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ALUMINUM EFFECTS IN ALLEATO 80 WG FUNGICIDE ON *EISENIA FOETIDA* SPECIES

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Abstract
The study highlights the influence of Alleato 80WG used in the laboratory chronic test, on an artificial soil according to OECD, on Eisenia foetida species, in terms of survival rate, biomass and content of bioaccumulated aluminum.50 mature individuals were used for each concentration in 5 repetitions (10 individuals / repetition). The toxic concentrations used were: LC_{50}= 500 mg·Kg^{-1}(V1); LC_{50}= 400 mg·Kg^{-1}(V2); LC_{50}= 300 mg·Kg^{-1}(V3); LC_{50}= 200 mg·Kg^{-1}(V4)/ dry soil, for 5 samples, the 5th (V5) being the control sample. The toxic concentrations in V1; V2 and V3, caused a significant decrease of the survival rate. The average values were 80%, as compared to V4 and control sample (V5). The correlations between Alleato 80 WG and the survival rate were negative ($p<0.0001$) while the increase of toxic concentration caused the decrease of the survival rate. After being subjected to toxic Alleato 80WG, biomass recorded for individual variants V1, V2, V3, and V4 does not differ significantly from version V5. The increase of toxic concentrations caused a significant increase of aluminum bioaccumulated by earthworms (R Square=0.304; p=0.004).

Keywords: *Alleato 80 WG, Eisenia foetida, survival, biomass, bioaccumulation.*

1. INTRODUCTION

OECD artificial soil is used as a standard in toxicity biological tests, but the possibility of extrapolation in natural soils is questionable. The response of earthworms to chemicals was much studied (Lukkari & Haimi, 2005, Garcia et al., 2008) suggesting that the tests on earthworms subject to different biocides may represent a great potential to obtain rapid and ecologically relevant information in assessing risk. The ecological characteristics (epigenous, anecic and endogeic species) of earthworms used in toxicity tests have often influenced the test result (Double et al., 1997 Lukkari & Haimi, 2005) but may also influence their sensitivity to contaminants in the soil.
Eisenia foetida - known as epigenous species is considered to be less sensitive to soil factors than other species of earthworms (Lukkari & Haimi, 2005 Owojori & Reinecke, 2009, Spurgeon et al., 2000). It is a standard species for ecotoxicity tests (Kobetičová et al., 2010), it is easily reproduced in laboratory conditions and has similar responses following exposure to soil contaminants, like other species living in agricultural soils such as *Lumbricus terrestris Aporrectodea caliginosa*, etc. This hypothesis has been reported in other research (Langdon et al., 2005), which determined the effect of lead on the survival of the species *Eisenia andrei*, *Lumbricus rubellus* and *Aporrectodea caliginosa* in a standard artificial soil.

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Bioaccumulation of pollutants in earthworms must also be taken into serious account for the environmental risk assessment, as this can lead to toxic effects in the food chains. Earthworms, as organisms that inhabit the soil, are known to be highly influenced by chemicals in the terrestrial environment. It was therefore considered to analyze the effect of xenobiotic substances, namely Alleato 80WG fungicide on the general parameters of earthworms as indicators of soil pollution.
The general objective of the study is to assess the influence of aluminum content in Alleato 80WG fungicide on *Eisenia foetida*, known as the standard species in toxicity tests for earthworms. The specific objectives have pursued: 1. analysis of survival rate (%) after applying toxic and correlations between survival rate and toxic concentrations; 2. analysis of the initial biomass (g) and biomass resulting (g) from intoxication; 3. analysis of aluminum bioaccumulation (ppm) in earthworms.

2. MATERIALS AND METHODS

Eisenia foetida individuals used in the experimental variants were mature and obtained from a farm in Romania practicing vermiculture.
Test underlayer. Preparation of OECD underlayer (OECD 207/1984, 222/2004) was conducted as follows: 10% peat moss; 20% kaolinite clay; 69% industrial quartz sand with a particle size from 0.05mm up to 0.2mm; 1% calcium carbonate ($CaCO_3$). All amounts were mixed, while plant debris, dirt and possible pebbles were removed. The water content of the underlayer was determined by bringing it to a constant weight while drying it in an oven at 105^0C for 8 hours. After determining humidity, the basic underlayer was filled with 1.5 liters of distilled water in order to bring it to the optimal humidity (approx. 25-42% dry weight of the basic underlayer). Calcium carbonate was added to bring the underlayer to a pH of 6.0.
Acclimatization
Samples were prepared for acclimatization before applying toxic. In this regard, the artificial underlayer was put in 800 g glass jars. The jars were weighed on the analytical balance before being filled with soil (1/3 of their capacity) after which they were weighed again. Five jars were filled with soil, each representing 5 samples, one sample for each concentration of toxic, the 5th being the control variant. There were 5 repetitions for each concentration. The earthworms were immersed in distilled water to remove excess soil on the body surface, then they were placed on filter paper to remove the water retained on the body surface; their individual biomass (g) was determined on the analytical balance, after which they were inserted in the samples. Ten specimens were inserted in each sample for each concentration. After all individuals have entered the soil, the samples were covered with previously drilled lids to allow air and to prevent water evaporation, then they were placed in the climate chamber for 7 days at a temperature 20^0 C and constant humidity.
After acclimatization, the samples were removed to check the soil humidity, the survival rate was analyzed, individuals were weighed and inserted again in the samples after applying the toxic on the soil surface in the form of a fine powder. The following toxic concentrations were used: V1= 500 $mg\cdot kg^{-1}$ dry soil, V2= 400 $mg.kg^{-1}$ dry soil, V3= 300 $mg\cdot kg^{-1}$ dry soil, V4=200 $mg\cdot kg^{-1}$ dry soil and V5 the control variant. Concentrations were determined according to specialized data starting from LC_{50} for lumbricidae. After applying the toxic, the samples were placed again in the climate chamber at a temperature of 20°C with constant humidity for 30 days. Earthworms were not fed during the test. After 30 days, the earthworms were removed from the climate chamber. Survival rate, individual biomass and bioaccumulation of aluminum were analyzed.
Alleato 80WG. In accordance with EU Regulation no.1907 / 2006, amended by EU Regulation no. 453/2010 and Regulation no.1272 / 2008, Alleato 80WG is a phosphonate compound with structure

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and action different from those of most other organophosphorus compounds used as pesticides. This fungicide is used on more than 100 crops. Alleato 80 WG contains 80 % Al fosetyl active substance.

Bioaccumulation. After completing the test it was determined aluminum bioaccumulation by atomic emission spectrometry with inductively coupled plasma (ICP-AES). Varian Liberty 110 spectrometer was used for the quantitative determination of aluminum. The instrument has a radio frequency generator of 40.68 MHz and Czerny-Turner monochromator of 0.75m. The instrument operating parameters were: plasma flow 12L / min, V-Groove nebulizer, pump rotations (15 rpm), 10 seconds integration time and automatic background. The wavelength at which aluminum was determined: Al-396.152nm. The reagents used for the mineralization of the samples were nitric acid (67% -75 ml), hydrogen peroxide (15ml) from Merck and distilled water to bring the 10 ml flasks to the sign, after mineralization of samples on the sand bath. Calibration of the spectrometer used five reference solutions of various concentrations obtained by the dilution of a multi-element standard solution (multi-element ICP-AES Merck, IV solution) with a concentration of 1000mg / l.

Statistical analysis. The statistical interpretation of the results was performed using SPSS 16 For Windows. The parameters analyzed applied Duncan test of variance for the significance threshold $p<0.05$; the trend line was drawn to calculate the coefficient of determination (R Square).

3. RESULTS AND DISCUSSIONS

Aluminum is a non-essential element for most animals on earth. Normally, the solubility of aluminum is low in neutral soil. Human actions increase the amount of this element in the soil through activities such as the use of fertilizers, poultry manure or under the action of rain which releases aluminum ions in the soil, thus affecting organisms such as earthworms, due to its toxicity. The present study has demonstrated that aluminum content in Alleato 80WG may affect survival and growth of earthworms and can be bioaccumulated by these bodies.

Figure 1 shows the values of survival rate in *Eisenia foetida* individuals in the experimental variants subject to toxic. The survival rate for control variant (V5) was 100%. The lowest concentration of Alleato 80 WG corresponding to V4 (200mg·kg^{-1}) did not produce changes in the survival rate (100%). The toxic concentration of 300 mg·kg^{-1}(V4) resulted in a significant decrease in the survival rate, with average values of 80%. Concentrations of 400mg·kg^{-1}(V2) and 500 mg·kg^{-1}(V1) have also determined significantly lower values of the survival rate compared with V4 and V5. There were no significant differences ($p<0.05$) between V2 and V3. However, the decrease of survival rate in V1 did not differ significantly from V2 ($p>0.05$).

Similar studies made by Annapoorani (2014) on *Eudrilus eugeniae* species, subject to treatment with aluminum in concentrations between 500 ppm-3000ppm, have shown that in concentrations higher than 2000ppm, individuals of this species developed general morphological abnormalities, from the body swelling in the clitellum to reducing the number of species individuals. Similar pathological symptoms resulting in reduced populations of earthworms in the soil have also been reported in other species of earthworms subject to various toxic substances (Ramalingam & Thilagar, 2000, Vijver et al., 2004).

The coefficient of determination between Alleato 80 WG concentration and survival of individuals in artificial soil has revealed a significant negative correlation ($p <0.0001$), meaning that an increase in the concentration of toxic resulted in a decrease of the survival rate (Figure 2). Other similar studies (Venkateswara et al., 2003) have shown that aluminum was more toxic for a pH of 3.4 and LC_{50} = 589 mg Al kg^{-1} dry soil.

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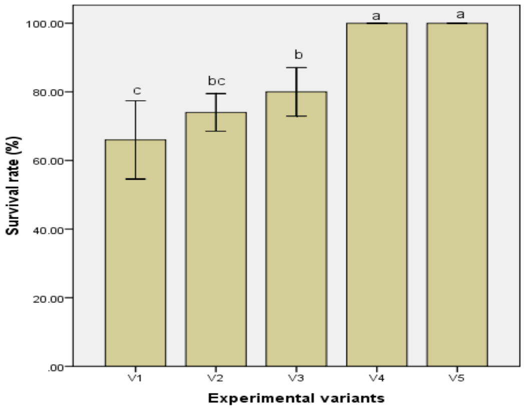

Figure 1. Survival rate (%) of Eisenia foetida individuals subject to Alleato 80WG toxic recorded in the five experimental variants V1 (500 mg·Kg^{-1}); V2 (400 mg·Kg^{-1}); V3 (300 mg·Kg^{1}); V4 (200 mg·Kg^{-1}); V5 (control variant)

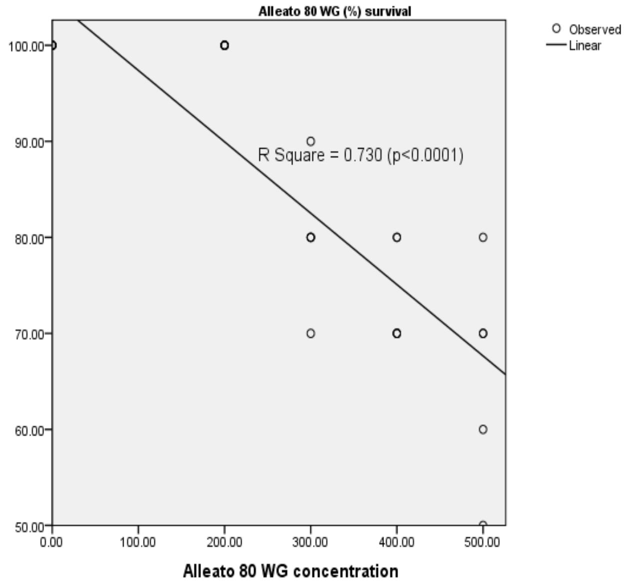

Figure 2. Correlation between survival rate (%) of Eisenia foetida individuals, represented by the trend line (R^2=0.730; p<0.0001), in the five experimental variants subject to toxic action (Alleato 80 WG): V1 (500 mg·Kg^{-1}); V2 (400 mg·Kg^{-1}); V3 (300 mg·Kg^{-1});V4 (200 mg·Kg^{-1}); V5 (control variant)

For the five experimental variants, individual biomass values before applying Alleato 80WG ranged between 2.713g and 3.371g with no significant differences between them (p> 0.05) (Figure 3).
After poisoning with Alleato 80WG (Figure 4), individual biomass for variants V1, V2, V3, and V4 did not differ significantly from the control variant V5. A decrease in biomass was however recorded in all experimental variants after applying the toxic (Figure 2).

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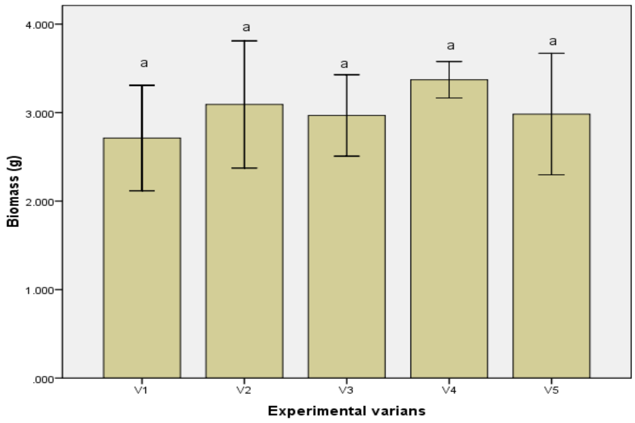

Figure 3. Biomass (g) of Eisenia foetida individuals in the five experimental variants before applying the toxic (Alleato 80WG)

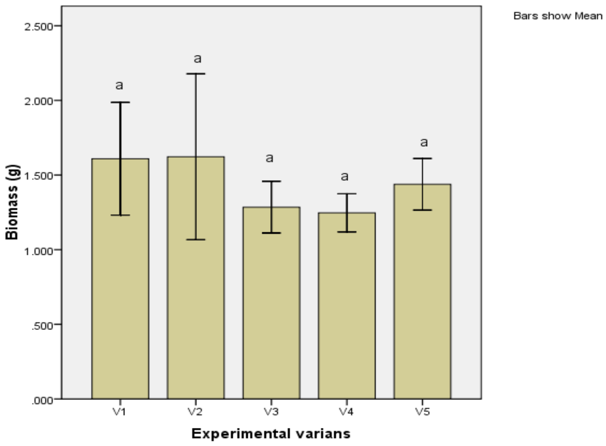

Figure 4. Biomass (g) of Eisenia foetida individuals in the five experimental variants V1 (500 mg·Kg^{-1}); V2 (400 mg·Kg^{-1}); V3 (300 mg·Kg^{-1}); V4 (200 mg·Kg^{-1}); V5 (control variant) after applying the toxic

Weight loss in individuals subject to Alleato 80WG toxic may be due to the fact that earthworms were not given any food during the experimental period (30 days). Lack of food, toxic action, and habitat change can lead to physiological stress followed by weight loss or population decrease. Relatively recent results have shown that growth, reproduction and enzymatic activity of earthworms were negatively affected with increasing Al concentration in soil (Owojori & Reinecke, 2009). The correlation between Alleato 80 WG concentration and Al bioaccumulated by earthworms is shown in Figure 5. It was stated that increasing concentration of toxic resulted in a significant increase of bioaccumulated aluminum (R Square = 0.304; p = 0.004).
As regards the bioaccumulation capacity of earthworms, some studies (van Gestel & Hoogerwerf, 2001, Vijver, 2004) have shown that species of earthworms can accumulate different amounts of the same metal, depending on the species, species behaviour, and their habitat. Other studies (Santorufo, 2012) show that bioaccumulation of metals by earthworms is associated with metal concentrations in soil.

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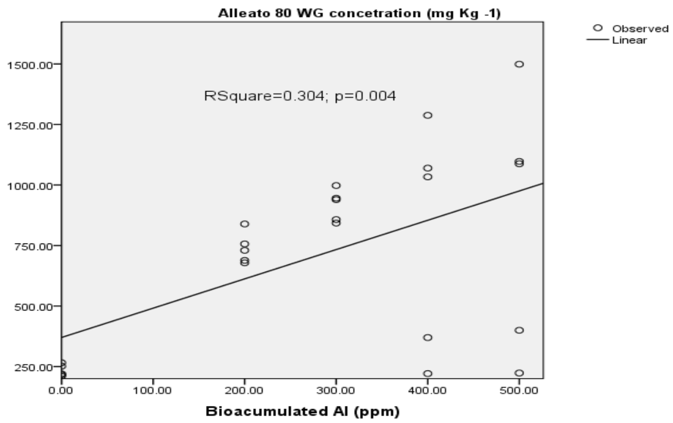

Figure 5. The trend line of aluminum bioaccumulated by earthworms depending on Alleato 80 WG concentration (V1 =500 mg·Kg^{-1}; V2 = 400 mg·Kg^{-1}; V3 = 300 mg·Kg^{-1}; V4= 200 mg·Kg^{-1}; V5 = control variant)

4. CONCLUSIONS

The study results have demonstrated that a higher concentration of Alleato 80WG in the soil (LC_{50}= 500 mg·Kg^{-1}) may affect survival, growth and development of lumbricidae especially if it is associated with lack of food. Alleato 80WG concentrations less than 300 mg·Kg^{-1} do not change the survival rate and individual biomass. Bioaccumulation may be associated with the amount of toxic on / in the soil. The results also suggest that under natural conditions, where there is a high degree of pollution with metals or other toxic substances, lumbricidae populations may undergo changes in their structure and functions, thus highlighting the importance of this type of study in environmental risk assessment.

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