

# Estimation Of Toxic Effect Of Lead And Cadmium Concentrations On *Perionyx excavatus* By Using Filter Paper Contact Method.

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## ABSTRACT

There is a growing public concern over the potential impact of accumulation of heavy metals in soil. Bioassays can be useful tools to gauge the potential toxicity of contaminants focusing on their bioavailable fraction. The detritivorous soil fauna are ideal model for assessment of soil health. The aim of this study was to determine the toxic effects of lead and cadmium concentrations, administered in the form of lead nitrate [Pb(NO<sub>3</sub>)<sub>2</sub>] and cadmium nitrate [Cd(NO<sub>3</sub>)<sub>2</sub>] solutions, on *Perionyx excavatus* by using filter paper contact method under laboratory conditions. Aqueous solutions of various concentrations were prepared by dissolving these test chemicals in deionized water. The concentrations were prepared in mg/ml and the toxicity was measured as µg/cm<sup>2</sup>. A geometric concentration series of test solution 0.25, 0.5, 1, 2, 4, 8, 16, and 32mg/1ml was prepared and tested. Significant toxic effects and deaths were observed with all tested heavy metal concentrations and were most marked at the concentration: 0.5 mg/1ml for Cd and 4 mg/1ml for Pb in case of *P. excavatus*. The lethal toxic concentration of Cd and Pb were thus evaluated as 7.15 µg/cm<sup>2</sup> and 57.16 µg/cm<sup>2</sup> for *P. excavatus*. Hence the relative toxicity grade were categorized as 'Extremely toxic' for Cd and 'very toxic' for Pb to *P. excavatus*. Evaluated results shows that Cd is more toxic than Pb to *P. excavatus*.

**Key words:** Lead nitrate, Cadmium nitrate, Earthworms, Acute toxicity, Filter paper contact method.

## 1. Introduction

Metals occur naturally in soils as a result of diverse geological processes. Beside this, a large number of industrial activities produce wastes and contaminants that reach the soil through direct disposal, spills, leaks, atmospheric deposition from air, and other pathways [Nadal M, Mari M, Schuhmacher M, Domingo JL. 2009]. However, the highest mean Cd concentrations were found from the textile industry, while the highest Pb from cement plant, battery production industry. In addition to the sources mentioned above, battery production and scrap battery recovery facilities, thermal power plants, and iron-steel industries are commonly found to be major industrial sources of Pb [Yaylali-Abanuz G., 2011]. The deposition of metals from the atmosphere and resulting accumulation in terrestrial ecosystems is a well-known phenomenon [Tyler, 1972]. To assess soil quality, bioassays can be useful tools to gauge the potential toxicity of contaminants focusing on their bioavailable fraction. Soil is a dynamic and complex system functioning as habitat for microorganisms, flora, animals and humans [Hund-Rinke et al., 2002]. Earthworms, called the "Intestine of Earth" by Aristotle, are very important soil organisms that help in the disintegration of plant litter, such as the thatch layer, and in recycling of nutrients enrich the soil. Earthworms can be used as a first screening tool for soil Environmental Risk Assessment. The aim of this study was to determine the toxic effects of lead and cadmium concentrations, administered in the form of lead nitrate and cadmium nitrate, on *Perionyx excavatus* by using filter paper contact method.

## 2. Material and Method

### 2.1. Test Animals

The present study was carried out on a species of soil fauna, *Perionyx excavatus*. Specimens *Perionyx excavatus* were collected from the grassland around Midnapore town (West Bengal, India). This species

was reared in the laboratory and adopted as a test species. We used adult earthworm which was above 250-300mg of body weight.

## 2.2. Test solutions

Cadmium nitrate [ $\text{Cd}(\text{NO}_3)_2$ ] and lead nitrate [ $\text{Pb}(\text{NO}_3)_2$ ] were used as test chemical. These chemicals were purchased from the scientific chemical shop, Purba Medinipur, West Bengal, India. Aqueous solutions of various concentrations were prepared by dissolving these test chemicals in deionized water. The concentrations were prepared in mg/ml and the toxicity was measured as  $\mu\text{g}/\text{cm}^2$ . A geometric concentration series of test solution 0.25, 0.5, 1, 2, 4, 8, 16, and 32mg/1ml was prepared and tested.

## 2.3. Acute toxicity test:

Acute toxicity test was performed following the method described in the OECD (1984) guideline for testing of chemicals no. 207. This is a simple screening test to identify the toxic potential of the chemical to earthworm. The test vial was a petridish (Wang *et al*, 2012) which was transparent of 14cm diameter and 2cm height. Round filter paper (Whatman No. 1) were cut to the suitable size and located in such a manner that sides were lined with filter paper. 10ml test solution was pipetted into each petridish in order to wet the filter paper. One blank test were prepared with 10ml of deionized water and used as a control. For each treatment, five replicates were used, each consisting of one live earthworm per petridish. Adult clitelated earthworms which had an individual wet weight of 250–300mg were selected for testing. Earthworms were washed briefly with deionized water, and were kept on moist filter paper for 3 h to devoid the gut content, after which it was rinsed again with deionized water, blotted on the filter paper and placed in a petridish. An earthworm was introduced to each petridish and the petridish were covered with plastic film that had been punched with small holes using needles. Trials were made out in the dark at  $28 \pm 2$  °C for 48 h. After 48 hours the earthworm was monitored for mortality by a gentle mechanical stimulus to the front part.

## 2.4. Statistical analysis

For the filter paper contact test method, based on the resulting 48-h LC50 values, the fertilizer will be classified as super toxic ( $<1.0 \mu\text{g}/\text{cm}^2$ ), extremely toxic ( $1-10 \mu\text{g}/\text{cm}^2$ ), very toxic ( $10-100 \mu\text{g}/\text{cm}^2$ ), moderately toxic ( $100-1000 \mu\text{g}/\text{cm}^2$ ) or relatively nontoxic ( $>1000 \mu\text{g}/\text{cm}^2$ ) (Roberts and Dorrough, 1984).

## 3. Result and Discussion

Observation Table1

Chemical	Concentration	Set 1	Set 2	Set 3	Set 4	Set 5	Most marked toxic Concentration
Lead nitrate [ $\text{Pb}(\text{NO}_3)_2$ ]	0.25 mg/ml	Live	Live	Live	Live	Live	
	0.50 mg/ml	Live	Live	Live	Live	Live	
	1mg/ml	Live	Live	Live	Live	Live	
	2mg/ml	Death	Live	Live	Live	Live	
	4mg/ml	Live	Death	Death	Death	Death	4mg/ml
	8mg/ml	Death	Death	Death	Death	Death	
	16mg/ml	Death	Death	Death	Death	Death	
	32mg/ml	Death	Death	Death	Death	Death	

Observation Table 2

Chemical	Concentration	Set 1	Set 2	Set 3	Set 4	Set 5	Most marked toxic Concentration
Cadmium nitrate [Cd(NO <sub>3</sub> ) <sub>2</sub> ]	0.25 mg/ml	Live	Live	Live	Death	Live	
	0.50 mg/ml	Death	Death	Live	Death	Death	0.50 mg/ml
	1mg/ml	Death	Death	Death	Death	Death	
	2mg/ml	Death	Death	Death	Death	Death	
	4mg/ml	Death	Death	Death	Death	Death	
	8mg/ml	Death	Death	Death	Death	Death	
	16mg/ml	Death	Death	Death	Death	Death	
	32mg/ml	Death	Death	Death	Death	Death	

In the present experiment, we used filter paper contact method for the assessment of toxic effects of Cd and Pb on *Perinoyx excavatus*. Pb and Cd are two poisonous heavy metals which affect on body of earthworm. Experiments show the mortality of *Perinoyx excavatus*.

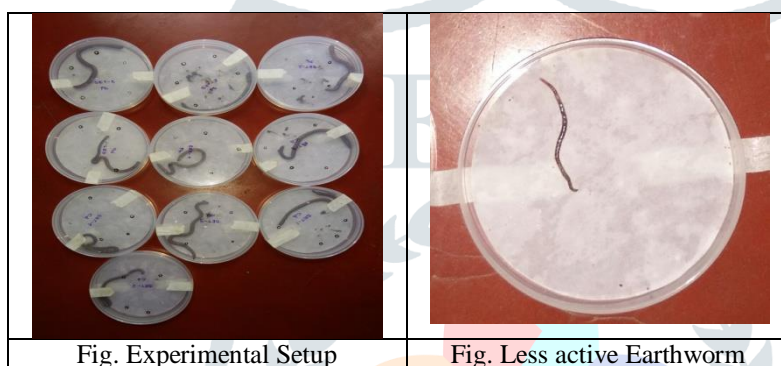


Fig. Experimental Setup

Fig. Less active Earthworm

With uniform area of contact exposure of Cadmium nitrate [Cd(NO<sub>3</sub>)<sub>2</sub>] and lead nitrate [Pb(NO<sub>3</sub>)<sub>2</sub>] to the earthworm in different concentrations showed various toxic effects in filter paper substrate medium. In the lead nitrate solutions of 0.25mg/ml, 0.50mg/ml and 1mg/ml, the earthworms were very active and moving. In lead nitrate solutions of 2mg/ml and Cadmium nitrate solution of 0.25mg/ml the earthworm were slightly less active and moving. Significant toxic effects and deaths were observed with all tested heavy metal concentrations and were most marked at the concentration: 0.5 mg/ml for Cd and 4 mg/ml for Pb in case of *P. excavatus*. In the concentration of 8mg/ml to 16mg/ml of lead nitrate and 1mg/ml to 2 mg/ml of Cadmium nitrate were very effected on earthworm's body. The body of an earthworm was folding and died and some part of body was broken down. In the concentration above 16mg/ml of lead nitrate and 2mg/ml of Cadmium nitrate, the earthworms have been melting and died.

#### 4. Conclusion

Contact filter paper test is an initial screening technique to assess the relative toxicity of chemicals to earthworms in which the chemicals are absorbed mainly by the skin. It is important to know the toxic status of a particular chemical, whether it is toxic or not. The lethal toxic concentration of Cd and Pb were thus evaluated as 7.15 µg/cm<sup>2</sup> and 57.16 µg/cm<sup>2</sup> for *P. excavatus*. Hence the relative toxicity grade were categorized as 'Extremely toxic' for Cd and 'very toxic' for Pb to *P. excavatus*. Thus, Cd is more toxic than Pb to *P. excavatus*.

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