

## **Assessment of acute toxicity of superphosphate to *Eisenia foetida* using paper contact method**

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### **ABSTRACT**

Superphosphate was the most widely used phosphate fertilizer in agricultural fields. It is applied to the soil to increase and to maintain soil phosphorus levels. The ultimate goal is of maximizing productivity and economic returns, while side effects on soil organisms are often neglected. Another important indirect effect especially of superphosphate (SP) fertilization is soil acidification, with considerable negative effects on earthworms. Earthworms are typically inferred as soil indicators without which soil fertility cannot be measured directly. An acute toxicity test of SP to earthworms (*Eisenia foetida*) was performed using paper contact method proposed by OECD (Organization for Economical and Cooperative Development) testing guideline no. 207. The worm was exposed to the deposit of SP kept uniform on filter paper for 48 h and the mortality was recorded. The lethal concentration was recorded as 210mg/5ml (i.e. 300 $\mu$ g/cm<sup>2</sup>). Based on the resulting LC<sub>50</sub> value, the SP was classified as "moderately toxic" to earthworm, *Eisenia foetida*. The result of this study further demonstrates that the inorganic mineral fertilizer can also be toxic to earthworms when contacted directly. Thus there arises an unavoidable need of monitoring the usage of fertilizer dosage on agricultural lands, particularly the superphosphate.

**Keywords:** 48h LC<sub>50</sub>; Acute toxicity; *Eisenia foetida*; Paper contact method; Superphosphate

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### **INTRODUCTION**

Superphosphate is a compound produced by treating rock phosphate with sulfuric acid or phosphoric acid, or a mixture of the two. It is the principal carrier of phosphate, the form of phosphorus usable by plants, and is one of the world's most important fertilizers. The employment of intensive crop production technologies in agriculture includes the application of inorganic mineral fertilizers in various forms. SP is a major ingredient in the most commonly used fertilizers. It exists in various forms like Single Superphosphate, Double Superphosphate and Triple Superphosphate. The compound mainly studied in this work was single SP. The agricultural Department of India recommends 60kgs per hectare of SP for paddy or wheat field and various concentrations for various other crops. But due to the interest in maximizing the crop productivity and economic return, farmers, overuse SP beyond the recommended dosage. Though the farmers' ultimate goal is achieved, the side effects on soil organisms, especially on earthworms are often ignored.

Among the organisms with their living activity in soil, the earthworms are recognized for their important role regarding the improvement of physical and chemical characteristics of soil, and thus increasing its fertility [1] [2] [6]. Knowing their dynamics in soil under the influence of different technological treatments, or as a result of fertilizers application, it is very important for soil fertility conservation [3] [5]. The influence of various inorganic pesticides applied in soil on earthworms was studied by many scientists. But no study was conducted on the

influence of direct conduct of fertilizers on earthworms. This was the first study on the effect of direct contact of the fertilizer, SP on earthworms.

As a good indicator of soil quality, earthworms were used as testing organisms by OECD in early 1980's for the registration of industrial fertilizers and pesticides before implementing them into the soil. Likewise, in this study, paper contact method was adopted to determine the toxic potential of SP on earthworms. Because if such a simple method could predict the toxicity of chemicals, it would be useful as a preliminary step to decide the concentration level of the particular chemical for the further study (acute or chronic). Also if the chemical is proved to be non-toxic in such paper contact method, decision can be made not to study further on it and its application to agricultural fields can also be recommended without any hesitation. Thus the aim of the work was to investigate the influence of direct contact of SP on the *Eisenia foetida* worms.

## MATERIALS AND METHODS

### 2.1. Earthworms

*Eisenia foetida* was adopted as the test species, because it is the recommended species in OECD (1984) [7] guideline for testing of chemicals no. 207, earthworm, acute toxicity tests. The earthworms were purchased from the Vermicomposting Unit, Annamalai University, Annamalai Nagar, Tamilnadu, India. They were all cultured under the same conditions, fed mainly on the excrement of milk cows. This culture was judged to be free for contaminants. Adult earthworms, which possessed clitellum and had an individual wet weight of  $200 \pm 30$  mg (after void of the gut content), were selected for testing.

### 2.2. Test chemicals and solutions

The commonly used inorganic mineral fertilizer SP was used as test chemical. It was purchased from the Agriculture office, Annamalai Nagar, Cuddalore district, Tamilnadu, India. Aqueous solutions of various concentrations were prepared by dissolving the SP in deionized water. The concentrations were prepared in mg/ml and the toxicity was measured as  $\mu\text{g}/\text{cm}^2$ .

### 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 petri dish [9] of 14cm diameter and 2cm height. Round filter paper (Whatman No. 1) was cut to the suitable size and placed in such a way that sides are lined with filter paper. 5ml test solution was pipetted into each vial in order to wet the filter paper. Blank tests were performed with 5ml of deionized water. For each treatment, ten replicates were used, each consisting of one earthworm per vial. Adult earthworms, which possessed clitellum and had an individual wet weight of 250–350mg, were selected for testing. Earthworms were washed briefly with deionized water, and were kept on moist filter paper for 3h to devoid the gut content, after which it was rinsed again with deionized water, blotted on the filter paper and placed in a test vial. An earthworm was introduced per vial and the vial was covered with plastic film that had been punched with small holes using needles. Tests were done in the dark at  $28 \pm 2^\circ\text{C}$  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 48h  $\text{LC}_{50}$  values, the fertilizer will be classified as supertoxic ( $<1.0 \mu\text{g}/\text{cm}^2$ ), 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 ( $>1000 \mu\text{g}/\text{cm}^2$ ) [8].

## RESULTS AND DISCUSSION

The lethal toxic concentration of SP was thus evaluated as  $300 \mu\text{g}/\text{cm}^2$ . Hence the relative toxicity grade was categorized as “moderately toxic” for SP to *Eisenia foetida*. A geometric concentration series of test solutions (70, 140, 210, 280 and 350mg/5ml) were prepared and tested, in which mortality of earthworms was observed in 280 and 350mg concentrations after 24 and 8h respectively. Only the 70 and 140mg/5ml concentrations were non-toxic to earthworm for 48 h. The lethal concentration for 48h to *Eisenia foetida* was 210mg/5ml concentration (**Fig. 1.**). The deleterious effects of SP on earthworm were lesions, inflammations and separation of the posterior body parts (**Fig. 2.**). The worm was not able to move after 40h, neural retention starts and the body parts separate leading to death.

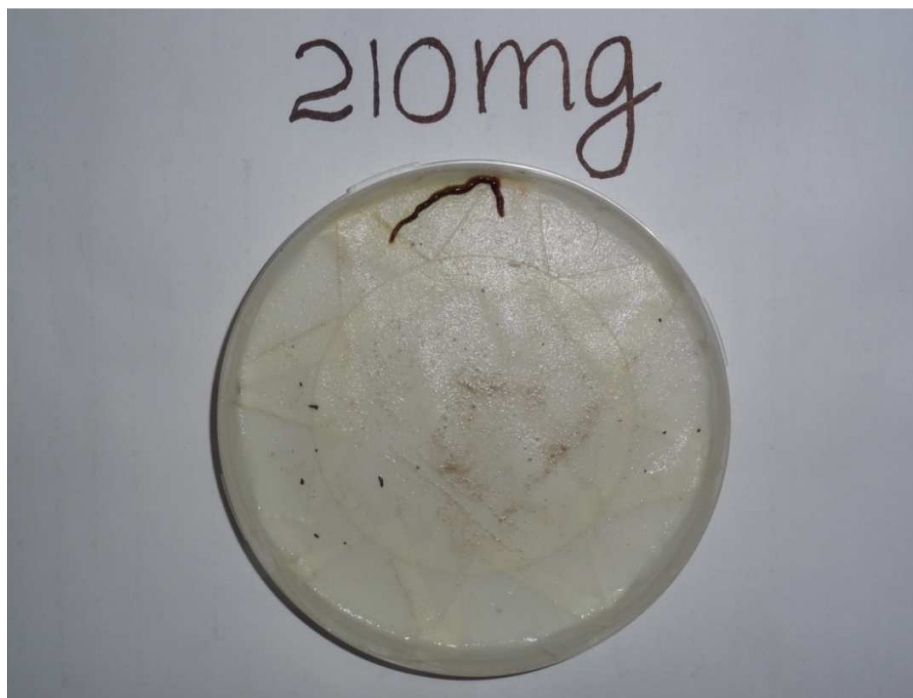


Figure 1: Mortality of *Eisenia foetida* at 210mg/5ml concentration of SP



Figure 2: Detrimental effects like lesions, inflammations and separation of body parts observed on *Eisenia foetida* in another replicate vial

Contact filter paper test is an initial screening technique to assess the relative toxicity of chemicals to earthworms. In this initial screening test, the chemicals are absorbed into the earthworm body mainly through the skin when it is

moving around the filter paper. Though the method fails to represent the situation in soil, it is important to know the toxic status of a particular chemical, whether it is toxic or not. If the chemical proved to be toxic, further extended study on artificial soil can be carried out and if not, there is no need for the extended study. It has been demonstrated for many decades that most of the inorganic mineral fertilizers are non-toxic to earthworms; however, it has been disproved in this study.

### CONCLUSION

India is the 3rd largest fertilizer producer in the world. There are about 139 fertilizer plants in operation in India, among which 80's are producing superphosphate (the maximum number). Superphosphate is the third largest consumption fertilizer after urea and diammonium phosphate. All-India capacity utilization of SP went up from 41.5% during April-February 2009-10 to 49.2% during April-February 2010-11 [4]. The general fertilizer recommendation of NPK dosage as per the department of agriculture, India is 120kg: 60kg: 30kg (4:2:1) per hectare. But this recommendation is not followed by Indian farmers as they aim at higher productivity and the fertilizers are overused. This attitude leads to more amount of fertilizers into the soil affecting the soil organisms.

From the above results, it may be concluded that the acute toxicity of SP on *Eisenia foetida* by using a simple paper contact method was really significant in confirming the toxic potential. The application of environmentally realistic doses of SP revealed the possible harmful effects on earthworms when contact directly. Thus, in future this method will be necessary to find a way to determine the sensitivity of the earthworm's acute toxicity before going for the evaluation in soil (i.e. acute and chronic toxicity tests in artificial soil). Soil being a very complex system, it is often difficult to compare toxicity data directly. Though the paper contact method ignores the contribution of soil components it has high advantages of reproducibility and the possible of direct comparison of results. Using the data obtained from this method, the range of test concentration for acute and chronic toxicity tests can also be determined.

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