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STUDYING THE TOXIC EFFECT OF CADMIUM AND HEXAVALENT CHROMIUM ON MICROBIAL ACTIVITY OF A SOIL AND PURE MICROBE
A microcalorimetric method

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Using TAM III multi-channel calorimetry combined with direct microorganism counting (bacteria, actinomycetes and fungi) under laboratory conditions, we determined the microbial population count, resistance and activity toward cadmium (Cd(II)) and hexavalent chromium (Cr(VI)) toxicity in soil. The thermokinetic parameters, which can represent soil microbial activity, were calculated from power-time curves of soil microbial activity obtained by microcalorimetric measurement. Simultaneous application of the two methods showed that growth rate constant (k), peak-heat output power (P_{max}) and the number of living microorganisms decreased with increasing concentration of Cd and Cr. The accumulation of Cr on *E. coli* was conducted by HPLC-ICP-MS. Cr^{6+} accumulation by *Escherichia coli* was increased steadily with increasing Cr^{6+} concentration.

The results revealed that the change in some thermo-kinetic parameters could have good corresponding relationship with metal accumulation. Our work also suggests that microcalorimetry is a fast, simple, more sensitive, on-line and in vitro method that can be easily performed to study the toxicity of different species of heavy metals on microorganism compared to other biological methods, and can combine with other analytic methods to study the interaction mechanism between environmental toxicants and microbes.

Keywords: *cadmium, Escherichia coli, hexavalent chromium, HPLC-ICP-MS, microcalorimetry, soil microbial activity*

Introduction

Environmental pollution in China has seriously increased the risks to human due to pollutants entering the food chain. The Food and Agriculture Organization of the United Nations (FAO) has assisted Hubei Province in central China to enhance its environmental data acquisition and management capacity by strengthening the Hubei Agro-Environmental Protection Station in identifying, analyzing, monitoring and reporting the levels of pollutants in water, soil and crops in the province. Industries contribute significantly to the organic loads, heavy metals (i.e., cadmium, chromium, lead, mercury and arsenic), volatile phenols, cyanide, hydrocarbons, sulfides and ammonia in soil and water bodies. Methods were developed to estimate the economic cost of both industrially and agriculturally generated pollution [1]. However, a correct approach to a monitoring plan should not only focus on the assessment of pollutant levels and remediation costs, but also on the capability of microbes in the soil and water ecosystems to recover their functionalities in the environment.

Soil microorganisms play an essential role in the environment due to their role in cycling nutrients and in the decomposition of organic material [2]. Nutrient cycling occurs as a consequence of microbial activity and is especially important in the ecosystems where the input of nutrients is low [3]. Addition of inorganic and organic matter promotes changes in chemical and physical properties of the soil and the biodiversity of the soil microbial community can be influenced by the added chemicals. Soil microorganisms and their controlled processes are essential for the long-term sustainability of ecological and agricultural systems [4].

Chromium pollution in some places of Wuhan city has been a serious problem due to the natural geological reasons together with the industrial pollution. Hexavalent chromium is directly correlated to carci-

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nogenicity in human and to acute toxicity of aquatic organisms, while its reduced form, Cr^{3+}, is an essential element for animals [5–8]. Also, extensive data suggests that cadmium is the most toxic heavy metal and it is listed as a priority pollutant by the US Environmental Protection 34 Agency [1, 5].

The acute toxicity test is very important, because an acute toxicity study can establish relationship between the dose of toxicant and its effect on the tested organism. Accurate measurement of effects of potential toxic materials depends on reproducibility of acute toxicity tests. Using growth metabolism of microbe as the environmental risk assessment process is attracting more interest. Bioenergetic investigations should be the most important in ecotoxicology for assessment of harmful properties of substances. Microcalorimetry can continuously quantify the microbial activity in real time, with the incubation time being the same as in actual soil conditions. This procedure is quicker than measuring separate component groups of microorganisms, and does not require optically transparent systems [9]. Therefore, the calorimetric method proves to be very sensitive toward changes in the microbial biomass which cannot be readily detected by some conventional methods [10].

Microcalorimetry would be an efficient and less expensive tool for fast monitoring of wide extensions of lands. Recently, microcalorimetry has been proposed to measure the quantitative indices of microbial growth rate constant, heat evolution process and heat yield in heterogeneous complex soil systems [9, 11–14].

In the present study, a multi-channel thermal activity monitor, a kind of heat conduction microcalorimeter, has been applied to investigate the inhibitory effects of different concentrations of hexavalent chromium and chromium on microbial activity in a Wuhan brown sandy soil and pure microbe –*Escherichia coli* (*E. coli*). Our objective is to evaluate the multi-channel thermal activity monitor as an instrument for determining toxicity. Our proposed microcalorimetric method is a fast, simple and more sensitive technique to investigate the toxicity of various species of heavy metals on microorganism; as a result, the understanding on the acute toxicity of heavy metal to the soil microbes can be easily acquired. Meanwhile, in order to understand interaction between heavy metals and microorganism, the accumulation of hexavalent chromium on pure microbe (*E. coli*) was carried out by high-performance liquid chromatography and inductively coupled plasma-mass spectrometry (HPLC-ICP-MS).

Experimental

Materials

Reagents

All chemicals such as $C_6H_{12}O_6$, $(NH_4)_2SO_4$, KCl, $K_2Cr_2O_7$ and $CdCl_2$ were analytical grade. They were supplied by the Shanghai Yuelong Chemical Factory (Shanghai, PR China). $K_2Cr_2O_7$ and $CdCl_2$ were used as a toxicant by exposing it to the test soil organism after which microbial activity was measured as described underneath. All the glassware, as well as the polyethylene and polypropylene laboratory ware, were soaked in 10 HNO_3 (*v*/*v*%) for at least 48 h before use. Deionized water was used throughout the study.

E. coli (CCTCC HB101) was provided by the China Center for Type Culture Collection, Wuhan University (Wuhan, China). The peptone culture medium was a solution contained per 1.0 L: 5.0 g peptone, 3.0 g beef extract and 5.0 g NaCl at pH of 7.2–7.4. It was sterilized in high-pressure steam at 120°C for 30 min before use.

Soil sample and its physico-chemical properties

A Wuhan brown sandy soil from the campus of the China University of Geosciences (30.581N and 114.281E) obtained from 5 to 15 cm depth was used. The soil was air dried for 10 days and homogenized by sieving to less than 2 mm, to eliminate roots and large particles. The soil was stored in polyethylene bags at 4°C and was used for all assays. Soil organic matter was determined by placing the dry soil (10.0 g) in a muffle furnace (600°C) and then monitoring the decrease in mass for 24 h [9, 15]. Under these conditions organic matter is combusted, leaving only the inorganic component of the soil. Carbon, nitrogen, hydrogen and sulfur content in the soil were determined with a Leeman Labs Model CE-440 Elemental Analyzer (Kreuztal, Germany). The pH was measured with a Leici PHS-25C pH meter (Shanghai, PR China) in a suspension of 2.0 g of soil sample with 5.0 mL of 1.0 M calcium chloride (i.e. a 1:2.5 soil:solution ratio) [9, 13, 15]. The analyses were conducted in triplicate.

Determination of biological properties

The number of living microorganisms was estimated by a spread plate method of viable count. A series of 10-fold dilutions for the soil sample was used after a 10.0 g dry soil was suspended in 90 mL sterile water and stirring the contents for 30 min. Aliquots of 1.0 mL of the suspension were taken and added to 9.0 mL sterile water. The sample was further diluted

five times with the sterile water. Finally, 0.1 mL serial aliquots of the diluted sample suspension were spread over the surface of an agar plate with Martin's medium for fungi, beef extract peptone medium for bacteria and Gause's No. 1 synthetic medium for actinomycetes, respectively. Each sample was plated in triplicates and the plates incubated at 28°C until the colonies appeared. The colony forming units (CFU) on each plate were counted. The number of bacteria, fungi, varying from 10 to 100 for fungi and 30 to 300 for prokaryotes and actinomycetes were calculated.

Instrumental methods

Microcalorimeter and measurement

A TAM III multi-channel thermal activity microcalorimeter (Thermometric, Järfälla, Sweden) was used to measure the heat output of the growth metabolism of soil microbes and *E. coli* under different concentrations of Cr and Cd at 25 and 37°C, respectively. It is designed to monitor continuously a wide variety of processes and complex systems such as the thermal activity of physical, chemical and biological processes in terms of heat, heat flow and heat capacity over a temperature range of 15–150°C. The voltage signal was recorded by a computer.

The thermal effect was obtained using 4.0 mL stainless steel ampoules with Teflon sealing discs to prevent evaporation of the sample solution. All determinations of the thermal effect were performed in ampoules containing 1.20 g of soil and 0.60 mL solution of 5.0 mg glucose and 5.0 mg ammonium sulfate with different concentrations of Cd and Cr for soil samples. Glucose and ammonium sulfate can stimulate soil microbial activity. Under these conditions, the applied moisture was maintained at 35% to maximize microbial activity [15].

For pure microbe, all 4.0 mL steel ampoules were cleaned, sterilized and filled with 2.0 mL of peptone culture medium containing *E. coli* at an initial density of $2 \cdot 10^6$ bacteria mL^{-1}. Various concentrations of Cr^{6+} were respectively added at the beginning of the experiment. The steel ampoules were then positioned in the microcalorimeter at 37°C.

The power–time curves of soil microorganisms and *E. coli* were recorded by a computer. All samples were done in triplicates.

HPLC-ICP-MS measurement system

For the measurement of Cr^{6+}, an HPLC system (GBC, Australia) consisting of a solvent delivery unit (model LC 1140) and an HPLC pump (model LC 1150) was coupled to the Thermo Element 2 HR-ICP-MS instrument (Thermo Finnigan, Germany) instrument.

A Hamilton 10 μm PRP-X100 column of 250×4.1 mm equipped with a pre-column of 4.6×4.1 mm and 20 mmol dm^{-3} $NH_4H_2PO_4$ (pH=5.6 with NH_3) as mobile phase were used for the anion-chromatographic measurements. A Supelcosil 10 μm LC SCX-100 250×4.1 mm column equipped with a pre-column of 4.6×4.1 mm and 25 mmol dm^{-3} pyridine (pH=2.7 with formic acid) as mobile phase were used for the cation-chromatographic measurements [16]. In both cases, isocratic elution was used. Flow rate was 1.5 cm^3 min^{-1}.

Statistical analyses

All the tests were conducted through three parallel experiments. The ANOVA method was used for statistical for determining the significance at the $P<0.05$ level of difference between treatments. Data were presented as arithmetic means±standard deviations (SDs).

Results and discussion

Properties of the soil

The microbial activity in a chosen system can be affected by the soil properties. Thus, the inherent physical and chemical properties such as pH, organic matter content and elemental composition are important features to be considered. The soil used was constituted of 4.35±0.12% organic matter, 3.97±0.21% carbon; 0.79±0.06% nitrogen and 3.56±0.21% hydrogen. It had a pH of 6.74±0.08 and its microbial population was very sensitive to the addition of nutrients. In general, metabolism is directly promoted by addition of the desired nutrients to the ecosystem. This stimulating source is normally composed of glucose, ammonium sulfate and water in certain mixture ratios.

Growth power–time curves

The metabolism of microorganisms in the Wuhan brown sandy soil in the absence and presence of distinct amounts of hexavalent chromium and cadmium is shown in a series of curves (Figs 1–2), and the power–time curves of *E. coli* growth under the action of various concentration of Cr^{6+} are shown in Fig. 3. As illustrated, the power–time curves are recorded for different amounts of Cr and Cd, in which a great variability of the activity can be visualized. The power–time curves show that the shapes of the thermogenic curves changed little when the low concentration of

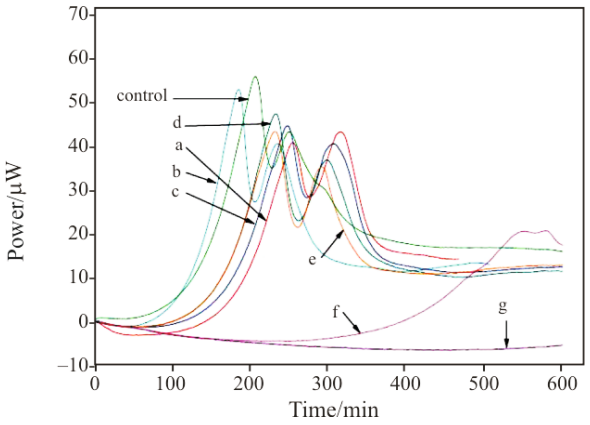

Fig. 1 The power–time curves of soil microbial activity at a moisture content of 35% in 1.20 mg of soil. The soil samples were incubated with 0.60 mL of solution containing 5.0 mg glucose together with 5.0 mg ammonium sulfate and different concentrations of Cr(VI). All samples were incubated at 25°C. The concentrations of Cr(VI) were a – 0.2, b – 0.4, c – 0.8, d – 1.6, e – 2.4, f – 5.0 and g – 6.0 μg mL^{-1} Cr(VI). The control treatment did not have any Cr(VI) added

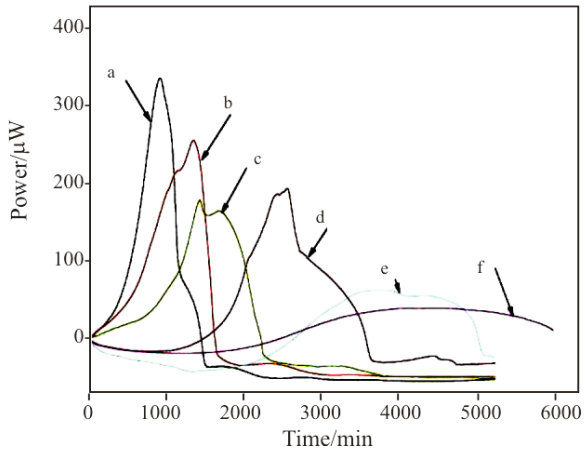

Fig. 2 The power-time curves of soil microbial activity at a moisture content of 35% in 1.20 g of soil. The soil samples were incubated with 0.60 mL of solution containing 5.0 mg glucose together with 5.0 mg ammonium sulfate and different concentrations of Cd(II). All samples were incubated at 25°C. The concentrations of Cd(II) were a – control, b – 100, c – 200, d – 400, e – 800 and f – 1600 μg mL^{-1} Cd(II). No Cd(II) was added to the control sample

hexavalent chromium and cadmium was in the soil suspension. But when higher than 5.0 μg mL^{-1} hexavalent chromium were added, the shapes changed obviously. When the concentration of Cr^{6+} reached up to 6.0 μg mL^{-1}, the power-time curve is a line, which indicated the growth of *E. coli* has been restrained completely. When Cd concentration was up to 1600 μg mL^{-1}, the microorganisms could not grow. Values of the peak–time, that is, time at which the microcalorimetric signal reaches its maximum amplitude, delayed with the increasing the concentration of heavy metal. It is also shown that the metabolic activity of microorganisms obeyed the dose-response rule.

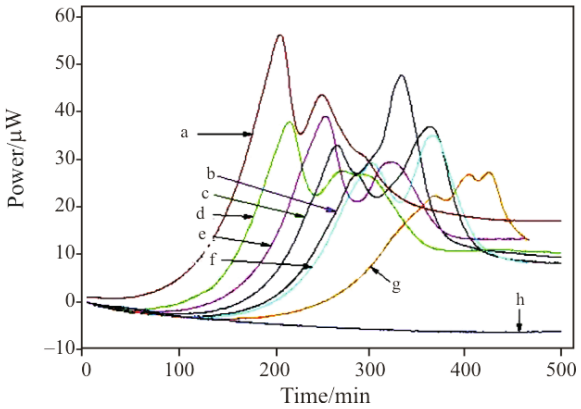

Fig. 3 The power-time curves of *E. coli* at 37°C in 2.0 mL peptone cultural medium. The concentrations of Cr(VI) were a – control, b – 0.1, c – 0.5, d – 1.0, e – 1.0, f – 2.0, g – 3.0, h – 6.0 μg mL^{-1} Cr(VI). No Cr(VI) was added to the control sample

For Cr, not only soil microbes but also pure microbe showed two typical peaks of the microbial activity curves. But, the metabolic curves had only one peak for Cd. These reasons should be studied further. The microorganism's metabolism is so complicated that different peaks may arise from the growth of different microbial communities. Comparing the thermogenic curves of soil microbes and *E. coli*, it can be proved that the specific metals influenced the microorganisms by the same mechanism. The tolerance of microorganisms towards specific metals often include binding of metals by cell wall or proteins and extracellular polymers, formation of insoluble metal sulfides, volatilization and enhancing export from cell [17].

Intracellular Cd or Cr could produce toxic effect on the microorganism through different routes. When Cd or Cr binds to the active sites of enzymes such as alkaline phosphatase, they are inactivated and the metabolisms are disrupted [18, 19]. Cadmium, besides being an enzyme inhibitor, can have deleterious effects on membrane structure and function by binding to ligands such as phosphate and the cysteinyl and histidyl groups of proteins [20].

Tables 1 and 2 display the effect of hexavalent chromium and cadmium on the numbers of living microorganisms consisting of bacteria, actinomycetes and fungi in the sandy soil samples. The higher the concentration of the hexavalent chromium and cadmium added to the soil samples, the smaller the number of the microorganisms was found, such as bacteria ($\cdot 10^7$), from 3.01±0.38 to 0.77±0.21 for Cd; from 3.01±0.38 to 0.31±0.09 for Cr^{6+}. This indicates that

Table 1 Effects of various concentrations of cadmium on microbial activity in Wuhan brown sandy soil

C, $CdCl_2$/μg mL^{-1}	0.00	100	200	400	800	1600
k/min^{-1}	0.00482± 0.0013	0.00319± 0.0015	0.00238± 0.0009	0.00178± 0.0011	0.00092± 0.0007	–
I/%	0	33.82±1.03	50.62±2.39	63.08±4.01	80.91±1.83	100
Q_{total}/J	7.83±0.11	9.25±1.32	9.26±1.07	10.40±1.02	5.93±0.88	–
P_{max}/μW	335.98±2.47	256.14±2.91	178.12±1.89	193.08±1.68	61.40±1.03	–
r	0.9912	0.9905	0.9900	0.9901	0.9930	–
IC_{50}/μg mL^{-1}			207			
Number of microorganisms/CFU g^{-1}						
Bacteria (·10^7)	3.01±0.38	2.23±0.17	1.72±0.18	1.01±0.09	0.77±0.21	–
Actinomycetes (·10^6)	4.67±0.17	3.18±0.45	2.13±0.20	3.89±0.14	2.83±0.13	–
Fungi (·10^5)	1.51±0.03	0.98±0.137	0.59±0.11	0.35±0.09	0.13±0.04	0.002

' – ': no detection; k, the growth rate constant; I, the inhibitory ratio; Q_{total}, the total thermal effect for the microbial metabolism generated by the microbial population in the whole process; P_{max}, the peak–heat output power

Table 2 Results of the effects of the concentration of Cr(VI) on microbial activity in Wuhan brown sandy soil

C, $K_2Cr_2O_7$/ μg mL^{-1}	0.00	0.20	0.40	0.80	1.60	2.40	5.0	6.0
k/min^{-1}	0.03070± 0.0009	0.02967± 0.0012	0.02942± 0.0008	0.02854± 0.0011	0.02516± 0.0008	0.02319± 0.0016	0.02319± 0.0016	0
I/%	0	3.36±0.26	4.17±0.07	7.04±0.29	18.05±0.13	24.46±0.31	60.65±0.22	100
Q_{total}/J	0.72±0.09	0.17±0.05	0.32±0.03	0.51±0.05	0.52±0.08	0.48±0.06	0.07±0.02	–
P_{max1}/μW	56.14±2.86	41.02±1.13	53.18±3.91	44.83±2.19	47.61±3.20	43.51±2.75	20.64±2.33	–
P_{max2}/μW	250.2±1.92	43.54±2.56	40.76±2.39	40.68±1.09	36.95±4.11	35.44±2.01	20.76±1.38	–
r	0.9966	0.9965	0.9975	0.9981	0.9969	0.9931	0.9985	–
IC_{50}/μg mL^{-1}				4.27				
Number of microorganisms/CFU g^{-1}								
Bacteria (·10^7)	3.01±0.38	2.78±0.14	2.43±0.28	2.01±0.08	1.57±0.21	0.82±0.16	0.31±0.09	–
Actinomycetes (·10^6)	4.67±0.17	3.98±0.55	2.23±0.23	3.37±0.11	1.88±0.09	1.37±0.04	1.69±0.14	–
Fungi (·10^5)	0.51±0.03	0.87±0.13	0.49±0.10	0.31±0.07	0.41±0.03	0.26±0.02	0.19±0.02	–

' – ': no detection; k, the growth rate constant; I, the inhibitory ratio; Q_{total}, the total thermal effect for the microbial metabolism generated by the microbial population in the whole process; P_{max1}, the first peak-heat output power; P_{max2}, the second peak–heat output power

hexavalent chromium and cadmium can suppress the growth of these microorganisms in the soil.

Growth rate constant, inhibition ratio and half inhibitory concentration

The power–time curves of soil microbes and *E. coli* show that microbial cell growth is exponential in the log phase of growth [21].

$$\ln P_t = \ln P_0 + kt \quad (1)$$

The growth power–time curves of the log phase correspond to Eq. (1). So, using the data $\ln P_t$ and t taken from the curves to fit a linear equation, the thermokinetic equation for soil microbial activity at different concentrations of Cd and Cr at 37°C can be obtained.

Inhibitory ratio is defined as the concentration of Cd and Cr that will inhibit microbial activity. The inhibitory ratio I obtained from the equation [22, 23] is:

$$I = [(k_0 - k_C)/k_0] \cdot 100\% \quad (2)$$

where k_0 is the rate constant of the control, and k_C is the rate constant for soil microbial activity inhibited by an inhibitor whose concentration is C. When the inhibitory ratio I is 50%, the corresponding concentration of the inhibitor is called the half inhibitory concentration (IC_{50}).

The inhibitory ratio of Cd is related to its bioavailability. Bioavailability is considered as the frac-

tion of the total contaminant in the interstitial water and soil particles that is available to the receptor organism [24].

Relationship among the growth rate constant, the number of microorganism and different concentrations of cadmium and chromium

Table 1 clearly depicts that the growth rate constant, the number of living bacteria and fungi (except actinomycetes) decrease with increasing concentration of Cd and further suggests that the soil microbial activity has been inhibited. The power–time curves of the soil microbial activity are good illustrations. When the $CdCl_2$ concentration reached 1600 μg mL^{-1}, the soil microorganisms stopped growing and their numbers dropped to almost zero. Although the growth rate constant and the number of living microorganisms decreased with increasing Cd concentration, they did not show a linear relationship as depicted in Fig. 4.

Table 2 clearly depicts that the growth rate constant (k) and the number of microorganisms, especially the number change of bacteria, decrease with increasing concentration of hexavalent chromium (Fig. 5), further indicating that the soil microbial activity has been inhibited according with increasing

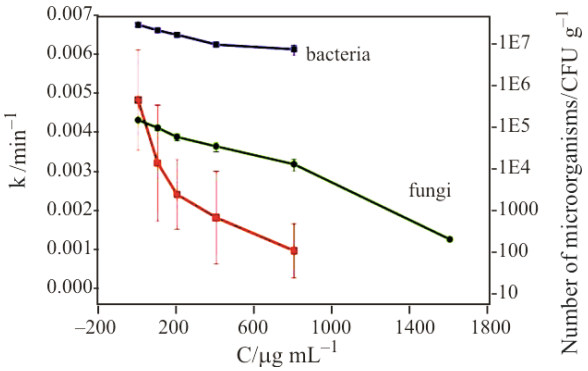

Fig. 4 Relationship between the growth rate constant k, the number of bacteria or fungi and concentration of Cd (C)

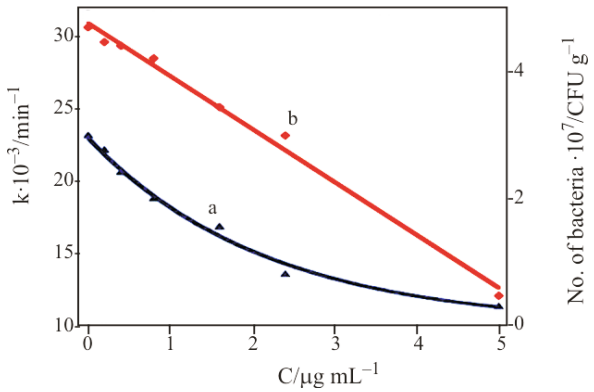

Fig. 5 a – Plot of the number of bacteria *vs.* concentration of $K_2Cr_2O_7$ (C). b – Relationship between the growth rate constant k and C. The linear relationship between k and C is k=0.0309–0.00364C with r= –0.9951

concentrations of hexavalent chromium. The metabolic results of growth of *E. coli* also show that the k decreased with the increasing the concentration of Cr, seen in Table 3.

Relationship among the total thermal effect in whole process Q_{total}, the number of microorganism and the concentration of hexavalent chromium and cadmium

From the power–time curves shown in Figs 1–3, the total thermal effect of microbial metabolism Q_{total} generated by the microbial population was obtained through integration of each curve from the beginning to the end. Thermal effect values are displayed in Table 2. It was found that the thermal effect decreased with the increase in the concentration of hexavalent chromium. This trend indicated toxic effect on the soil microbial activity due to the addition of hexavalent chromium. However, this trend is not linear since the concentration of 0.2–0.4 μg $K_2Cr_2O_7$ per mL has abnormal phenomena with the total thermal effect of microbial metabolism Q_{total} not following the general trend as shown in Table 2. The thermal effect decreased with the increase in Cd concentration and the

Table 3 Experimental data of the growth of *E. coli* under the effects of various concentrations of Cr^{6+}

C, $K_2Cr_2O_7$/ μg mL^{-1}	0.00	0.1	0.5	1.0	2.0	3.0	4.0	6.0
k/min^{-1}	0.03070± 0.0009	0.02860± 0.0032	0.02685± 0.0018	0.02592± 0.0036	0.02477± 0.0050	0.02213± 0.0031	0.01902± 0.0014	0
I/%	0	6.84±1.11	12.54±2.93	15.57±2.19	19.32±3.18	27.92±2.06	38.04±4.08	100
Q_{total}/J	0.72±0.09	0.26±0.02	0.32±0.02	0.35±0.03	0.15±0.02	0.13±0.03	0.06±0.01	–
P_{max}/μW	56.14±2.86	26.57±2.67	32.84±1.90	37.73±3.99	38.96±4.01	29.03±3.16	22.00±2.10	–
r	0.9966	0.9927	0.9931	0.9936	0.9938	0.9926	0.9916	–
IC_{50}/μg mL^{-1}				3.96				

' – ': no detection; k, the growth rate constant; I, the inhibitory ratio; Q_{total}, the total thermal effect for the microbial metabolism generated by the microbial population in the whole process; P_{max}, the first peak–heat output power

Table 4 Cr^{6+} accumulation on *E. coli* measured by HPLC-ICP-MS

Chemicals	Cr^{6+}							
Cr^{6+} in media/ μg mL^{-1}	0	0.1	0.5	1.0	2.0	3.0	4.0	6.0
Cr^{6+} accumulated in cell/μg g^{-1}	0	0.035±0.012	0.63±0.06	1.47±0.02	1.80±0.33	2.08±0.27	2.28±0.50	2.71±0.45

decrease in the number of microorganisms. The thermal effect is the sum of catabolic and anaerobic processes that occur during inorganic and organic material degradation and accumulation. It reflects the ability of the community present in soil to facilitate these processes. So, the total thermal effect of microbial metabolism can be one of the indices of soil microbial activity.

Metal accumulation of E. coli to hexavalent chromium

In order to explain the change of thermal parameters with the increasing the concentration of heavy metals, the pure microorganism was used to study its accumulation on the heavy metals. The results of the sets with Cr^{6+} revealed that Cr^{6+} accumulation by *E. coli* were increased steadily as the corresponding Cr^{6+} concentration in culture media increased from 0.1–6.0 μg mL^{-1}. The results were shown in Table 4. Metal resistance is defined as the ability of an organism to survive metal toxicity by means of a mechanism produced in direct response to metal species concerned [25]. However, the tolerance mechanism is not yet completely understood, not even the exact location of metal accumulation in the microbes. This can prove that the toxic effect of heavy metals on soil or pure microorganism obey dose-response relationship at a certain dose.

Conclusions

The evaluation of the toxic effect of Cd and Cr(VI) on soil microbial activities was carried out both by microcalorimetric measurement and microorganisms counting. In addition, the effect of Cr(VI) on *E. coli* also was conducted by microcalorimetry. In order to understand the interaction between the heavy metals and pure microorganism, HPLC-GC-MS was used to measure the accumulation of Cr(VI) on *E. coli*. Cr^{6+} accumulation by *E. coli* were increased steadily as the corresponding Cr^{6+} concentration. This can be deduced that soil microorganisms also accumulated the heavy metals like *E. coli*. Through the thermodynamic parameters changes, the relationship between microbial metabolism and concentration of heavy metals showed clearly dose-response rule.

The calorimetric data and microorganism population showed similar trends with increasing Cr and Cd concentration. The IC_{50} value for the poisonous species of Cd(II) and Cr(VI) against soil microbe was 206.96 and 4.27 μg mL^{-1}, respectively. Cr showed more toxic to microorganism than Cd. In brief, microcalorimetric technique is promising in analyzing the mechanisms and interactions between toxicants (both inorganic and organic species) and soil microorganisms, when supplemented with other analytical techniques [26].

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