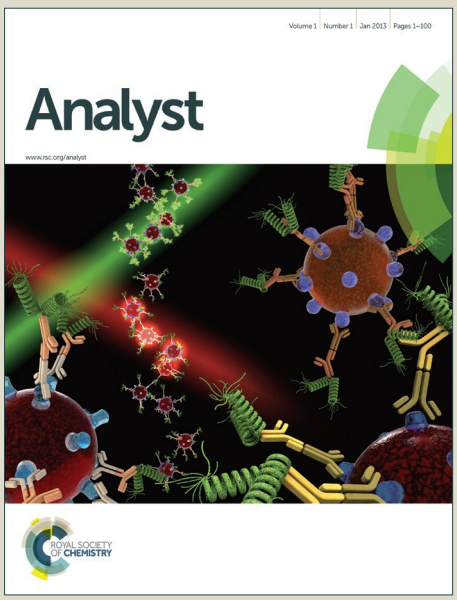
Analyst

Accepted Manuscript



ROYAL SOCIETY OF CHEMISTRY

www.rsc.org/analyst

Journal Name

ARTICLE

Adaptive use of personal glucose meter (PGM) for acute biotoxicity assessment based on the glucose consumption of microbes

Received 00th January 20xx,
Accepted 00th January 20xx

DOI: 10.1039/x0xx00000x

www.rsc.org/

Deyu Fang, [a, b] Guanyue Gao, [a, b] Yuan Yu, [a] Jie Shen, [a] Jinfang Zhi [a, *]

In this study, a new method for acute biotoxicity assessment was proposed by measuring the glucose consumption of microbes with personal glucose meter (PGM). To obtain an ideal biotoxicity assessment performance, appropriate microbe was selected first, and then the relevant parameters, such as temperature, microbial concentration were optimized. Under the optimized parameters, acute biotoxicity of four environmental pollutants (As^{3+}, Ni^{2+}, 4-chlorophenol, 2,4-dichlorophenol), three wastewater samples and three soil samples were evaluated. This technology breakthrough will help us develop a low cost, easy to use water-environmental early-warning kit.

Introduction

Due to the increasing severity of environmental pollution, assessing the acute biotoxicity of environmental pollutants has been playing an important role in environment monitoring and early warning systems. Traditional biotoxicity evaluations mainly rely on living organisms such as plants,[1, 2] invertebrates[3, 4] and fish.[5] Unfortunately, most of these methods are expensive and time consuming. The ubiquitous presence microbes offer an alternative for addressing these limitations because of their short life cycle, rapid response to toxicants and low cost.[6, 7] The past few decades have witnessed the trend of utilizing microbes for toxicity assessment and some research activities have been carried out in this area.[8-10]

In brief, microbes-based biotoxicity assessment methodologies can be subdivided into two main approaches, *i.e.*, optical methods and electrochemical methods. Optical methods are based on enzyme-related colour reactions[11, 12] or the bioluminescence produced naturally by bioluminescent microbes. The presence of toxicants inhibits the enzyme activity or the respiratory activity of bioluminescent microbes, which results in the decline of colour depth or bioluminescence intensity. However, optical methods are of high cost due to their need of sophisticated optical instruments and rare microbial strains (bioluminescent microbes, or microbes possessing specific enzymes), which hinder their practical applications and popularization. Electrochemical methods are usually used to evaluate biotoxicity by transforming the microbial respiration magnitude to electricity signals. When toxicants exist, the respiration of microbes is inhibited and it can be reflected by a change in electricity, *i.e*, a decline of electricity can be observed. The early biosensor based on microbial respiration metabolism employed dissolved oxygen as the electron acceptor.[13, 14] However, the solubility of oxygen in water is low and easily affected by temperature, pressure and salinity,[15] it is difficult to measure the dissolved oxygen precisely. The improved biosensors utilized some redox mediators, such as potassium ferricyanide or benzoquinone, to improve the sensitivity of the biosensors.[9, 16] The mediators can participate in the respiration process of microbes and replace the dissolved oxygen as the electron acceptor. But mediators are usually toxic to microbes,[17] consequently, they interfere with the biotoxicity results. In addition, just like the optical methods, the high costs are also great challenges to these methods due to sophisticated electrochemical instruments are indispensable for electricity measurement. Therefore, developing low cost and easy to use biotoxicity assessment technologies are still challenging.

On the other hand, a new trend, *i.e.*, adaptive use,[18, 19] has been concerned recently. It is implies using materials or instruments that already designed and produced in large quantity with high quality at a low cost, for purpose other than those for which they were originally intended. Some systems based on 'adaptive use', such as cell phone cameras as colorimetric detectors for paper-based microfluidic devices,[20] pins and thread used as electroanalytical devices,[21] have been developed. Among the commercial available instruments, personal glucose meters (PGMs), which are the most successful electrochemical biosensor, have been widely used by worldwide diabetic patients for blood glucose monitoring.[22, 23] Compared to the sophisticated optical instruments and electrochemical workstation, PGMs are cheap ($4-50), portable (pocket-size)

[a] *Key Laboratory of Photochemical Conversion and Optoelectronic Materials, Technical Institute of Physics and Chemistry, Chinese Academy of Sciences, Beijing 100190, PR China*
[b] *University of Chinese Academy of Sciences, Beijing 100190, PR China*
Electronic Supplementary Information (ESI) available: [details of any supplementary information available should be included here]. See DOI: 10.1039/x0xx00000x

and widely available in pharmacies.[24-26] Since the first proposition of Lu's group to utilize PGM to detect non-glucose targets in 2011, PGM has drawn much attention due to its potential usage in areas other than blood glucose monitoring, such as the detection of biological molecules,[27-29] metal ions,[25, 30] microorganisms,[31, 32] et. al. In present work, a new solution is proposed for the biotoxicity assessment by elucidating the activity change of microbial glucose metabolism that affected by the toxicants, where the commercial PGM was used as readout for the evaluation of glucose concentration. To obtain an ideal acute biotoxicity performance, appropriate microbe was selected first, and then the relevant parameters such as temperature, microbial concentration and incubation time were optimized. Under the optimized parameters, acute biotoxicity of four environmental pollutants (As^{3+}, Ni^{2+}, 4-chlorophenol, 2,4-dichlorophenol), three wastewater samples and three soil samples were evaluated. All the results suggest our new solution is a low-cost and practical alternative for acute biotoxicity assessment. This technology breakthrough will help us develop a low cost, easy to use water-environmental early-warning kit.

Materials and methods

Materials and Reagents

Escherichia coli (ATCC 25922), *Bacillus subtilis* (CGMCC 1.1086) and *Saccharomyces cerevisiae* (ATCC S288C) were obtained from China General Microbiological Culture Collection Center (CGMCC). The ACCU-CHEK® Performa personal glucose meter (PGM) and corresponding test-strips were purchased from Roche Diagnostics. Filter membranes (pore size=0.22 μm) were provided by Shanghai Xinya purification devices factory, China. Peptone, beef extract and yeast extract were purchased from Beijing Aoboxing Bio-tech Co., Ltd., China. All other reagents (analytical grade) were obtained from Beijing Lanyi Chemical Products Co., Ltd., China, and used without further purification. All solutions were prepared with deionized water (18.0 MΩ•cm, Milli-Q Gradient System, Millipore). All toxicants (Cu^{2+}, Cd^{2+}, Pb^{2+}, As^{3+}, Ni^{2+}, 3,5-dichlorophenol, 2-phenylphenol, hydroxylbenzene, 4-chlorophenol, 2,4-dichlorophenol) were freshly prepared in and stored in a 4℃ refrigerator. For phenol compounds that have low solubility in water, 0.5% (v/v) dimethyl sulfoxide (DMSO) was added to increase their solubility.

Three kinds of polluted water samples (effluents from landfill, electroplating wastewater and wastewater from chemical laboratory) were provided by Technical Institute of Physics and Chemistry, Chinese Academy of Sciences and used directly. The three soil samples (polycyclic aromatic hydrocarbons polluted soil from foundry, soil from farm land and heavy metal ions polluted soil from lead zinc mine area) were kindly provided by Environmental Protection Research Institute of Light Industry, China. Soil extracts were obtained by shaking 10 g of soil (dry weight) for 2.5 h at 200 rpm with 100 ml certain solutions. The certain solutions were DMSO (v):H2O (v) =1:4 solution for polycyclic aromatic hydrocarbons polluted soil, pure water for soil from farm land and HCl (0.1 M) for heavy metal ions contaminated soil, respectively. The slurries were then centrifuged for 5 min at 3000 rpm and extracts (supernatants) were filtered through filter membranes (pore size=0.22 μm). For acid leaching heavy metal ions contaminated soil extract, the pH was adjusted with NaOH solution (2 M) to 6.5 before use.

Lysogeny broth (5 g/L beef extract, 10 g/L peptone, 5 g/L NaCl) for *E. coli* and broth (3 g/L beef extract, 1 g/L peptone, 5 g/L NaCl) for *B. subtilis* were adjusted to pH=7.4-7.8 with NaOH solution (2 M) and sterilized in high-pressure steam at 120℃ for 20 min. YEPD (yeast extract peptone dextrose) broth (20 g/L peptone, 10 g/L yeast extract, 20 g/L glucose) for *S. cerevisiae* was prepared by sterilizing peptone and yeast solution in high-pressure steam at 120 ℃ for 20 min firstly, then followed by adding glucose.

Microbial cultures

All microbes were maintained on nutrient agar plates at 4 ℃ .The suitable medium and incubation conditions were confirmed according to the guide of CGMCC. *E. coli* was grown aerobically in a shaker bath (180 rpm) at 37 ℃ for 16 h, *B. subtilis* was grown aerobically in a shaker bath (180 rpm) at 37 ℃ for 24 h，and *S. cerevisiae* was grown aerobically in a shaker bath (180 rpm) at 30 ℃ for 24 h. Cells were harvested by first centrifuging at 6, 000 rpm for 5 min at room temperature, then washed twice with 0.85% (w/V) saline solution, and finally suspended in saline solution. The concentrations of cells were adjusted with saline solution and determined by measuring the optical density at 600 nm (OD_{600}) using a SECOMAM UVIKONXL UV-vis Spectrophotometer. The microbial suspensions were kept at 4 ℃ for less than 3 h before the cells were used for experiments.

Acute biotoxicity assessment

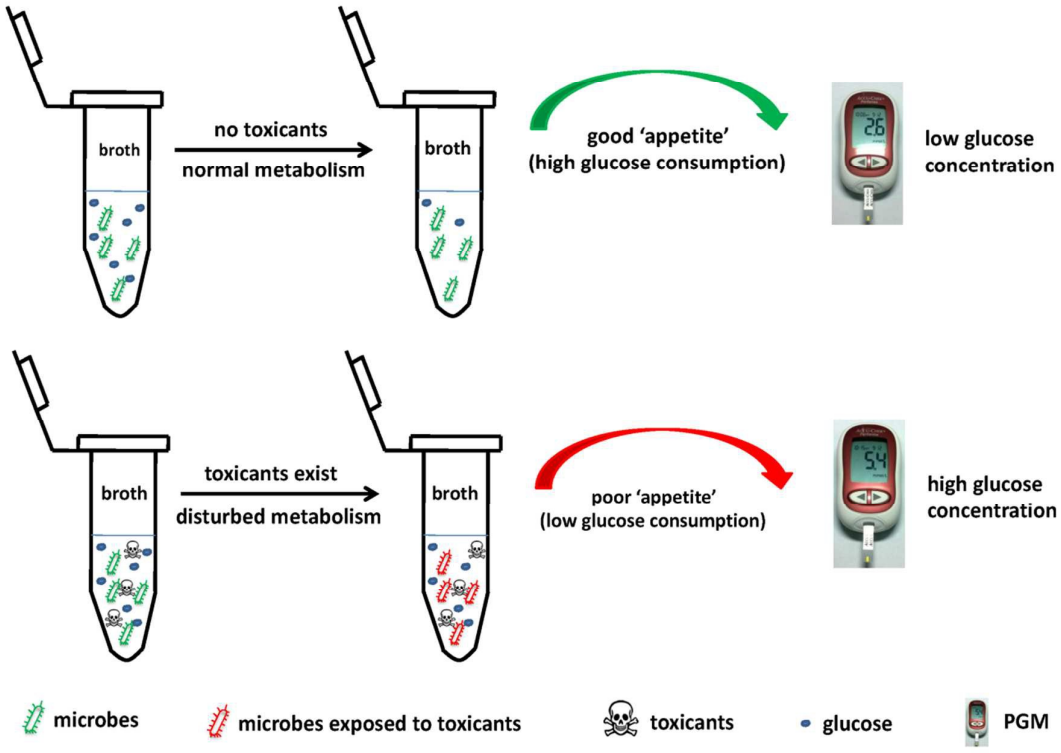
It is well known that glucose is a primary nutrient for most organisms, and glucose metabolism of microbes is severely affected when microbes are exposed to most environmental toxicants. Therefore, the microbial glucose consumption would decrease when toxic materials exist in their living environment. Consequently, we hypothesized that this concept can be applied for the biotoxicity assessment of pollutants in water and soil, *i.e.*, we can utilize the glucose consumptions of microbes to evaluate the total acute biotoxicity of toxicants.

A schematic of this proposed concept is presented in Scheme 1. Samples were prepared by mixing 100 μL of broth, 10 μL of glucose solution, 10 μL of toxicants solution and 80 μL of microbes suspension thoroughly. The control samples contained 0.85% (w/v) saline solution in place of the toxic chemical solution. After incubation at 30 ℃ (*S. cerevisiae*) or 35℃ (*E. coli* and *B. subtilis*) for a period of time, the samples were first centrifuged at 12,000 rpm for 1 min, and then 5 μL of the supernatants was taken out for glucose concentration measurement by PGM. For each toxicant concentration, the glucose concentration can be converted to equivalent inhibitory percentage values according to the Eq. 1.

$$\text{Inhibition } (\%)=(C_e-C_c)/(C_i-C_c) \times 100\% \qquad (1)$$

Journal Name

ARTICLE



Scheme 1. A schematic illustration of experimental procedure by using PGM for total acute toxicity assessment basing on glucose consumptions of microbes.

Where C_i is the initial glucose concentration of all samples, C_e is the final glucose concentration when toxicants exist, and C_c is the final glucose concentration in the control group solution, respectively.

Joint toxicity assessment

The joint toxicity assessments were conducted according to the toxic unit (TU) approach, which has been widely used to test the response addition model for the chemical mixtures. [33, 34] In the TU model, concentrations in the mixtures are expressed as TU, which was calculated as fractions of their half maximal inhibitory concentration (IC_{50}). The sum of TU can be expressed by the Eq. 2.

$$TU_{summation}=c_1/IC_{50(1)}+c_2/IC_{50(2)}+\ldots+c_i/IC_{50(i)} \quad (2)$$

where c_i is the concentration of a toxicant in the mixture and $IC_{50(i)}$ is the IC_{50} value for respective component chemicals of the mixture from 1 to *i*.

In joint toxicity assessment, toxicants were mixed in different toxic units. The mixtures were prepared by adding the appropriate amount of each toxicant at the same concentration as in their individual experiments, but their toxic units varied. IC_{50mix} is defined as the sum of TU values at 50% inhibition for the mixtures.

Results and discussion

Principle verification

PGMs are initially designed to detect the glucose in blood samples, which contain red blood cells, enzymes, etc,. Given that the physical and chemical properties of 0.85% (w/V) sodium chloride solution, which was selected and used throughout our experiment, are different from the bloods, it is essential to verify the accuracy of using PGMs to detect glucose in it. Moreover, the test strips of PGMs contain enzymes such as glucose oxidase or glucose dehydrogenase,[23, 35] which may be inhibited by some pollutants, especially the heavy metal ions. [36, 37] Hence, the feasibility of using PGMs to detect the acute toxicity of heavy metal ions is also need to be verified. Firstly, five glucose solutions with different concentrations were prepared with 0.85% (w/v) sodium chloride solution. As shown in Fig. S1a, the glucose concentrations detected by the PGM were in accord with the actual glucose concentrations. Furthermore, when metals toxicants, Cu^{2+}, Cd^{2+} and Pb^{2+} with

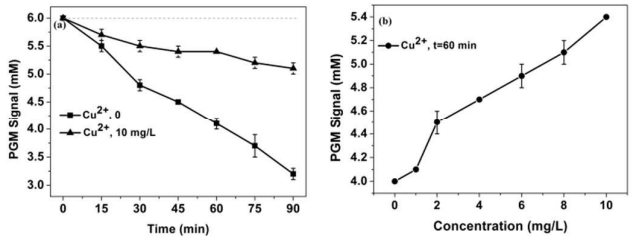


Figure 1. Feasibility study of toxicity assessment based on the microbial glucose metabolism: (a) The glucose concentration-time curve with or without the existence of Cu^{2+} (10 mg/L); (b) the final glucose concentration-Cu^{2+} concentration curve when samples were incubated for 60 min. In all samples, the initial glucose concentration was 6 mM. The microbe was *E. coli* and its concentration was OD_{600}=2.5. The incubation temperature was 35 ℃. Data points represent the average of three replicates.

three different concentrations were added into the glucose/sodium chloride solution, the glucose concentrations detected by the PGM also meet well with the actual glucose concentrations (Fig. S1b), indicating that the toxic effect of heavy metal ions to the test strips can be ignored. It has been recognized that the immobilization matrix can reduce the inactivation of heavy metal ions to enzymes.[37, 38] Moreover, the test strips of ACCU-CHEK® Performa PGM are not only contain active enzyme and mediator,[35] but also many kinds of additives such as KOH, K_2HPO_4, disodium succinate, etc.,[39] which may also contribute to the resistance of test strips to heavy metal ions. All these results suggest ACCU-CHEK® Performa PGM can be used to detect the glucose in 0.85% (w/V) sodium chloride solution accurately no matter heavy metal ions exist or not. In addition to these three typical heavy metal ions (Cu^{2+}, Cd^{2+}, Pb^{2+}), the interference effects of As^{3+}, Ni^{2+}, 4-chlorophenol, 2,4-dichlorophenol and hydroxybenzene on the PGM were also studied (Fig. S2), and no interference effect was observed.

On the other hand, one doubt should be cleared up prior to toxicity assessment: whether glucose concentration can be used as a toxicity indicator, in other words, are there a glucose consumption differences when toxicants exist or not? Firstly, *E. coli*, a general microbe, and Cu^{2+}, a typical environmental pollutant was utilized to investigate the feasibility. Fig. 1 shows the glucose concentration changes with or without the existence of Cu^{2+}. As expected, we can observe that the glucose concentration decreased dramatically with the increase of incubation time when no Cu^{2+} existed, while the glucose concentration decreased slightly when Cu^{2+} (10 mg/L) was introduced (Fig. 1a). Obviously, comparing to *E. coli* exposed to toxic environment, *E. coli* under nontoxic environment consumed more glucose. In addition, as shown in Figure 1b, a higher final glucose concentration was detected with the increase of Cu^{2+} concentration under the same incubation time (t=60 min), implying that the higher concentration of Cu^{2+} exhibits higher inhibition for the glucose metabolic of *E. coli*. Because (1) the pH of all samples before and after incubation were almost neutral (Table S1) and the PGM were quite stable and precise at neutral pH range (Table S2), and (2) there was no remarkable change of ionic strength of samples (Fig. S3), it can be concluded that the observed glucose concentration

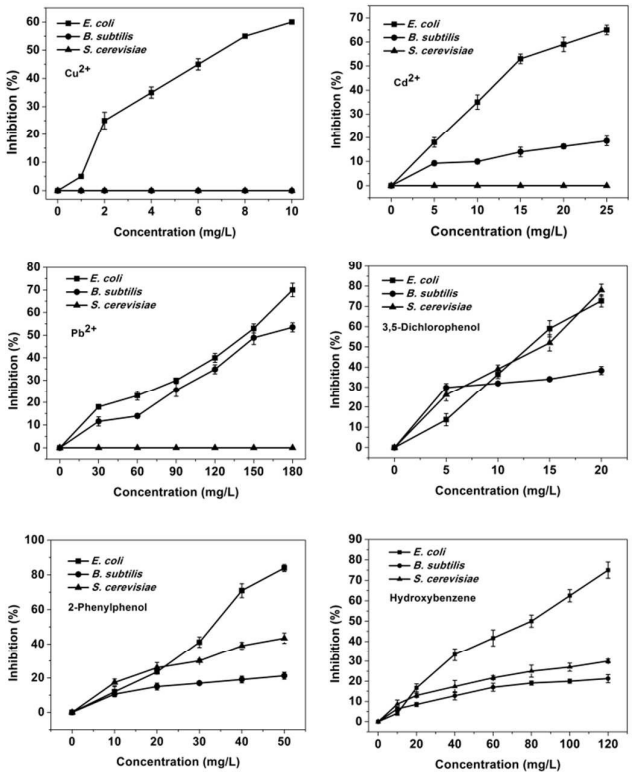


Figure 2. Inhibition responses of *E. coli*, *B. subtilis* and *S. cerevisiae* to three heavy metal ions (Cu^{2+}, Cd^{2+}, Pb^{2+}) and three phenolic compounds (3,5-dichlorophenol, 2-phenylphenol, hydroxybenzene), respectively. Data points represent the average of three replicates. The concentrations of these three microbes were the same, i. e., OD_{600}=2.5.

differences were solely because of the microbial glucose consumption rather than the change of pH and ionic strength of the solutions after microbial metabolism. Thus, the present results suggest that: (1) Cu^{2+} is toxic to *E. coli* glucose metabolic system; and (2) the glucose consumption of *E. coli* can be evaluated just by measuring the glucose concentration by PGM for the acute toxicity assessment.

Optimization of experimental conditions

Experimental conditions, including microbial species, microbial concentration and incubation temperature were optimized so as to obtain an excellent biotoxicity assessment performance.

Microbial species

No single microbial bioassay can detect all the categories of environmental toxicants with equal sensitivity; different microbes may show different sensitivities to the different toxicants. Moreover, since most real water samples contain complex and multiple chemicals, it is necessary to select microbe which exhibit broader sensitivity to toxicants for biotoxicity assessment. We studied the responses of *E. coli* (a typical kind of gram-negative bacteria), *B. subtilis* (a classic example of gram-positive bacteria) and *S. cerevisiae* (a typical kind of fungus) to three heavy metal ions (Cu^{2+}, Cd^{2+}, Pb^{2+}) and three phenolic compounds (3,5-dichlorophenol, 2-phenylphenol,hydroxybenzene). As shown in the inhibition-toxicants concentration curves (Fig. 2a, b and c), when exposed

to heavy metal ions (Cu^{2+}, Cd^{2+} and Pb^{2+}), *E. coli* showed greatest inhibition, followed by *B. subtilis*, and almost no inhibition response was observed for *S. cerevisiae*, suggesting that the fungus is insensitive to heavy metal ions. Besides, it should be noted that Cu^{2+} (0-10 mg/L) was only toxic to *E. coli*. However, when exposed to phenolic compounds (3,5-dichlorophenol, 2-phenylphenol, hydroxybenzene), all these three microbes showed obvious inhibition (Fig. 2d, e and f). The *S. cerevisiae* showed the greatest inhibition when exposed to phenolic compounds at low concentrations; while with the increase of the concentration of phenolic compounds, the most inhibited bacterium was *E. coli*. As a result, for toxicity assessment of phenolic compounds, it is essential to select microbe according to the phenolic concentrations. Considering its high sensitivity to heavy metal ions and phenolic compounds at the same time, *E. coli* was selected as the experimental microbe for following studies.

Incubation temperature

Given that the metabolic activities of microbes are sensitive to incubation temperature, the effect of incubation temperature on acute biotoxicity assessment between 25 ℃ and 45 ℃ was evaluated. As shown in Fig. S4a, the inhibitions engendered by Cu^{2+} (6 mg/L) went up significantly with the increase of incubation temperature from 25 ℃ to 35 ℃, with the further increase of incubation temperature from 35 ℃ to 45 ℃, the inhibitions showed a downward trend. The highest inhibition was obtained at 35℃. As a result, 35 ℃ was adopted as the optimal temperature for acute toxicity assessment and used for further studies.

Microbial concentration

For total biotoxicity assessment, rapid detection and high sensitivity are two important goals. Previous works have shown the microbial concentration affect the sensitivity of biotoxicity assessment.[40, 41] Fig. S4b illustrated the influence of microbial concentrations (OD_{600}) for inhibition response caused by Cu^{2+} (6 mg/L) to *E. coli*. It is easy to understand that decreasing

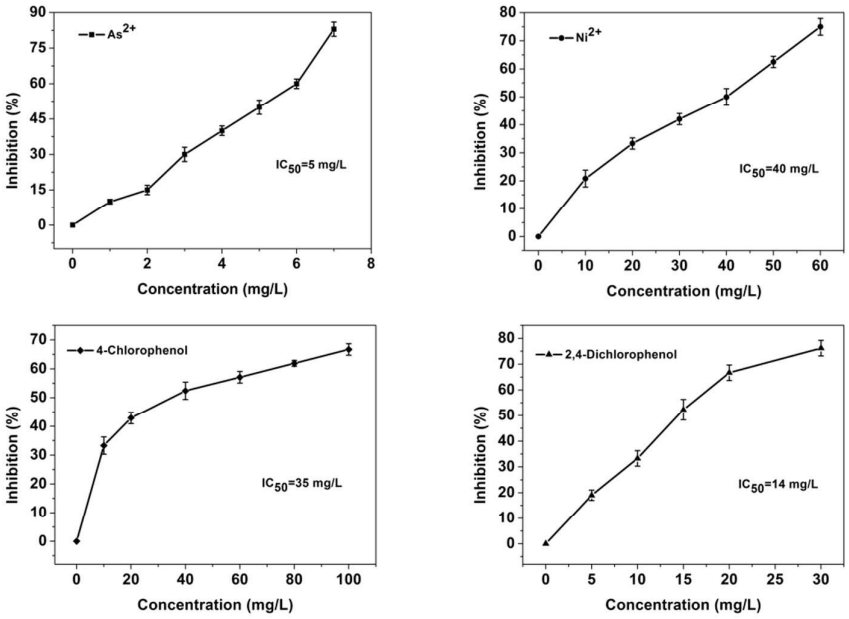


Figure 3. Responses of *E. coli* to four typical environmental pollutants (As^{3+}, Ni^{2+}, 4-chlorophenol, 2,4-dichlorophenol). Data points represent the average of three replicates.

Table 1. Comparison of IC_{50} values by measuring glucose consumption with PGM with other methods obtained in references.

Methods	Environmental pollutants, IC_{50} (mg/L)					Ref.
	As^{3+}	Ni^{2+}	Cd^{2+}	4-chlorophenol	2,4-dichlorophenol	
Glucose consumption inhibition	5	40	14.2	35	14	Present study
Respirometry inhibition	-	-	-	175	42	42
Respirometry inhibition	15	>60	-	-	-	7
Luminescence inhibition	-	-		89.7	26	43
Luminescence inhibition	18.8		21.8	-	-	44
Nitrification inhibition	-	-	20	-	-	45

Journal Name

ARTICLE

microbial concentration is equivalent to 'increase' the concentration of toxicants under the condition that the initial toxicant concentration is fixed. As shown in Figure S4b, the inhibition increased from 14% to 45% with the decrease of OD_{600} from 3.5 to 2.5 when the concentration of Cu^{2+} was fixed at 6 mg/L. However, with the further decrease of OD_{600} from 2.5 to 2.0, a decrease of the inhibition was observed. The inhibition reached the maximum when OD_{600}=2.5, indicating *E. coli* showed highest sensitivity to toxicants under present concentration. Therefore, OD_{600}=2.5 was chosen as the optimal microbial concentration for biotoxicity assessment and used for further studies.

Acute biotoxicity assessments of four environmental pollutants

Biotoxicity of four typical environmental pollutants (As^{3+}, Ni^{2+}, 4-chlorophenol, 2,4-dichlorophenol) were evaluated under the optimized conditions and the results were shown in Fig. 3. We also calculated the IC_{50} values for As^{3+}, Ni^{2+}, 4-chlorophenol, 2,4-dichlorophenol according to Eq. 1, they are 5 mg/L, 40 mg/L, 35 mg/L and 14 mg/L, respectively. Based on the magnitude of IC_{50} values, the acute toxicity sequence was As^{3+}>2,4-dichlorophenol>4-chlorophenol>Ni^{2+}. The IC_{50} values obtained were further compared with those of the published data obtained by other methods, as listed in Table 1. As can be seen in Table 1, the IC_{50} values obtained by measuring glucose concentration with PGM were comparable or even better than those of other methods. Besides, some significant differences could be observed in the sensitivities of various methods, which indicated the fact that different microbes and experimental procedures have their own sensitivity patterns to toxicants.[7] All the results suggest that measuring the glucose consumption of *E. coli* with PGM for acute toxicity assessment is promising and competitive.

Joint biotoxicity assessment of binary toxicants

For the real wastewater, chemicals often exist as mixtures, it is essential to study the joint toxicity of multiple toxicants. In the previous part, we evaluated the acute toxicity of single toxicant, such as As^{3+}, Ni^{2+}, 4-chlorophenol and 2,4-dichlorophenol. To simple show that our method can also be applied in joint biotoxicity assessment, the binary combinations of As^{3+} and Ni^{2+}, As^{3+} and 4-chlorophenol, 4-chlorophenol and 2,4-dichlorophenol were selected, which belong to the metal-metal mixture, metal-organic mixture and organic-organic mixture, respectively, and their joint toxicity at different TUs were evaluated. The TUs were calculated based on the IC_{50} value of each individual toxicant according to the Eq. 2. Fig. S5a, b, c shows the dose-response curves of As^{3+}+Ni^{2+}, As^{3+}+4-chlorophenol and 4-chlorophenol+2,4-dichlorophenol, respectively. From these dose-response relationships, IC_{50mix}, sum of toxic unit ($TU_{summation}$) at 50% inhibition for the mixtures, were calculated. The combined effect was defined as being concentration additive (IC_{50mix}=1TU), synergistic (IC_{50mix}< 1TU) or antagonistic (IC_{50mix}>1TU).[33, 34] As shown in Figure S5d, binary combinations of the three kinds of toxicants produced all three types of interactions: synergistic, concentration additive, and antagonistic. The IC_{50mix} was 0.91TU for As^{3+}+Ni^{2+}, indicating the combination of As^{3+} and Ni^{2+} produced synergistic effect. When 4-chlorophenol and 2,4-dichlorophenol were mixed, the IC_{50mix} was 1.27TU, which was significantly greater than 1TU, showing that an antagonistic response existed. The IC_{50mix} was approximately equal to 1TU for the combination of As^{3+} and 4-chlorophenol, indicating that the concentration additive effect occurred.

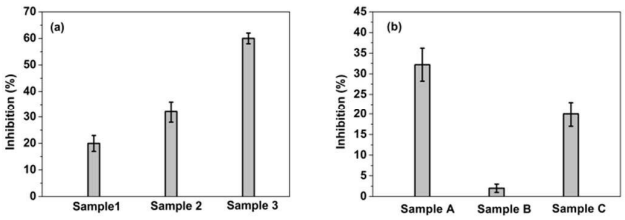


Figure 4. Acute biotoxicity assessments of real samples: (a) Sample 1, Sample 2, Sample 3 were effluents from landfill, wastewater from chemical laboratory and electroplating wastewater, respectively; (b) Sample A, Sample B, Sample C were polycyclic aromatic hydrocarbons polluted soil from foundry, soil from farm land and heavy metal ions polluted soil from lead zinc mine area, respectively. Data columns represent the average of three replicates.

It should be noted that the joint toxicity of mixtures containing multiple toxicants were not evaluated due to our aim was just to show the potential application of our method in joint biotoxicity assessment rather than study the joint biotoxicity comprehensively. Besides, the achieved joint biotoxicity results of these three binary combinations (As^{3+}+Ni^{2+}, As^{3+}+4-chlorophenol and 4-chlorophenol+2,4-dichlorophenol) to *E. coli* are not suitable to all metal-metal mixtures, metal-organic mixtures and organic-organic mixtures or other organisms. For example, the combination of Cd and Cu shows concentration additive effect on *Cucumis sativus*,[34] but it shows synergistic effect on *Caenorhabditis elegans*.[46] As a result, it may be wrong to expect the combination of As^{3+} and Ni^{2+} or any metal-metal toxicant mixture should give a synergistic interaction to organisms other than *E. coli*, which is also the same for the combination of As^{3+} and 4-chlorophenol, 4-chlorophenol and 2,4-dichlorophenol.

Journal Name

ARTICLE

Evaluation of the acute toxicity of real samples

The acute biotoxicity of three polluted water samples and three soil samples were also evaluated by the method we proposed. As can be seen in Fig. 4, three water samples and three soil samples exhibited acute toxicity to *E. coli*. The inhibitions caused by effluents from landfill, wastewater from chemical laboratory and electroplating wastewater were 20%, 32% and 60%, respectively, which indicated electroplating wastewater was most toxic and effluents from landfill was least toxic among these three water samples. The electroplating wastewater showing strong toxicity was within our expectation because it is well known that electroplating industry produce highly toxic heavy metals and cyanide ions. The inhibitions engendered by polycyclic aromatic hydrocarbons polluted soil from foundry, soil from farm land, heavy metal ions polluted soil were 32.2%, 2.3% and 20%, respectively. The successful total acute toxicity assessments of these six real samples proved the practicability of our method.

There still exist two limitations of our method: (1) the usage of centrifuge and thermostat incubator makes it difficult to assess biotoxicity on site; (2) the time to complete the whole experiment is a little long (1 h). Hence, there are much more to be done in our future work. Besides, it should be pointed out that acute biotoxicity assessment is focusing on the acute toxic effect of a substance on living organisms rather than detecting this substance specifically and analytically. Therefore, our method can reflect the acute biotoxicity of samples if only there exist substances which may affect the microbial glucose metabolism.

Conclusions

In this work, a new method for acute biotoxicity assessment was proposed, *i. e.*, measuring the microbial glucose consumption with personal glucose meter (PGM). *E. coli* was chosen as the target microbe for acute biotoxicity assessment due to its high sensitivity to heavy metal ions and phenolic compounds. Acute toxicity of As^{3+}, Ni^{2+}, 4-chlorophenol, 2,4-dichlorophenol were evaluated under the optimized conditions. The IC_{50} values determined were 5 mg/L for As^{3+}, 40 mg/L for Ni^{2+}, 35 mg/L for 4-chlorophenol and 14 mg/L for 2,4-dichlorophenol. These results obtained were comparable or even better than those of other reported acute biotoxicity assessment methods. Besides, the joint biotoxicity of these four toxicants was also investigated. Moreover, the total acute biotoxicity of three water samples and three soil samples were successfully evaluated by our method. All the results suggest our method offering a simple, low-cost and practical alternative for total acute biotoxicity assessment.

Acknowledgements

The authors appreciate the supports of the Key Project of Beijing Natural Science Foundation (No.2120002), the international Science & Technology Cooperation Program of China (Most-Japan joint project, No. 2013DFG50150), and the Natural Foundation of Sciences of the People's Republic of China (No.21175144).

References

1 S. Sharma, S. Sharma, N. Upreti and K. P. Sharma, *Toxicological and Environmental Chemistry*, 2009, 91, 109-120.
2 T. Tatsuma, Y. Yoshida, I. Shitanda and H. Notsu, *Analyst*, 2009, 134, 223-225.
3 R. Weltens, R. Goossens and S. Van Puymbroeck, *Archives of Environmental Contamination and Toxicology*, 2000, 39, 315-323.
4 R. Dagan, B. Dubey, G. Bitton and T. Townsend, *Archives of Environmental Contamination and Toxicology*, 2007, 53, 168-173.
5 W. H. van der Schalie, T. R. Shedd, P. L. Knechtges and M. W. Widder, *Biosensors & bioelectronics*, 2001, 16, 457-465.
6 C. Liu, T. Sun, X. Xu and S. Dong, *Analytica chimica acta*, 2009, 641, 59-63.
7 X. Wang, M. Liu, X. Wang, Z. Wu, L. Yang, S. Xia, L. Chen and J. Zhao, *Biosensors & bioelectronics*, 2013, 41, 557-562.
8 S. Girotti, E. N. Ferri, M. G. Fumo and E. Maiolini, *Analytica chimica acta*, 2008, 608, 2-29.
9 A. Tizzard, J. Webber, R. Gooneratne, R. John, J. Hay and N. Pasco, *Analytica chimica acta*, 2004, 522, 197-205.
10 J. Qian, J. Li, D. Fang, Y. Yu and J. Zhi, *RSC Advances*, 2014, 4, 55473-55482.
11 A. Reinhartz, I. Lampert, M. Herzberg and F. Fish, *Toxicity Assessment* 1987, 193-206.
12 R. J. Dutton, G. Bitton, B. Koopman and O. Agami, *Archives of Environmental Contamination and Toxicology*, 1990, 19, 395-398.
13 Z. Kong, P. A. Vanrolleghem and W. Verstraete, *Biosensors and Bioelectronics*, 1993, 8, 49-58.
14 Z. Filipovic-Kovacevic, M. Miksaj and D. Salamon, *European Food Research and Technology*, 2002, 215, 347-352.
15 P. A. Ruffieux, U. von Stockar and I. W. Marison, *Journal of biotechnology*, 1998, 63, 85-95.
16 D. Yu, J. Zhai, D. Yong and S. Dong, *Analyst*, 2013, 138, 3297-3302.
17 C. Liu, T. Sun, Y. Zhai and S. Dong, *Talanta*, 2009, 78, 613-617.
18 D. K. Bwambok, D. C. Christodouleas, S. A. Morin, H. Lange, S. T. Phillips and G. M. Whitesides, *Analytical Chemistry*, 2014, 86, 7478-7485.
19 A. W. Martinez, S. T. Phillips, M. J. Butte and G. M. Whitesides, *Angewandte Chemie-International Edition*, 2007, 46, 1318-1320.

20 E. Petryayeva and W. R. Algar, The Analyst, 2015, 140, 4037-4045.
21 A. C. Glavan, A. Ainla, M. M. Hamedi, M. Teresa Fernandez-Abedul and G. M. Whitesides, *Lab on a Chip*, 2016, 16, 112-119.
22 L. C. Clark and C. Lyons, *Annals of the New York Academy of Sciences*, 1962, 102, 29-&.
23 J. Wang, *Chemical Reviews*, 2008, 108, 814-825.
24 S. K. Vashist, D. Zheng, K. Al-Rubeaan, J. H. T. Luong and F.-S. Sheu, *Analytica chimica acta*, 2011, 703, 124-136.
25 Y. Xiang and Y. Lu, *Nature Chemistry*, 2011, 3, 697-703.
26 R. Chavali, N. S. K. Gunda, S. Naicker and S. K. Mitra, *Analytical Methods*, 2014, 6, 6223-6227. asd
27 C. Gu, T. Lan, H. Shi and Y. Lu, *Analytical Chemistry*, 2015, 87, 7676-7682.
28 J. Zhang, Y. Xiang, D. E. Novak, G. E. Hoganson, J. Zhu and Y. Lu, *Chemistry-an Asian Journal*, 2015, 10, 2221-2227.
29 L. Yan, Z. Zhu, Y. Zou, Y. Huang, D. Liu, S. Jia, D. Xu, M. Wu, Y. Zhou, S. Zhou and C. J. Yang, *Journal of the American Chemical Society*, 2013, 135, 3748-3751.
30 Y. Xiang and Y. Lu, Chemical Communications, 2013, 49, 585-587.
31 J. Joo, D. Kwon, H. H. Shin, K.-H. Park, H. J. Cha and S. Jeon, Sensor Actuat B-Chem, 2013, 188, 1250-1254.
32 Z. Wang, Z. Chen, N. Gao, J. Ren and X. Qu, Small, 2015, 11, 4970-4975.
33 P. A. PapeLindstrom and M. J. Lydy, *Environmental Toxicology and Chemistry*, 1997, 16, 2415-2420.
34 Y. J. An, Y. M. Kim, T. I. Kwon and S. W. Jeong, *Science of the Total Environment*, 2004, 326, 85-93.
35 K. H. Cha, G. C. Jensen, A. S. Balijepalli, B. E. Cohan and M. E. Meyerhoff, *Analytical Chemistry*, 2014, 86, 1902-1908.
36 S. Nakamura and Y. Ogura, *Journal of Biochemistry*, 1968, 64, 439-&.
37 C. Chen, Q. Xie, L. Wang, C. Qin, F. Xie, S. Yao and J. Chen, *Analytical Chemistry*, 2011, 83, 2660-2666.
38 S. Fennouh, V. Casimiri, A. Geloso-Meyer and C. Burstein, *Biosensors & bioelectronics*, 1998, 13, 903-909.
39 US Patent, 2004, International Application Number: PCT/US2004/019687.
40 D. Fang, Y. Yu, L. Wu, Y. Wang, J. Zhang and J. Zhi, *RSC Advances*, 2015, 5, 59472-59479.
41 D. Yong, C. Liu, C. Zhu, D. Yu, L. Liu, J. Zhai and S. Dong, *Chemosphere*, 2015, 139, 109-116.
42 N. F. Pasco, R. Goonerate, R. M. Daniel, A. Czollner and A. J. Scott, *International Journal of Environmental Analytical Chemistry*, 2008, 88, 1063-1075.
43 S. H. Choi and M. B. Gu, *Biosensors & bioelectronics*, 2002, 17, 433-440.
44 J. C. Cho, K. J. Park, H. S. Ihm, J. E. Park, S. Y. Kim, I. Kang, K. H. Lee, D. Jahng, D. H. Lee and S. J. Kim, *Biosensors & bioelectronics*, 2004, 20, 338-344.
45 D. J. B. Dalzell, S. Alte, E. Aspichueta, A. de la Sota, J. Etxebarria, M. Gutierrez, C. C. Hoffmann, D. Sales, U. Obst and N. Christofi, *Chemosphere*, 2002, 47, 535-545.
46 K. W. Chu and K. L. Chow, Aquatic Toxicology, 2002, 61, 53-64.