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Determination of acute toxicity of potash to *Eisenia foetida* using a simple paper contact method

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ABSTRACT

Fertilizers are intentionally added to the soil to improve the crop yield. But their application may affect the soil organisms, particularly earthworms and thus the soil fertility may end in risk. Soil fertility is the ability of soil to function within natural and managed ecosystems and depends on physical, chemical and biological properties of the soil. Most of these qualities cannot be measured directly, but are typically inferred from soil properties that serve as indicators. Earthworm is one such indicator which is used for monitoring the soil fertility. Potash is a worldwide used mineral fertilizer that is applied to crops for supplying potassium. An acute toxicity test of potash 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 potash kept uniform on filter paper for 48 h and the mortality was recorded. The concentrations were expressed in $\mu g/cm^2$. Based on the resulting LC_{50} value, the potash was classified as "very toxic" to earthworm. The result of this study further demonstrates that the inorganic chemical 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 potash.

Keywords: Potash; Acute toxicity; 48 h LC_{50}; *Eisenia foetida;* Paper contact method.

INTRODUCTION

The employment of intensive crop production technologies in agriculture includes the application of inorganic mineral fertilizers in various forms. Potash is a major ingredient in the most commonly used fertilizers. International potash institute recommends using 40 to 60 kg of potash per hectare in various agricultural lands. But due to the interest in maximizing the crop productivity and economic return, farmers, overuse potash beyond the recommended dosage. Though the farmers' ultimate goal is achieved, the side effects on soil organisms, especially on earthworms are often ignored.

As modern science considers the soil as a living or endowed with life planetary system which can accumulate and transform the chemical substances, it becomes evident that ecotoxicological evaluation of their effects on soil biota is a must in every concrete case of their use. It is important to elucidate how they affect the organisms, particularly the earthworms famous for their useful role in soil formation (Bouche, 1992; Edwards and Bohlen, 1996).

There is a strong and growing interest in soil organisms among environmental scientists and regulators in recent years. This reflects the concerns about soil fertility in agricultural land and its effects on soil organisms. Soil organisms can be adopted as valuable indicators by which these issues can be studied. Recent developments in national and international legislation have sharpened the need for reliable, sensitive indicator organisms to use in research, monitoring, and regulatory testing (Edwards and Bater, 1992; Edwards and Bohlen, 1992).

Earthworms are a major component of the animal biomass of terrestrial ecosystems where they play several biological roles; as food for other organisms, interactions with plant roots and soil micro-organisms, chemical and physical functions which affect soil fertility and conservation. They are the main or occasional food for about 200 species of birds, mammals, amphibian and reptiles, plus many fishes and invertebrates. They eat and crush an average of 300 Tones/ha of soil, mixing mineral layers and organic compounds to produce soil crumbs. The burrows they produce provide channels through which soils respire. These channels also play a key role in infiltration of water allowing a mean through fall of 16 cm of water per hour. The protection of earthworms which in turn will contribute to better control of soil erosion, flooding, nitrate production and also an improvement of alternative methods such as minimum cultivation which need the earthworm task force as a substitute for most of the mechanical cultivation (Edwards and Bohlen, 1996).

Thus the need for protecting earthworms has become inevitable. 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, a 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. Thus the aim of the work was to investigate the influence of potash on the *Eisenia foetida* worms.

MATERIALS AND METHODS

1.1. 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 300–400 mg, were selected for testing.

1.2. Test chemicals and solutions

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

1.3. 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. 10ml test solution was pipetted into each vial in order to wet the filter paper. Blank tests were performed with 10ml 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 300–450mg, were selected for testing. Earthworm was washed briefly with deionized water, and was 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 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 ± 2^0C for 48 h. After 48 hours the earthworm was monitored for mortality by a gentle mechanical stimulus to the front part.

1.4. Statistical analysis

For the filter paper contact test method, based on the resulting 48-h LC_{50} values, the fertilizer will be classified as supertoxic (<1.0 µg/cm^2), extremely toxic (1–10 µg/cm^2), very toxic (10–100 µg/cm^2), moderately toxic (100–1000 µg/cm^2) or relatively nontoxic (>1000 µg/cm^2) (Roberts and Dorough, 1984).

RESULTS AND DISCUSSION

Figure 1 shows the mortality of earthworm, *Eisenia foetida* at 40mg/10ml concentration of potash. The deleterious effects of potash on earthworm were lesions and inflammations (Figure 2). The lethal toxic concentration of potash was thus evaluated as 57µg/cm^2. Hence the relative toxicity grade was categorized as "very toxic" for potash to *Eisenia foetida*.

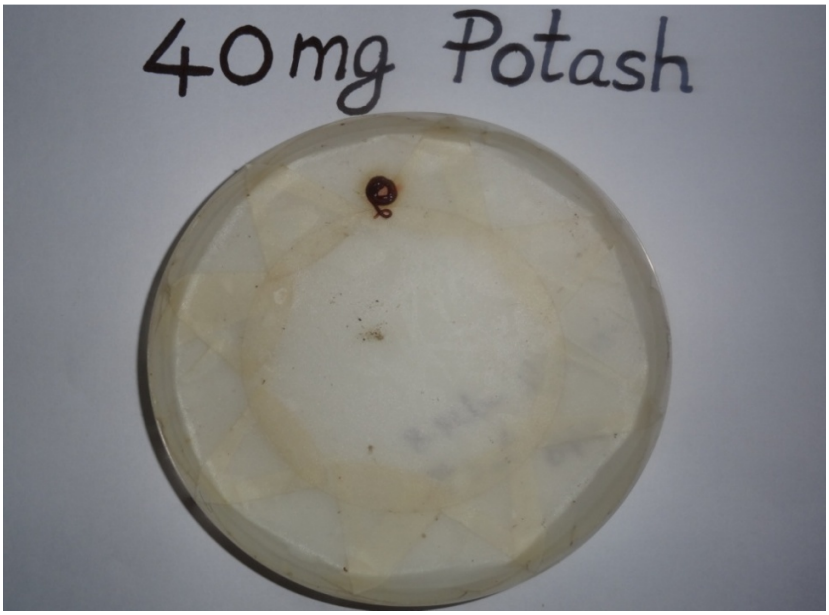

Figure 1: Mortality of *Eisenia foetida* to 40mg/10ml concentration of potash solution

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

With uniform area of contact exposure of potash to the earthworm in different concentrations showed various toxic effects in filter paper substrate medium. A geometric concentration series of test solution (10, 20, 40, 80 and 160mg/10ml) was prepared and tested, in which immediate mortality of earthworms were observed in 80 and 160mg concentrations. Only the 10 and 20 mg/10ml 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. 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

Potash that is applied for plant growth is proved to be toxic to earthworms at certain concentration, which infers the need of limited usage of potash. 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 datas gained from it can be used for extended study using artificial soil (as recommended by OECD) during range finding tests and for comparing the toxic potential.

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