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Short-time effect of heavy metals upon microbial community activity

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ABSTRACT

Microcalorimetry was applied to assess and compare the toxic effect of heavy metals, such as As, Cu, Cd, Cr, Co, Pb and Zn, on the soil microbial activities and community. About 1.0 g soil spiked 5.0 mg glucose and 5.0 mg ammonium sulfate, the microbial activities were recorded as power–time curves, and their indices, microbial growth rate constant k, total heat evolution Q_T, metabolic enthalpy ΔH_{met} and mass specific heat rate $J_{Q/S}$, were calculated. Comparing these thermodynamic parameters associated with growth yield, a general order of toxicity to the soil was found to be Cr > Pb > As > Co > Zn > Cd > Cu. When soil was exposed to heavy metals, the amount of bacteria and fungi decreased with the incubation time, and the bacterial number diminished sharply. It illustrates that fungi are more tolerant, and bacteria–fungi ratio would be altered under metal stress. To determine the status of the glucose consumed, a glucose biosensor with eggshell membrane was used to measure the remaining glucose in soil sample. Results showed that the time at which glucose was consumed completely was agreed with the microcalorimetric time to a large extent, and depended on the toxicity of heavy metals as well.

1. Introduction

Heavy metal contamination of soil is widespread due to metal processing industries, tannery, combustion of wood, coal and mineral oil, traffic, and plant protection [1]. Metals can exist in the soil solution as free cations (e.g. Cu^{2+}, Cd^{2+}, Zn^{2+}), as soluble complexes with inorganic or organic ligands (e.g. $ZnCl^{+}$, $CdCl_3^{-}$, metal citrates) and associated with colloidal material. However, metal toxicity is greatly affected by the physico–chemical nature of the environment and the chemical behavior of the particular metal species in question [2,3].

Soil microorganisms, as an essential component of most terrestrial ecosystems, play a critical role in the environment due to their role in cycling mineral compounds and in the decomposition of organic material [4]. Environmental stress caused by heavy metals generally decreases the diversity and activity of soil microbial populations, and upsets the ecological balance of population interactions within the community. The toxic effects of heavy metals result mainly from the interaction of metals with proteins (enzymes) and inhibition of metabolic processes [5]. So far, many researchers have measured the soil microbial activities as indicators of soil degradation by heavy metals [6–10].

Undoubtedly, metal effects on natural soil communities are complex and difficult to characterize because of the complex array of contributing factors. The existence of a highly heterogeneous population in soils implies that any methodology to measure its activity must be largely non-specific in order to include the contributions from all the diverse groups of soil microorganisms [11].

Meanwhile, soil microorganisms will release heat when decomposing the substrate. It has also been reported that heat released closely correlates with the CO_2 evolution [12]. CO_2 was considered as one of the most important indicator of soil microbial activity [13]. Consequently, microcalorimetric technique has the advantage to be specific to assess the overall biomass and activity of soil, which demands only the knowledge of the initial and final energetic states and also it's independent on the organisms and reaction pathways [14]. Currently, microcalorimetry is sensitive enough to detect very low values of heat and its application in studies of soil microbial activity [11,15,16]. Microcalorimetry permits us to monitor the soil microbial activity continuously during long periods of time without disrupting the system, giving qualitative and quantitative indicators that inform us about the soil state and the soil degradation [17]. Besides, it constitutes a very suitable method to obtain several indices simultaneously, such as microbial growth rate constant k, the heat yield of the microbial growth Q_T, the metabolic enthalpy ΔH_{met} etc. The enormous advantage made microcalorimetry successfully applied to study the effects of cop-

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per sulphate and ammonium-iron(II) phosphate monohydrate on soil microbial activities [18,19].

Microcalorimetric method has been used to assess the effect of heavy metals on soil microbial activities in soils [20,21]. In addition, most knowledge about effects of these toxic metals on soil microorganisms is derived from data relating to only a few toxic metals, or from studies on sewage sludge applications that contain metal mixtures at relatively low concentrations [22]. On the other hand, due to differences in methodology and experimental approaches it was not possible to compare directly and compile results from different studies although researchers initially attempted to establish relationships between metal toxicity and soil microbial activities.

For the growth rate, metabolic enthalpy and biomass, it is important to note that it reflect to those population which can utilized glucose as a substrate, i.e. substrate responsive microbes [23]. So far, most of traditional methods for glucose analysis, such as spectrophotometry, HPLC, amperometry, are lengthy or costly. Thus, new methods of glucose analysis appropriate for rapid filed tests are needed [24]. Recently, different biosensors have been developed and applied to measure glucose in different media [25,26].

This investigation aimed to evaluate the toxic effect of heavy metals (As, Pb, Cr, Cd, Cu, Zn and Co) on the soil microbial activities and community. Meanwhile, the glucose degradation in soil sample was determined by a dissolved oxygen sensor. Through these studies, we can better understand the change of microbial activity and population in short-term contaminated soil, and biota tolerance to heavy metals.

2. Materials and methods

2.1. Materials

Salts of heavy metals such as Cr (K_2CrO_4), Cu ($CuSO_4$), Cd ($Cd(NO_3)_2$), Co ($Co(NO_3)_2$), Pb ($Pb(NO_3)_2$), Zn ($ZnSO_4{\cdot}7H_2O$) and As ($NaAsO_2$) were purchased commercially.

Glucose oxidase (EC 1.1.3.4. from *Aspergillus*) with a specific activity of 245,900 units g^{-1} of solid and glutaraldehyde solution (25%, w/w) in water was obtained from the Department of Chemistry, Hong Kong Baptist University, Hong Kong SAR, China. The buffer solution for preparing glucose standards was monosodium dihydrogen phosphate–disodium hydrogen phosphate solution (200 mmol L^{-1}) at pH 7.0. All solutions were prepared with deionized water.

All chemical reagents were of analytical-reagent grade.

2.2. Soil sampling and analysis

The soil sample was collected from an orchard soil in Wuhan, Hubei Province, central China (where there is a typical subtropical zone), at a depth of 5–10 cm. After the removal of the surface layer, it was air-dried and sieved (mesh size $2 \times 2\,mm^2$) to remove root fragments and large particles. The soil was stored in polyethylene bags at 4 °C [27].

Soil pH value was determined with a pH-meter (Beckman φ690). The measurement was performed by dipping the electrode in the supernatant solution prepared, from 10.0 g of soil and 25.0 mL of distilled water. Organic matter (OM) content of each sample was determined by titrating the samples in an acidic medium, with the end point followed by a redox reaction [28]. The elements C, H, N were analyzed by element analyzer (VARIO EL3, Germany), using a previously obtained calibration curve.

The determinations of Na, K, Mg, Ca and soluble P were performed by extracting a percolated subsample of 5.0 g of soil with 50.0 mL Mehlich 3 solution (0.2 mol L^{-1} acetic acid, 0.25 mol L^{-1} ammonium nitrate, 0.015 mol L^{-1} ammonium fluoride, 0.013 mol L^{-1} nitric acid and 0.001 mol L^{-1} EDTA, pH 2.5) [29]. Na and K were determined by flame photometry and P by photometry, using a previously obtained calibration curve. Mg and Ca were obtained by atomic absorption spectrometry (WFX-1F2B, Beijing Ruili) [27].

Table 1
Main soil characteristics.

Organic matter	52.28 g kg^{-1}	P	33.17 mg kg^{-1}
C	34.88 g kg^{-1}	Na	27.53 mg kg^{-1}
H	8.16 g kg^{-1}	Ca	1157.61 mg kg^{-1}
N	1.89 g kg^{-1}	Mg	414.74 mg kg^{-1}
K	168.86 mg kg^{-1}	pH	5.83 ± 0.03
Soil texture[a]	Clay-loam	Sand	25.3%
Clay	29.8%	Silt	44.9%

[a] Soil textures were determined by the sedimentation method.

Physicochemical properties of soil used in this study were shown in Table 1.

2.3. Determination and immobilization of glucose oxidase

The basic principle of glucose biosensor is following:

$$\beta\text{-D-glucose} + O_2 + H_2O \xrightarrow{\text{glucose oxidase}} \text{D-gluconic acid} + H_2O_2 \quad (1)$$

Glucose oxidase (GOx) is used to catalyze the oxidation of glucose by oxygen to produce gluconic acid and hydrogen peroxide [24,25]. The construction of a glucose biosensor based on the action of GOx offers a suitable approach to determine the glucose content in liquid sample.

An eggshell membrane was carefully peeled from a broken fresh eggshell after removing albumen and yolk. It was then cleaned with a copious amount of deionized water several times in order to remove the albumen from the eggshell membrane completely. After that, it was cut into a circle of a diameter of 1.5 cm and then placed on a clean glass. 100 μL of 0.8% (w/v) GOx solution in a pH 7.0 phosphate buffer (25 mM) was added. After about 2 min, 10 μL of 2.5% (w/w) glutaraldehyde solution as a cross-linking agent was dropped onto the surface of the membrane. A glass rod was gently used to spread the glutaraldehyde solution thoroughly on the membrane surface. After about 1 h, the membrane was immersed and washed with a pH 7.0 phosphate buffer for 5 min. Finally, the GOx immobilized eggshell membrane was stored in a pH 7.0 phosphate buffer at 4 °C until use [26].

2.4. Instrument

Microcalorimeter (Thermometric Järfälla, Sweden) was used to determine the thermal effect of microbes in the given system. Each thermal power value is determined and analyzed from the calorimetric curve. The instrument was calibrated by the release of electrical energy in a resistor and the thermal effect of the sample ampoule was adjusted to the electrical calibration [30]. This microcalorimeter has precise control of the isothermal conditions in the thermostated bath and of the detection of the thermal events in the system [31].

Pasco CI-6542 dissolved oxygen sensor and Pasco CI-6400 Science Workshop 500 interface (purchased from Pasco Scientific, Roseville, CA, USA) were also used in order to determine the glucose degradation. The prepared GOx immobilized eggshell membrane was positioned on the surface of oxygen sensor's probe, and kept in a steady position by an *O*-ring. The electrode was immersed into a magnetically stirred fixed volume of a phosphate buffer solution. Various volumes of standard solutions were injected into the phosphate buffer with the use of a syringe. The dissolved oxygen signal was captured and processed by a datalogger system consisting of a Pasco Scientific Workshop 500 interface, serial cables, a power

supply, and control software. The real-time data were recorded by a computer.

The CJJ 79-1 thermostated magnetic stirrer was purchased from Automated Instruments Plant of Jintan City (Jiangsu province, China).

2.5. Experimental design

The calorimetric curve was obtained by the 4.0 mL stainless steel ampoules, which were hermetically closed by Teflon sealing discs to control evaporation and energy loss. All experiments, carried out at 28 °C, were performed in ampoules containing 1.0 g of soil and 0.2 mL of aqueous solutions of glucose (5.0 mg) and ammonium sulfate (5.0 mg) in a 1:1 proportion that avoided soil samples submergence and offered ample nutrition for microorganism growth [32]. The thermal effect associated with nutrient degradation was recorded by a computer as a function of time. The heavy metals (As, Cu, Cr, Co, Cd, Pb and Zn) were added into soil samples with the same dose of 1.0 mg g^{-1} soil, except for Cr (0.01 mg), because Cr inhibited the soil microbial growth completely at the dose of 1.0 mg g^{-1} soil.

Glucose degraded by soil microorganisms was measured by dissolved oxygen sensor. These experiments were kept under the same conditions as those of the calorimetric tests. Parallel eight 10.0 mLglass tubes with 1.0 g of soil were hermetically closed. The tubes were placed into the incubator at 28 °C. At about 3 h intervals, one tube was selected to measure the glucose concentration in soil. The tube was injected with 9.0 mLphosphate buffer, and then placed into a supersonic oscillator for 20 min. After that, soil solution was filtered to remove the soil particle. Finally, the filtrate was left for 10 min; and 1.0 μL upper filtrate was taken by syringe to measure the glucose concentration. The glucose concentration was calculated according to the calibration curve.

2.6. Calculation of parameters

In order to interpret the changes of metabolic activity of soil microorganisms exposed to various heavy metals in more quantitative ways, thermodynamic parameters, such as total heat evolution of samples Q_T and microbial growth rate constant k were calculated according to the thermogenic curves. From the areas limited by the power–time curves, Q_T in J g^{-1} soil, was calculated for all the samples [15]. While, microbial growth rate constant, k in min^{-1}, could be obtained from the semilogarithmic conversion of heat flow rate, according to the modified model of Monod [33]:

$$\ln P_t = \ln P_0 + kt \quad (2)$$

As the heat evolution is proportional to the quantity of nutrient added [34,35], the metabolic enthalpy change per mole of glucose degraded by microorganisms, ΔH_{met}, can be calculated from the equation [36]:

$$Q_t = \Delta H_{met}(S_0 - S_t) \quad (3)$$

where Q_t is the total heat evolved to time t; S_t is the quantity of nutrient at time t; S_0 is the initial quantity of nutrient. Since, it is assumed that glucose has been completely degraded when the power–time curve returns to the initial base line after microbial exponential growth [37], in this case, it is possible to quantify ΔH_{met} from the equation:

$$\Delta H_{met} = \frac{Q_T}{S_0} \quad (4)$$

Microbial biomass C is an important index in studies of soil microbial activity. It is associated closely with the heat flow rate measured by microcalorimeter. Based on the relationship, Sparling established an equation to depict their relation [38].

$$y = 0.6970 + 1.025x (r = 0.98, P < 0.001) \quad (5)$$

where $y = \log_{10}$ biomass C, μgC g^{-1}; $x = \log_{10}$ rate of heat output (P), μW g^{-1}.

The mass specific heat rate of soil, $J_{Q/S}$ in Joules per gram soil and hour J g^{-1} h^{-1}, is defined as the heat released by the basal respiration of the soil microorganisms.

The numbers of bacteria and fungi in soil samples were calculated by the plate-counting method as colony forming unit (CFU), using beef extract peptone agar medium and Martin's agar medium respectively [39].

2.7. Statistical analyses

The microbial growth rate constant, total heat evolution, metabolic enthalpy, biomass C, and mass specific heat rate from calorimetric experiments, and number of bacteria and fungi were obtained through three parallel experiments. The glucose determination was processed in triplicate for each soil sample. The ANOVA method was used to statistically determinate the significance at the $P < 0.05$ level of difference between treatments. Data were presented as arithmetic means ± standard deviations (SDs).

3. Results and discussion

3.1. Thermogenic curves

The set of distinguishable profiles obtained from thermal/power–time curves from soil samples amended with glucose and ammonium sulphate are shown in Fig. 1. Thermogenic profiles were different in samples exposed to different metals. Differences were attributed to different levels of metal-induced inhibition of the microbial activities. It demonstrates that the heavy metals ion restricted the soil microbial activities to some extent, and that a soil community has different susceptibility to effects of different heavy metals due to their different functions in microbial life processes.

Metals play an integral role in the life processes of microorganisms. Some metals, such as Co, Cr, Cu, Fe, As, Ni, and Zn, are required nutrients and are essential. Others have no biological role (Al, Cd, Au, Pb, and Ag) and are nonessential [40]. Essential metals play an important role in regulating gene expression and the activity of

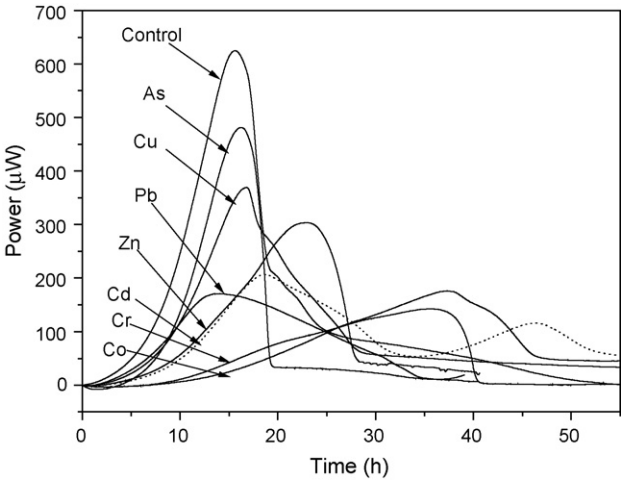

Fig. 1. Power–time curves for soil amended with glucose and ammonium sulphate and different selected heavy metals (1.0 mg g^{-1} As, Cu, Pb, Zn, Cd, Co and 0.01 mg g^{-1} Cr).

Table 2
Calorimetric parameters in the orchard soil amended with glucose, ammonium sulfate and heavy metals.

Heavy metal ion	Q_T[a] (J g^{-1} soil)	ΔH_{met}[b] (kJ mol^{-1})	$J_{Q/S}$[c] (J g^{-1} h^{-1})	k[d] (h^{-1})	p_{max}[e] (μW g^{-1})	Biomass C (mgC g^{-1})
Control	26.15 ± 4.21	−941.4 ± 33.3	0.47 ± 0.05	0.302 ± 0.041	624.4 ± 20.5	3.63 ± 0.53
As^{3+}	13.5 ± 2.19	−486 ± 21.5	0.34 ± 0.04	0.287 ± 0.025	481.3 ± 19.6	2.82 ± 0.14
Cu^{2+}	19.43 ± 1.97	−699.48 ± 27.3	0.35 ± 0.05	0.261 ± 0.014	369.2 ± 18.6	2.14 ± 0.24
Cr^{6+}	9.91 ± 0.95	−356.76 ± 24.5	0.22 ± 0.04	0.043 ± 0.005	142.0 ± 15.3	0.79 ± 0.07
Co^{2+}	13.97 ± 1.83	−502.92 ± 29.1	0.25 ± 0.03	0.085 ± 0.008	175.9 ± 12.7	1.0 ± 0.06
Cd^{2+}	17.82 ± 2.52	−641.52 ± 30.2	0.32 ± 0.05	0.220 ± 0.047	206.4 ± 19.1	2.82 ± 0.45
				0.082 ± 0.009	116.1 ± 15.5	
Pb^{2+}	11.19 ± 1.53	−402.84 ± 20.4	0.32 ± 0.04	0.191 ± 0.024	170.7 ± 13.8	0.95 ± 0.05
Zn^{2+}	14.13 ± 1.04	−508.68 ± 21.5	0.40 ± 0.02	0.163 ± 0.032	303.4 ± 19.5	1.74 ± 0.19

[a] Total heat released in Joules per gram of soil sample calculated from the area limited by the power–time curves obtained from soil samples amended with glucose
[b] Metabolic enthalpy change per mole of glucose degraded by soil microorganisms in kilojoules per mole of glucose
[c] Heat released by the basal respiration of the soil microorganism in Joules per gram of soil and day
[d] Microbial growth rate constant calculated from the slope of the lines obtained plotting the logarithm of the heat flow rate against time
[e] Rate of heat flow in μW per soil sample

biomolecules. They are also part of enzymes or cofactors for critical biochemical reactions. As a result of the distinction of these metals' functions, they exhibit a range of toxicities towards microorganisms, depending on physico–chemical factors, speciation etc., and while toxic effects on microbial communities are more commonly associated with anthropogenic contamination or redistribution of toxic metals in terrestrial ecosystems [41].

It is obvious that all power–time curves have only one thermogenic peak for each of the heavy metals except for Cd. Two peaks appeared when Cd was added into the soil. This may be the fact that there are tolerant microbes to Cd in the soil, and that the shift of sensitive microorganisms to tolerant microorganisms. It is the reason that an increase in metal tolerance of bacterial community after metal addition may be attributed to the immediate death of sensitive species followed by differing competitive abilities and adaptation of bacterial survivors [42]. On the other hand, numerous studies have shown that microbial population responses to toxic metals are characterized by a population shift from bacteria to fungi [43–46].

3.2. Comparing the toxicity of heavy metals

Toxic metals may have a considerable impact on populations of soil microorganisms and their activities [6,7,21]. The microbial activity indices, i.e., microbial growth rate constant k, total heat evolution Q_T, metabolic enthalpy ΔH_{met}, and mass specific heat rate $J_{Q/S}$ of soil sample obtained from power–time curves, are represented in Table 2. In order to visualize the parameters, some were depicted in Fig. 2. It is obvious that the metabolic enthalpy (-941.4 ± 33.3 kJ mol^{-1}), microbial growth rate constant (0.302 ± 0.041 h^{-1}) and microbial biomass C (3.63 ± 0.53 mgC g^{-1}) shows the highest values for soils without any heavy metal. Whereas, when the soil was exposed to various heavy metals, the decrease of these parameters was observed due to their toxicity [47].

Herein, the microbial activities were affected to different levels. However, the tendencies of changes of these indices were not kept the same for any heavy metal. For As, the value of ΔH_{met} was -486 ± 21.5 kJ mol^{-1}, lower than those of Cu

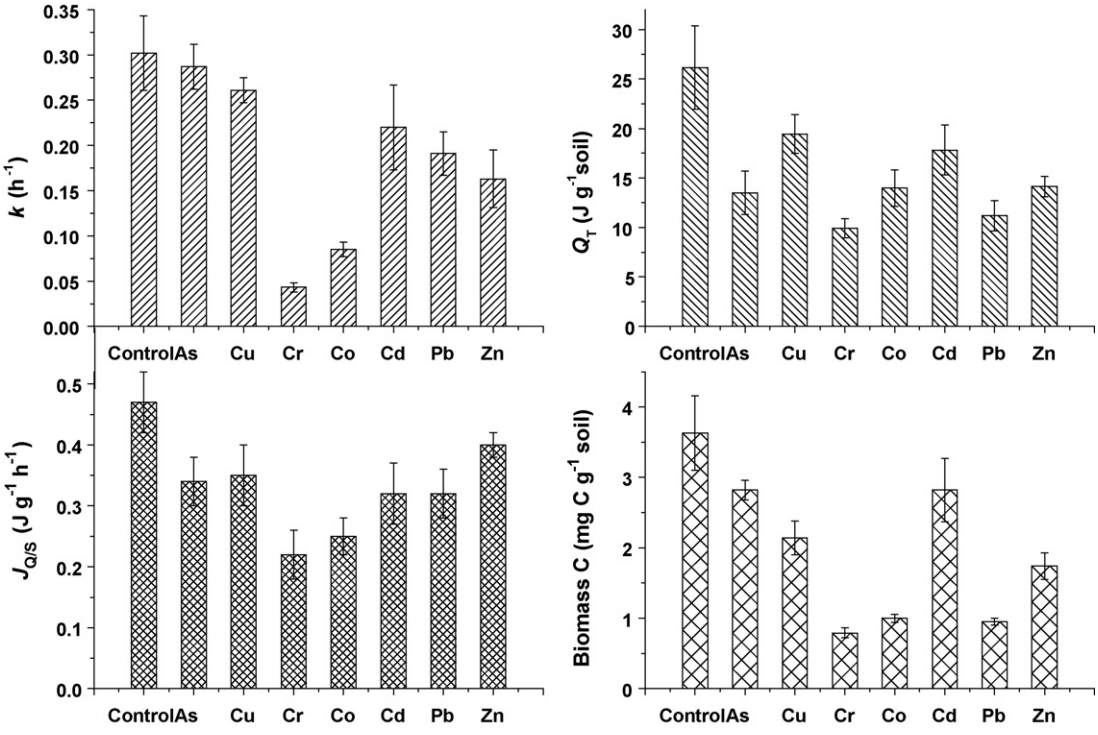

Fig. 2. Microbial growth rate constant k, total heat evolution Q_T, mass specific heat rate $J_{Q/S}$, and microbial biomass C of soil samples were obtained from power–time curves.

(-699.48 ± 27.3 kJ mol^{-1}), Co (-502.92 ± 29.1 kJ mol^{-1}) and Zn (-508.68 ± 21.5 kJ mol^{-1}), but its k (0.287 ± 0.025 h^{-1}) and biomass C (2.82 ± 0.14 mgC g^{-1}) showed relatively higher values than those of other heavy metals. Similar results were reported as well, that the decrease of the value of ΔH_{met} is accompanied by an increase of the microbial growth rate constant [36]. We have demonstrated when soil microorganisms are exposed to heavy metals initially; the toxic effect is slight due to limited interaction. In the beginning, the acute toxic effect could be represented by microbial growth rate constant. But the toxic effects of heavy metals on microbes were enhanced with time. The normal metabolic activities were restrained step by step. So, the metabolic enthalpy could reflect the whole interaction process. This illustrates that other metals can enter into the microbes much easier than As, but once As entered into the cell, its toxicity was released, and stronger that others. Comparing the values of k, Co (with lowest k value, 0.085 ± 0.008 h^{-1}) could inhibit the soil microbial metabolic activities faster than the other metals initially, except for Cr.

However, the Cr was made an exception with the lowest values of ΔH_{met} and k at the dose of 0.01 mg g^{-1} soil. This may be the fact that the acute toxicity of heavy metals not only influenced the microbial metabolic activities but also changed the number of soil microorganisms [48]. This brought about the decrease of these parameters at the same time. The values of parameters showed significant differences at the same doses of metals, indicating that microbial groups with different growth rate involved in response to different metals. The main processes of regulating intracellular metal concentration refer to membrane transport mechanisms. Under normal conditions, both nonessential and essential metal ions are transported into an organism by nonspecific uptake systems [49]. However, in situations where metal ions are in excess, synthesis of specific ion efflux systems can occur to exclude nonessential metals [40]. According to the indices of the microbial activities, for Cd and Pb as nonessential metals, the values of ΔH_{met} and biomass C showed large differences, -641.52 ± 30.2 kJ mol^{-1} and 2.82 ± 0.45 mgC g^{-1} for Cd, -402.84 ± 20.4 kJ mol^{-1} and 0.95 ± 0.05 mgC g^{-1} for Pb, respectively. The lowest value implies that Pb was the most toxic element except for Cr. This contributed to their biochemical properties. Pb at high concentrations can bind to DNA and change its conformation [50,51]. Whereas, Cd as a soft Lewis acid is very likely to combine with easily oxidized soft ligands; among these, sulfur-containing ones are of major importance in biology. Therefore, Zn is expected to be displaced by Cd in the many proteins and enzymes in which Zn has a sulfur-dominated coordination sphere. The relatively simple cadmium chemistry indicates that the toxic form of Cd is almost exclusively the di-cation [52].

According to the values of metabolic enthalpies, a general order of inhibition was found to be Cr > Pb > As > Co > Zn > Cd > Cu.

3.3. Analysis of change of soil microbial population

Individual heavy metals differ in their degrees of toxicity to soil microbial population [53]. The CFU of bacteria and fungi was obtained by plate-counting method. The changes of their numbers with incubation time were depicted in Fig. 3, when soil microbes were exposed to various heavy metals. It is obvious that the number of bacteria and fungi decreased at different extents for different heavy metals, as compared with control sample. For bacteria, CFU reduced with time, and the extent of decrease was larger than that of fungi. After 24 h, the number of fungi increased slightly for Cu, Co, etc. It elucidates that fungi are more tolerant under metal stress, and that shifts in the microbial population led to a more tolerant population. Similar findings were previously reported that prokaryotes (bacteria) are more sensitive than eukaryotes (fungi) to heavy

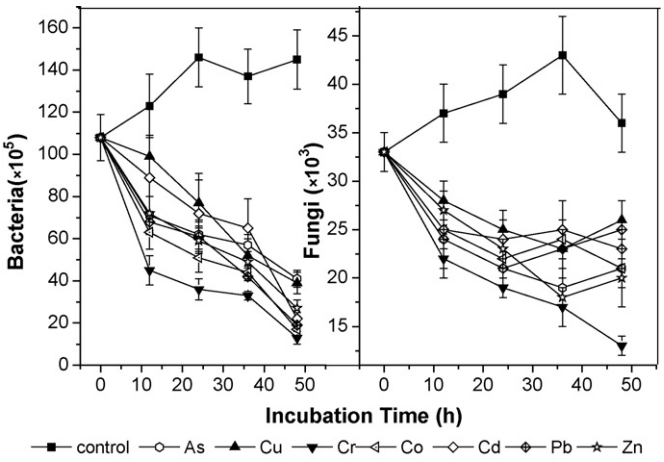

Fig. 3. CFU numbers of bacteria and fungi in metal-exposed samples.

metal pollution of soil. While, Gram-positive bacteria are more sensitive than Gram-negative bacteria [54,55]. Díaz-Raviña and Bååth [42] also observed an increase in the metal tolerance of the bacterial community with time after adding metals to the soil. They expected that this might be attributed to an acute effect due to the death of sensitive bacterial species and a late effect due to the increase in resistant microbes in the soil based on the results of measuring microbial activity over a time course [42]. According to the results, two exothermic peaks for soil sample with Cd obtained from microcalorimetry may be due to the above fact. It is probable that there are more Cd-tolerant microorganisms in the used soil.

The shift of bacteria population was not observed from the change in number of CFU. This may be due to the limited incubation time. There is not enough time to evolve into tolerant bacteria. The possibility would be that the metal tolerant bacteria that initially colonized the soil did not show any decreased competitive abilities due to their metal tolerance and thus could persist in the soil during the subsequent incubation time [56].

Comparing the results obtained from microcalorimetric and plate-counting methods, some differences could be found as well, especially tolerance degree of soil microbe to heavy metals. This may be due to the differences in the two methods. Microcalorimetry takes place in a closed incubation condition with limited air (3.0 mL), but plate-counting method offers a relatively on-limits situation. In general, the change of bacterial and fungal CFU could illustrate the change of metabolic enthalpy of soil microbial population to some extent by analyzing their change. Molecular methods would provide high-resolution information about the effects of heavy metals on soil microbial community.

3.4. Determining the glucose degradation in soil by dissolved oxygen biosensor

When calculating the metabolic enthalpy, it was assumed that glucose has been completely degraded when the power–time curve returns to the initial base line. But it is difficult to demonstrate that glucose is totally degraded in all soil samples by the microcalorimetric method directly. So, some analytical techniques are necessary to support the results. Dissolved oxygen biosensor was employed to measure the glucose concentration in real samples with high sensitivity and speed [24,57]. The calibration curve was shown in Fig. 4, with high correlation coefficient ($R^2 = 0.99804$). Soil samples with different heavy metals were measured by the biosensor at 3 h intervals. The results were presented in Fig. 5, and according to this figure the glucose was totally degraded during the incubation time. For soil without heavy metal, the glucose con-

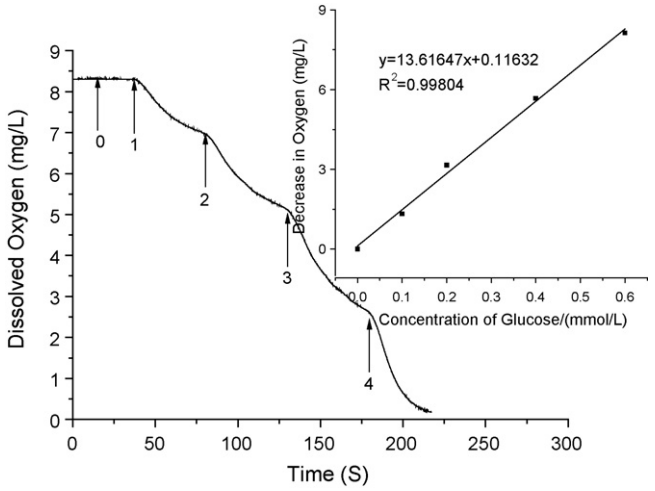

Fig. 4. Response of glucose electrode to successive additions of glucose standard solution. Inset, calibration curve.

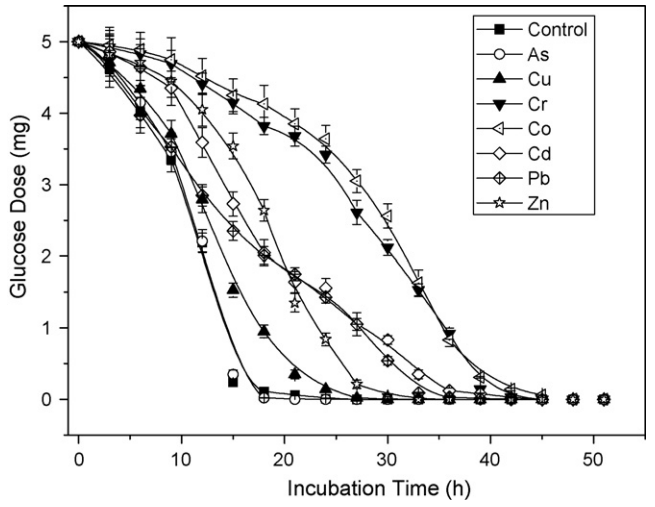

Fig. 5. Residual glucose in soil samples. Control, soil without metal addition.

centration decreased sharply. In contrast, residual glucose reduced slowly for soil samples with heavy metal except for As. The profiles coincided with the microbial growth rate constants obtained from microcalorimetry. These results were in good agreement with previous report [37]. Hence, dissolved oxygen biosensor can be used as an alternative method to analyze glucose dose in environmental samples in vitro.

4. Conclusion

By analyzing the microbial growth rate constant k, total heat evolution Q_T, metabolic enthalpy ΔH_{met}, and mass specific heat rate $J_{Q/S}$, and microbial biomass C of soil samples, these parameters can act as indicators to reflect the changes in soils polluted by heavy metals. Among these metals, Cr exhibited the strongest toxicity to soil microbes. According to the values of ΔH_{met}, a general order of inhibition was found to be Cr > Pb > As > Co > Zn > Cd > Cu. Through analyzing the number of cultivable bacteria and fungi, we can understand the change of bacteria-to-fungi ratio in soil when soil is polluted by excessive organic or inorganic substances.

As shown in this work, microcalorimetry is a powerful tool to provide qualitative and quantitative data to study microbial activities in soils. Anyway, to avoid problems in the quantification of the total heat evolution involved in the degradation of a carbon source, it is suggested here to measure its concentration remaining in samples by sensitive glucose biosensor. Therefore, these two techniques can be combined to assess the soil status in view of metabolic activity and glucose degradation kinetics.

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