Assessment of soil contamination using ToxAlert test

Nóra Kováts[1], Andrea Reichel[1], Tímea Szalay[2], Gábor Bakonyi[3] and Péter Nagy[3]

[1]University of Veszprém, School of Environmental Engineering and Chemical Technology, Veszprém, Hungary. E-Mail: kovats@almos.vein.hu
[2]University of Veszprém, Department of Zoology, Veszprém, Hungary. E-Mail: szalayt@freemail.hu
[3]Szent István University, Department of Zoology and Ecology, Gödöllő, Hungary. E-Mail: bakonyi@fau.gau.hu

Abstract

During the analysis of environmental risk posed by hazardous waste disposal sites numerous questions should be addressed. First of all, whole ecosystems are impacted therefore a complex testing scheme is required where surrogate species represent key elements of the ecosystem. Secondly, considering different exposure routes, direct tests and tests using elutriates should be implied simultaneously. In the first case the organisms are in direct contact with the contaminated soil while in the second case the elutriate might represent the mechanism of runoff or leaching from the soil. The third problem, however, rests in the very complex nature of the medium tested. Soil shows an extremely high spatial heterogeneity, increasing considerably the required number of sampling spots. Our main effort was to test the reliability of a very rapid and cost-effective test, ToxAlert ®100 versus direct tests in the environment of a hazardous waste disposal site and to develop an effective strategy to assess environmental risk posed by hazardous waste of similar composition disposed.

Keywords: soil contamination, superfund, ToxAlert, bioluminescence inhibition, direct tests

1. INTRODUCTION

In the United States detailed methodology is available to assess the environmental risk posed by superfund sites (USEPA, 1997). In Hungary a complex assessment program was initiated in 1996 by the Ministry of Environmental Protection.
Toxicity tests are useful tools in the assessment of superfund sites as they measure the aggregate effect of all the contaminants and furthermore they indicate whether the contaminants are in bioavailable form. However, there are some methodological difficulties implied. First of all, physical variance (texture and chemistry) is an actual property of soils. In order to represent spatial heterogeneity, both vertical and horizontal, sampling spots should be densely located which increases sampling costs.

Another problem is the selection of appropriate endpoints. Assessment endpoints are ecological characteristics which may be adversely affected by the contamination supposed to occur at the site in question (USEPA, 1992). As in the environment of superfund sites whole ecosystems are impacted, ecological risk assessment should evaluate the adverse impact on ecosystem level. Therefore assessment endpoints involve several species which are likely to be exposed to differing degrees and also, they might have different reaction upon the same contaminant. It requires a complex testing scheme where key elements of the ecosystem (mostly trophic levels) are represented by surrogate species (for example Daphnids represent planktonic crustaceans playing an important role in the aquatic food web). Nevertheless, test results do not necessarily correlate with each other in every case.

Also, toxicity tests vary as to the media they analyse. Many standardised tests have been developed for the regulation of aqueous discharges. They do not only use aquatic organisms but the medium is also the elutriate. Toxicity tests using elutriate is useful tool to get information about how different contaminants are transferred from sediment or soil to water. In this way effects of runoff or leaching from soil can be modelled and predicted (USEPA, 1994).

There are tests available which use terrestrial organisms in their original medium. In this case the organisms are in direct contact with the contaminated soil. For example the ISO 11267 standard test is used for assessing the effects of chemicals on the reproductive output of *Folsomia candida* (Collembola).

Such direct tests, however representative they are, have a significant negative aspect: they are time and labour-consuming. Therefore our primary goal was to investigate to what extent a very cost-effective aquatic test, ToxAlert can be used for assessing soil contamination. The survey area was the environment of a hazardous waste disposal site, where contamination caused mainly by heavy metals as well as cyanide and fluoride could be predicted.

The ToxAlert ®100 developed by Merck uses bioluminescence of the bacterium *Vibrio fischeri* as the end-point. Bioluminescence is a natural phenomenon in which visible light is generated by an organism as a result of a chemical reaction. These reactions can be reconstructed outside the organisms from which they originate, thereby enabling exploitation of this natural process. There are diverse types of organisms that display bioluminescence: bacteria, protozoa, fungi, sponges, crustaceans, insects, fish, squid, jellyfish, and lower plants. Bioluminescent organisms occur in a variety of habitats, particularly the deep sea, where light is employed for functions including defence, reproduction and feeding. The enzymes involved in the luminescent (lux) system, including luciferase, as well as the corresponding lux genes, have been most extensively studied from the marine bacteria in the Vibrio and Photobacterium genera and from terrestrial bacteria in the Xenorhabdus genus. It has been found that the light-emitting reactions are quite distinct for different organisms with the only common component being molecular oxygen.

The light output of luminescent microorganisms which emit light as a normal consequence of respiration is read by a luminometer. Chemicals or chemical mixtures, which are toxic to the bacteria, cause changes in some cellular structures or functions such as the electron transport system, cytoplasmic constituents or the cell membrane, resulting in a reduction in light output proportional to the strength of the toxin.

The use of bacteria as test organism has several advantages comparing to conventional toxicity testing. The test is rapid (exposure takes only 30 minutes in contrary to the 24-72 hour period being necessary for other aquatic ecotoxicological tests) and causes no ethical problems. This is a very important issue as in the European Union more and more

effort is made to reduce the number of test animals and to develop alternative test methods (Worth and Balls, 2002). The Chemicals Policy of the EU (European Commission, 2001) explicitly states that among others, the political objective of this policy involves the promotion of non-animal testing.

In order to determine the reliability and accuracy of the test (accuracy in this aspect means to what extent the measured value approaches the real value of a given parameter) results obtained by ToxAlert were compared and correlated to direct ecotoxicological tests and zoological bioindication methods.

Direct tests applied were *Folsomia candida* and *Enchytraeus albidus* reproduction tests.

There are several laboratory test protocols to study pollutant effects on collembolan mortality. One of the first technique was elaborated by Kiss and Bakonyi (1992). A novel handbook (Lokke and van Gestel, 1998) contains soil zoological laboratory test methods, including those for collembolans. Based on the collected knowledge an ISO standard (No. 11267) has been accepted that aims at reproduction studies with *Folsomia candida*.

For enchytraeid worms, or potworms (Oligochaeta: Annelida) there is no generally accepted standard for testing contaminated soils (however, there is an OECD proposal for a new guideline considering *Enchytraeidae* reproduction test). Nevertheless, enchytraeid worms are widely used for toxicological testing in Europe. The generally applied technique studies whether the tested soil inhibits (if yes, to what extent) the multiplication of *Enchytraeus albidus* worms.

Biological indication techniques (including zoological methods) provide information on the extent of toxic and other adverse effects of the tested soils. Terrestrial animals in tests may exhibit contamination effects even when chemical soil pollution levels are below the background values. Thus soil animals can be considered as sensitive biological indicators.

Free-living terrestrial nematodes are important indicators of soil processes, including various disturbances (Samoiloff, 1987; Bongers, 1990; Bongers, 1999; Bongers and Ferris, 1999). Due to several properties (e.g. complexity, high taxonomic variability, a considerable diversity of feeding types and life strategies, different sensitivity to pollutants, etc.) this group is highly suitable for field studies of disturbance effects, including inorganic contamination (heavy metals, microelements), as demonstrated by various authors (Zullini and Peretti, 1986; Weiss and Larink, 1991; Nagy, 1999; Georgieva et al., 2002; Bakonyi et al., 2003; Nagy et al., in press). Therefore the structure of a soil nematode assemblage may serve as an important reference point in soil pollution studies.

2. MATERIALS AND METHODS

30 soil samples were collected in the environment of a hazardous waste disposal site (Fig. 1), from the upper 0.1 m. Each sample consisted of 15-20 composite subsamples serving for the purposes of nematological studies, collembolan and potworm tests as well as ToxAlert probes.

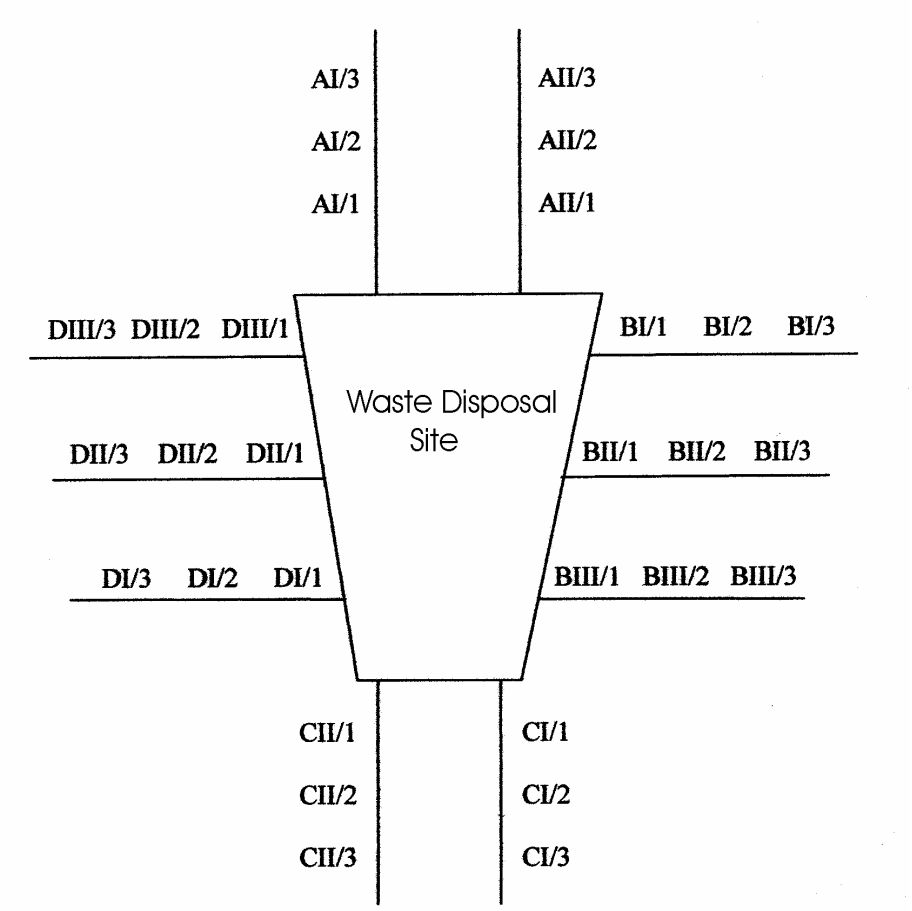

Fig.1. Location of the sampling spots around the waste disposal site.

2.1 ToxAlert test

Elutriate was prepared using 0.1 % dimethyl-sulphoxide (DMSO). In order to provide optimal conditions for the test pH of the samples was adjusted to pH 7.0.
At the beginning of the measurement firstly the test suspension was prepared, using liquid-dried bacteria and reconstitution solution, also provided by Merck. The luminescence intensity of the test suspension was measured in RLU (relative luminescence unit), then the control as well as the sample solutions were added to the test suspension. Luminescence intensity was measured again after the previously set incubation (exposure) time. In our case it was the maximum, 30 minutes.
The ToxAlert ®100 luminometer calculates the inhibition effect (H_t) of the samples automatically in % values. Firstly the f_{kt} correction factor is calculated from the measured luminescence (Equation [1]).

$f_{kt} = I_{kt} / I_0$ (t = 30 min in our test) [1]

where

f_{kt} the correction factor for the contact time
I_{kt} luminescence intensity in the control sample measured in RLU (relative luminescence units), after the contact time
I_0 luminescence intensity of the control test suspension.

Using the correction factor, than the corrected value of for every test sample cuvettes are calculated (Equation [2]).

$I_{ct} = I_0 \times f_{kt}$ [2]

where

f_{kt} mean of f_{kt} of the two control samples

I_0 luminescence intensity of the control test suspension

I_{ct} corrected value of for test sample cuvettes immediately before the addition of the test sample

Then the inhibitory effect H_t of the test sample is calculated (Equation [3]).

$H_t = [(I_{ct} - I_{Tt}/I_{ct})] \times 100$ [3]

where

H_t the inhibitory effect of the test sample after the contact time, in%

I_{ct} corrected value of for test sample cuvettes immediately before the addition of the test sample

I_{Tt} luminescence intensity of the test sample after the contact time, in RLU.

2.2 Collembolan (*Folsomia candida*) test

Folsomia candida is among the most common beneficial springtail species. It has a white colour and no eyes. Adults are 1,5-3,0 mm long. Its furca is long and massive. This species is widely spread throughout Europe in organic soils. Under laboratory conditions it shows parthenogenetic multiplication. It is easy to culture.

In present study the ISO 11267 standard was applied that is meant to test toxicity of contaminants through the reproductivity of *F. candida* under laboratory conditions. During the present experiment two modifications were applied. First, the tests were performed with the exposure of 5, 6 and 7 weeks instead of 4, as demanded by the standard. The reason for this was to obtain a surplus of information, regarding the reproduction rate of the population. Second, the tests were performed on 15 ^{o}C instead of 20 ^{o}C, which also explained the longer duration. The aim of the tests were counting the offspring numbers after the above periods and comparing the collembolan numbers on the different soil samples.

2.3 Potworm (*Enchytraeus albidus*) test

This method provides information on the degree of inhibition of potworm reproduction in the soil samples tested. *Enchytraeus albidus* is a relatively large (max. 15 mm long) member of the annelid group Oligochaeta. It is spread all over the world, especially in organic soils. This species is also widely used and cultured as fish food in aquaria. In our tests adults were used that contained mature eggs. Mature eggs are easy to observe in the clitellum region under a microscope.

Duration of the experiments were 5, 6 and 7 weeks. As an endpoint, potworm numbers were read under a stereomicroscope and adult counts were separated from those of juveniles.

2.4 Nematological analysis

Nematodes were extracted from soil using Cobb's decanting and sieving method (modified according to s'Jacob and van Bezooijen, 1984). Abundance values were estimated under a transmission stereomicroscope (30-40x magnification). After fixing with

80-90 °C hot formalin (cc. 8%) coenological processing meant the identification of at least 150 specimens per sample to genus level.

2.5 Statistical evaluation

In testing environmental media toxicity it provides baseline information to determine whether the sample shows significant toxicity. Toxicity is not significant if the effects do not differ statistically significantly from controls and/or the effects relative to controls are less than 20% (Suter, 1996). According to this, in case of ToxAlert test samples showing bioluminescence inhibition higher than 20% were considered toxic.
For the comparison of animal abundance values, t-tests were applied. Subsequently, exponential curves were fitted to juvenile numbers. Based on this technique population growth rates were read. For curve fitting we used CurveExpert 1.3. (D. Hyams, 112B Crossgate St., Starkville, MS 39759, USA).
Nematode assemblages were studied by the following basic parameters: abundance and taxon numbers. To study significant effects, one-way ANOVA was performed after ln(x+1) transformation of basic data (Statistica for Windows software, Statsoft Inc., Tulsa, USA). As a coenological parameter, Maturity Index (MI) was used (Bongers, 1990).
Correlation amongst different tests was calculated using SPSS for Windows 9.0.

3. RESULTS AND CONCLUSIONS

3.1 Analysis of the soil samples collected in the environment of the waste disposal site

Table 1 gives the results of ToxAlert test for soil samples shown in Fig.1.

Table 1: Results of ToxAlert (Vibrio fischeri) test.

TOXALERT 100		**TOXALERT 100**	
soil sample	inhibition %	soil sample	inhibition %
AI/1	5.8	CI/1	12.65
AI/2	-4.95	CI/2	17.5
AI/3	0.35	CI/3	30.75
AII/1	-2.7	CII/1	0.35
AII/2	-4.55	CII/2	-6.55
AII/3	-7.35	CII/3	36.6
BI/1	-1.1	DI/1	38.65
BI/2	-2.75	DI/2	19.7
BI/3	-1.75	DI/3	-3.0
BII/1	35.1	DII/1	-6.4
BII/2	39.45	DII/2	28.65
BII/3	26.7	DII/3	36.35
BIII/1	-2.7	DIII/1	38.6
BIII/2	26.1	DIII/2	28.95
BIII/3	2.45	DIII/3	29.6

Table 2 gives the results of *Folsomia candida* reproduction tests for soil samples shown in Fig.1.

Table 2: Results of Folsomia candida reproduction test. Numbers refer to juvenile numbers at the end of tests with various treatments. x: unusable samples n: no exponential curve could be fitted

Soil sample	Duration of the test (exposure)			Growth rate (b)	Correlation coefficient (r)
	5 weeks	6 weeks	7 weeks		
AI/1	116	389	493	0.52	0.92
AI/2	139	371	412	0.40	0.88
AI/3	107	416	795	0.80	0.99
AII/1	56	464	907	0.89	0.98
AII/2	77	83	342	1.10	0.97
AII/3	72	394	143	n	-
BI/1	131	311	885	1.0	0.99
BI/2	384	391	832	0.49	0.93
BI/3	181	402	852	0.76	0.99
BII/1	312	493	656	1.01	0.99
BII/2	330	309	720	0.51	0.91
BII/3	228	236	607	0.65	0.95
BIII/1	362	402	883	0.55	0.95
BIII/2	348	411	909	0.58	0.97
BIII/3	363	326	629	0.35	0.86
CI/1	X	97	198	0.61	0.99
CI/2	X	49	474	1.79	0.98
CI/3	X	61	302	1.25	0.97
CII/1	31	345	532	0.61	0.99
CII/2	13	285	548	1.79	0.98
CII/3	156	358	605	1.25	0.97
DI/1	63	103	258	081	0.99
DI/2	48	98	308	1.06	0.99
DI/3	179	168	288	0.14	0.79
DII/1	33	62	108	0.58	0.99
DII/2	22	72	125	0.70	0.78
DII/3	49	63	54	n	-
DIII/1	132	179	324	0.49	0.99
DIII/2	43	92	309	1.13	0.99
DIII/3	52	96	389	1.28	0.99

Table 3 gives the results of Enchytraeus *albidus* reproduction test for soil samples shown in Fig.1.

*Table 3: Results of Enchytraeus albidus reproduction test. Numbers refer to juvenile numbers at the end of tests with various treatments. *: curves were fitted to data, n: no exponential curve could be fitted.*

Soil sample	Duration of the test (exposure)			Growth rate (b)	Correlation coefficient (r)
	5 weeks	6 weeks	7 weeks		
AI/1	0	18	24	12*	0.96
AI/2	0	42	33	16.5*	0.75
AI/3	0	18	34	17*	0.99
AII/1	0	8	54	27*	0.93
AII/2	0	10	39	19.5*	0.96
AII/3	0	3	10	5*	0.97
BI/1	0	27	17	8.5*	0.62
BI/2	5	5	16	0.83	0.95
BI/3	4	52	29	0.31	0.44
BII/1	7	48	10	n	
BII/2	9	11	78	1.68	0.99
BII/3	23	76	53	n	
BIII/1	15	37	59	0.59	0.99
BIII/2	9	23	41	0.67	0.99
BIII/3	5	12	79	1.78	0.99
CI/1	10	12	83	1.67	0.99
CII/2	18	58	102	0.71	0.99
CI/3	27	18	32	0.11	0.38
CII/1	11	10	102	1.88	0.99
CII/2	25	5	162	2.11	0.98
CII/3	23	6	126	1.57	0.96
DI/1	5	6	261	2.46	0.99
DI/2	43	25	189	1.55	0.96
DI/3	29	49	201	1.27	0.99
DII/1	25	13	153	1.84	0.98
DII/2	31	15	158	1.73	0.97
DII/3	33	50	74	0.4	0.99
DIII/1	73	67	57	n	
DIII/2	10	26	210	1.9	0.99
DIII/3	13	65	198	1.18	0.99

Table 4 gives basic data for nematode assemblages in soil samples shown in Fig.1.

Table 4: Basic data for nematode assemblages. MI: Maturity Index, PPI: index for plant feeders. Richness: number of nematode taxa, Abundance: number of nematodes in the sample.

	MI	PPI	Richness	Abundance
AI/1	2.73	2.94	21	2410
AI/2	3.01	2.87	22	1719
AI/3	2.74	X	27	473
AII/1	2.81	2.86	19	976
AII/2	2.39	2.25	19	1163
AII/3	2.93	X	19	1180
BI/1	2.75	2.21	19	1180
BI/2	2.99	X	22	1404
BI/3	2.8	2.68	22	1269
BII/1	2.93	2.63	24	604
BII/2	2.74	2.86	20	1791
BII/3	2.64	X	22	1325
BIII/1	2.65	2.77	20	3059
BIII/2	2.39	2.96	23	918
BIII/3	2.88	2.98	22	1010
CI/1	2.47	X	22	2045
CI/2	2.39	2.47	23	1666
CI/3	2.6	2.31	21	2007
CII/1	2.37	2.13	21	3318
CII/2	2.57	2.71	21	643
CII/3	2.3	2.38	16	3278
DI/1	2.36	2.6	27	2666
DI/2	2.26	X	25	1097
DI/3	2.6	2.65	27	1106
DII/1	2.34	2.33	23	2188
DII/2	X	X	X	2259
DII/3	2.37	2.87	25	1442
DIII/1	2.31	2.24	27	1887
DIII/2	2.59	2.73	24	1193
DIII/3	2.58	2.18	25	1479

3.2 Assessment of soil toxicity

Of soil samples tested the following ones showed bioluminescence inhibition exceeding 20%: BII/1 (35.1%), BII/2 (39.45%), BII/3 (26.7%), BIII/2 (26.1%), CI/3 (30.75%), CII/3 (36.6%), DI/1 (38.65%), DII/2 (28.65%), DII/3 (36.35%), DIII/1 (38.6), DII/2 (28.95%) és DIII/3 (29.65). When plotting them, contaminated areas could be delineated (Fig. 2).
Areas DIII/1 - DII/2 - DIII/3 and BII/1 – BIII/2 – BII/3 are adjacent to the waste disposal site, this fact and their continuity indicate that the disposal site might have really posed an ecological risk. However, toxicity of CI/3 – CII/3 cannot be directly linked to the disposal site and therefore it is difficult to give a clear interpretation. DI/1 shows toxicity while DII/1 does not: it might be caused by either spatial heterogeneity or analytical error.
The maximum inhibition value is 38.65 (DI/1). It indicates that contamination is actually present but the ecological effect on soil fauna cannot be considered as severe.

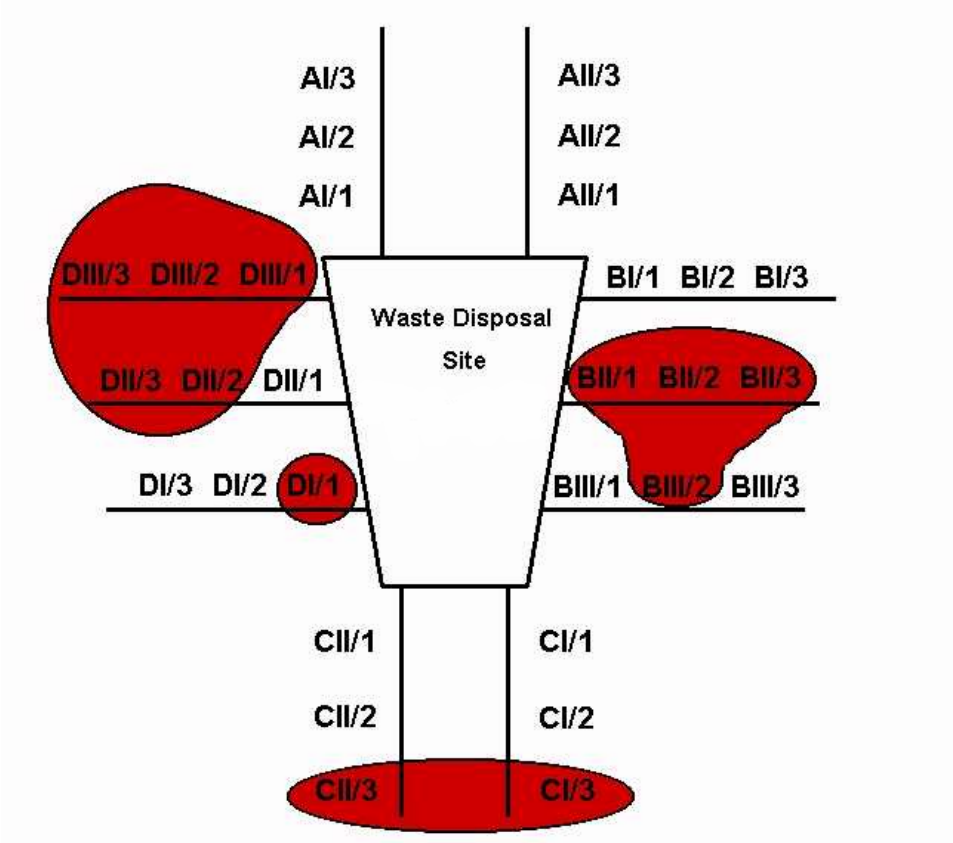

Fig.2. Results of ToxAlert test, red colour indicates samples exceeding 20% bioluminescence inhibition delineating contaminated area.

Based on the *Folsomia candida* test it can be concluded that soil samples taken from the four sides of the hazardous waste disposal site influenced springtail multiplication according to a pattern respective to the localities (Fig. 3).

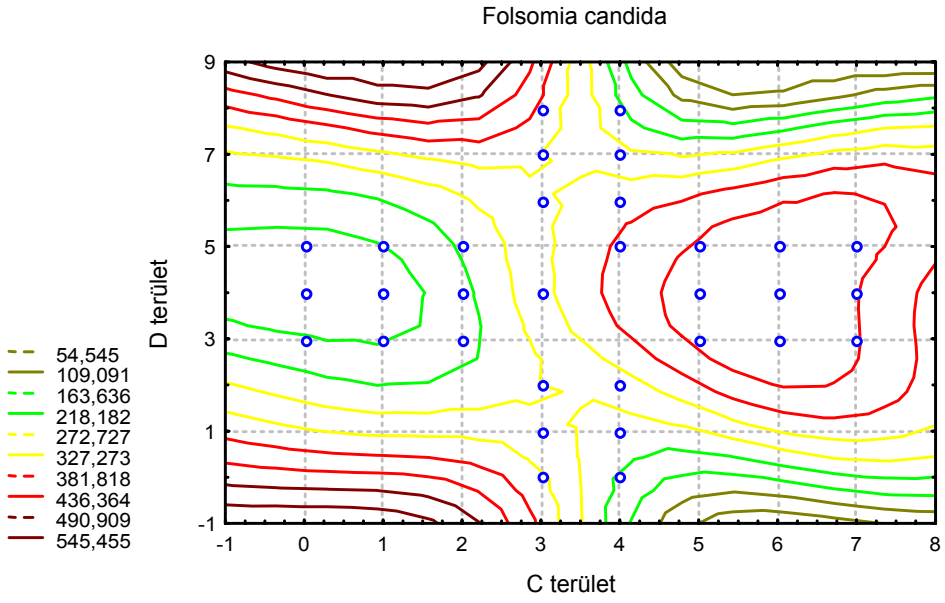

Fig.3. Izoclines derived from *Folsomia candida* offspring numbers.

Most of the collembolans were recorded from the B-side (n=477.5 in average). For the further sides (A, C and D) the average values were 320.9, 258.0 and 137.7, respectively. All these values differed significantly from each other at a minimum level of $p < 0,05$. Population growth rates showed a different pattern. The lowest growth rates could be found in the line of sides B and D also extending over the smelter area, while in samples from sides A and C faster growth could be measured. (Fig. 4).

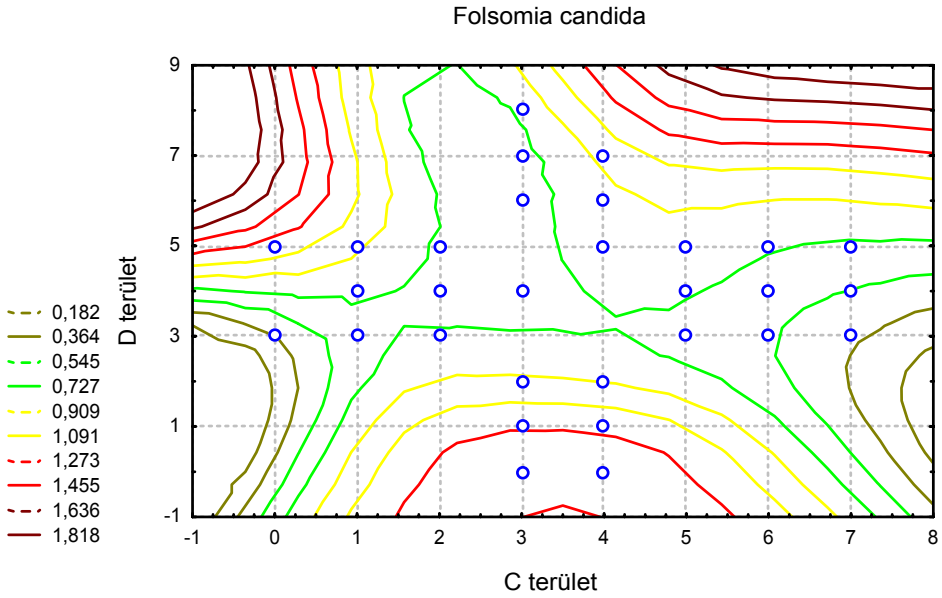

Fig.4. Izoclines derived from *Folsomia candida* growth rates.

Enchytraeus reproductivity tests resulted in the maximum numbers in side D (n=77.0 in average). The following sites were C, B and A with n= 46.1, 27.8 and 16.3, respectively. The differences were significant between sides A and C, A and D as well as B and D, at a minimum level of $p < 0,05$. (Fig. 5).

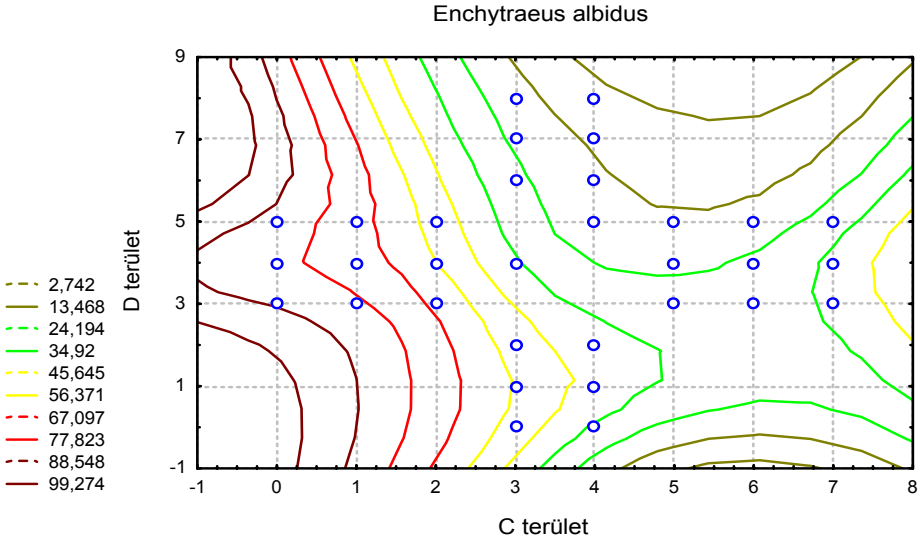

Fig.5. Izoclines derived from *Enchytraeus albidus* offspring numbers.

Population growth rates were the highest in samples from side D, adjacent to the smelter and decreased with the increasing distance (Fig. 6).

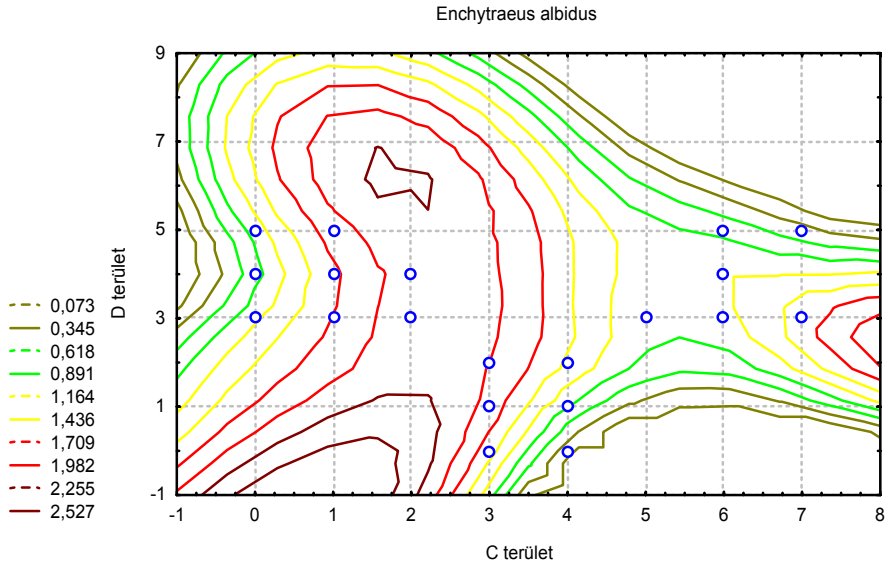

Fig.6. Izoclines derived from *Enchytraeus albidus* growth rates.

It has to be pointed out that in the laboratory tests it was not possible to apply an absolute control. The soil type-related effects being often higher than treatment effects it was not reasonable to involve such a control (e.g. OECD soil).
Nematode abundance values give no sign of possible distance-dependent effects. Neither could be determined any systematic side-dependent effect. Only data from side C were higher than those from other samples. The abundance values for the zone closest to the smelter are in average slightly higher than the others. However, this difference is not significant. Taxon numbers show no distance dependence either, but there appears a significant difference ($p < 0,01$) with the highest values in samples from side D.
In terms of Maturity Indices there is a pair-wise difference: values in samples from sides A and B are higher than those in C and D. The difference over 10% measured in this study can be termed considerable in case of such a derived index. This is supported by the statistically significant difference ($p < 0,05$) among the various side areas. Along the distance gradient an insignificant increase of the MI was detected.
Regarding PPI, samples from all but C side show high values (PPI=2.8 and over). However, occurrence of these values is absolutely sporadic. Moreover, in PPI values the nutrient supply capacity of the plant cover plays an important role besides the pollution. Therefore results of this parameter could not be evaluated consistently in this study.
Both nematode abundance and taxon richness values correspond to data measured in comparable agroecosystems. Thus these parameters give no alert of any possible disturbance. MI values in sides A and B are definitely high, while in the two other localities are within the range observed in comparable agricultural fields in Hungary (Bakonyi et al., 2003, Nagy et al., in press.).
We might come to the final conclusion that by ToxAlert contaminated areas could be delineated to some extent, while zoological parameters are not sensitive enough to

measure minor differences between soil samples with low or negligible of pollution. This is indicated by the fact that parameters measured in *Folsomia candida* and *Enchytraeus albidus* laboratory reproductivity tests as well as nematological indices show no clear distance-dependent effects, i.e. the growth of reproduction rate with the increasing distance from the source of contamination.
Even though contaminated areas delineated by ToxAlert cannot be clearly seen from other numerical test results, a certain correlation was found between ToxAlert and *Enchytraeus albidus* reproduction as well as between ToxAlert and the Maturity Index (Table 5). The correlation is negative, as increase in bioluminescence inhibition correlates with the decrease in *Enchytraeus albidus* reproduction and in Maturity Index, all meaning a certain degree of toxic contamination in the respective samples.

Table 5: Correlation amongst ToxAlert, Folsomia candida and Enchytraeus albidus reproduction as well as certain nematological parameters. FC reprod.: Folsomia candida reproduction, EA reprod.: Enchytraeus albidus reproduction, MI: Maturity Index, PPI: index for plant feeders (Plant Parasite Index), Taxon: number of nematode taxa, Abund.: Nematode abundance in the samples. Bold figures refer to a significant correlation at the 0.01 level (2-tailed).

		Tox Alert	**FC reprod.**	**EA reprod.**	**MI**	**PPI**	**Taxon**	**Abund.**
Tox Alert	Pearson Correlation	1.000	0.292	**-0.416**	**-0.392**	-0.048	0.283	0.180
	Sign (two-tailed)		0.131	0.031	0.036	0.826	0.137	0.341
	N	30	28	27	29	23	29	30
FC reprod.	Pearson Correlation	0.292	1.000	-0.075	-0.222	0.008	0.328	0.251
	Sign (two-tailed)	0.131		0.722	0.265	0.972	0.095	0.198
	N	28	28	25	27	22	27	28
EA reprod.	Pearson Correlation	**-0.416**	-0.075	1.000	0.323	0.106	-0.210	-0.283
	Sign (two-tailed)	0.031	0.722		0.107	0.649	0.304	0.152
	N	27	25	27	26	21	26	27
MI	Pearson Correlation	**-0.392**	-0.222	0.323	1.000	**0.475**	-0.206	**-0.383**
	Sign (two-tailed)	0.036	0.265	0.107		0.022	0.283	0.040
	N	29	27	26	29	23	29	29
PPI	Pearson Correlation	-0.048	0.008	0.106	**0.475**	1.000	0.030	-0.298
	Sign (two-tailed)	0.826	0.972	0.649	0.022		0.892	0.167
	N	23	22	21	23	23	23	23
Taxon	Pearson Correlation	0.283	0.328	-0.210	-0.206	0.030	1.000	-0.271
	Sign (two-tailed)	0.137	0.095	0.304	0.283	0.892		0.156
	N	29	27	26	29	23	29	29
Abund.	Pearson Correlation	0.180	0.251	-0.283	**-0.383**	-0.298	-0.271	1.000
	Sign (two-tailed)	0.341	0.198	0.152	0.040	0.167	0.156	
	N	30	28	27	29	23	29	30

ToxAlert proved to be effective in assessing risk posed by this hazardous waste disposal site. *Vibrio fischeri* test has widely been successfully applied to detect soil and sediment contamination (e.g. Bennett and Cubbage, 1992; Svenson et al., 1996; Johnson and Long, 1998). Doherty (2001) concludes that for many chemicals tested, the results obtained through in laboratory testing with the *Vibrio fischeri* test are often consistent with the results of other ecotoxicological tests and with analytically derived concentration of the contaminant. However, Bennett and Cubbage (1992) recommend the test to be used separately to screen many samples and for inclusion in a multispecies test where risk is evaluated based on the results of several different bioassays.
This test is not only reliable but very rapid: preparation of the elutriate takes app. 45 minutes, performing the test takes another 45 minutes (in case the maximum exposure,

30 minutes is selected, but for screening-level tests even 5 minutes exposure can be enough).
Another advantage of the test is its precision. Precision – defined as measurement of variability in the data collection process (USEPA 1997) – is one of the key components of quality assurance in risk assessment. In case of ecotoxicological tests variability among parallel measurements is meant. The main cause of variability is that individuals normally show different sensitivity to the same toxicant. It follows a predictable statistical distribution with most organisms having approximately the same (average) sensitivity and few being more sensitive or less sensitive. The frequency distribution of sensitivity is assumed to follow a log-normal distribution. However, in case of ToxAlert the number of test organisms is of order of million therefore this source of error can be minimised.
Considering the accuracy, reliability and easy-to-perform nature of the test, it can be recommended to be used in the diagnostic phase of superfund management, significantly reducing the costs.

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