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The toxicity of chromium and cadmium in the earthworm *Eisenia fetida* after exposure

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ABSTRACT

The objective of this research was to determine: 1) the individual toxicity of cadmium and chromium on E. fetida on protein content; 2) the combined toxicity of cadmium and cadmium on E. fetida in lou soil. In individual toxicity, the increased heavy metal concentration can decrease the protein content ($P<0.01$); the protein content increased as the time goes by ($P<0.05$). Different concentrations of Cd were the main factors influencing in individual Cd toxicity. Time was the main factor influencing in individual Cr toxicity. Combination of metals, no obvious regulations of time toxicological effects were identified, and the protein contents were significantly affected by Cd concentrations.

KEYWORDS

Eisenia fetida; Cadmium; Chromium; Toxicity; Protein content.

INTRODUCTION

It is becoming a serious problem that the disposal of a large amount of wastewater and sludge produced by anthropogenic sources (Suthar 2009). For many years, the discharge of untreated or semi-treated sewage into water bodies or soil has resulted in ground water and atmospheric pollution (Lukkari et al. 2005). Cadmium and cadmium were chosen to be used in this study among the various metals that contaminate terrestrial ecosystems. Earthworms are an important ecological component of many soils, and consequently, a wide variety of tests has been developed to determine the effects of pollutants on these animals (Bouwman 2007). Cadmium and cadmium were labelled as non-essential metals (Depledge et al., 1994). The toxicity of chromium in soil organisms is less studied. The effects of the mixture of cadmium and chromium on soil fauna is even less. Relative to other types of investigations, only a few studies had been undertaken to investigate the effects of mixed metals on soil fauna (Khalil et al., 1996; Van Gestel and Hensbergen, 1997; Korthals et al., 2000; Witzel, 2000).

The objective of this work is to obtain the individual and mixed toxicity of Cd and Cr on *E. fetida* by soil expose test. This would give an indication whether cadmium and zinc influence each other's toxicity. *E. fetida* was chosen in this paper because it is easy to culture, and there are relatively large datasets available in the literature on the biology and ecotoxicology of this species.

MATERIALS AND METHODS

Earthworms

Earthworm plays an important role in soil system. They resides organic rich habitats such as compost and manure heaps (Bouche´, 1972). In this experiment, earthworms were collected manually from earthworm farms in Xianyang, Shanxi, China, and brought to the laboratory within one weak in a plastic container. Earthworms were maintained in plastic boxes containing sterilized loam. Cow dung was provided as feed daily, and earthworms were free to feed ad libitum. Healthy earthworms of about 60 days old, with a well-developed clitellum, were used for exposure experiments. They were starved for one day to allow depuration of their gut contents before being used in experiments.

Reagents

The exposure solutions of Cd and/or Cr were prepared by dissolving the $CdCl_2$ (reagent grade, Xian Chemical Reagent Co.) and CrO_3 (purity >99%, Xian Chemical Reagent Co.) into deionized water. Solutions were prepared weekly or daily depending on their concentrations. All reagents were of residue analysis grade (purity >99%). All glassware and plastic containers were soaked in 5% (v/v) HNO_3 for at least 12 h and thoroughly rinsed initially with tap water and subsequently with deionized water before use.

Soil collection and earthworm exposures

TABLE 1 : Physical and chemical properties of the tested soil

Soil texture	Organic matter (g/kg)	Total nitrogen (g/kg)	Total phosphorus (g/kg)	CEC (mmol/kg)	Mechanical composition (%)			pH
					sand	silt	clay	
Lou soil	11.2	1.17	0.85	206.0	36.08	51.73	12.19	8.03

The site to collect soil is a lou soil experimental plot northwest A&F university of Yangling, Shanxi, China. After removing the grasses and stones, the top soil (0–20 cm) was collected. The soils were sieved (4 mm) and stored in a dry place. Physicochemical properties of the soil were showed as TABLE 1.

Before exposure experiment, the earthworms were acclimated in non-spiked soil for one week. Then, earthworms were applied to perform metal exposure for each group. The contaminated soil with different concentrations and earthworms was placed in plastic pots. The plastic pots were covered with gauze in order to prevent escape. The soil moisture is 50% of the maximum. Three replicates and eight controls were used in the experiment. The concentrations of heavy metal are 1/80-1/50 of the LC_{50} (Shehata etal 1999).

Three replicates and five controls were used in the experiment. Sixty earthworms were placed into each treatment. In the individual acute toxicity experiments, the concentrations of Cd were 0, 50, 100, 120 and 180 mg/kg (dry soil). The concentrations of Cr were 0, 50, 100, 150 and 210 mg/kg (dry soil). The sampling times were 4, 8, 16, and 32 days after polluted. In the combined toxicity experiments, the concentrations of Cd were 0, 20, 60 and 80 mg/kg (dry soil). The concentrations of Cr were 0, 20, 60 and 110 mg/kg (dry soil). The sampling times were 8 and 16 days after polluted.

Homogenization buffer of earthworm

The preparation methods of homogenization buffer was reference to Osman etal (2003). Tris is short for tris (hydroxymethyl) aminomethane. After preparation, the homogenates buffer of earthworm preserved in refrigerator, which the temperature was 4℃.

TABLE 2 : Physical and chemical properties of the tested soil

Composition	Concentration	Ph
Tris	50 mmol/ L	
DTT	1 mmol/ L	
EDTA	1 mmol/ L	7.6
sucrose	250 mmol/ L	

The preparation and determination of protein standard solution

The preparation of protein standard solution: Put 100 mg bovine serum albumin in a 100 ml volumetric flask, and dissolved with distilled water. Then the concentration of bovine serum albumin is 1000 μg/ mL.

The preparation of coomassie brilliant blue G-250 reagent: Dissolved 100 mg coomassie brilliant blue G – 250 with 50 ml 90% ethanol, add 100 mL 85% (W/V) phosphate, and diluted with distilled water to 1000 mL.

TABLE 3 : The content of standard curve to protein

	1	2	3	4	5	6
Bovine serum albumin (mL)	0	0.05	0.1	0.2	0.5	1.0
Distilled water (mL)	1.0	0.95	0.9	0.8	0.5	0
Protein content (μg)	0	50	100	200	500	1000

0.1 mL solution mixed with 5 mL coomassie brilliant blue G-250, to measure the absorbance in 595 nm. The sample processing methods were the same.

RESULTS

All data were processed by SPSS, univariate analysis of variance was used to analysis.

Protein determination standard curve

TABLE 4 : The result of standard curve

Protein concentration (mg/kg)	0	50	100	200	500	1000
Absorbance value (A)	0.000	0.046	0.115	0.237	0.734	1.750

Standard curve is shown in Figure 1. The standard curve equation is y = 0.0018x-0.0628, the R^2 is 0.9927. The protein content in the range of 0-1000μg/mL, Absorbance value and protein content has a good linear relationship.

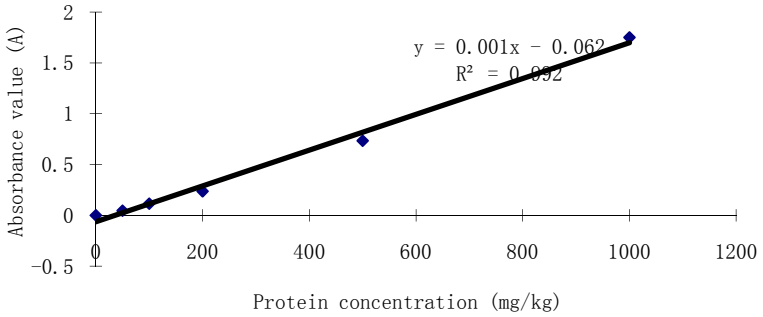

Figure 1 : The standard of protein curve

Individual toxicity assay on protein content

The variance analysis results are shown in Table 6. The F statistics of Cd concentration is 50.484; the F statistics of time is 98.442. The F statistics of Cr concentration is 137.812; the F statistics of time is 98.442. Therefore the impact of time,

Cd and Cr concentration on protein content is extremely significant under the 0.05 significance level. Interaction between heavy metal and time is extremely remarkable.

The correlation coefficient of Cd concentration is -0.452, means the increased Cd concentration can decrease the protein content (P<0.01); the correlation coefficient of time is 0.279, means the protein content increased as the time goes by(P<0.05).

The correlation coefficient of Cr concentration is -0.265, means the increased Cr concentration can decrease the protein content (P<0.05); the correlation coefficient of time is 0.360, means the protein content increased as the time goes by(P<0.01). The partial correlation coefficient Cd (0.470) > time (0.313), showed that different concentrations of Cd were the main factors influencing in individual Cd toxicity. The partial correlation coefficient Cr (0.284) < time (0.373), showed that time was the main factor influencing in individual Cr toxicity.

TABLE 5 : Individual toxicity on protein content

	Cd content (mg/kg)	Protein content[a]	Cr content (mg/kg)	Protein content[a]
4d	0	1.001±0.009	0	1.064±0.012
	50	0.937±0.002	50	1.012±0.008
	100	0.964±0.009	100	0.910±0.017
	120	0.897±0.012	150	0.892±0.009
	180	0.963±0.017	210	0.880±0.010
8d	0	1.008±0.014	0	1.012±0.014
	50	1.108±0.009	50	1.105±0.018
	100	0.991±0.006	100	0.972±0.014
	120	1.073±0.017	150	0.984±0.024
	180	0.972±0.014	210	1.123±0.016
16d	0	1.104±0.020	0	1.104±0.020
	50	1.121±0.012	50	1.340±0.020
	100	1.076±0.017	100	1.028±0.014
	120	1.007±0.024	150	1.118±0.017
	180	1.044±0.019	210	1.127±0.022
32d	0	1.076±0.020	0	1.076±0.020
	50	1.117±0.015	50	1.199±0.004
	100	1.010±0.017	100	1.081±0.023
	120	0.980±0.002	150	0.988±0.009
	180	0.945±0.013	210	0.992±0.008

[a]Protein contents were shown in terms of ratio of measured data in experimental groups and control group (Mean±S.E.).

TABLE 6 : The variance analysis results (dependent variable: protein content)

Pollutant	Cd			Time			Cd join Time	
	P	F	PC	P	F	PC	F	P
Protein	-0.452**	50.484	-0.470	0.279*	98.442	0.313	17.032	.000**
	a. R^2= .946 （adjusted R^2 = .920）							
Pollutant	Cr			Time			Cr join Time	
	P	F	PC	P	F	PC	F	P
Protein	-0.265*	137.812	-0.284	0.360**	253.090	0.373	26.927	.000**
	a. R^2= .976 （adjusted R^2 = .965）							

P: Pearson correlation; PC: Partial correlation coefficient; *: cases where the concentration of exposure had a significant effect (*P<0.05, ** P<0.01)

The changes of protein content in *E. foetida* exposed to different Cd or Cr concentrations under different days were shown in Figure 2. The changes of protein content in E. foetida exposed to different days under different Cd or Cr concentrations were shown in Figure 3.

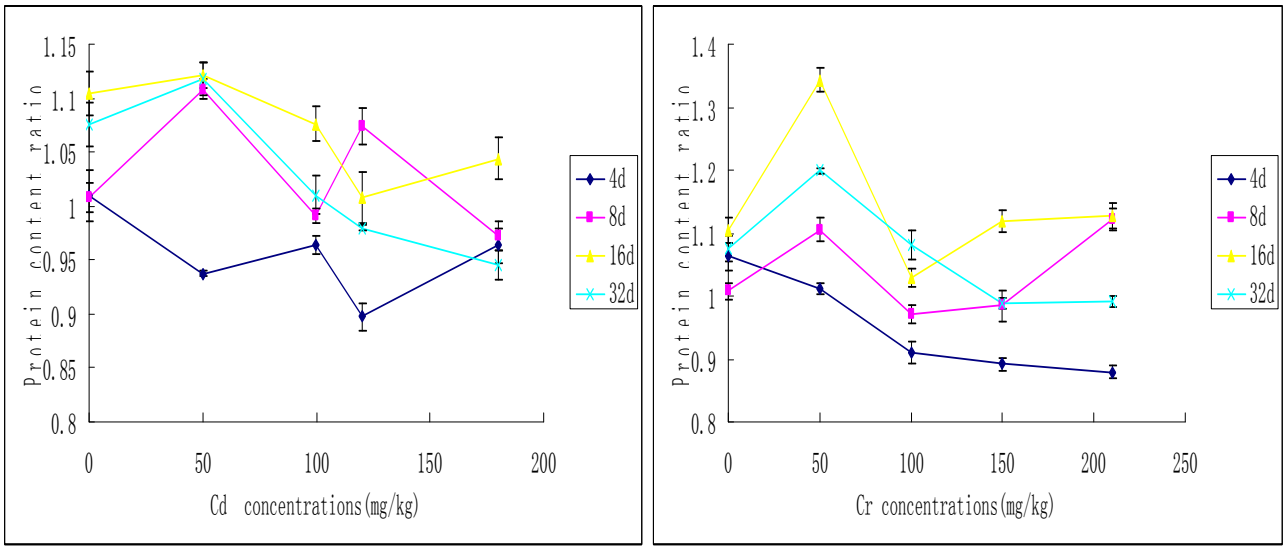

Figure 2 : The influences of different days on the protein content under different Cd or Cr concentrations

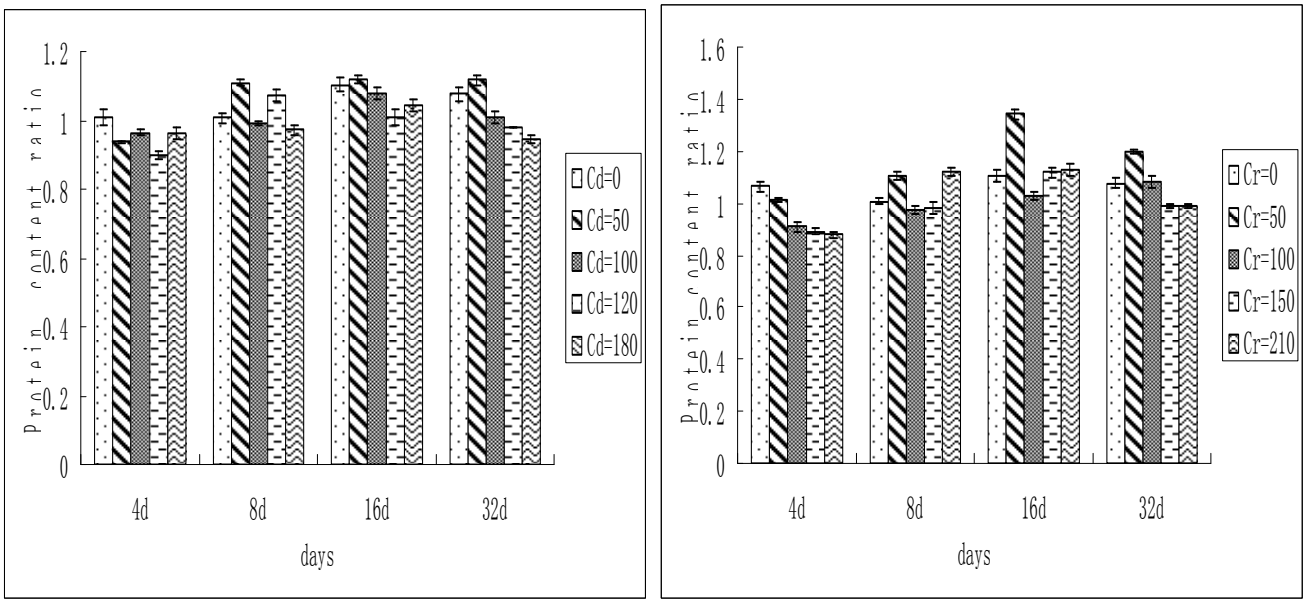

Figure 3 : The influences of different Cd or Cr concentrations on the protein content under different days

Combined toxicity assay on Protein content

TABLE 7 shows the changes of protein content in *E. foetida* exposed to combination of different Cd and Cr concentrations. As shown in the table, Cd concentrations (p=0.387) and Cr concentrations (p=0.011) showed significant difference compared with the control. However, no obvious regulations of time (p=0.669) toxicological effects were identified.

In combined toxicity, the protein contents were significantly affected by Cd concentrations ($p < 0.05$), but Cr concentrations and time have no significant influence.

TABLE 7 : Combined toxicity on protein content

	Cd content (mg/kg)	Cr content (mg/kg)	Protein content[a]		Cd content (mg/kg)	Cr content (mg/kg)	Protein content[a]
4d	0	0	1.015±0.012	8d	0	0	1.120±0.09
	0	20	0.947±0.012		0	20	1.005 ±0.017
	0	60	1.004±0.028		0	60	1.109±0.012
	0	110	0.992±0.018		0	110	1.103±0.014
	20	0	1.113±0.016		20	0	1.215±0.011
	20	20	0.899±0.027		20	20	0.998±0.009
	20	60	1.092±0.011		20	60	1.21 ±0.018
	20	110	0.825±0.005		20	110	0.942±0.017
	60	0	1.123±0.007		60	0	1.383±0.013
	60	20	0.985±0.010		60	20	1.034±0.020
	60	60	1.093±0.018		60	60	1.306±0.022
	60	110	1.109±0.011		60	110	1.312±0.027
	80	0	0.936±0.015		80	0	1.004±0.002
	80	20	1.033±0.011		80	20	1.085±0.022
	80	60	1.014±0.017		80	60	1.101±0.017
	80	110	1.015±0.021		80	110	1.075±0.010
16d	0	0	1.125±0.007	32d	0	0	1.022±0.007
	0	20	0.998±0.011		0	20	0.987±0.021
	0	60	1.109±0.012		0	60	1.009±0.015
	0	110	1.175±0.012		0	110	1.041±0.017
	20	0	1.307±0.012		20	0	1.152±0.007
	20	20	1.011±0.021		20	20	0.992±0.016
	20	60	1.244±0.012		20	60	1.160±0.022
	20	110	1.001±0.016		20	110	1.005±0.009
	60	0	1.441±0.017		60	0	1.135±0.008
	60	20	1.057±0.006		60	20	1.007±0.007
	60	60	1.255±0.021		60	60	1.165±0.017
	60	110	1.271±0.026		60	110	1.193±0.018
	80	0	1.023±0.018		80	0	0.996±0.012
	80	20	1.115±0.011		80	20	1.111±0.012
	80	60	1.134±0.011		80	60	1.043±0.005
	80	110	1.177±0.024		80	110	1.028±0.019

[a]Protein contents were shown in terms of ratio of measured data in experimental groups and control group (Mean±S.E.).

CONCLUSIONS

Interaction between heavy metal and time is extremely remarkable. The increased heavy metal concentration can decrease the protein content ($P<0.01$); the protein content increased as the time goes by ($P<0.05$). Different concentrations of Cd were the main factors influencing in individual Cd toxicity. Time was the main factor influencing in individual Cr toxicity. Combination of metals, no obvious regulations of time toxicological effects were identified, and the protein contents were significantly affected by Cd concentrations.

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