ECOTOXICOLOGICAL ASSESSMENT OF THE EFFECT OF SOME NEW ORGANIC-MINERAL FERTILIZERS ON EISENIA FOETIDA EARTHWORMS

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Abstract

Ecotoxicity tests provide a direct measure of the bioavailability of toxicants and help to establish linkages between site contamination and adverse ecological effects. There are more current laboratory test methods outlined, but it is very important the major advantages/disadvantages of each of them. The present paper uses as research methodology the OECD Guideline for testing of chemicals no. 207 which meets the most criteria expected for ecotoxicological testing. Earthworms *Eisenia foetida* (Savigny, 1826) had been exposed for 14 days to a geometrical series range of concentrations of the test substances (organic-mineral fertilizers L-200-Hum and SH-120). The performed study showed that the fertilizer L-200-Hum determined lower earthworm mortality comparing to SH-120 for all tested concentration levels and the fertilizer L-200-Hum positively influenced the earthworm rate of biomass accumulation. DL 50 it was established to be the concentration 4 g for the SH-120 fertilizer at the end of the test (14 days).
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Key words: earthworms, ecotoxicology test, organic-mineral fertilizers, mortality, reproduction

The concern regarding the effect of the toxic substances on the environment is growing rapidly in whole world and the efforts to develop more reliable methods for evaluating side-effects on non-target organisms are being increased. In Europe, there were elaborated several test methods which use a large variety of organisms, from aquatic or terrestrial environments, like algae, superior plants, fish and in the last time earthworms which became the most preferred organisms for such tests.

Toxicity tests provide a direct measure of the bioavailability of toxicants, and, when combined with chemical analyses and field surveys, can help establish linkages between site contamination and adverse ecological effects. Toxicity tests evaluate acute, subchronic, and chronic exposures and measure biological endpoints such as mortality, reproductive performance, growth, and behavioural changes (Lee B.T. et al., 2007). Also by using toxicity tests, the relative toxicity of a mixture of chemicals can be assessed by taking into account synergistic or antagonistic interactions among chemicals.

For the general screening of most chemicals, laboratory testing appears to be the most realistic option (Christensen O.M., Mather J.G., 1994).

Earthworms are considered efficient test organisms for assessing ecotoxicological effects of chemicals, in order to elaborate recommendations and risk assessment schemes earthworms not only by considerations of reproducibility and practicability but also because of ecotoxicological relevance (Christensen O.M., Mather J.G., 1994).

In order to test the ecotoxicity of some substances on earthworms, an essential importance is attributed to the test soil, because earthworms are organisms whose life environment is represented by soil. The differences appeared in the soil composition influence the bioavailability and the toxicity of the pollutants (Stephenson G.L. et al., 1998).

Earthworms are often used as test organisms to assess soil processes because of they accomplish important function, e.g. as decomposer (Edwards C.A., Bohlen P.J., 1996), their high biomass in soil, between 10-200 g fresh weight m^{-2} (Hund-Rinke K., Wiechering H., 2001) and their sensitive reactions towards environmental influences.

MATERIAL AND METHOD

There are more current laboratory test methods outlined, but it is very important the major advantages/disadvantages of each of them. OECD (Organisation for Economic and Cooperation Development) founded in 1961 in Paris, France, provided the method of artificial soil test (Christensen O.M., Mather J.G., 1994) which meets the most criteria expected for ecotoxicological testing known as OECD Guideline for testing of chemicals 207 adopted in April 1981.

The equipment and the materials are those stipulated by OECD Guidelines 207/1984: earthworms from the species of *Eisenia foetida*

(Savigny, 1826) (belonging to Phyllum *Annelida*, Class *Oligochaeta*, Family *Lumbricidae*) and artificial soil consisting of: 10 per cent sphagnum peat (as close to pH 5.5 to 6.0 as possible, no visible plant remains, finely ground, dried to measured moisture content), 20 per cent kaolin clay (kaolinite content preferably above 30 per cent), 70 per cent industrial sand (fine sand should be dominant with more than 50 per cent of the particles between 50 and 200 microns). The pH must be adjusted to 6.0 ± 0.5 by addition of calcium carbonate.

The characteristics that make the species of *Eisenia foetida* (Savigny, 1826) suitable for such ecotoxicological tests are: its susceptibility to chemicals because it is a true soil-inhabiting species, it has a short life cycle, hatching from cocoons in 3 to 4 weeks, and reaching maturity in seven to eight weeks at 20°C. It is very prolific, each worm producing two to five cocoons per week from each of which emerge several worms. It is available commercially and can be bred readily in a wide range of organic waste materials.

There were tested two types of organic-mineral fertilizers which had been coated with potassium humic acids, noted as L-200-Hum and SH-120. The chemical composition and the chemical and physical properties of these fertilizers are listed in the *table 1*.

Table 1

Chemical composition and chemical and physical properties of the organic-mineral fertilizers tested on earthworms

No. crt.	Chemical composition	Organic-mineral fertilizers	
		L-200-Hum	SH-120
Chemical compositions			
1	Humic acids	29.9%	26.4%
2	Nitrogen (Nt)	23.49%	10.47%
3	Phosphorous (P_2O_5)	-	16.50%
4	Potassium (K_2O)	0.255%	0.307%
Chemical and physical properties			
5	Cationic exchange capacity	96.3 me/100g	100.3 me/100g
6	pH in aqueous solution 1:2.5	7.3	7.0
7	Apparent density	0.823 g/cm^3	0.852g/cm^3
8	Granulation (1-5 mm)	89.9%	93.5%

RESULTS AND DISCUSSIONS

The earthworms *Eisenia foetida* had been exposed for 14 days to a geometrical series range of concentrations of the test substances (the fertilizers) as following: 1, 2, 4, 8, 16 g active substance/kg dry weight artificial soil). There were realized 4 replicates according to OECD methodology. All earthworms were adults, at least 2 months old and with weight ranging between 400 – 500 mg. At the test start and at the end there was established their biomass, and before starting the testing the worms were washed with tap water and incubated on wet paper for 24h to empty their guts. The monitoring of worms and data analyzing had been realized in the days 7 and 14 in order to establish the earthworm mortality, the reproduction capacity (number of cocoons) and the weight changes. In the *tables 2* and *3* there are presented the data regarding the mortality and the reproduction activity of earthworms under exposure to the fertilizer L-200-Hum.

Table 2

Earthworm mortality after exposure to the fertilizer L-200-Hum

Earthworms remained alive (mean values)	Fertilizer concentration (g/kg dry weight)					Control
	1	2	4	8	16	
After 7 days	10	9	8	8	7	10
After 14 days	10	9	8	7	7	10

Table 3

Earthworm reproduction index after exposure to the fertilizer L-200-Hum

Number of cocoons (mean values)	Fertilizer concentration (g/kg dry weight)					Control
	1	2	4	8	16	
After 7 days	38	33	26	28	22	47
After 14 days	42	36	27	25	22	41

In the *tables 4* and *5* there are shown the data regarding the mortality and the reproduction activity of earthworms under exposure to the fertilizer SH-120.

In the study there was also monitored the changes appeared in the earthworm weight after one week (7 days) and then after another week (14 days) of test. The results for the two tested fertilizer are shown in the *tables 6 and 7*.

Comparing to the control variant, it can be observed that there is no mortality under L-200-Hum exposure for 1 g concentration, after 14 day of testing, after what the mortality start to increase as concentration level is higher (*fig. 1, 2*).

Instead, the reproduction rate of earthworms decreases under L-200-Hum action comparing to the control pots in the same time with concentration increasing level (*fig. 3, 4*).

Regarding the fertilizer SH-120 it was observed that it manifests a negative influence on mortality and reproduction rate of earthworms, being found 50% mortality for 4 g concentration and 100% mortality for 8 and 16 g concentration (*fig. 1,2,3,4*).

As well, the cocoon number drastically decreases after 14 days of SH-120 exposure to 1/2 comparing to L-200-Hum and to 1/3 related to the control pots for 2 g concentration and even to zero values (because of 100% mortality for 8 and 16 g concentration).

Table 4

Earthworm mortality after exposure to the fertilizer SH-120

Earthworms remained alive (mean values)	Fertilizer concentration (g/kg dry weight)					Control
	1	2	4	8	16	
After 7 days	10	9	7	5	4	10
After 14 days	9	7	5	0	0	10

Table 5

Earthworm reproduction index after exposure to the fertilizer SH-120

Number of cocoons (mean values)	Fertilizer concentration (g/kg dry weight)					Control
	1	2	4	8	16	
After 7 days	36	31	22	17	8	47
After 14 days	23	18	10	0	0	41

Table 6

Earthworm weight after exposure to the fertilizer L-200-Hum

Earthworms weight (mg)	Fertilizer concentration (g/kg dry weight)					Control
	1	2	4	8	16	
After 7 days	471	439	394	353	311	457
After 14 days	462	427	379	347	324	431

Table 7

Earthworm weight after exposure to the fertilizer SH-120

Earthworms weight (mg)	Fertilizer concentration (g/kg dry weight)					Control
	1	2	4	8	16	
After 7 days	467	426	327	238	178	457
After 14 days	449	294	216	0	0	431

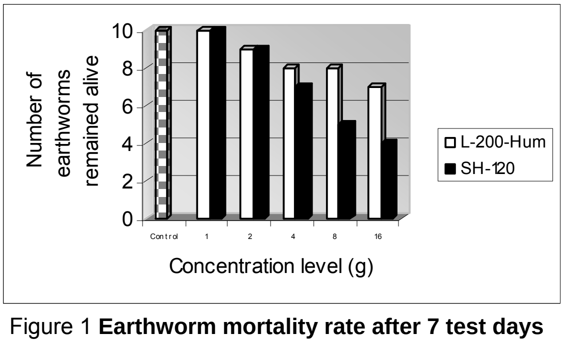

Figure 1 **Earthworm mortality rate after 7 test days**

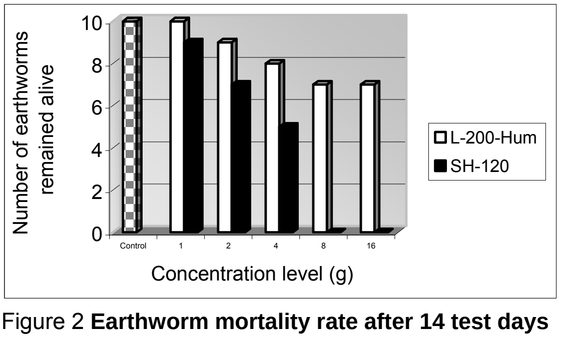

Figure 2 **Earthworm mortality rate after 14 test days**

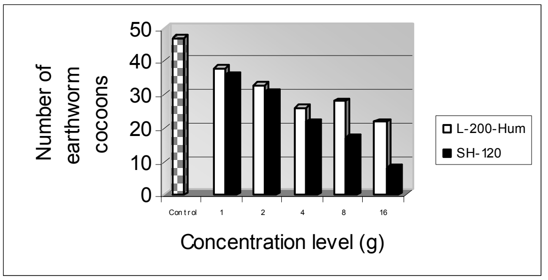

Figure 3 **Earthworm reproduction rate after 7 test days**

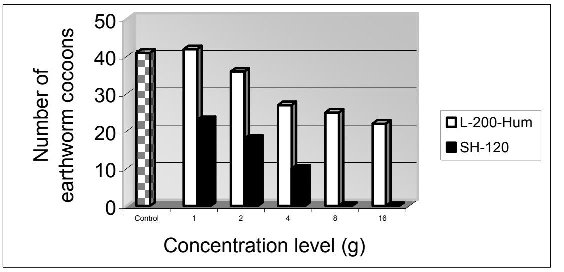

Figure 4 **Earthworm reproduction rate after 14 test days**

Referring to the earthworm weight evolution (biomass) it was noticed that it increased for 1 g concentration under L-200-Hum exposure comparing to the control pots. This fact is explained by the high content in nitrogen of the fertilizer L-200-Hum which is an essential element for earthworm surviving, because it means trophic resource and it can be found in large amount in the chemical composition of the mucus that is excreted by these organisms. The fertilizer SH-120 exerts in this case too a negative influence on earthworms, their weight decreasing with 50% for 4 g concentration related to the control pots (*fig. 5; 6*).

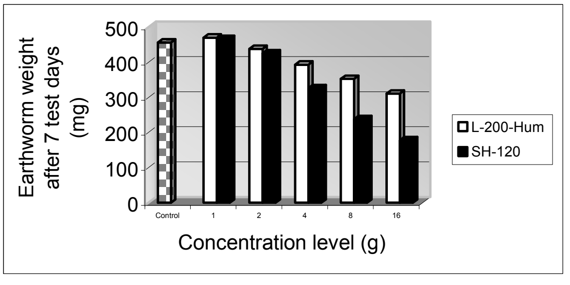

Figure 5 **Earthworm weight after 7 test days**

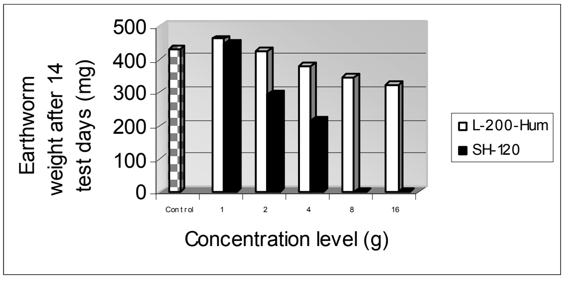

Figure 6 **Earthworm weight after 14 test days**

CONCLUSIONS

The performed study shows the following conclusions:

The fertilizer L-200-Hum determined lower earthworm mortality comparing to SH-120 for all tested concentration levels.

The fertilizer L-200-Hum positively influenced the earthworm rate of biomass accumulation, being found its increasing for 1 g concentration for the entire test period comparing to the control variants.

The fertilizer SH-120 determined a 50% mortality of earthworms for 4 g concentration and 100% mortality for 8 and 16 g concentration, which requires a precaution attitude regarding its use in agriculture, and it is recommended the attentive establishment of the amounts applied into the field to not affect the earthworm populations.

DL 50 it was established to be the concentration 4 g for the SH-120 fertilizer at the end of the test (14 days).

The highest concentrations without causing mortality were: L-200-Hum, 1 g, 7 days; L-200-Hum, 1 g, 14 days; SH-120, 1 g, 7 days.

The lowest concentration causing 100% mortality was SH-120, 8 g, 14 days.

Due to the limitative factors which appear in the laboratory tests it is recommended their completion with field tests in order to obtain more realistic results.

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