



## **Study on Single Toxicity of Pb, Cd and Cr to Earthworms**

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**Abstract:** In this paper, the ecotoxicology research method combines field investigation and indoor simulation experiment. Sampling and analysis of heavy metal pollution of solid soil samples from lead-acid battery factory, identified Pb, Cd and Cr as the pollution research objects, discussed the biological reaction mechanism of Eiseniafetida under the stress of heavy metals Pb, Cd and Cr, and obtained the results. The results of the lethal and sublethal effects of Pb, Cd and Cr on Pb, Cd and Cr and the effect of biomarkers. The main research results are as follows: In the acute toxicity test of Aisheng worm, the theoretical half-lethal concentrations (LC50) of single Pb, Cd, and Cr exposure for 14 days were 2620.93, 362.83, and 229.44 mg/kg, respectively, and their toxicity was Cr>Cd>Pb .

**Keywords:** toxicity; heavy metal.

### **1. Introduction**

Soil is one of the most important material bases on which organisms live, and it is also an important medium for the great cycle of the ecosystem. While the rapid development of industrial zones has improved the living standards of local residents, it also has an increasingly significant impact on local regional ecological environment pollution. Among them, heavy metals produced by production activities in industrial

zones have become one of the important pollutants affecting the regional soil environment.

Soil can not only provide the material basis for plant growth and development, but also provide a venue for soil animals. The vast majority of pollutants in the environment can enter the soil from different ways, including chemical substances released into the atmosphere or attached to dust, and as a result, they are integrated into the soil or into the sediment through surface weathering and erosion. Therefore, the final carrier of pollutants in the environment is soil [1]. Due to the rapid development of science and technology, the rapid development of industrialization promotes a substantial increase in economic benefits, and at the same time, it has an increasingly significant impact on local and regional ecological environment pollution, which is harmful to human health. Soil heavy metal pollution has the characteristics of large coverage, long-term, stable, latent and irreversible, and is easily absorbed by organisms and stored in the body during the food chain transmission process, and finally enters the human body and poses a hazard to health [3,4]. Therefore, it is very urgent and necessary to evaluate the biological test of the toxic effects of heavy metals in soil, to explore the mechanism of toxic effects on organisms, to find prevention and control methods, and to protect the environment on which we live and the health of human beings.

Earthworms play an extremely critical role in terrestrial ecosystems. They are the key link through which toxic substances can be absorbed by plants through the soil and ultimately delivered to consumers through the food chain. They are considered to be the main living organisms in soil ecotoxicology research. At present, acute and chronic toxicity tests of *Eisenia fetida* have been standardized and are considered to be a suitable model species. Earthworms account for the largest biomass (>80%) of all terrestrial invertebrates, and some of its activities can affect soil structure and nutrient composition [5]. In addition, it also participates in the cycling process of chemical substances in the soil ecosystem and affects the interaction between other components of the soil ecosystem [6]. Therefore, some physiological and biochemical changes of earthworms and the content of heavy metals in the body can be used as the basis for soil environmental risk assessment, providing early warning and indication for soil environmental pollution [7].

## **2. Materials and Methods**

### **2.1.1 Tested earthworm**

The Aisheng worm, the purest biological product, was purchased from Beijing Lvhuang Jingyu Science and Trade Co., Ltd. After returning to the laboratory, screened for more than 2 months, the healthy adult Aisheng worms with a body weight between 300-

500 mg and a clear reproductive ring were acclimated in an incubator (45cm×30cm×20cm) for at least one week. Feeding on cow dung, the temperature is controlled at (20±1) °C, and the light is about 600lx (the light ratio is 12h: 12h) under the condition of adding water to maintain a certain humidity, providing a stable living environment for the growth and reproduction of the worm.

48h before the test, take out the baby worms needed for the test, wash them with deionized water, put them into the prepared artificial soil environment for one day, and feed on cow dung. During the acclimation period, there are no dead baby worms. . Twenty-four hours before the experiment, the acclimated Aisheng worms were taken out, rinsed with deionized water, and placed in a Petri dish covered with moist filter paper (the best covering filter paper). To encourage earthworms to drain impurities from their bodies, seal the petri dish with plastic wrap and poke a few small holes. Put it in a dark environment for a day, take out the earthworms and wash them, and dry the water outside the earthworms with filter paper.

#### 2.1.2 Test soil

The artificial soil was prepared with reference to the method of the Acute Toxicity Test of Aisheng Vermi in the OECD Test Methods for Chemicals. The artificial soil is uniformly mixed with 70% (by dry mass) quartz sand, 20% kaolin, and 10% peat. The pH is adjusted between 6.0 and 6.5 with calcium carbonate, and the soil moisture is adjusted to the maximum with ultrapure water. 40% of water holding capacity. The peat soil was purchased from the flower market, pH 6.0.

#### 2.2 Experimental Method

The earthworm acute toxicity test in the OECD chemical test method is used as a reference. Arrange the required materials before the test is officially carried out.

Purchase the same type of plastic lunch boxes, put 500 grams of artificial soil into each lunch box, and prepare the required number of groups for the test.

In the pre-test, several concentration groups were designed with reference to the geometric series of three single heavy metals. Based on the results, the concentration range of the toxic effect of a single heavy metal on earthworms was estimated, and the concentration gradient was designed within the estimated range.

According to the pre-test results, 8 experimental groups were set with the concentration of single heavy metal Pb as 0, 1000, 1400, 1800, 2200, 2500, 2800, 3200 mg/kg, and the concentration of single heavy metal Cd as 0, 250, 300, 350, 400, 450, 500 , 8 experimental groups of 550 mg/kg, 7 experimental groups with single heavy metal Cr concentration of 0, 120, 160, 200, 240, 280, 320 mg/kg, 4 parallels for each concentration, repeated 3 times. Ultrapure water to maintain soil moisture to 40% of the maximum water holding capacity, cover the box, and move to an artificial climate with a temperature of (20±1) °C, a humidity of 80% and a light of about 600lx

(light ratio of 12h:12h). Place in the box for 1 day to prepare for subsequent experiments.

Randomly put 10 acclimated, similar body weight, and bowel-cleansed Aisheng worms into each 500 g of artificial soil prepared and balanced according to the concentration of heavy metals, until all the earthworms have completely burrowed into the soil, sealed with plastic wrap and A rubber band is placed over the container, and the anatomical needle is pricked to prevent worm escape, water evaporation, and to provide gas exchange. The acute toxicity test was carried out in an artificial climate incubator with a temperature of (20±1) °C, a relative humidity of 80% and a light of about 600lx (light ratio of 12h:12h), with a period of 14d. On each day of the experiment, all the soil in the container was poured onto the glass plate, and all the worms were found, the response of the front and tail of the worms to mechanical stimulation was checked, the dead worms were taken out, and the number of dead worms was recorded. After the end, the soil and Aisheng worms were re-cultivated in the container. At the end of 14 days, the recorded results were calculated according to the standard method to obtain LC50. LC50 is based on experimental data and obtained through mathematical statistics. It has high accuracy and less sampling error and can more accurately reflect the acute toxicity of a certain pollutant, which is more reliable.

### 3. Results

The 14-day acute toxicity test of soil heavy metal Pb, Cd and Cr single pollution to *A. chinensis* was calculated by SPSS. The dose-effect relationship was obvious. It is 2526.05mg/kg, 362.83mg/kg, 229.4mg/kg, and its toxicity is Cr>Cd>Pb.

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