

## Earthworms as biomarkers for detecting soil pollution around swine farms in Timiș County

### Râmele ca bioindicatori în detectarea poluării solului din jurul fermelor de suine din județul Timiș

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**Key words:** earthworms, pollution, mortality, soil sample  
**Cuvinte cheie:** râme, poluare, mortalitate, probă de sol

#### Abstract

By using several samples collected from different farms distances it can be determined the degree of farms pollution and their effects on ecosystems. In order to avoid any error the soil samples were tested in comparison with a control soil sample, used as a reference, considered animal waste pollution free. The registration of survival rate for each testing recipient and for each soil along five repeats was a critical control point of testing. The 11 identified soil types were tested in comparison with a clean reference soil, by using 275 test earthworms and 55 control earthworms. According to normality Kolmogorov- Smirnov test the mortality was analyzed and results were significant ( $p=0.046$ ).

#### Rezumat

Prin utilizarea mai multor probe prelevate de la distanțe diferite de ferme se poate determina gradul de poluare a fermelor și efectele asupra ecosistemelor. Pentru a evita orice eroare probele prelevate din locațiile alese s-au testat în paralel cu cele de sol de referință, lipsit de poluare cu dejectii. Înregistrarea ratei supraviețuirii în fiecare recipient de testare și pe fiecare tip de sol de-a lungul celor cinci repetări, a reprezentat un punct critic biologic al testărilor. Cele 11 tipuri de sol identificate s-au testat în comparație cu un sol curat, de referință, prin utilizarea a 275 de râme plus 55 râme de control. Conform testului de normalitate Kolmogorov- Smirnov mortalitatea a fost analizată iar rezultatele au fost semnificative ( $p=0,046$ ).

#### Introduction

Earthworms represent 94 % of biomass fauna in soil (Fig.1). Each soil and climate area is characterized by a certain structure of earthworms species and mesofauna.

The number of individuals in certain areas varies, from a few thousand to a few million per hectare.

Earthworms transport soil from upper layers, humus, to lower layers and with this, is transported an impressive number of microorganisms or spores and sclerotia.

The passage of organic and inorganic substances through earthworms digestive tract and also other mesofauna components

facilitates the formation of clay – humic complexes leading to an increase of substances resistance to the decomposing action of microorganisms [1].

Many organisms in soil have an important role in stimulating microbial activity and also in their transport in soil [9].

The spores of fungi (*Trichoderma*, *Penicillium*, *Fusarium*, *Cephalosporium*) were found in earthworms digestive tract (Figures 2-4), and also spores from genre *Torula*, *Rhizopus*, *Mucor* and *Cladosporium* [5].

Their voluminous coprolites represent a good environment for microbiological activity and especially in leading to elaborate humic materials.

This in a physicochemical relation with clay is forming organic complexes.

Next to the ingestion activity of soil material, earthworms also have a role in soil transport between layers (from surface, between layers) (Table 1).

Transported material from surface layer can contain humic substances and microorganisms or their resistant forms. Soil mega fauna activity is also important in soil surfacing,

opening galleries and thereby influencing soil physico-chemical properties.

Their abandoned galleries are filled with soil from humus layers and represents a preferred circulation area for soil organisms, soil materials from this galleries has a high quantity of organic matter, is good structured by its biological activity, is aerated thus being populated with organisms and microorganisms [8].

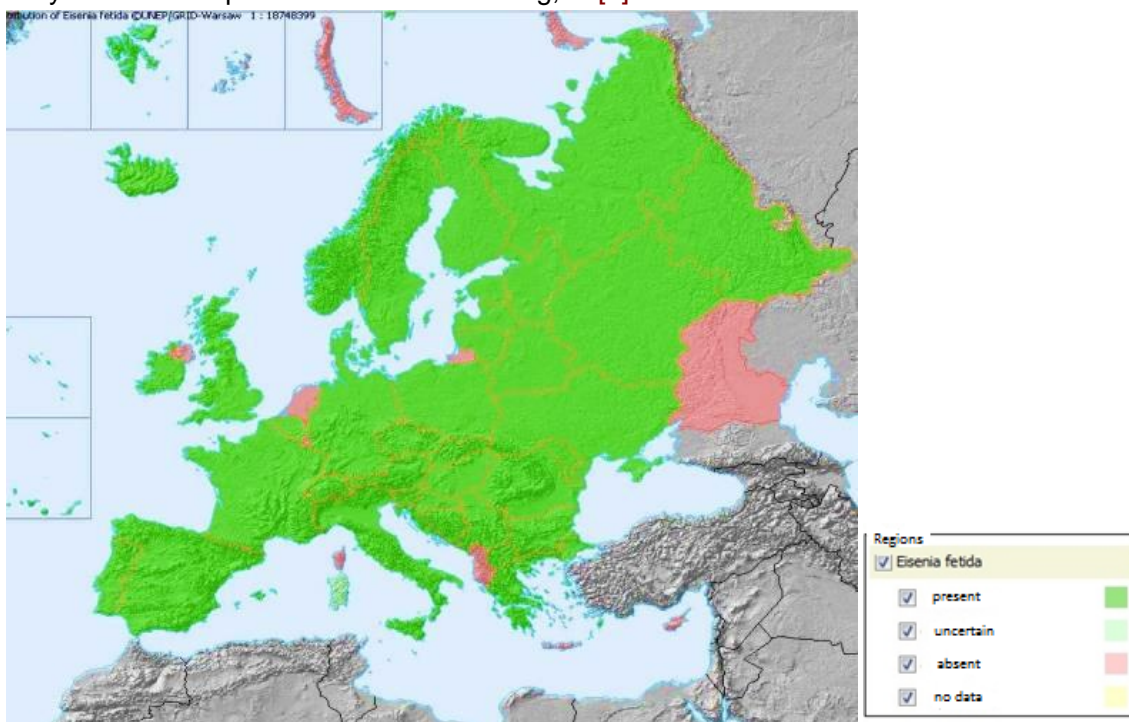


Fig.1. Eisenia fetida distribution in Europe [11]

Table 1.

Eisenia fetida population dynamics according to season [6]

Crt no.	Specification	Season			
		Autumn	Winter	Spring	Summer
1	Total number of individuals (per m <sup>2</sup> )	2450	3965	8460	4888
2	Number of adult individuals (per m <sup>2</sup> )	1680	1760	3810	432
3	Number of preclitellated individuals (per m <sup>2</sup> )	540	540	790	504
4	Number of juvenile individuals (per m <sup>2</sup> )	150	1250	2280	3488
5	Number of larvae (per m <sup>2</sup> )	unmonitored	410	1650	464
6	Number of cocoons (per m <sup>2</sup> )	unmonitored	850	3970	184
7	Total biomass of individuals (g/m <sup>2</sup> )	775	980	1570	527
8	Biomass of adult individuals (g/individual)	0.33	0.40	0.32	0.29
9	Biomass of preclitellated individuals (g/individual)	0.28	0.25	0.21	0.22
10	Biomass of juvenile individuals (g/individual)	0.15	0.10	0.09	0.08
11	Coccons weight (mg/cocoon)	unmonitored	12.03	22.57	19.12
12	Mating activity (%)	10.5	3.8	1.1	4.9

## Earthworm taxonomy classification

Eartworms were categorized in [11]:

Kingdom *Animalia*  
 Subkingdom *Eumetazoa*  
 Phylum *Annelida*  
 Class *Oligochaeta*  
 Subclass *Diplotesticulata*  
 Order *Opisthopora*  
 Suborder *Lumbricina*  
 Family *Lumbricidae*  
 Genre *Eisenia*  
 Species *fetida*

## Earthworm anatomy aspects

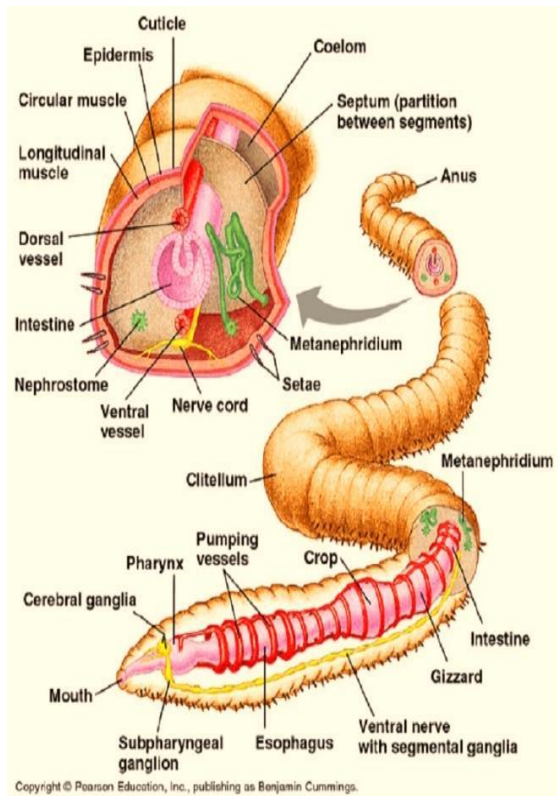


Fig. 2. *Eisenia fetida* earthworm anatomy [12]



Fig.3 - 4. Anatomical structures in adult earthworms (original)

## Aim of research

Evaluation of earthworm mortality in acute tests of soils from swine farms vicinity in Timiș County.

By using several collected samples from different distances from farms it can be determined the degree of pollution in farms and their effects on ecosystems.

In order to avoid any error the collected samples from selected locations were tested in comparison with reference soil, free of pollution with animal waste.

## Research objectives

Monitoring earthworm mortality with acute ecotoxicity tests on soil samples with a potential risk of pollution collected from areas with animal waste risk of contamination.

Registration of survival rate in each test container and for each soil type used for five repeats, thus being a critical control point in biological testing.

Highlighting the number of living earthworms in reference soil and comparing results with the ones for the tested soils.

The testing method was a toxicity test for contaminated soils by using earthworms (*Eisenia andrei*, *Eisenia fetida* or *Lumbricus terrestris*) EPS 1/RM/43- June 2004.

## Materials and method

It was used an acute test for evaluating mortality: Toxicity test for evaluating contaminated soils by using earthworms (*Eisenia andrei*, *Eisenia fetida* or *Lumbricus terrestris*) EPS 1/RM/43- June 2004, Canada [13], a static test, accomplished without soil renewal during 14 days of testing.

According to methodology there were used five testing organisms for each soil types and for each soil needed five repeats in comparison with a reference soil sample with five earthworms inside for each test.

The 11 identified soil types were tested in comparison with a clean soil, a reference soil, by using 275 test earthworms and 55 control earthworms.

Test organisms, *Eisenia fetida* had a body weight between 250-600 mg/ individual and there were used 5 earthworms/ testing chamber, without being fed during the entire testing.

In testing were used 500 ml capacity glass containers, covered with transparent foils with holes for ventilation attached with rubber bands to facilitate observation without damaging the foils.

There were used soil samples collected from several locations in quantity of 350 ml and a reference soil with several parameters:

- Soil relative humidity of aprox. 70% was maintained by soil moistening at different time periods in correlation with tested soil water capacity.
- Temperature was kept in limits of 20 ± 2°C for the entire tests.
- Photoperiod was of 16 / 8 (hours / day light-dark).

**Ecotoxicity tests for soil samples in Pădureni area**

Soil samples were collected from the vicinity of swine farms in Pădureni, at a distance of 100-300m for sample 1a and 500-1000 m for sample 1b.

Before testing earthworms were weighted individually and registered in tables (Table 2, Graph 1).

**Table 2.**

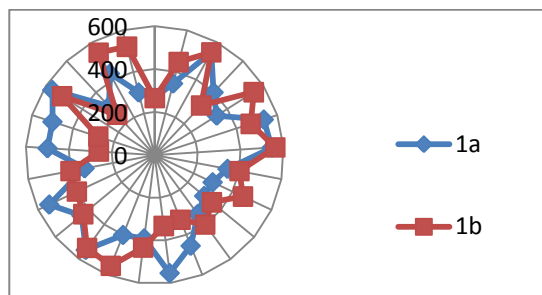
**Earthworm individual weighting for test**

Pădureni Test	Earthworm weight (mg)	
B.1	Test1	Test2
	265.4	264.4
	343.5	446.4
	545.1	545.2
	400.1	315.6
B.2	342.0	545.6
	340.0	400.1
	454.6	324.1
	554.1	333.4
	391.1	434.3
B.3	400.5	555.4
	533.6	468.9
	545.7	562.2
	344.9	400.9
	298.0	454.1
B.4	299.0	345.6
	546.2	535.0
	442.3	432.0
	542.2	400.3
	332.2	399.9
B.5	499.9	261.0
	500.0	276.8
	564.3	511.1
	311.1	256.7
	432.2	543.1
	300.1	520.2

Where: B1, B2, B3, B4, B5 –testing containers

**Graph 1.**

Graphical representation of earthworm weight used in tests on soils from Pădureni



**Fig. 5.** Soil sample appearance in testing containers (original)



**Fig. 6.** Earthworm appearance at the end of testing (original)

**Table 3.**

Earthworm survival rate in tested soils from Pădureni

Pădureni Soil Sample 1a					
Container	1	2	3	4	5
Number of living earthworms /test	5	4	5	5	5
Pădureni Soil Sample 1b					
Container	1	2	3	4	5
Number of living earthworms /test	4	5	4	5	5
C Sample					
Number of living earthworms /test	Test1		Test2		
	5		5		

Samples were examined on day 0 at about an hour from earthworm insertion in order to observe their presence or absence on soil surface.

At seven days from the beginning of test mortality is evaluated by touching the earthworm leading end and an absence of a reaction proves an organism lethality.

A final evaluation is registered at 14 days from the beginning of test with mortality monitorization and any developmental changes (Figures 5 - 6).

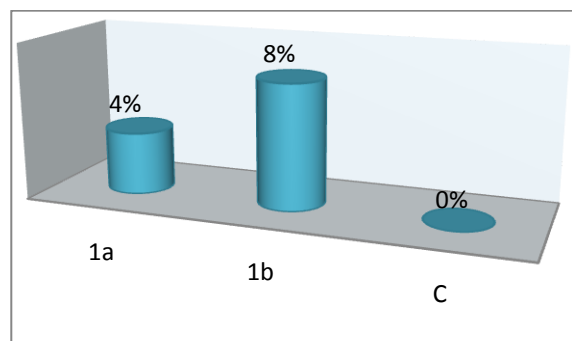
In tests on soils from Pădureni weren't registered developmental changes and also zero earthworm mortality at seven days of testing.

After testing sample 1a collected from a distance of 100-300 m from swine farm in Pădureni was registered an earthworm survival rate of 96% and for sample 1b, taken from a distance of 500-1000 m from farm was registered an earthworm survival rate of 92% (Table 3).

In testing sample C, considered reference sample, was not registered any earthworm mortality during testing in comparison with samples from Pădureni.

**Graph 2.**

Graphical representation of earthworm mortality at the end of two tests



Mortality for sample 1a was 4%, for sample a 1b of 8% and for sample C it wasn't revealed any dead organism (Graph 2.).

### Ecotoxicity tests for soil samples in Voiteni

Soil samples were collected from the vicinity of swine farms in Voiteni, at a distance of 100-300m for sample 2a and 500-1000 m for sample 2b.

Before testing earthworms were weighted individually and registered in tables (Table 4, graph 3).

In soil testing from Voiteni weren't any behavioural changes and any mortality at seven days from test beginning.

After testing sample 2a collected from a distance of 100-300 m from swine farm in Voiteni was registered an earthworm survival rate of 92% and for sample 2b, taken from a distance of 500-1000 m from farm was registered an earthworm survival rate of 96% (Table 5).

In testing sample C, considered reference sample, it wasn't registered any mortality during testing in comparison with the ones from Voiteni.

Mortality for sample 2a was 8%, for sample 2b of 4% and for sample C it wasn't revealed any dead organism (Graph 4.).

**Table 4.**

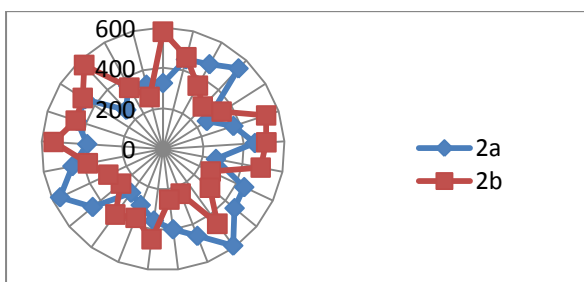
Earthworm individual weighting for test

Voiteni Test	Earthworm weight (mg)	
	Test1	Test2
<b>B.1</b>	Test1	Test2
	324.7	578.7
	455.6	466.7
	476.6	356.7
	544.6	287.6
256.7	343.4	
<b>B.2</b>	589.9	456.7
	461.7	237.8
	400.2	252.0
	356.9	450.0
	298.0	365.7
<b>B.3</b>	365.6	535.1
	454.5	511.1
	266.3	490.9
	443.1	260.7
	458.8	300.9
<b>B.4</b>	267.8	400.3
	450.0	269.1
	561.1	298.0
	454.6	377.7
	374.4	541.0
<b>B.5</b>	446.5	453.5
	456.4	468.8
	264.4	568.0
	355.4	344.6
	326.7	264.3

Where: B1, B2, B3, B4, B5 –testing containers

**Graph 3.**

Graphical representation of earthworm weight used in tests on soils from Voiteni



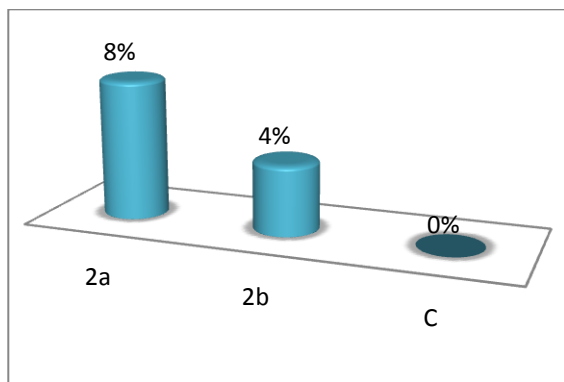
**Table 5.**

Earthworm survival rate in soil tests from Voiteni

Voiteni Soil Sample 2a					
Container	1	2	3	4	5
Number of living earthworms /test	3	5	5	5	5
Voiteni Soil Sample 2b					
Container	1	2	3	4	5
Number of living earthworms /test	5	5	5	5	4
C Sample					
Number of living earthworms /test	Test1	Test2			
	5	5			

**Graph 4.**

Graphical representation of earthworm mortality at the end of the two tests



**Ecotoxicity tests of soil samples from Ciacova**

Soil samples were collected from the vicinity of swine farms in Ciacova, at a distance of 100-300m for sample 3a and 500-1000 m for sample 3b.

Before testing earthworms were weighted individually and registered in tables (Table 6, graph 5).

**Table 6.**

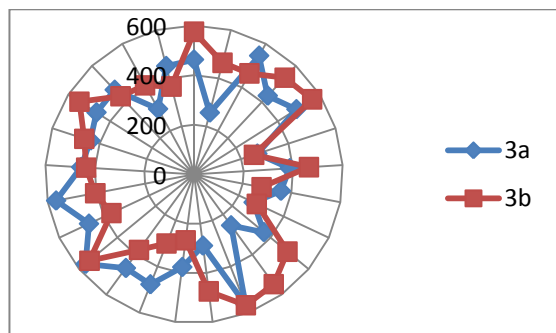
Earthworm individual weighting for test

Ciacova Test	Earthworm weight (mg)	
	Test1	Test2
<b>B.1</b>	462.1	574.6
	256.3	464.3
	545.2	464.4
	434.4	534.5
	488.9	564.5
<b>B.2</b>	254.5	546.7
	564.6	567.6
	289.9	475.3
	376.8	267.6
	476.8	300.1
<b>B.3</b>	268.5	254.4
	386.8	464.6
	356.6	277.5
	265.5	280.0
	365.4	488.7
<b>B.4</b>	466.9	376.7
	566.7	546.5
	467.8	367.5
	565.6	405.0
	455.4	435.7
<b>B.5</b>	438.7	464.7
	465.4	546.7
	466.8	432.2
	300.6	409.0
	450.1	365.4

Where: B1, B2, B3, B4, B5 –testing containers

**Graph 5.**

Graphical representation of earthworm weight used in tests on soils from Ciacova



**Table 7.**

Earthworm survival rate in testing soil from Ciacova

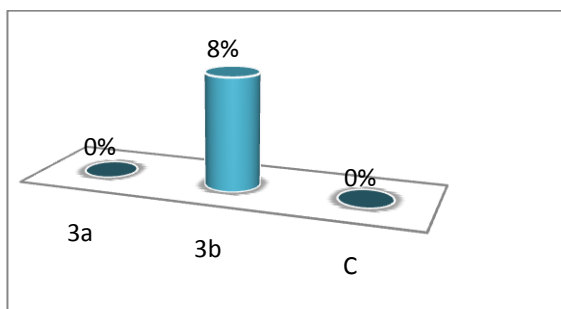
Ciacova Soil Sample 3a					
Container	1	2	3	4	5
Number of living earthworms /test	5	5	5	5	5
Ciacova Soil Sample 3b					
Container	1	2	3	4	5
Number of living earthworms /test	5	4	5	5	4
C Sample					
Number of living earthworms /test	<b>Test1</b>				<b>Test2</b>
	5				5

After testing sample 3a collected from a distance of 100-300 m from swine farm in Ciacova was registered an earthworm survival rate of 100% and for sample 3b, taken from a distance of 500-1000 m from farm was registered an earthworm survival rate of 92% (Table 7).

In testing sample C, considered reference sample, it wasn't registered any mortality during testing in comparison with the ones from Ciacova.

**Graph 6.**

Graphical representation of earthworm mortality at the end of the two tests



Mortality for sample 3a was 0%, for sample 3b of 8% and for sample C it wasn't revealed any dead organism (Graph 6.).

### Ecotoxicity tests of soil samples from Peciu Nou

Soil samples were collected from the vicinity of swine farms in Peciu Nou, at a distance of 100-300m for sample 4a, 500-1000 m for sample 4b and from over 1000 m for sample 4c.

Before testing earthworms were weighted individually and registered in tables (Table 8, graph 7).

**Table 8.**

Individual weighting of testing earthworms

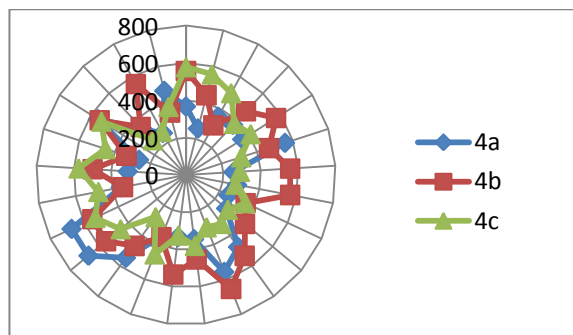
Peciu Nou Test	Earthworm weight (mg)		
B.1	Test1	Test2	Test3
	366.4	556.3	577.7
	256.7	440.1	556.6
	355.6	303.4	500.8
	367.6	467.8	377.1
B.2	356.6	570.7	409.9
	474.5	533.5	322.0
	556.1	654.6	299.9
	345.4	454.3	381.1
	333.3	535.6	325.5
B.3	350.0	356.4	455.6
	556.3	467.4	311.0
	254.6	557.8	288.9
	277.9	567.3	270.0
	250.5	352.2	350.7
B.4	279.1	409.0	290.2
	546.6	467.6	276.7
	675.4	554.5	453.3
	675.4	553.2	535.4
	354.4	343.4	477.0
B.5	311.1	500.1	575.6
	263.6	334.5	453.3
	556.7	546.6	535.3
	334.5	353.4	256.8
	256.6	555.6	266.0
	464.4	345.4	377.4

Where: B1, B2, B3, B4, B5 –testing containers

After testing sample 4a collected from a distance of 100-300 m from swine farm in Peciu Nou was registered an earthworm survival rate of 96% and for sample 4b, taken from a distance of 500-1000 m from farm was registered an earthworm survival rate of 92% being identical as for sample 4c of 92% (Table 9).

**Graph 7.**

Graphical representation of earthworm weight used in Peciu Nou soil testing



**Table 9.**

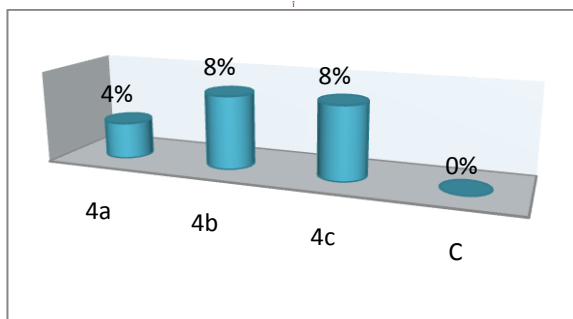
Survival rate of earthworms in testing soils from Peciu Nou

Peciu Nou Soil Sample 4a						
	c	1	2	3	4	5
Number of living earthworms /test	5	4	5	5	5	5
Peciu Nou Soil Sample 4b						
Container	1	2	3	4	5	
Number of living earthworms /test	5	5	3	5	5	
Peciu Nou Soil Sample 4c						
Container	1	2	3	4	5	
Number of living earthworms /test	5	4	4	5	5	
C Sample						
Number of living earthworms /test	5	5	5	5	5	

In testing sample C, considered reference sample, it wasn't registered any mortality during testing in comparison with the ones from Peciu Nou.

**Graph 8.**

Graphical representation of earthworm mortality at the end of the three tests



Mortality for sample 4a was 4%, for sample 4b of 8%, sample 4c of 8% and for sample C it wasn't revealed any dead organism (Graph 8.).

### Ecotoxicity tests of soil samples from Parța

Soil samples were collected from the vicinity of swine farms in Parța, at a distance of 100-300m for sample 5a and 500-1000 m for sample 5b.

Before testing earthworms were weighted individually and registered in tables (Table 10, graph 9).

**Table 10.**

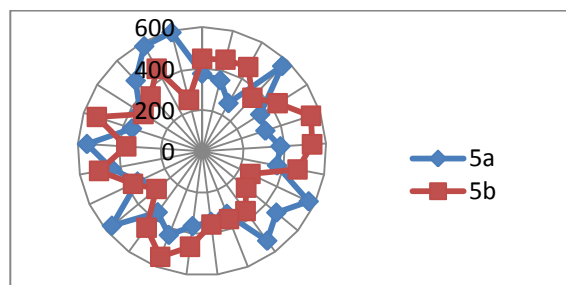
Individual weighting of testing earthworms

Parța Test	Earthworm weight (mg)	
B.1	Test1	Test2
	374.7	446.7
	353.4	456.4
	264.6	463.7
	564.5	354.6
B.2	332.1	432.2
	534.5	356.7
	325.4	352.5
	343.5	356.8
	364.7	464.6
B.3	435.2	547.8
	321.2	553.2
	378.8	532.2
	368.6	468.8
	568.6	257.5
B.4	465.4	276.3
	364.2	455.7
	564.6	285.3
	344.5	368.8
	445.4	506.4
B.5	556.4	366.0
	355.6	534.5
	353.8	335.6
	467.8	362.2
	578.8	454.2
	592,3	256,6

Where: B1, B2,B3,B4,B5 –testing containers

**Graph 9.**

Graphical representation of earthworm weight used in Parța soil testing



After testing sample 5a collected from a distance of 100-300 m from the swine farm in Parța it was registered an earthworm survival



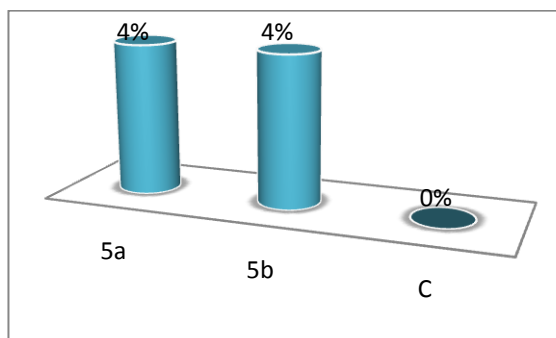
rate of 96% and for sample 5b, collected from a distance of 500-1000 m from farm a survival rate of 96% (Table 11).

**Table 11.**  
Survival rate of earthworms in testing soils from Parța

Parța Soil Sample 5a					
Container	1	2	3	4	5
Number of living earthworms /test	4	5	5	5	5
Parța Soil Sample 5b					
Container	1	2	3	4	5
Number of living earthworms /test	5	5	5	5	4
C Sample					
Number of living earthworms /test	Test1	Test2			
	5	5			

In testing sample C, considered as reference, it wasn't recorded any earthworm mortality in comparison with the ones taken from Parța.

**Graph 10.**  
Graphical representation of earthworm mortality at the end of the two tests



Mortality for sample 5a was 4%, for sample 5b also 4% and for sample C the mortality rate was zero (Graph 10).

Mortality registered for samples 1b, 3b, 4b and 4c was 8%, this being collected from a distance of 500-1000 m from farm, only sample 4c was collected from a distance over 1000 m from farm.

For samples 1a, 2b, 4a, 5a and 5b it was obtained a mortality of 4%, samples 1a, 4a and 5a collected from a 100-300 m distance from farm and samples 2b and 5b from 500-1000 m distance from potential risk farms.

Sample 3a taken from 100-300 m distance from swine farm in Ciacova didn't recorded any mortality rate.

## Discussions

Shin (2001) proved that chemical substances toxic effects on earthworms can be used to determine limits and in what way pollution can be reduced, the acute testing method having a high specificity towards chronic tests [10].

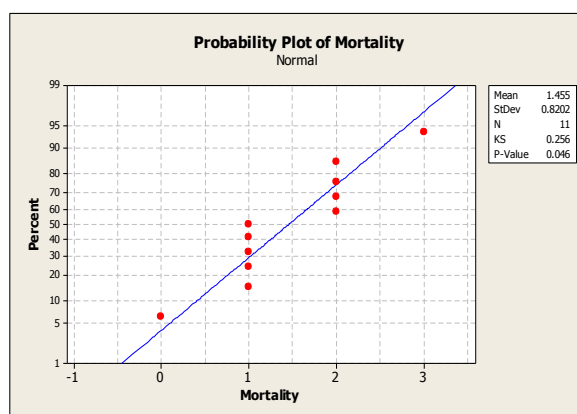
Gunadi (2003) observed that body development of *E. Fetida* in swine waste substrate was superior to the one where it was used cow waste substrate. Mortality was 25% in tests accomplished with swine wastes from fattening swine units [4].

Acute lethal effects and sublethal of animal wastes require a positive correlation between substance concentration and mortality, the negative correlation being with earthworm growth rate [7].

Gibbs (2009) tested an acute earthworm method made by monitoring isolated earthworm pairs and generating a high volume of data in comparison with other tests, including adult development, with a high sensitivity of the method [3].

Faheem (2010) accomplished acute earthworm tests by using pesticides obtaining a mortality between 20-80% correlated with the tested substance concentration [2].

**Graph 11.**  
Statistical registration of mortality



According to Kolmogorov-Smirnov normality test, mortality was analysed and results were significant (p=0.046) (Graph 11).

Mortality value for the entire experiment was 5.45% (n=15).

## Conclusions

- The highest rate of mortality was registered for samples collected from a distance of 500-1000 m from farms in Pădureni, Ciacova and Peciu Nou.
- Also a high mortality was registered for sample from Peciu Nou collected from a distance over 1000m from farm.
- Sample taken from Ciacova from a distance of 100-300 m registered a zero mortality level.
- There weren't registered any behavioural disorders of living earthworms on records from days 0, 7 and 14 of testing.
- Acute mortality test is realized with low costs and a high importance in achieving useful results in earthworm ecotoxicity tests. Results interpretation of tests gives a foundation for clear assessments with statistical significance which can be compared with results obtained from other researchers in the domain.

## Acknowledgements

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