

## Determination of Acute Toxicity of NPK Fertilizers to *Eisenia Foetida* Using a Simple Paper Contact Method



### Zoology

**KEYWORDS :** NPK fertilizers; Acute toxicity; 48h LC50; *Eisenia foetida*; Paper contact method.

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### ABSTRACT

*NPK fertilizers were the most widely used growth promoters in agricultural fields. They were applied to the soil to increase and to maintain soil nutrient levels. The ultimate goal was of maximizing productivity and economic returns, while side effects on soil organisms were often neglected. Another important indirect effect especially of NPK fertilization was 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 NPK fertilizers to earthworms (*Eisenia foetida*) was performed using a simple paper contact method proposed by OECD (Organization for Economical and Cooperative Development) testing guideline no. 207. The worm was exposed to the deposit of NPK fertilizers (4:2:1 ratio) kept uniform on filter paper for 48 h and the mortality was recorded. The concentrations were expressed in  $\mu\text{g}/\text{cm}^2$ . Based on the resulting LC50 value, the NPK fertilizers combined were classified as "very toxic" to earthworm. The result of this study further demonstrates that the inorganic chemical fertilizers can also be toxic to earthworms when contacted directly. Thus there arises an unavoidable need of monitoring the usage of fertilizer dosages on agricultural lands, particularly the NPK.*

### 1.1. INTRODUCTION

Fertilizers are soil amendments applied to promote plant growth, the main nutrients added in fertilizer are nitrogen, phosphorus, potassium and other nutrients are added in smaller amounts. Collectively, the main nutrients vital to plants by weight are called macronutrients, including: nitrogen (N), phosphorus (P), and potassium (K) (i.e. NPK). Urea is the main product for making nitrogen available to plant. Phosphorous is made available in form of superphosphate. Muriate of Potash (Potassium Chloride) is used for supply of potassium. Nitrogen percentage is reported directly, however phosphorus is reported as the mass fraction of phosphorus pentoxide and potassium is reported as the mass fraction of potassium oxide (K<sub>2</sub>O).

India is the 3rd largest fertilizer producer in the world. In India chemical fertilizers played a significant role which is well recognized and established for the success in agriculture sector, not only in terms of meeting total requirement of food grains but also generating exportable surpluses. According to fertilizers association of India during the year 2008-09 the production of fertilizers in India was 143 lakhs tons and India had to import over 100 lakhs tons to meet the domestic requirement. Of the imported products 23% or (23 lakhs tons) were NPK Grade fertilizers. To attain the self-reliance in food-grain production, the Government of India has been consistently pursuing policies conducive to increased availability and consumption of fertilizers in the country. As a result, the annual consumption of fertilizers in nutrient terms (N, P & K), has increased from 0.7 lakh MT in 1951-52 to 264.86 lakh MT 2009-10, while per hectare consumption of fertilizers, which was less than 1 Kg in 1951-52 has risen to the level of 135.27 Kg (estimated) in 2009-10 (Anonymous, 2011). This intensive use of chemical fertilizers results in threatening the soil quality and the soil dwelling beneficial organisms..

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 (Aina, 1984; Edwards and Bohlen, 1996; Abdul Rida and Bouché, 1997). 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 (Aira et al., 2006; Asawalam, 2006).

The influence of various inorganic fertilizers on earthworms was studied since 1982 by Edwards and Loftly in three long-term experiments and two short-term field experiments, one on grass and one on wheat, in conditions of annual fertilizer treatments with various forms of inorganic nitrogen (48, 96, 144 and 192 kg N/ha). Here the positive correlation between

the amounts of inorganic nitrogen applied and populations of earthworms was studied well. The effects of long-term use of nitrogenous fertilizers were studied with 3 different amounts (60, 120 and 180 kg N/ha/year) from 6 different sources (mineral ammonium sulphate, nitrochalk, synthetic sulphur-coated urea, organic-coated urea, isobutylidenediurea, and ureaformaldehyde) in uncultivated turfgrass on loamy sand soil. Results showed that mineral ammonium sulphate and sulphur-coated urea drastically decreased earthworm numbers and biomass and lowered pH of soil.

Other studies evaluated the effect of 14 years of treatment with NPKMg fertilizer on earthworm populations, on a clay loam (Humic Gleysol) situated at St-Lambert, Quebec. The results established that mineral fertilizer application had no significant effect on earthworms, and compared to mineral fertilizer treatment, long-term application of manure increased earthworm populations (Estevez et al., 1996). Numerous studies concerning the evolution of earthworm abundance and biomass focused on differences that appear between organic fertilization of soil and inorganic fertilization. Toxicity studies were also made with pesticides and herbicides on earthworms (De Silva, 2004, Eijsackers, 1978; Subagja and Snider, 1981; Eijsackers, 1991; Van Gestel et al., 1992; Xiao, 2006). But studies on toxic effect of fertilization of soil on earthworms were very rare. Thus this study aimed at studying the acute toxicity of NPK fertilizer on earthworm *Eisenia foetida* using the standard paper contact test proposed by OECD (1984).

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. In this study, the simple paper contact method was adopted as the testing method, because if such a simple method could predict the toxicity of chemicals for earthworms, it would be useful as a preliminary step for remediation of contaminated soil and for the further toxicity studies (acute and chronic toxicity in soil). Thus the aim of the work was to investigate the influence of NPK fertilizers on the *Eisenia foetida* worms.

### 2. Materials and methods

#### 2.1. Earthworms

*Eisenia foetida* was adopted as the test species, because it is the recommended species in OECD (1984) guideline for testing of chemicals no. 207, earthworm, acute toxicity tests. The earthworms were purchased from the Vermicomposting Unit, Annamalai University, Annamalainagar. 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 fertilizers, urea, superphosphate and potash were used as test chemicals. They were purchased from the Agriculture office, Annamalainagar, Cuddalore district, Tamilnadu, India. Aqueous solutions of various concentrations were prepared by dissolving the NPK fertilizers taken in 4:2:1 ratio in deionized water. This is the generally recommended ratio to agricultural soil mainly for paddy and wheat by the Department of Agriculture, Ministry of Agriculture, India in the soil testing manual (2011). 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 (Wang et al, 2012) 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 48-h  $\text{LC}_{50}$  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 ( $>1000 \mu\text{g}/\text{cm}^2$ ) (Roberts and Dorough, 1984).

## 3. Results and Discussion

Figure 1 shows the mortality of earthworm, *Eisenia foetida* at 8:4:2mg/5ml concentration of NPK fertilizers. The deleterious effects of NPK fertilizers on earthworm were lesions and inflammations (Figure 2). The clitellum part of the body was very much affected. The lethal toxic concentration of NPK fertilizers were thus evaluated as  $40 \mu\text{g}/\text{cm}^2$ . Hence the relative toxicity grade was categorized as "very toxic" for NPK fertilizers to *Eisenia foetida*.



**Figure 1: Mortality of *Eisenia foetida* to 8:4:2mg/5ml concentration of NPK fertilizers solution**



**Figure 2: Deleterious effects of NPK fertilizers to *Eisenia foetida*, a nearer view**

With uniform area of contact exposure of NPK fertilizers to the earthworm in different concentrations showed various toxic effects in filter paper substrate medium. A geometric concentration series of test solution of NPK fertilizers (2:1:0.5, 4:2:1, 8:4:2, 16:4:2 and 32:16:8mg/5ml) was prepared and tested, in which immediate mortality of earthworms were observed in 32:16:8mg/5ml concentration. Only the 2:1:0.5 and 4:2:1mg/5ml was non-toxic to earthworm for 48 h. 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 an initial screening test to prove the toxicity of chemicals to earthworms. Though it 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.

## 4. Conclusion

NPK fertilizers that are applied to promote plant growth are proved to be toxic to earthworms at certain concentration, which infers the need of limited usage of NPK fertilizers. Many authors – such as Larson and Pierce (1991) and Doran and Parkin (1996) have proposed several minimum data sets on the use of earthworms as soil quality indicators. However, to date, there is no universally accepted standard data set, nor are their universal critical values of soil quality parameters. This is because the magnitude and direction of change in soil quality and the equilibrium contents of parameters are dependent on climate, mineralogy, soil conditions and land-use practices which vary from region to region (Sanchez-Maranon et al, 2002; Sparling et al, 2003). Thus frequent monitoring of soil quality is a must. Though the filter paper contact method does not represent soil quality, the data gained from it can be used for extended study (using artificial soil as recommended by OECD) and for comparing the toxic potential. sublethal and lethal criteria for nine different chemicals in standardized toxicity tests using the earthworm *Eisenia andrei*. *Ecotox. Environ. Safety*, 23: 206-220.

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