Pollutants impact bioassay from waters and soils in Banat region

Bioanaliza impactului poluanților proveniți din ape și soluri din regiunea Banat

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Abstract

Analyses of water and soil samples by chemical methods identified the quantities of chlorides, nitrates and phosphates by comparison with the maximum limits of law. Acute toxicity tests on zebra fish embryos is an alternative test of water samples around swine farms in Banat region, because embryos are not subject to animal protection legislation during experiments. The use of *Eisenia fetida* earthworms as pollution indicators allowed assessment of avoidance behavior of potentially polluting soils collected from different distances from farms.

Rezumat

Analizele prin metode chimice a probelor de apă și sol au identificat cantitățile de cloruri, nitrați și fosfați prin compararea cu limitele maxime admise de legislația în vigoare. Testele de toxicitate acută pe embrioni de pește zebră reprezintă o alternativă de testare a probelor de apă din jurul fermelor de suine din regiunea Banatului, datorită faptului că embrionii nu se supun legislației de protecție a animalelor pe parcursul experimentelor. Utilizarea râmelor *Eisenia fetida* ca indicatori de poluare a permis aprecierea comportamentului de evitare a solurilor cu potential poluant prelevate de la distanțe diferite de ferme.

Introduction

Acute toxicity tests on zebrafish are an approved alternative at an international level for adult fish toxicity tests [7].

Fertilization rate of zebrafish eggs is reduced significant in polluted waters [8].

The chorion has the capacity to protect the zebrafish embryo in the first 24 h post-fecundation and a certain degradation of it could lead to a low rate of survival (< 10%) [5].

Zebrafish abnormalities vary depending on the pollutant substances concentration and the embryo stage of development [1, 4].

During testing zebrafish suffers alterations like anxiety, lethargy and that they don't avoid capture [12].

According to approved tests, mortality in control must not exceed 10% at the end of testing [16, 17].

The degree of accumulation of heavy metals in fish tissues, both in muscles and liver can have adverse effects for the main consumer: the human [14].

Phosphorus applied in soil in high quantities can lead to eutrophication of surface waters.

The application of swine slurry increases phosphorus level at the place of injection in soil.

A controlled injection of slurry in correlation with detection limits of phosphorus can affect farm economy [10].

Nitrates pollution can also lead to eutrophication, toxic effects on aquatic organisms and also harmful effects on human health [2].

Animals manure is a major source of organic wastes that applied on soil in high quantities can affect balance leading to toxin bioaccumulation in fodder, acting on animal-human food chain [3, 6, 9, 11, 13, 15].

Chemical analysis of water and soil samples

Determination of chlorides quantity in collected samples

For chlorides determination it was used a titrimetric method and results were expressed in mg / L (ppm).

Obtained results are shown in Table 1:

Table 1

Chlorides value in testing samples

Water and soil samples	Chlorides level determined in testing (ppm)	
P1(Pădureni)	29	40
P2 (Parța)	40	51
P3 (Peciu Nou)	37	32
P4 (Voiteni)	30	44
P5 (Ciacova)	75	79

From the presented data it is observed that chlorides values in water have results within limits permitted by current legislation.

Determination of nitrates quantity in collected samples

It was used a spectrophotometric method based on the characteristic of nitrates to react with phenol disulphonic acid forming yellow nitro compounds with an intensity proportional with nitrates concentration in samples.

In order to achieve calibration curve there were chosen standards for water and soil samples, of which absorbance was read at the spectrophotometer with the samples and the results presented in tables (Tables 2 to 5).

The absorbance reading was made for each sample / standard in double.

Table 2

Absorbance and nitrates value in water

Samples	Absorbance		Registered nitrates value in water (mg/dm^3)	
5	0.009	0.009	0.225	0.225
10	0.022	0.022	0.550	0.550
50	0.013	0.011	0.325	0.275
P1	0.078	0.081	1.950	2.025
P2	0.095	0.095	0.375	2.375
P3	0.085	0.086	2.125	2.150
P4	0.124	0.120	3.100	3.000
P5	0.094	0.100	2.350	2.500

Table 3

Absorbance and phosphates value in water

Samples	Absorbance		Phosphates value registered in water (mg/dm^3)	
1	0.002	0.001	0.050	0.025
2.5	0.001	0.002	0.025	0.050
5	0.002	0.001	0.050	0.025
P1	0.696	0.643	0.057	0.030
P2	0.526	0.538	0.027	0.021
P3	0.429	0.432	0.076	0.074
P4	0.578	0.582	0.001	0.001
P5	0.577	0.564	0.001	0.008

Table 4

Absorbance and nitrates value in soil

Samples	Absorbance		Nitrates value registered in soil (ppm)	
5	0.005	0.008	0.125	0.200
10	0.002	0.005	0.050	0.125
50	0.004	0.001	0.100	0.002
P1a	0.251	0.248	6.275	6.200
P1b	0.238	0.243	5.950	6.075
P2a	0.217	0.218	5.425	5.450
P2b	0.215	0.219	5.375	5.475
P3a	0.235	0.235	5.875	5.875
P3b	0.229	0.226	5.725	5.650
P4a	0.119	0.115	2.975	2.875
P4b	0.113	0.116	2.825	2.900
P4c	0.120	0.122	3.000	3.050
P5a	0.138	0.140	3.450	3.500
P5b	0.149	0.148	3.725	3.700
C	0.146	0.145	3.650	3.625

Table 5

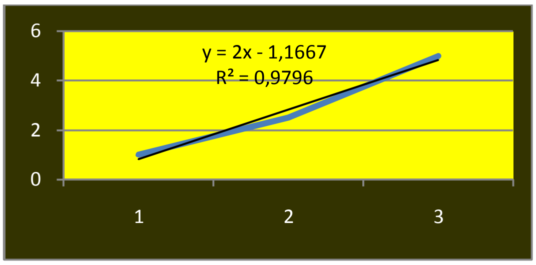
Absorbance and phosphates value in soil

Samples	Absorbance		Registered phosphates value in soil (ppm)	
0.05	0.010	0.010	5.700	5.700
0.1	0.010	0.015	5.700	5.677
0.3	0.090	0.092	5.315	5.381
0.5	0.181	0.187	4.877	4.923
0.7	0.205	0.203	4.836	4.846
1.0	0.026	0.026	5.699	5.699
P1a	0.022	0.028	5.718	5.689
P1b	0.025	0.029	5.704	5.685
P2a	0.017	0.018	5.743	5.738
P2b	0.020	0.022	5.728	5.718
P3a	0.033	0.034	5.665	5.661
P3b	0.027	0.027	5.694	5.694
P4a	0.022	0.023	5.718	5.714
P4b	0.027	0.025	5.694	5.704
P4c	0.021	0.026	5.723	5.699
P5a	0.043	0.051	5.617	5.579
P5b	0.039	0.039	5.636	5.636
C	0.015	0.017	5.677	5.743

With Excel 2010 program was drawn the calibration curve, it was calculated the line equation (y) and correlation coefficient (R^2) (Graph 1->2).

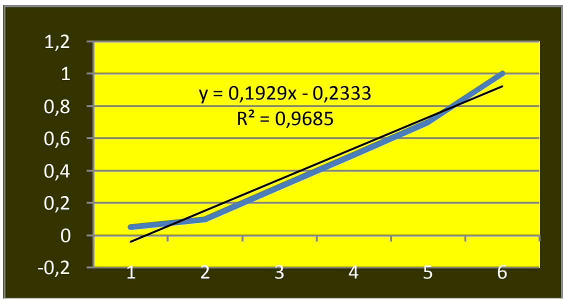
Graph 1

Phosphate standards calibration curve in water



Graph 2

Calibration curve of phosphates standards in soil



Chemical testing results for determination of nitrates, phosphates and chlorides in water and soil reveal that they are in limits of current legislation.

Acute ecotoxicity tests on zebrafish embryos

It was used during testing a number of 500 zebrafish embryos and 100 control embryos. Embryos tests were based on **European Standard ISO 15088:2007** in order to assess the mortality by appreciating acute toxicity of waters in testing on the developmental stages of zebrafish [16].

Test endpoints that allow an appreciation of acute toxicity signs were: lack of tail detachment, no heartbeat and coagulated eggs. A presence of an endpoint is a clue of pollution, embryos thus being included in the mortality index monitored during testing.

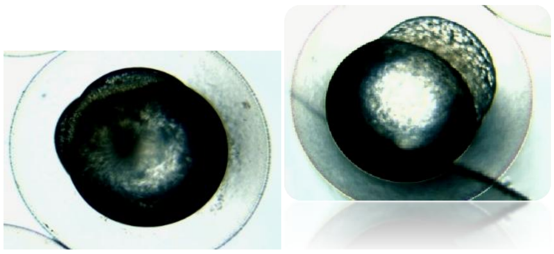


Figure 1. Stages of cell division of embryos in the forst hours post-fertilization

Figure 2. Fully developed zebrafish embryo at 48 h postfertilization (10×)

Figure 3. Zebrafish embryo at 24 h post-fertilization–developmental abnormalities of head and tail

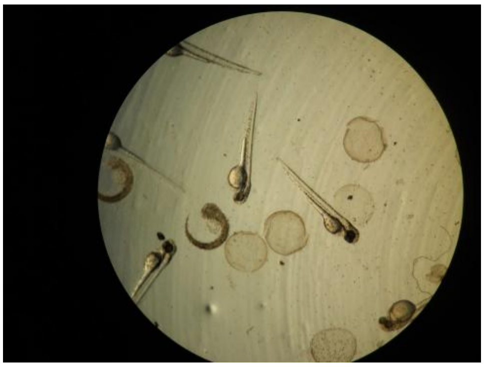


Figure 4. Embryo development overview at 60 h postfetilization – under magnifier (2.5 ×)

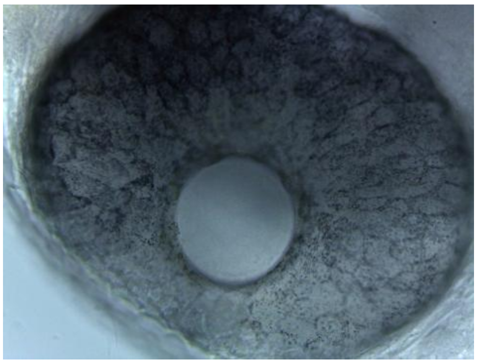


Figure 5. Embryo eye appearance at the end of testing (40 ×)

Figure 6. Zebrafish tail appearance at 60 h post-fertilization (40 ×)

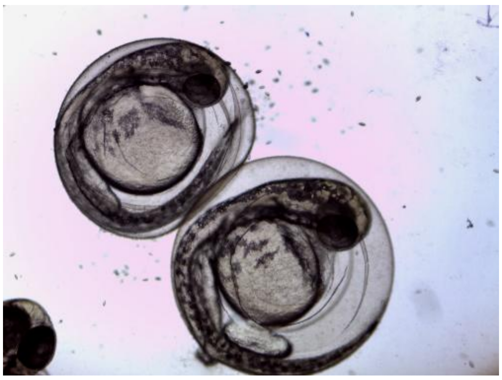


Figure 7. Embryos inside chorionic membrane (10 ×)

Figure 8. Hatching moment in zebrafish (10X)

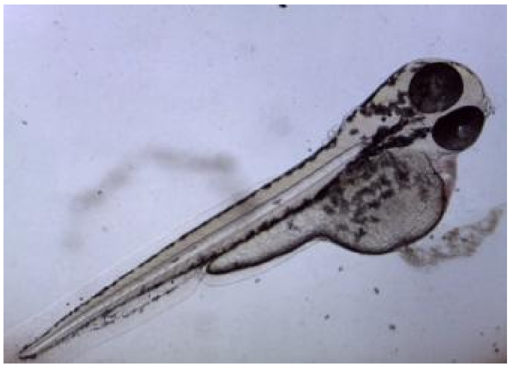


Figure 9. Bleeding appearance in the pericardial sac (10 ×)

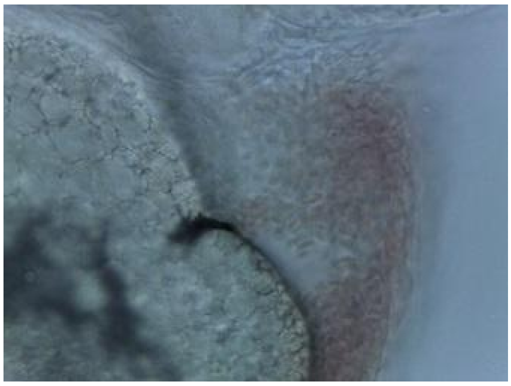
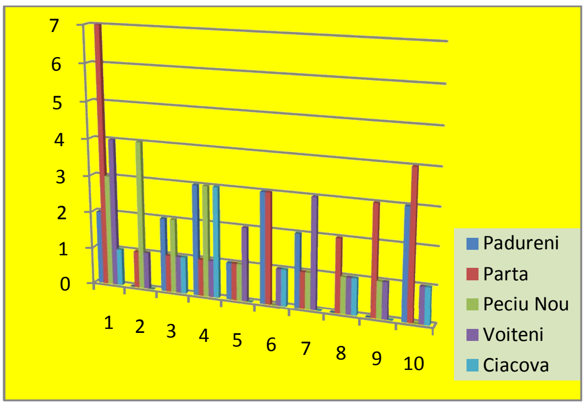


Figure 10. Bleeding appearance in the pericardial sac (40 ×)

Graph 3

Embryo mortality registration after acute ecotoxicity testing on water samples collected from Timiș County



A highest mortality was registered for dilutions 4×, 8× and 32× (Pădureni), dilutions 1× and 32× (Parța), 2× (Peciu Nou), dilution 1× (Voiteni) and dilution 4× (Ciacova).

Mortality was registered for the controls at dilutions: 4× and 8× (Parța), 2× and 32× (Peciu Nou), 1× (Ciacova).

Experiments using *Eisenia fetida* pollution indicators

Soil samples were collected from the vicinity of swine farms in Pădureni, Parța, Peciu Nou, Voiteni and Ciacova at a distance of 100-300m and from 500-1000 m.

It was also collected a sample from a distance higher than 1000 m from the farm in Peciu Nou. In comparison there were used clean soil samples, as controls, collected from areas with swine manure pollutants (Park Pădurice).

Experiments on soil pollution indicators (*Eisenia fetida* earthworms) were held in Pharmaco - toxicology laboratory from Impact Platform Timișoara with the avoidance test **EPS 1 / RM / 43 / June 2004** [18].

During *Eisenia fetida* earthworm tests there were used 250 organisms with a body weight registered individually using a KERN AEJ 220-4M analytical balance (KERN & SOHN Germany).

Figures 11-12. Metallic avoidance device with walls and cover protected from light in order to prevent excessive drying of tested soils

In case of earthworms used in avoidance tests of soils collected from Pădureni it was shown this avoid potentially polluted soils taken from a distance of 100-300 m from farm in comparison with soils collected from a distance of 500-1000 m where it was found a higher number of earthworms unlike in control soils considered clean.

Table 6

***Eisenia fetida* population dynamics in tested soils from Pădureni**

Pădureni 1 soil sample					
DT	a	b	c	d	e
Earthworms number / compartment					
P1	5	1	-	1	4
P2	-	-	-	3	1
P3	2	2	3	1	2
C1	-	3	1	2	1
C2	1	1	4	1	1
C3	2	3	2	2	1
Pădureni 2 soil sample					
DT	A2	B2	C2	D2	E2
Earthworms number / compartment					
P1	3	1	2	2	1
P2	1	1	5	1	4
P3	1	3	-	2	1
C1	1	5	1	2	1
C2	1	-	1	2	3
C3	3	-	1	1	-

Table 7

***Eisenia fetida* population dynamics in tested soils from Parța**

Parța 1 Soil samples					
DT	f	g	h	i	j
Earthworms number / compartment					
P1	-	1	2	-	2
P2	4	-	1	1	1
P3	-	-	2	4	1
C1	2	4	1	1	4
C2	2	1	2	3	1
C3	2	4	2	1	1
Parța 2 Soil samples					
DT	F2	G2	H2	I2	J2
Earthworms number / compartment					
P1	1	2	3	-	6
P2	1	3	1	4	-
P3	1	5	1	1	-
C1	-	-	1	-	-
C2	7	-	2	5	2
C3	-	-	2	-	2

In case of tested soils from Parța it was revealed that organisms - indicators of pollution avoid soils collected from a distance of 100 - 300 m from the farm in the area in comparison with soils from a distance between 500 - 1000 m from farm where the earthworms' number was higher.

Table 8

Eisenia fetida population dynamics in tested soils from Peciu Nou

Peciu Nou 1 soil samples					
DT	k	l	m	n	o
Number of earthworms / compartment					
P1	2	-	3	1	6
P2	5	1	-	3	1
P3	1	1	1	2	1
C1	-	1	2	1	-
C2	1	5	1	2	2
C3	1	2	3	1	-
Peciu Nou 2 soil samples					
DT	k2	l2	m2	n2	o2
Number of earthworms / compartment					
P1	1	2	4	1	-
P2	2	-	1	1	3
P3	3	2	-	1	1
C1	4	1	-	1	1
C2	-	1	-	3	5
C3	-	4	5	3	-
Peciu Nou 3 soil samples					
DT	k3	l3	m3	n3	o3
Number of earthworms /compartment					
P1	5	1	-	1	-
P2	1	3	-	-	-
P3	3	3	1	1	-
C1	1	-	1	7	1
C2	-	2	3	1	1
C3	-	1	5	-	8

For samples collected from Peciu Nou it was registered a high number of earthworms in soils from a distance of 100-300 m from farm and in tests on samples from a distance of 500-1000 m and from over 1000 m from farm it was found that earthworms avoid tested soils in comparison with control soils.

Table 9

Eisenia fetida population dynamics in tested soils from Voiteni

Voiteni 1 soil samples					
DT	p	q	r	s	t
Number of earthworms / compartment					
P1	-	2	1	3	-
P2	2	1	6	4	2
P3	1	2	-	1	-
C1	2	2	1	1	2
C2	2	3	-	-	4
C3	3	-	2	1	2
Voiteni 2 soil samples					
DT	p2	q2	r2	s2	t2
Number of earthworms / compartment					
P1	2	3	2	2	1
P2	2	-	5	1	-
P3	2	-	1	1	-
C1	-	3	1	-	1
C2	1	4	1	1	4
C3	3	-	-	5	4

With the regard to avoidance test, results by using soils collected from Voiteni, from a distance of 100 - 300 m of farm it was shown that pollution indicator organisms don't have a certain preference for one type of soil in comparison with control soils. Earthworm tests on soils collected from a distance of 500-1000 m from Voiteni farm avoid potentially polluted soils.

Table 10

Eisenia fetida population dynamics in tested soils from Ciacova

Ciacova 1 soil samples					
DT	u	v	w	x	y
Number of earthworms / compartment					
P1	-	1	3	5	1
P2	4	4	-	-	1
P3	1	1	2	-	1
C1	2	1	1	3	2
C2	2	1	3	2	2
C3	1	2	1	-	3
Ciacova 2 soil samples					
DT	u2	v2	w2	x2	y2
Number of earthworms / compartment					
P1	1	2	-	2	-
P2	1	5	-	1	1
P3	1	1	2	3	4
C1	4	1	6	1	1
C2	2	1	1	1	1
C3	1	-	1	2	3

Test results of samples collected from Ciacova revealed that earthworms avoid polluted soils in comparison with control soil samples, regardless of the sampling distance from the farm in the area.

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