35 cm	1.19	552.97	17.61	10.55	27.72	3.18	0.43	0.22	20.26	0.64	0.005
	35-50 cm	1.27	505.60	14.68	10.25	20.97	3.10	0.44	0.16	20.36	0.64	0.003
10 m	**0-20 cm**	1.55	802.77	29.38	21.67	23.05	5.26	1.00	0.22	18.05	1.48	0.002
	20-35 cm	1.58	620.79	21.71	16.52	29.26	5.60	1.19	0.24	30.31	1.20	0.001
	35-50 cm	1.26	525.79	15.80	9.74	17.53	3.00	0.29	0.15	21.44	0.56	0.001
50 m	**0-20 cm**	1.16	508.43	13.80	8.93	28.10	3.66	0.36	0.14	20.08	0.54	0.033
	20-35 cm	1.12	482.68	12.29	8.09	16.19	2.62	0.41	0.13	19.88	0.45	0.001
	35-50 cm	1.12	628.89	15.03	9.35	18.22	3.08	0.46	0.14	20.17	0.53	0.001
80 m	**0-20 cm**	1.15	483.48	13.26	9.03	18.61	3.22	0.30	0.16	20.01	0.53	0.001
	20-35 cm	1.19	502.34	13.84	9.31	19.76	3.35	0.45	0.18	21.03	0.57	0.001
	35-50 cm	1.29	613.57	19.76	15.32	29.44	4.01	0.43	0.25	21.28	0.86	0.001
150 m	**0-20 cm**	1.10	494.60	13.56	8.90	19.03	3.24	0.58	0.15	19.99	0.56	0.001
	20-35 cm	1.24	474.09	16.46	10.87	24.61	4.48	0.43	0.22	20.51	0.76	0.001
	35-50 cm	1.25	448.66	18.50	13.00	28.81	5.08	0.41	0.22	21.70	0.90	0.001

Note: The analytical instrument is Hg analyzer (DMA-80) for Hg and ICP-MS (X series II) fir other elements.

Supplementary Material

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Biographies

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