Acute biotoxicity assessment of heavy metals in sewage sludge based on the glucose consumption of \textit{Escherichia coli}

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As a simple and feasible method for acute biotoxicity assessment, personal glucose meter (PGM) can be successfully applied in the early warning of environmental pollutants in sewage. In this paper, the acute biotoxicity of single and joint heavy metals in sewage and real sludge samples was systematically described based on the glucose metabolism of \textit{Escherichia coli} (\textit{E. coli}). Results indicated that the biotoxicity order of five single heavy metals in sewage was Hg$^{2+}$ > As$^{3+}$ > Cu$^{2+}$ > Zn$^{2+}$ > Cd$^{2+}$. The joint heavy metals of Cu$^{2+}$ + Zn$^{2+}$, Cu$^{2+}$ + Cd$^{2+}$, and Cu$^{2+}$ + Hg$^{2+}$ produced synergistic effects, while Cu$^{2+}$ + As$^{3+}$ and Cd$^{2+}$ + Zn$^{2+}$ possessed antagonistic effects for the combined biotoxicity. In spiked sludge, Cd$^{2+}$ and Zn$^{2+}$ owned higher biotoxicity than Cu$^{2+}$ and As$^{3+}$. Notably, the electroplate factory and housing estate sludge respectively showed the highest and lowest inhibition rates as 57.4% and 17.7% under the real sludge biotoxicity assessment. These results demonstrated that PGM was a sensitive and portable method, which could be widely used for acute biotoxicity assessment of heavy metals in sewage sludge.

1. Introduction

According to the China Environment Statistical Yearbook (2017), approximately 135.20 million cubic metres per day of urban sewage was produced in China in 2016 [1]. The various applications of sewage sludge administered to agricultural soil can improve the physical, chemical and biological properties of the soil, such as N, P, K and other micronutrients [2,3], and in some cases, can contribute to causing harm to humans and animals after entering food chain [4,5]. One of the main
constraints of sewage sludge use on agricultural soil was its high biotoxicity of heavy metals (As, Cu, Cd, Pb, Zn, Hg, etc.) [6]. Concentrations of Zn, Cu, Pb and Cd heavy metals were measured to monitor the instant pollution in the Golden Horn sediment sludge. The total heavy metal concentrations and range of ratios in sludge samples were critical for potential biotoxicity [7]. Otherwise, Cr$^{3+}$ was 6.41 times higher toxicity than the baseline in aquatic ecotoxicity while Cu$^{2+}$ has the major contribution to terrestrial ecotoxicity in the tannery sludge in Bangladesh [8]. Therefore, it is a challenge to rapidly detect biotoxicity of environmental pollutants in sewage sludge.

In the past decades, many researches of biotoxicity assessment have been mainly focused on the sensitivity and pollutant degradation analysis of aquatic organisms and invertebrates [9–12], toxicity assessment of bioluminescent bacteria in water and atmospheric particulate matter [13,14] and electrochemical detection of microbial respiratory inhibition [15–17]. For example, higher concentration of triclosan has produced great oxidative stress to goldfish under the acidic condition [18]. The uninterrupted application of plasma gas can decrease the bioluminescence of Photobacterium phosphoreum [14]. The study of Hg$^{2+}$ ion on DC electrical properties has found that HgCl$_2$ salt acted as an inhibitor to Escherichia coli (E. coli) [16]. In addition, those methods were also characterized by high cost and time-consuming. It is necessary to develop a timely and effectively method for acute biotoxicity assessment, which can play an important role in the early warning of environmental pollutants in sewage sludge.

As a successful electrochemical biosensor, personal glucose meter (PGM) has been widely used to monitor the blood glucose of diabetic patients around world [19]. This system was based on the electric flow of glucose produced in the blood samples, which can be measured by PGM. Nowadays, PGM has also been used to detected biological molecules and microorganisms with other instruments or technologies. For example, PGM coupled with aptamer–invertase biosensor can quantify detected quinine in reclaimed wastewater [20]. PGM combination with UO$_2$$^{2+}$-specific DNAzyme was a sensitive and specific method to quantify Pb$^{2+}$ and UO$_2$$^{2+}$ ions [21]. Based on the combined effect of PGM and DNAszyme-capped mesoporous silica nanoparticles (MSNs), this as-prepared sensing platform has been successfully allowed to detect Pb$^{2+}$ at 1.0 ppm level [22]. PGM combination with monoclonal antibody-functionalized magnetic nanoparticle clusters (MNCs) was a simple and sensitive method for the detection of Salmonella bacteria in milk [23]. Moreover, PGM can also determine chloramphenicol (CAP) in animal-derived food, and this method has been successfully applied in the on-site assay [24]. PGM also has a certain development prospect for acute biotoxicity assessment of pollutants (such as As$^{3+}$, Ni$^{2+}$, 2,4-dichlorophenol and 4-chlorophenol), and it can be popularly considered due to the low cost and simple operation characteristics [25,26].

Herein, we optimized the experimental parameters and assessed the acute biotoxicity of heavy metal ions (Cu$^{2+}$, Zn$^{2+}$, Cd$^{2+}$, Hg$^{2+}$ and As$^{3+}$) separately as well as joint mixtures by using PGM method. The microorganisms include E. coli and Bacillus subtilis (B. subtilis). The inhibition rate and toxicity unit (TU) values of this study have successfully testified that PGM method can reflect the genuine biotoxicity of real sludge samples and it was a reliable and simple alternative for acute biotoxicity assessment in sewage sludge.

2. Material and methods

2.1. Materials and reagents

E. coli and B. subtilis were bought from China General Microbiological Culture Collection Center (CGMCC). The serial numbers were CGMCC 1.1564 and 1.7740 for E. coli and B. subtilis, respectively. Copper (II) sulphate pentahydrate (CuSO$_4$·5H$_2$O; 99.0%), zinc sulphate heptahydrate (ZnSO$_4$·7H$_2$O; 99.5%), cadmium sulphate 8/3hydrate (CdSO$_4$·8/3H$_2$O; 99.0%), sodium arsenite solution (NaAsO$_2$; 90.0%) and mercurous nitrate dihydrate (Hg$_2$(NO$_3$)$_2$·2H$_2$O) were bought from Sinopharm Chemical Reagent Co. Ltd (Shanghai, China). PGM was purchased from Changsha Sinocare Biosensing Incorporated Company, China. All the chemicals and reagents were stored at 4°C in a refrigerator.

The sewage containing five heavy metals (Cu$^{2+}$, Zn$^{2+}$, Cd$^{2+}$, As$^{3+}$ and Hg$^{2+}$) was real-time prepared by the industrial wastewater of sewage treatment plant and deionized water. Each kind of heavy metal sewage was prepared in at least five concentrates gradients. The joint heavy metal sewages of Cu$^{2+}$ + Zn$^{2+}$, Cu$^{2+}$ + Cd$^{2+}$, Cu$^{2+}$ + Hg$^{2+}$, Cu$^{2+}$ + As$^{3+}$ and Cd$^{2+}$ + Zn$^{2+}$ were prepared by adding each heavy metals as the same concentration as in their single experiment. All the concentrates gradients of
heavy metal sewage were realized by those reagents (CuSO₄·5H₂O, ZnSO₄·7H₂O, CdSO₄·8/3H₂O, NaAsO₂ and Hg₂(NO₃)₂·2H₂O) and deionized water and repeated in triplicate.

2.2. Microbial cultures

Five vaccination rings of *E. coli* and *B. subtilis* were inoculated in lysogeny broth (LB) medium, respectively. The LB medium was put into oscillation incubator (180 r.p.m.) and shaken at 30°C for 24 h to obtain 100 ml bacterial saline suspension solution. Bacterial saline suspension solution (10 ml) was taken for subculture three times with the same method as above. The last subculture of *E. coli* and *B. subtilis* was incubated to the post stabilization period. Then 20 ml 0.85% (w/v) saline was added into the bacterial saline suspension solution and centrifuged at 6000 r.p.m. for 5 min. The *E. coli* and *B. subtilis* suspension solution was diluted by saline (0.85(w/v)). The UV spectrophotometer (TU1901, Shanghai) has been used to determine the optical density of bacterial suspension solution at 600 nm (OD₆₀₀) for 2.3. The bacterial suspension solutions were stored at 4°C in a refrigerator for less than 3 h until the experiment started [27,28].

2.3. Acute biotoxicity assessment in sewage

The sewage samples were prepared by evenly mixing 1 ml LB medium, 0.1 ml glucose, 0.1 ml heavy metal liquid and 0.8 ml *E. coli* suspension solution. Saline (0.85% (w/v)) was selected as the control sample. All the samples were cultivated at 30°C after a period of time, then centrifuged at 9000 r.p.m. for 1 min. Supernatants (5 µl) were taken out from the suspension solution and the glucose concentration measured by PGM. Based on the initial and final glucose concentration, the equivalent inhibition rate was calculated by equation (2.1) [29].

\[
\text{Inhibition(%) = } \left( \frac{C_e - C_i}{C_i} \right) \times 100\%,
\]

where, \(C_i\) is the initial glucose concentration of all samples; \(C_e\) is the final glucose concentration of control group samples; \(C_c\) is the final glucose concentration when heavy metal existed.

2.4. Joint biotoxicity assessment in sewage

The joint biotoxicity assessment was determined by the TU, which has been widely used to test the reaction of chemical mixture. TU values were calculated by equation (2.2) [25].

\[
\sum \text{TU}_i = \sum_{i=1}^{n} \frac{C_i}{IC_{50,i}}
\]

where, \(C_i\) is the concentration of mixture component; \(n\) is the type of toxic substance in the sample; \(IC_{50,i}\) is the half maximal inhibitory concentration of component \(i\). Among them, half maximal inhibitory concentration of mixture \((IC_{50\text{mix}}) < 1\text{TU}\) means synergistic effect; \(IC_{50\text{mix}} = 1\text{TU}\) means additive effect; \(IC_{50\text{mix}} > 1\text{TU}\) means antagonistic effect.

2.5. Biotoxicity assessment of heavy metals in sludge

Due to the high content and strong biotoxicity of heavy metals in the sewage sludge, the toxicants have exceeded the detection range by microbial method. To improve the sensitivity of the PGM method, spiked sludge was prepared by industrial park sludge and fresh soil with different mixed ratio. Then, added the reagents (CuSO₄·5H₂O, ZnSO₄·7H₂O, CdSO₄·8/3H₂O and NaAsO₂) were added into the heavy metal extract solution to obtain different concentrates of spiked sludge, respectively.

The heavy metal composition analysis of four real sludge samples (industrial park, electroplate factory, steelworks and housing estate) was performed by inductively coupled plasma-atomic emission spectrometry (ICP-AES) (ICP-5000, Zhejiang). Real sludge samples were directly prepared by four real sludge and fresh soil using the same process as above. All spiked or real sludge samples were air-dried and milled by 2 mm screen.

Samples of air-dried sludge (2 g) and 0.1 mol l⁻¹ hydrochloric acid were taken into centrifuge tube, then centrifuged (180 r.p.m.) for 2 h to obtain the heavy metal extract solution. Using the same process as the acute biotoxicity assessment in sewage, biotoxicity assessment of heavy metals in sludge samples...
were detected by PGM and calculated by equations (2.1) and (2.2). The parameter optimization process of the heavy metal extract solution is shown in electronic supplementary material, figure S1.

2.6. Statistical analysis

The statistics box figure and line chart was analysed by Origin 9.0. Data were presented as mean ± standard error of mean (s.e.m.). SPSS 19.0 was used for statistics analysis and the significant difference was at 0.05 probability level.

3. Results

3.1. Principle verification

Glucose oxidase or glucose dehydrogenase of bacteria could be inhibited by heavy metal, which was related with glucose production. As shown in figure 1, when E. coli was suspended with glucose and heavy metal was not added, lots of glucose was consumed in normal metabolism and PGM showed the low glucose concentration as 3.0 mM. If heavy metal existed in the suspended solution, glucose will be less consumed in disturbed metabolism. High glucose concentration was displayed in PGM as 7.0 mM.

In order to verify the glucose concentration, PGM can be applied in acute biotoxicity assessment as an indicator; the PGM signal of E. coli and glucose suspended with or without Cu_{2}^{+} is shown in figure 2.

Figure 2a is the glucose concentration–time curve of the microbial glucose metabolism. PGM signals were 5.03 ± 0.15 mM with no Cu_{2}^{+} and 6.63 ± 0.06 mM with 20 mg l^{-1} Cu_{2}^{+} at 60 min, which has the significant difference between each other. In the non-toxic environment, E. coli can consume more glucose than in a toxic environment. In addition, the final glucose concentration–Cu_{2}^{+} concentration curve was observed when samples were incubated for 60 min, as shown in figure 2b.

3.2. Optimization of experimental conditions

Parameter optimization of bioassay in terms of E. coli and B. subtilis concentration, culture temperature and OD_{600} values for inhibition of glucose metabolism was studied to obtain a better condition for biotoxicity assessment of Cu_{2}^{+} and Zn_{2}^{+}. In figure 3a, from the responses of E. coli and B. subtilis to two heavy metal ions, Cu_{2}^{+} and Zn_{2}^{+} had a relatively high inhibitory effect on the glucose metabolism of E. coli, while the glucose metabolism of B. subtilis was almost not affected. It was observed that with incubation temperature and microbial concentration increased, the inhibition of glucose metabolism on E. coli by Cu_{2}^{+} (10 mg l^{-1}) increased firstly and then decreased. The highest inhibition was obtained at 30°C and the inhibition rate was 47.6% in figure 3b. And figure 3c shows that the largest inhibition rate of E. coli was 57.4% when microbial concentration (OD_{600} value) was 2.3.

3.3. Acute biotoxicity assessment of single heavy metal in sewage

Biotoxicity of five single heavy metals was assessed after optimization of experimental parameters. As shown in figure 4, with the concentration of heavy metal increased, the inhibition of glucose metabolism on E. coli increased. The inhibitory curves of five single heavy metals were adapted to
linear fitting. All the R square of linear fitting correlation was greater than 0.9200. The IC50 values of Cu2+\(,\) Zn2+, As3+, Cd2+ and Hg2+ were 10.3, 12.9, 6.9, 24.3 and 2.8 mg l\(^{-1}\), respectively. The biotoxicity order of single heavy metal to \(E. coli\) was Hg2+. As3+. Cu2+. Zn2+. Cd2+. Hg2+.

Comparing the IC50 values of heavy metal in this paper with other methods, many IC50 values of Cd2+, Zn2+, Cu2+ and Hg2+ to \(E. coli\) by using glucose consumption inhibition method were lower than other methods [29–32], as shown in table 1. From the comparison of single heavy metal toxicity assessment, it can be seen that Hg2+ had the highest biological toxicity for different microbial species with different methods.

### 3.4. Joint biotoxicity assessment of binary heavy metals in sewage

For real wastewater or other contaminants, chemical substances often existed as mixtures. It was crucial to study the mixture poisons biotoxicity. As shown in table 2, the IC50 value of each heavy metal was treated as 1 TU in the mixture for subsequent joint toxicity evaluation. In order to facilitate the setting of binary heavy metal concentration, the concentrations of Cu2+, Zn2+, Cd2+, Hg2+ and As3+ were defined as A at 10, 20, 20, 10 and 3 mg l\(^{-1}\), respectively.

For the combined effect experiment, the combined toxicity was measured through the TU of binary compounds in table 3. Based on the response relation of the obtained dose (TU-based), combined effect of heavy metals was defined as synergy effect (IC50\(_{\text{mix}}<1\) TU), additive effect (IC50\(_{\text{mix}}=1\) TU) or antagonism effect (IC50\(_{\text{mix}}>1\) TU).

In this study, five binary heavy metals mainly produced as synergistic and antagonistic effects in figure 5. The dose (TU-based) response relationship curves of the binary heavy metals at different concentrations were adapted to logarithmic function fitting. All R square of logarithmic function fitting correlation was greater than 0.9750. Synergistic response to \(E. coli\) occurred when Cu2+ + Zn2+, Cu2+ + Cd2+ and Cu2+ + Hg2+ mixed together because the IC50\(_{\text{mix}}\) values were detected to be 0.86,
0.98 and 0.71, respectively. Joint heavy metals of Cu$^{2+}$, As$^{3+}$, and Cd$^{2+}$ produced antagonistic response for the biotoxicity as the IC$_{50}$ values were 1.85 and 1.43, respectively.

3.5. Acute biotoxicity assessment of heavy metals in sludge

To assess the acute biotoxicity of sewage sludge, the types and concentrations of heavy metals in the sludge were spiked according to the concentration of main heavy metals in industrial park sludge in Table 1.

| methods                                | Cu$^{2+}$ (mg L$^{-1}$) | Zn$^{2+}$ (mg L$^{-1}$) | As$^{3+}$ (mg L$^{-1}$) | Cd$^{2+}$ (mg L$^{-1}$) | Hg$^{2+}$ (mg L$^{-1}$) | references |
|----------------------------------------|-------------------------|-------------------------|-------------------------|-------------------------|-------------------------|------------|
| glucose consumption inhibition, E. coli| 10.3                    | 12.9                    | 6.9                     | 24.3                    | 2.8                     | present    |
| glucose consumption inhibition, E. coli| —                       | —                       | 5.0                     | 14.2                    | —                       | study      |
| amperometry mixed microbes             | 16.5                    | —                       | —                       | 20.5                    | —                       | [25]       |
| colorimetric bioassay, B. subtilis     | 5.0                     | 9.3                     | —                       | 21.3                    | —                       | [29]       |
| amperometry, E. coli                  | 44.0                    | —                       | —                       | 79.0                    | 21.2                    | [30]       |
| amperometry, Psychrobacter sp.        | 20.2                    | 53.2                    | —                       | 36.2                    | —                       | [31]       |
| amperometry, S. cerevisiae            | 10.12                   | 10.9                    | —                       | 47.3                    | 0.8                     | [32]       |
| nitrification inhibition              | 41.5                    | 22.6                    | —                       | 79.0                    | —                       | [33]       |
| Photobacterium phosphoreum            | 1.905$^a$               | —                       | 0.537$^a$               | —                       | —                       | [34]       |
| Photobacterium phosphoreum            | 0.5$^a$                 | —                       | —                       | —                       | —                       | [35]       |

$^a$15 min IC$_{50}$ (mg L$^{-1}$).

0.98 and 0.71, respectively. Joint heavy metals of Cu$^{2+}$ + As$^{3+}$ and Cd$^{2+}$ + Zn$^{2+}$ produced antagonistic response for the biotoxicity as the IC$_{50mix}$ values were 1.85 and 1.43, respectively.
our designed study. The mixed ratio of real sludge and fresh soil was 1 : 9. Different from sewage, it could produce acute biotoxicity to the bacteria when heavy metal dissolved from the sludge. The higher *E. coli* inhibition in spiked sludge was done by hydrochloric acid than acetic acid and deionized water, as shown in electronic supplementary material, figure S1(a).

### Table 2. The TU values of single heavy metal in different concentration gradients. Note: the concentrations of Cu$^{2+}$, Zn$^{2+}$, Cd$^{2+}$, Hg$^{2+}$ and As$^{3+}$ were defined as A at 10, 20, 20, 10 and 3 mg l$^{-1}$, respectively.

| concentration (mg l$^{-1}$) | TU$_{Cu^{2+}}$ | TU$_{Zn^{2+}}$ | TU$_{Cd^{2+}}$ | TU$_{Hg^{2+}}$ | TU$_{As^{3+}}$ |
|---------------------------|----------------|----------------|----------------|----------------|----------------|
| 0                         | 0              | 0              | 0              | 0              | 0              |
| 0.2A                      | 0.19           | 0.31           | 0.17           | 0.29           | 0.21           |
| 0.4A                      | 0.39           | 0.63           | 0.33           | 0.58           | 0.43           |
| 0.6A                      | 0.58           | 0.94           | 0.50           | 0.87           | 0.64           |
| 0.8A                      | 0.78           | 1.25           | 0.66           | 1.16           | 0.86           |
| A                         | 0.97           | 1.56           | 0.82           | 1.45           | 1.07           |

### Table 3. The sum of TU of binary heavy metals. Note: A has the same means as in table 2.

| concentration (mg l$^{-1}$) | TU$_{Cu^{2+}+Zn^{2+}}$ | TU$_{Cu^{2+}+Cd^{2+}}$ | TU$_{Cu^{2+}+Hg^{2+}}$ | TU$_{Cu^{2+}+As^{3+}}$ | TU$_{Cd^{2+}+Zn^{2+}}$ | TU$_{Cd^{2+}+Hg^{2+}}$ |
|---------------------------|-------------------------|-------------------------|-------------------------|-------------------------|-------------------------|-------------------------|
| 0                         | 0                       | 0                       | 0                       | 0                       | 0                       | 0                       |
| 0.2A                      | 0.50                    | 0.48                    | 0.40                    | 0.48                    | 0.36                    | 0.36                    |
| 0.4A                      | 0.99                    | 0.97                    | 0.82                    | 0.96                    | 0.72                    | 0.72                    |
| 0.6A                      | 1.52                    | 1.45                    | 1.22                    | 1.44                    | 1.08                    | 1.08                    |
| 0.8A                      | 2.03                    | 1.94                    | 1.64                    | 1.91                    | 1.44                    | 1.44                    |
| A                         | 2.53                    | 2.42                    | 2.04                    | 2.38                    | 1.79                    | 1.79                    |
As shown in figure 6, the biotoxicity of spiked sludge of Cu$^{2+}$ and As$^{3+}$ was relatively weak to *E. coli*. When the contents of Cu$^{2+}$ and As$^{3+}$ were respectively 1000 and 200 mg kg$^{-1}$, the growth inhibition rate was lower than 25%. However, when the contents of Cd$^{2+}$ and Zn$^{2+}$ were respectively 50 and 2000 mg kg$^{-1}$, the growth inhibition rates were 38.2% and 47.2% to *E. coli*.

### 3.6. Acute biotoxicity assessment of real sludge samples

The PGM method was applied in real sludge samples, including industrial park, electroplate factory, steelworks and housing estate. In figure 7, for the sludge from electroplate factory, steelworks, housing estate and industrial park, the inhibition rates on the growth of *E. coli* were 57.4%, 35.6%,
17.7% and 48.3%, respectively. The biotoxicity order of sludge was: electroplate factory > industrial park > steelworks > housing estate. Comparing the inhibition rates on the growth of *E. coli* in real sludge samples with ICP-AES method, electroplate factory and industrial park sludge also have more seriously heavy metal pollution, as shown in table 4.

### 4. Discussion

In recent years, some researches have indicated that heavy metals and other pollutants inhibited the glucose oxidase or glucose dehydrogenase of bacteria, which were related with glucose production [30,38,39]. A higher PGM signal was detected with the increase of Cu²⁺ and Zn²⁺ concentration [40]. Under the consistent parameter optimization of bioassay in terms of *E. coli* and *B. subtilis* concentration, culture temperature and OD₆₀₀ values [41,42], *E. coli* were more sensitive and suitable for heavy metal biotoxicity assessment in the following studies.

Comparing the IC₅₀ values of single heavy metal with the integrated biosensor, which was prepared by benzoquinone (BQ) redox mediator and gelatin-hybrid hydrogel (GSH), the detected IC₅₀ values of Cu²⁺, Cd²⁺ and Hg²⁺ to *E. coli* were respectively 44.0, 79.0 and 21.2 mg l⁻¹ [33]. Combined with p-benzoquinone-mediated amperometric biosensor, the IC₅₀ values of Cu²⁺, Zn²⁺, Cd²⁺ and Hg²⁺ to *Psychrobacter* sp. were 2.6, 10.9, 47.3 and 0.8 mg l⁻¹, respectively [31]. It was noted that glucose consumption method was more sensitive to heavy metals than various other methods. At the same time, Hg²⁺ had the highest biological toxicity for microbial in different methods [43]. Catterall et al. had studied the toxic effect of Hg²⁺ on *E. coli* through respiratory method, and showed that the IC₅₀ value was 2.03 mg l⁻¹, which was basically consistent with our study [44]. Otherwise, the research work of industrial wastewater under the *Photobacterium phosphoreum* method had shown that Cd²⁺ had a higher biotoxicity than Cu²⁺ [36]. The main reason for the result different from our findings might be that different microorganisms and test procedure had different sensitivity to pollutants.

According to the binary heavy metal biotoxicity research, the combined effects of Cd²⁺ + Zn²⁺ and Cu²⁺ + Cd²⁺ have existence conclusions in different methods [45,46]. In our studies, combined toxicity of Cu²⁺ + Cd²⁺ also produced synergistic effect, while Cd²⁺ + Zn²⁺ showed as antagonistic effect. This might be ascribed to Cu²⁺ being more bioavailable than other ions, which could enlarged the cell membrane permeability when Cu²⁺ and Cd²⁺ coexist in binary mixture. On the other hand, Zn²⁺ and Cd²⁺ coexistence would decrease the system toxicity due to the formation of less bioavailable complex than both single heavy metals [27].

Finally, we have applied the glucose consumption inhibition method to measure the biotoxicity of heavy metal to *E. coli* in spiked sludge and real samples. Biotoxicity of spiked sludge of Cu²⁺ and As³⁺ was weaker to *E. coli* than Zn²⁺ and Cd²⁺. This might be related to the low proportion of effective state and low biological toxicity of Cu²⁺ and As³⁺ found in sludge by many existing studies. Moreover, the form of Cu was mainly associated with the organic matter and Zn showed the higher proportion of exchangeable and reducible fractions in sludge, which had higher biotoxicity to *E. coli* [8,47]. For the biotoxicity of heavy metal in real samples, colorimetric method, electrochemical biosensor and other methods were applied to the acute biotoxicity detection of real heavy metals, and showed that electroplate factory and industrial park sludge had the higher biotoxicity to bacterial, which was basically consistent with the research results in our work [25,30,35].

On the whole results, it was successfully testified that heavy metal pollution had higher inhibition on the microbial glucose metabolism in this study. Based on the glucose consumption by *E. coli*, acute biotoxicity assessment can be consistent and accurate reflected by using PGM.

| real sludge        | pH  | Cu   | Zn   | Cd   | As | Hg |
|--------------------|-----|------|------|------|----|----|
| electroplate factory | 7.32 | 1283.2 | 1526.3 | 2.3 | 38.9 | — |
| steelworks         | 5.07 | 604.1 | 790.2 | 1.4  | 14.8 | — |
| housing estate     | 6.48 | 212.4 | 304.1 | —    | 2.5 | — |
| industrial park    | 6.23 | 427.8 | 5233.8 | 2.41 | 147.4 | — |

Table 4. The pH values and heavy metal contents of real sludge by ICP-AES method. Note: — undetected.
5. Conclusion

The systems of PGM and E. coli can detect the single and joint biotoxicity of heavy metal ions in sewage sludge. The IC_{50} values of Cu^{2+}, Zn^{2+}, Cd^{2+}, Hg^{2+} and As^{3+} were 10.3, 12.9, 24.3, 2.8 and 6.9 mg l^{-1} in sewage, respectively, revealing that Hg^{2+} was the most toxic ion to E. coli. The biotoxicity of binary heavy metals and four real sludge samples were also successfully assessed. In conclusion, our study offered an economic, timely and sensitive alternative for acute biotoxicity assessment of pollutants monitoring in sewage sludge.

Ethics. Escherichia coli and B. subtilis were bought from China General Microbiological Culture Collection Center (CGMCC). Experiments related to the plants and animals were not involved in this work.

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