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Individual and combined toxicity of chromium and cadmium on the earthworm *Eisenia fetida*

Jingjing Dou[1], Shibin Hu[1*]
[1]Department of Resources and Environment, Northwest A & F University, Yangling Shaanxi 712100, (P.R.CHINA)
E-mail: 1326801980 @qq.com; doudou861231@sina.com

ABSTRACT

The objective of this research was to determine: 1) the individual acute toxicity of cadmium and chromium on *E. fetida* by filter paper contact test and soil expose test; 2) the avoidance response of *E. fetida*; 3) the combined toxicity of cadmium and cadmium on *E. fetida* in lou soil. The site to collect soil is a lou soil experimental plot northwest A&F university of Yangling, Shaanxi, China. It was shown that the toxicity of cadmium and chromium through skin in nature soil are moderately (200~2000 mg·kg^{-1}). when Cd and Cr were the same concentrations, avoidance rate under Cd pollution is lower than under Cr pollution. It is also concluded that the interaction type of Cr and Cd combined toxicity is antagonistic.

KEYWORDS

Earthworms; Cadmium; Chromium; Metal interaction; Lou soil.

INTRODUCTION

The heavy metal contamination can be produced by tannery, electroplating, battery manufacturing and metal processing. In China, the heavy metal content in soil can reach an appreciable level. High cadmium and chromium concentrations are typically found in soil of Xianyang, Shaanxi Province, China. Cadmium is labeled as a non-essential metal, does not occur naturally in organism and may be potentially toxic[5]. Chromium is one of the major soil pollutants, but its toxicity in soil organism is less studied. So among the various metals that contaminate terrestrial ecosystems, cadmium and chromium were chosen to be used in this study.

Earthworms are the dominant macro fauna in most temperate and tropical grassland soils [10, 13]. They modify soil organic matter[12], influence plants[1] and microorganisms[11]. Because of their importance in soil, earthworm studies have increasingly been focused on[8]. Earthworm is well studied as a model for heavy metal toxicity. There were many literatures concerned with metal uptake and accumulation in earthworms. Much of them measured metal content, growth, worms density[19], accumulation rate[21] and excretion rate[15]. 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.

The objective of this research was to determine: 1) the individual acute toxicity of Cd and Cr on *E. fetida* by filter paper contact test and soil expose test; 2) the avoidance response of *E. fetida* ; 3) the combined toxicity of Cd and Cr on *E. fetida* in lou soil.

MATERIALS AND METHODS

Earthworms

Earthworm resides organic rich habitats such as compost and manure heaps[3], plays an important role in soil system. 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.

Acute toxicity test

According to the OECD guideline No. 207, paper contact toxicity assay was used to test the acute toxicity of Cd and Cr to earthworms. In order to evacuate the earthworms gut content, the depuration period is 24 h under dark condition. Then earthworms were rinsed and dried by absorbent paper cautiously. The concentrations of Cd on filter papers were 0, 0.13, 0.25, 0.38, 0.5, 0.63, 0.75 and 1 $mg{\cdot}cm^{-1}$. The concentrations of Cr on filter papers were 0, 0.10, 0.21, 0.30, 0.40, 0.50, 0.61 and 0.70 $mg{\cdot}cm^{-1}$. The expose times were 24h and 48h.

Soil collection and earthworm exposures

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.

TABLE 1 : physical and chemical properties of the tested soil

Soil texture	Organic matter ($g{\cdot}kg^{-1}$)	Total nitrogen ($g{\cdot}kg^{-1}$)	Total phosphorus ($g{\cdot}kg^{-1}$)	CEC ($mmol{\cdot}kg^{-1}$)	Mechanical composition (%)			pH
					sand	silt	clay	
Lou soil	11.2	1.17	0.85	206.0	36.08	51.73	12.19	8.03

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. The expose times were 7d and 14d.

Three replicates and eight controls were used in the experiment. The concentrations of Cd on individual toxicity were 0, 300, 600, 900, 1200, 1500, 1800 and 2100 $mg{\cdot}kg^{-1}$(dry soil). The concentrations of Cr on individual toxicity were 0, 400, 800, 1200, 1600, 2000, 2400 and 2800 $mg \cdot kg^{-1}$(dry soil). The combined toxicity were 5×5 complete orthogonal. Three replicates and five controls were used in the experiment. Forty earthworms were placed into each treatment. The expose times were 14d. The concentrations of Cd were 0, 300, 600, 900 and 1200 $mg \cdot kg^{-1}$(dry soil). The concentrations of Cr were 0, 400, 800, 1200 and 1600 $mg \cdot kg^{-1}$(dry soil).

Avoidance response test

The experiment was carried out in apparatus which was a plastic container with two chambers. The chambers were separated from one another with plastic slides (without perforations), and were covered with gauze to prevent worms from escaping. One chamber was placed into 200 g clean soil, another one was placed into 200 g heavy metal contaminated soil. Twenty earthworms were placed into the central area where the plastic slides placed after remove the slides. After an incubation of 48 hour, slide was placed between the chambers to prevent further migrations and the number of worms from each chamber was counted. Three replicates and five controls were used in the experiment. The concentrations of Cd were 10, 50, 100, 150 and 200 $mg \cdot kg^{-1}$ (dry soil), The concentrations of Cr on individual toxicity were 10, 25, 50, 100 and 125 $mg \cdot kg^{-1}$(dry soil).

Statistical analysis

Heterogeneity was assessed with chi-square statistics. Probit analysis was used for assessing the acute toxicity of contaminants to the earthworms. All these statistical procedures were performed using SPSS 18.0.

RESULTS

Individual acute toxicity assay

There are two routes for earthworms to take up chemicals: one through their skin and the other from their food in gut[7]. In this paper, both of these routes were investigated. The filter paper contact test is the acute toxicity through skin and the soil expose test is the acute toxicity through gut.

The LC_{50} values for Cd and Cr were listed, as shown in TABLE 2. Significant differences (Chi-square test, Sig.＞0.05) were observed in LC_{50} between the Cd and Cr. The results indicated that the acute toxicity of Cd and Cr to earthworm were different as time changes. Also, the toxicity through filter paper contact test and soil expose test were different. According to classification of chemical's acute toxicity in China, the toxicity of Cd and Cr through skin in nature soil are moderately (200~2000 $mg \cdot kg^{-1}$).

TABLE 2 : Calculated LC_{50} values for Cd and Cr

	Expose time	Chemicals	LC_{50}	95% Confidence intervals	X^2	Sig.
Filter paper contact test	24h	Cd	0.65 $mg \cdot cm^{-1}$	0.57 ~0.76 $mg \cdot cm^{-1}$	2.020	0.846
	48h	Cd	0.48 $mg \cdot cm^{-1}$	0.42 ~0.55 $mg \cdot cm^{-1}$	2.828	0.726
	24h	Cr	0.53 $mg \cdot cm^{-1}$	0.48~0.60 $mg \cdot cm^{-1}$	2.214	0.819
	48h	Cr	0.34 $mg \cdot cm^{-1}$	0.34 ~0.38 $mg \cdot cm^{-1}$	3.078	0.688
Soil expose test	7d	Cd	1395 $mg \cdot kg^{-1}$	1284 ~ 1520 $mg \cdot kg^{-1}$	1.211	0.944
	14d	Cd	810 $mg \cdot kg^{-1}$	701~916 $mg \cdot kg^{-1}$	4.707	0.453
	7d	Cr	889 $mg \cdot kg^{-1}$	761 ~1045 $mg \cdot kg^{-1}$	8.163	0.147
	14d	Cr	582 $mg \cdot kg^{-1}$	515~648 $mg \cdot kg^{-1}$	6.219	0.285

Avoidance response test assay

Earthworms can produce avoidance response towards undesirable contaminants because of the chemoreceptor on their body surface. The avoidance behavior was consistent with the results from other response experiments[20].

When the number of earthworm in clean soil reached 80% of placed, which named avoidance behavior. Avoidance rate calculation method is as follows:

$$NR = (C - T)/N \times 100$$

Where NR is avoidance rate, C is earthworms in clean soil, T is earthworms in polluted soil, N is total number of earthworms placed in.

Figure 1 illustrated avoidance response of earthworms in Cd and Cr polluted soil. During the study, significant avoidance response was observed when the Cd was 200 $mg \cdot kg^{-1}$ and the Cr was 125 $mg \cdot kg^{-1}$. Earthworms have no death occurred within 48 h. when Cd and Cr were the same concentrations, avoidance rate under Cd pollution is lower than under Cr pollution.

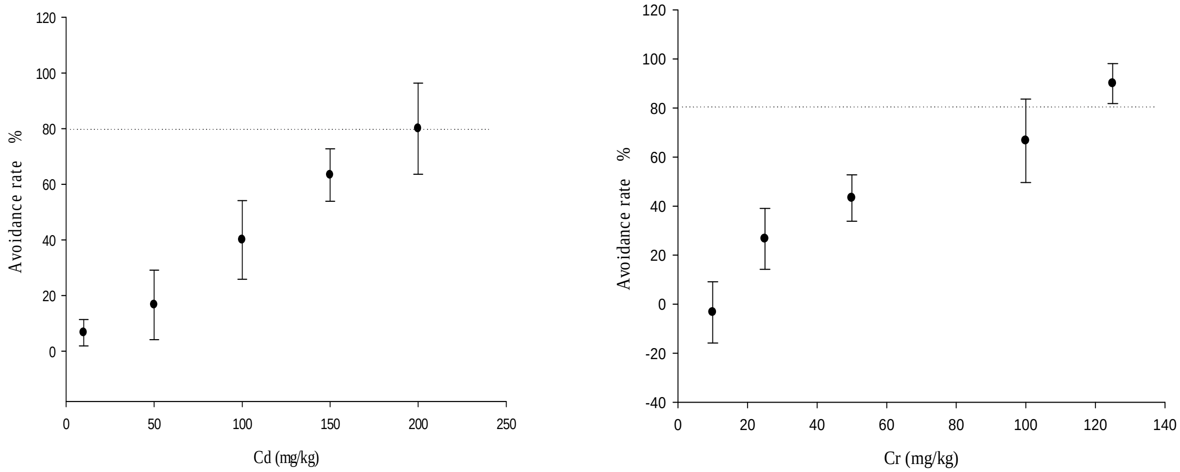

Figure 1 : Earthworms' avoidance test under Cd/Cr

Combined acute toxicity assay

The mixed heavy metal of Cd and Cr showed a significant influence on the mortality of earthworms than that of their separate use. Figure 2 showed the result which was Cr (Cd) concentrations as treatment group, Cd (Cr) concentrations as the control group. Without adding Cr, the mortality rate increase as the Cd concentration increasing. Under the same concentration of Cd, earthworm mortality rates rise as Cr concentrations rise. Without adding Cd, the mortality rate increase as Cr concentration increase. When Cd concentration is 300 mg·kg^{-1}, the mortality is less than the control group. When Cd concentration is 600 mg·kg^{-1}, Cr concentration is below 800 mg·kg^{-1}, the mortality rate are higher than the control group; when the concentration of Cr is above 800 mg·kg^{-1}, the mortality rate is lower than the control group. When Cd concentration are higher than 600 mg·kg^{-1}, the mortality of different treatments group are higher than the control group.

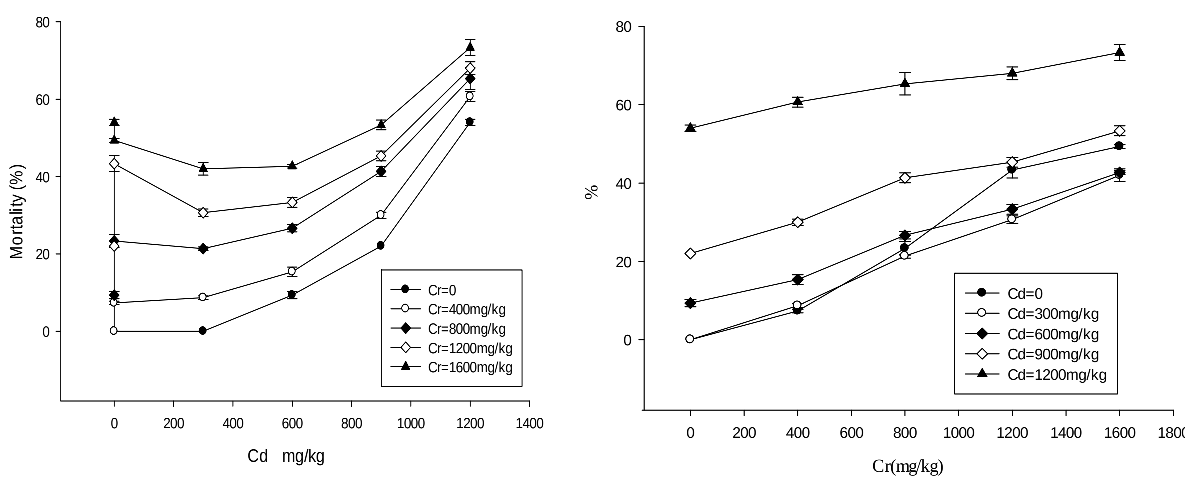

Figure 2 : Combined acute toxicity

All data were processed by SPSS, univariate analysis of variance and general linear correlation was used to analysis.

TABLE 3 : The variance analysis results of Cd and Cr combined acute toxicity (dependent variable: mortality)

Pollutant	Cd			Cr			Cd join Cr		
	df	F	P	df	F	P	df	F	P
Mortality	4	506.285	.000**	4	322.062	.000**	16	9.880	.000**

a. R^2= .986（adjusted R^2 = .979）

df: degree of freedom; *: cases where the concentration of exposure had a significant effect (* P<0.05, ** P<0.01)

The F statistics of Cd concentration is 506.285; the F statistics of Cr concentration is 322.062. Therefore the impact of Cd and Cr concentration on mortality is extremely significant under the 0.05 significance level. The F statistics of interaction is 9.880, the significance level is under 0.01, and interaction is extremely remarkable. The combination of Cd and Cr has a significant influence on mortality.

Statistics for combined toxicity experimental result was using linear regression model. The equation of Cd (X_{Cd}) and Cr (X_{Cr}) to mortality (Y) was $Y= 0.032X_{Cd}+ 0.022X_{Cr} - 2.720$. The partial correlation coefficient of Cd concentration and Cr concentration reached extremely significant level. The partial correlation coefficient Cd (0.818) > Cr (0.796), showed that different concentrations of Cd were the main factors influencing combined toxicity.

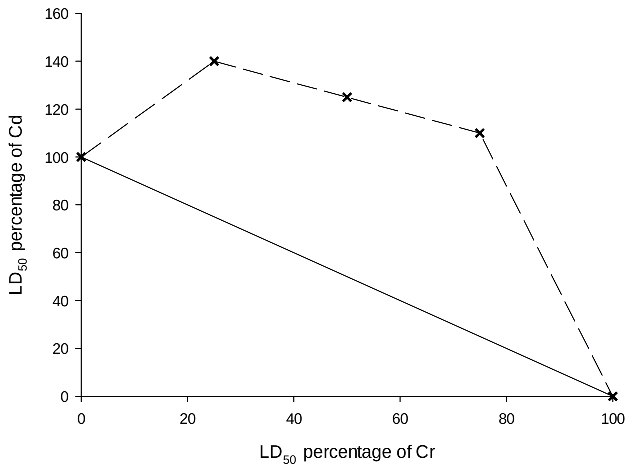

Figure 3 : Interaction of Cr to Cd with various doses

On the basis of Sarriselkä agreement, interaction is defined as three types: antagonistic, addition and synergistic[6]. According to the dose - effect equation, equivalent line was shown as Figure 4. First set up an equivalent line of additive effect for two heavy metals, that is the straight line connect 100% LD_{50} for Cr and 100% LD_{50} for Cd. From equivalent line of additive effect, equivalent Cd concentration can be calculated if Cr concentration reduces a certain proportion. Actual equivalent line is the curved line. Actual equivalent line indicates that Cr concentration reduces a certain proportion, actual Cd is higher than equivalent Cr concentration. The interaction type of Cr and Cd combined toxicity is antagonistic.

DISCUSSION

LD_{50} and EC_{50} are traditional methods carried out on the basis of the same indices of two compounds for assessing the combined toxicity of pollutants in environment.[4, 9, 16]. In this study, we chose the individual LC_{50} on filter paper, EC_{50} in nature soil, combined mortality rates and avoidance rate on earthworm as the toxicity endpoints. Factorial experiment could provide a greater precision in estimating the overall main factor effects, and explore the interaction among different factors[14, 18]. A single factorial experiment was designed to study the effects of Cd and Cr on the mortality rates. The combined toxicity of Cd and Cr was also designed with factorial analysis.

The individual toxicity tests on earthworm showed that the toxicity of Cd and Cr in nature soil is moderately. When heavy metals coexist in the environment, it is important to confirm the exact interaction among these metals because it will significantly affect their bioaccumulation processes in organisms and toxicological effects on different biological levels[2]. Some studies on interaction between heavy metals have been carried out. Pan and Yu (2011) found that the combination of Cd and Pb had synergistic toxicological effects on enzymes activities in soil. Jonker et al. (2004) found that more cadmium in the mixture decreased the toxicity and more copper increased the toxicity to the nematode *Caenorhabditis elegans*. The combined toxicity tests on earthworm in this study showed that the interaction type of Cr and Cd is antagonistic. The results of factorial analysis showed that the Cd had the highest influences on combined effects of both metals. The mechanisms of combined effects between Cd and Cr are complex, which might be influenced by the competition adsorption of both metals in soil and biomembrane and their bioavailability. Further studies are needed to intensively identify the mechanism of the combined toxicological effects.

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