



Szent István University
Ph.D. School of Environmental Sciences

Soil ecotoxicological investigations with potworm
(Enchytraeus albidus)

Doctoral dissertation theses

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BACKGROUND AND OBJECTIVES

The increasing contamination of the soil, water and air is an emerging issue of humanity. The development of our society is very much indeed based on the use of different chemical elements. To satisfy the increasing needs and claims of the growing population has led to the exponential growth of production and consumption. The extraction and use of mineral resources just up to recently have happened at such a rate and extent, as if the resources were inexhaustible on Earth.

In the last century, the economic structure of Hungary has undergone a significant change as well. Following the Second World War in particular, industrialisation accelerated, the chemical processing and mechanisation of the agriculture increased.

The contamination of the different environmental elements, such as the air, water and the soil in particular suddenly increased. The results of the researches carried out since the 1960s clearly show that particularly in the industrial and agricultural zones the microelement and the heavy metal concentration of the soil have abruptly grown. It is true that in numerous cases these levels do not reach the values that affect people in an acute way, however accumulated within the food chain may cause demonstrable health damage in the long run.

Mankind is decisively reliant on the food deriving from plants grown in soil. There exists a dynamic balance between the soils and the environmental factors influencing them. Due to the increasing human activity, this balance is becoming more vulnerable. The earthly living systems, such as the soil, are unable to adapt in the short run to the drastic environmental transformations. Soils contaminated with different microelements and heavy metals mean fundamental environmental problem because numerous elements remaining in the topsoil may keep their potential toxic effects for long decades. Thus special attention should be paid to the already contaminated areas. Efforts should be made to widely get to know the chemical processes of these elements occurring within the soil, their absorption by the plants, their role within the food chain and their behaviour.

A substance is considered to be toxic (chemical element, their compounds, organic substance) in case it has adverse effects on the soil, the plant, the animal and the human. Numerous mineral elements are indispensable or at least have beneficial physiological effects but become toxic or harmful in case of predominance. Thus the contamination of the soil is the function of the dose, the load and the concentration. From another side, toxicity is also a

relative concept. Its extent could be measured by the specific, which is the negative effect concentration per unit (yield loss, disease). This effect is however not independent of the presence or absence of and the possible interactions with other substances, chemical elements occurring in the environment. This effect is also dependent on the exposure time. The regular, lasting, low-dose load may be sneakier because accumulation in this case is harder to detect. The increasing load may cause chronic disorders, while the one-time high-dose acute illness and the lethal dose mortality. The damage occurs differently in the different stages of development, it may vary by gender, species and individuals. The form in which the harmful substance is found may be of importance. The criteria of toxicity are that the substance is easily soluble and absorbable. To sum up, it can be established that the problem of toxicity is highly complex. The toxic or harmful effect may depend on numerous factors, such as the concentration, the ion status or the degree of oxidation, the exposure time, the compounds form, the physical distribution and the specific surface area, the presence of other elements in the system and the interactions with them, the contact mode with the living system and the access conditions (via the surface, food, directly to the blood stream or to the respiratory organs.)

The objectives of the research

When establishing the contamination of the soil we need to be aware of the extent of the sources of error resulting from the sampling and analysis. Even if we examined the area and estimated the concentrations within the soil with due care it should not be forgotten that concentration on its own does not say much. Decisive is the impact on the environment, the hazards, which is the function of the utilisation and the soil conditions. Thus, the analytical data may only be interpreted in the knowledge of the habitat's characteristics.

The aim of the researches described in the doctoral dissertation was to determine that in the effect investigation of a micro element:

- how the mortality and the reproductive ability of a test animal was influenced by – of the 13 heavy metals and micro elements applied in the effect investigation of the MTA Institute for Soil Sciences and Agricultural Chemistry at the Nagyhörcsök experimental site - our chosen heavy metals and micro elements of cadmium (Cd), chrome (CR), copper (Cu), mercury (Hg), lead (Pb), zinc (Zn), Selenium (Se) seven years after their application.
- In case their effects can be detected, in what form (inhibitory or stimulating)?

- If the effect can be detected in case of more elements then in which is the most significant seven years following the application?

The aim of the acute effect investigation planned on the basis of the evaluation of the results of the researches was to determine:

- the effect of selenium (Se) applied to the soil as a new contaminant on the earlier examined parameters within a laboratory environment;
- whether there is any difference in the effect of the two absorbable selenium forms - selenite and selenate - occurring in the soil;
- whether the differences in the effect are influenced by the soils originating from different agriculturally cultivated areas.

MATERIALS AND METHODS

The experimental (test) animal

Our experiments were carried out with the application of the common potworm (*Enchytraeus albidus*). The stock culture of the experimental animal was provided to us by Jörg Roembke, one of the owners of the ECT Oekotoxikologie GmbH (D-65439 Floersheim am Main, Germany). The purity of the culture was verified by Madame Professor Dózsa-Farkas Klára, teacher of the ELTE Department of Systematic Zoology and Ecology.

For the maintenance of the stock culture, the animals were kept in perforated lid plastic boxes, light-tight in a thermostat, on the mixture of traditional, general potting soil and powdered peat available in stores. Prior to usage, the soil material was sterilised in autoclaves. The temperature of the thermostat was set between 17-18°C, the humidity between 70-80%. The water content of the soil was adjusted to 55-60% water capacity. We carefully stirred the soil in the boxes once a week; at this time we checked the medium's moisture content and at this point feeding was also carried out by the mixture of powdered oat meal or powdered cereal. The soil of the stock cultures had to be refreshed in varying intervals, but average of 3-4 months, due to the large number of proliferating animals.

The origin of the soil used during the micro element effect investigation

The soil used in the investigation was collected from the Nagyhörcsök experimental site of the MTA Institute for Soil Sciences and Agricultural Chemistry. The site is located at the southern part of Fejér County just 20 km northwest from Sárbogárd, at the part of Mezőföld, the Transdanubian side of the large part of the Great Plains, within the West Mezőföld „Bozót-sárvíz közti löszhát” geomorphologic landscape. The altitude is 140-150 metres; the soil-forming rock is 15-20 metres thick loess. The hydrologic, climatic and phytogeographical relationships of the area are similar to the Great Plains. On the basis of detailed soil geographic explorations the experimental area is a version of the medium and deeper humus layer calcareous chernozems of the Danube Valley with 0,5-1 meter humus layer.

The contamination of the soil used during the micro element effect investigation

The effect investigation was set in 1991 at a fenced experimental site, at divided parcels in 4 load levels, with 2 repetitions. The experimental treatments and the forms of the used salts as well as the quantity of the applied agents are described in Table 1.

Table 1: The one-time loads applied during the investigation in 1991

Sign of the element	Dosage in spring of 1991 (mg/kg)				Form of applied salts
	0/10	30	90	270	
Cd	10	30	90	270	CdSO ₄ ·8/3H ₂ O
Cr	0	30	90	270	K ₂ CrO ₄
Cu	0	30	90	270	CuSO ₄ ·5H ₂ O
Hg	10	30	90	270	HgCl ₂
Pb	0	30	90	270	Pb(NO ₃) ₂
Se	10	30	90	270	Na ₂ SeO ₃
Zn	0	30	90	270	ZnSO ₄ ·7H ₂ O

The changes that took place in the micro element and heavy metal content of the soils since the set of the investigation until the sampling is shown in Table 2.

The set of the micro element effect investigation

Our experiments were planned considering the regulations of the “Enchytraeus Reproduktion Test” (ERT) taking into account the results of similar experiments carried out earlier with other animals at the Department of Zoology and Animal Ecology of the Szent István University. In case of Se we

tested the full range of the contamination, in case of the other elements (Cd, Cr, Cu, Hg, Pb, Zn) we commenced with the examination of the soils of the parcels with the highest applied contamination level (270 mg/kg) and on the basis of the results obtained we continued with the testing of the lower (90 mg/kg) load level.

The soils used in the investigation were brought in from the Nagyhörcsök experimental site in the summer of 1998 by the colleagues of the department. The soil samples for later use were stored dried, closed, at 5°C temperature. Prior to the launch of the investigation the first task was to warm up the cooled and dried soil and to set the moisture content. The 100% water capacity of the soil from Nagyhörcsök is 53,13 g water per 100 g soil.

Table 2: The effect of the changes over time on the NH₄ acetate + EDTA soluble element content of the plowed layer

Year of the sampling	Load in spring of 1991(mg/kg) (total element content)			
	0/10	30	90	270
Cadmium(CD)				
1991	0	30	89	228
1994	0	14	44	164
1998	0	23,7	71	168
Chrome (Cr)				
1991	0	2	6	30
1994	0	1	2	4
1998	0	0,4	0,9	1,5
Copper (Cu)				
1991	7	24	49	110
1994	4	23	65	192
1998	4	19,5	51	131
Mercury (Hg)				
1991	0	4	49	189
1994	0	2	12	41
1998	0	0,1	2,2	17
Lead (Pb) mg/kg				
1991	5	29	56	158
1994	5	29	101	260
1998	4	31	87	167
Selenium mg/kg				
1991	0	7	23	123
1994	0	8	33	89
1998	0	2	7	28
Zink (Zn) mg/kg				
1991	2	13	55	153
1994	1	19	44	147
1998	2	20	47	124

The examination of selenium

In case of selenium, beside the control soil, we carried out examinations at four load levels. For the simpler identification, we identified the load levels with the applied concentrations. We worked with three replications per tests, so altogether we used 15 breeding pots. Individually weighed, we mixed 20 g dry soil, 5,85 g water (water content is 55% of the water capacity), 0,6 g powdered oat meal in the pots and on the top of this we placed 10 mature (adult) breeding animals. We closed the pots with parafilm. We placed such set experimental pots randomly in the thermostat according to the same circumstances as the maintenance conditions of the stock culture.

The examination of heavy metals

Compared to the previous experiment, in the examination of the heavy metals (Cd, Cr, Cu, Hg, Pb, Zn) there was a change as we observed only one load level at a time (the 270 mg/kg load, which corresponds to the 810 kg/ha quantity of applied agents) with five replications per tests. Thus we used 35 breeding pots. Concerning the quantity of the soil, the water, the oat meal and the animals as well as the method of closing and placing the pots we did not change anything.

The breakdown of the micro element effect investigation

The duration of the experiment was 6 weeks, followed by the breakdown. At this action we took advantage of the relative density between the soil composition elements and the bodies of the potworms. We prepared a concentrated 20 V% sugar solution. Of this, we poured 80cm³ to the just studied culture then we carefully stirred the soil with a narrow glass stick. Due to the combined effect of the sugar solution and the stirring, all animals within the soil (adult and juvenile) came to the surface of the solution. This action was followed by a 5-minute decanting, the aim of which was for the stirred, but heavier specific gravity of soil particles somewhat to subside. Following this, we poured the solution together with the animals contained therein into a clean pot and then we repeated the whole process once again with the soil once already washed and left in the experimental pot. We analysed the result of the experiments with the Statistica 5.0 software based on the LSD test (smallest significant difference).

The used soils and the investigation materials applied at the acute effect investigations of the different selenium forms

During our experiments it was important for us that we worked with soils that originate from areas under constant agricultural cultivation with inspected technical parameters. Accordingly, soils were collected from the experimental sites of the Károly Róbert College, Fleischmann Rudolf Agricultural Research Centre – Kompolt site, the MTA Institute for Soil Sciences and Agricultural Chemistry - Nagyhörcsök site and the University of Debrecen, Centre for Agricultural Sciences, Research Centrum of Karcag – Karcag site. Soil samplings were always taken from the plowed layer. Following the sampling the soils were air-dried to constant weight then were reworked on a 2*2 mm sieve. The resulting soil samples were hermetically sealed and maintained in a refrigerator at 5°C temperature until the beginning of the investigations. In our experiments, we investigated the effects of two water-soluble selenium forms, the Na-selenite and the Na-selenate.

Method

The basis of our experiments' methodology was given by the OECD standard on the reproduction test of the potworm. For the setting, we used the 150 ml sterile sample containers proven during the effect investigation of the micro elements. We measured 20 g dry soil to the pots which was then mixed with some powdered oatmeal. We set the water capacity of the soils between 55-60%. The application of the selenium used as a contaminant, together with the necessary amount of water required for the proper soil moisture conditions was dissolved. In case of the examination of a soil type we applied six different concentrations beside the control soil. In case of the control soil we worked with ten, in case of the contaminated soils we worked with five replications per concentrations. The measurement of the exact quantity of the different selenium forms applied was carried out in the ICP laboratory of the MTA Institute for Soil Sciences and Agricultural Chemistry.

We put 10 mature (adult) animals onto the prepared soil then we closed the pots. We placed the pots light-tight randomly in the thermostat at 18°C temperature at 60% of humidity. One experiment lasted for 42 days. During this period of time, once a week we checked the state of the experimental pots and the moisture content of the soils and fed the animals. Following the 6 weeks, instead of the originally suggested alcoholic devastation and staining by the ERT we applied the "cone execution" used by O'Connor first in the years of the 1960s.

The essence of the changes is that at the exploitation of the animals it bases on the active movement of the animals and their escape from the light and heat, thus only the actually, by the end of the experiment survived animals are present in the statistical evaluation.

We examined the relationship between the selenium contents of all the (ccHNO₃ + cc H₂O₂ soluble Se) and the absorbable (NH₄-acetate + EDTA soluble Se) on the different soil types and the way the soils' selenium content had changed during the time of the experiment with the help of the ICP laboratory of the MTA Institute for Soil Sciences and Agricultural Chemistry.

The statistical evaluation of the results

The endpoints of the experiments were the mortality and reproductive ability values. From the results obtained we calculated the values of LC₅₀, EC₅₀, LC₁₀, EC₁₀ and NOEC with the ToxRat Light 2.08 (ToxRat) software. We carried out the tests in relation to both the total and the absorbable element content. The program was explicitly developed for the statistical evaluation of ecotoxicological bio tests. The tests we performed with bilateral trials with a given 5% significance limit. As a first step, the presence or absence of the effect was determined with a one-way ANOVA analysis. Following this, the homogeneity of the data was ascertained with the help of the Cochran's test. If the data showed a homogenous distribution, we carried out the Williams' kind multiple, sequential T-test for the determination of the NOEC value. In case of inhomogeneous distribution, we conducted the Welch' kind T-test with Bonferroni correction. The values of EC_x, LC_x were determined with probit analyses.

RESULTS

The results of the micro element effect investigation – Heavy metals

Examining the mortality of the mature (adult) animals, the results of the experiment showed that the survival of the mature specimens of the test animal (*Enchytraeus albidus*) were not substantially influenced by neither the 90 mg/kg, nor the highest 270 mg/kg applied concentration of our chosen heavy metals seven years following the application.

However, the reproductive ability, the change in the number of individuals of the young (juvenilis) animals demonstrated a highly significant effect. Analysing the results it was found that by examining the highest applied concentration (270 mg/kg) – seven years following the application – all heavy metals significantly decreased the number of individuals of the young animals. Examining the lower concentration (90 mg/kg) – seven years following the application- only zinc (ZN) had significantly demonstrated reducing effect on the reproductive ability.

Results of the micro element effect investigation – Selenium

At the examinations seven years following the contamination we found detectable effect both among the mature (adult) and the young (juvenilis) animals. Compared to the results of the control soil, even at the lowest contamination level (10 mg/kg) we found reduction in the population and the progeny, which became more and more significant with the increase of the contamination concentration. At the 30 mg/ concentration level already a substantial reduction in the reproductive ability was seen. In the soil contaminated with 90 mg/kg selenium – seven years following the application – beside a high level of mature (adult) animal mortality, progeny was not able to be detected. At an even higher level of contamination level (270 mg/kg) after the sixth week of the experiment we did not find any live specimens.

The results of the acute effect investigation of the different selenium forms

The results of the experiments carried out on the soil of Kompolt – Na-selenite

Examining the element content of the solutions applied on the soils during the experiment it can be concluded that the treatment resulted in a significant effect on the mortality of the animals. The ratio of the survived animals in the soils treated with the highest concentration decreased to 14,6% (ANOVA $p < 0,001$). The NOEC value was defined in 8,89 mg/kg by the program. As a result of the probit analysis, LC_{10} value was 6,13 mg/kg, LC_{50} value was 55,13 mg/kg.

The effect of the examined element on the reproductive ability is stronger. In the soils treated with the highest concentration, the number of young (juvenilis) individuals decreased to zero (ANOVA $p < 0,001$). In case of reproductive ability the NOEC value was defined in 3,88 mg/kg by the program. As a result of the probit analysis, EC_{10} value was 3,44 mg/kg, EC_{50} value was 17,06 mg/kg.

When examining the absorbable selenium content, the calculated NOEC value for mortality was 0,61mg/kg. As a result of the probit analysis LC₁₀ value was 0,36mg/kg, LC₅₀ value was 7,42mg/kg. The obtained NOEC value at the reproductive ability examination was 0,26mg/kg, while as a result of the probit analysis, EC₁₀ value was 0,19mg/kg, EC₅₀ value was 1,48mg/kg.

Examining the development of the selenium content during the experiments we obtained the following results: of the applied total selenium content measured at the application 19,93% was absorbable, of the measured at the end of the experiment it was 14,46%. During the time period of the experiment the total selenium quantity decreased by nearly 10%. During the same period the quantity of absorbable selenium decreased by almost 35%.

The results of the experiments carried out on the soil of Kompolt – Na-selenate

As a result of the application the mortality of the test animal significantly increased (ANOVA $p < 0,001$). In the soil treated with the highest concentration level no living animal was detected. The examined lowest concentration is also resulted in statistically proven effect. As a result of the probit analysis, LC₁₀ value was 1,49mg/kg, LC₅₀ value was 6,92mg/kg.

The effect of the applied selenium in the form of selenate on the reproductive ability manifested in strong inhibition (ANOVA $p < 0,001$). Neither in the soils treated with the highest nor with the preceding concentration were any offspring. Due to the strong effect, the NOEC value could not be defined. As a result of the probit analysis, EC₁₀ value was 0,63 mg/kg, EC₅₀ value was 1,79 mg/kg.

At the examination of the absorbable selenium content LC₁₀ value calculated for mortality was 1,45 mg/kg, LC₅₀ value was 6,69 mg/kg. At the examination of the reproductive ability, on the basis of the results of the probit analysis EC₁₀ value was 0,56 mg/kg, EC₅₀ value was 1,68 mg/kg.

Examining the development of the selenium content during the experiments we obtained the following results: of the applied total selenium content measured at the application 93,27% was absorbable, of the measured at the end of the experiment it was 93,85%. During the time period of the experiment the ratio of the total and the absorbable selenium content did not change.

The results of the experiments carried out on the soil of Nagyhörcsök – Na selenite

Considering the total element content, due to the treatment the mortality of the test animal significantly increased. In the soil contaminated with the highest concentration of selenium, no animal survived (ANOVA $p < 0,001$). The NOEC value was defined in 6,21mg/kg by the program. As a result of the probit analysis LC₁₀ value was 7,3mg/kg, LC₅₀ value was 22,5mg/kg.

As a result of the effect of Na-selenite on the reproductive ability, in our examination with the highest concentration the number of offspring was reduced to 1,1% (ANOVA $p < 0,001$). The NOEC value was 2,35 mg/kg. The probit analysis calculated EC₁₀ value was 2,8 mg/kg, the EC₅₀ value was 7,3 mg/kg (Figure 24).

At the examination of the absorbable selenium content the NOEC value for mortality calculated by the program was defined in 1,83mg/kg. As a result of the probit analysis LC₁₀ value was 2,05mg/kg, LC₅₀ value was 8,01mg/kg. At the examination of the reproductive ability, the NOEC value was 0,8mg/kg, the probit analyses calculated EC₁₀ value was 0,82 mg/kg, EC₅₀ value was 2,46mg/kg.

Examining the development of the selenium content during the experiments we obtained the following results: of the applied total selenium content measured at the application 57,8% was absorbable. By the end of the experimental period this ratio decreased to 17,85%. During the 6-week time period of the experiments the total selenium content decreased by 9,41%, while the absorbable element content by its multiple, 72,03%.

The results of the experiments carried out on the soil of Nagyhörcsök – Na selenate

The selenium applied in the form of Na-selenate on the soil of Nagyhörcsök had an extremely strong and significant effect. It resulted in the total mortality of our test animals not only at the highest but also at the preceding concentration level. Statistically proven effect already occurred at the examination of the first concentration thus it was not possible to define the NOEC value. As a result of the probit analysis LC₁₀ value was 0,97 mg/kg, LC₅₀ value was 5,69 mg/kg.

Despite the carefully set concentration – up to the detection limit of the testing laboratory - the NOEC value for the effect of Na-selenate on the reproductive ability could not be defined. Already the first set concentration re-

sulted in statistically proven decrease in the reproductive ability. The probit analysis calculated EC₁₀ value was 0,29 mg/kg, EC₅₀ value was 0,41 mg/kg.

At the examination of the absorbable selenium content we could not define the NOEC value for mortality. As a result of the probit analysis LC₁₀ value was 0,67mg/kg, LC₅₀ value was 4,74mg/kg. Examining for reproductive ability EC₁₀ value was 0,19mg/kg, EC₅₀ value was 0,28mg/kg.

Examining the selenium content it was found that during the six-week time period of the experiment the ratio of the total and the absorbable element content remained unchanged. Of the applied total selenium content measured at the beginning of the experiment, 98,32% was absorbable, at the end of the experiment it was 97,6%. During the time of the experiment the total selenium content decreased by 17,13%, the absorbable one decreased by 17,74%.

The results of the experiments carried out on the soil of Karcag – Na-selenite

Considering the total element content, due to the treatment the mortality of the test animal significantly increased. In the soil treated with the highest concentration level no animal survived ($p < 0,001$). The NOEC value was defined in 8,39mg/kg by the program. As a result of the probit analyses LC₁₀ value was 4,29mg/kg, LC₅₀ value was 19,77mg/kg.

At the examination of the effect on the reproductive ability, following a significantly not justifiable increase in the number of offspring seen in the first concentration, in the next concentration a significant decrease in the reproductive ability occurred. Similarly to the mortality analysis, the NOEC value by the probit analysis was defined in 8,39mg/kg. The EC₁₀ value was 4,61mg/kg, EC₅₀ value was 14,64mg/kg.

At the examination of the absorbable selenium content, the effect on mortality was established as follows: the NOEC value was defined in 2,50mg/kg by the program. As a result of the probit analysis, LC₁₀ value was 1,02mg/kg, LC₅₀ value was 7,18mg/kg. At the examination of the absorbable selenium content, the effect on reproductive ability was established as follows: NOEC value was 2,50mg/kg, EC₁₀ value was 1,08mg/kg, EC₅₀ value was 4,85mg/kg.

Examining the development of the selenium content a great extent to which the absorbable selenium content of the soil decreased could be observed. Of the applied total selenium content measured at the beginning of the experiment, 58,9% was absorbable. By the end of the experimental period this ratio changed to 17,38%. During the 6-week time period of the experiment the

total selenium content decreased by 12,82%, while the absorbable element content by its multiple, 74,28%.

The results of the experiments carried out on the soil of Karcag – Na-selenate

Considering the total element content, due to the treatment the mortality of the test animal significantly increased ($p < 0,001$). In the soil treated with the highest concentration level no living animal was detected. The NOEC value was defined in 3,41mg/kg by the program. As a result of the probit analyses LC_{10} value was 3,49mg/kg, LC_{50} value was 6,8mg/kg.

The statistical analysis of the effect of selenium in the form of selenate on the reproductive ability resulted in the followings: NOEC value was 3,41 mg/kg, EC_{10} value was 2,81 mg/kg, EC_{50} value was 5,04 mg/kg.

At the examination of the absorbable selenium content, the effect on mortality was established as follows: NOEC value was 2,56mg/kg, LC_{10} value was 2,90mg/kg, LC_{50} value was 6,23mg/kg. Analysing the effect on the reproductive ability NOEC value was 2,56mg/kg, EC_{10} value was 2,54mg/kg, EC_{50} value was 4,64mg/kg.

Examining the total and absorbable element content it was found that during the 42 days of the experiment their ratio practically did not change. Of the applied total selenium content measured at the beginning of the experiment, 95,9% was absorbable. By the end of the experimental period this ratio did not decrease under 89,8%. During the 6-week time period the total selenium content decreased by 8,88%, while the absorbable element content decreased by not more than twice, by 17,74%.

NEW SCIENTIFIC RESULTS

1. Analysing the long-term effects of selenium I found that at the Nagy-hörcsök effect investigation in 1991 the application of an agent in 90kg/ha concentration had a significantly reducing effect on the reproductive ability even after seven years of the appliance. Examining the higher concentrations applied (270 kg/ha, 810 kg/ha) the substantial increase in mortality could also be demonstrated.

2. Analysing the long-term effects of different heavy metals (Cd, Cr, Cu, Hg, Pb, Zn) I concluded that at the Nagyhörcsök effect investigation the application of the concentrations did not influence the mortality of the test animal demonstrably seven years following the appliance, however at the highest concentration level, all heavy metals significantly decreased the number of individuals of the young animals. At the examination of lower applied concentrations (270 kg/ha) – in contrary to prior assumptions – only zinc had an extensively decreasing effect on the reproductive ability.
3. Examining the short-term effects of the different selenium forms (Na-selenite, Na-selenate) on three different soil types it can be concluded that projected to the total element content (dissolved element content in cc.HNO₃+ccH₂O₂-ban) independent of the soil type, Na-selenate had a more significant toxic effect on the *Enchytraeus albidus* test animal both in terms of mortality and reproductive ability. When examining the same parameters for absorbable element content (dissolved element content in NH₄-acetate+EDTA) we demonstrated that the toxicity of the element forms showed a soil-dependent effect. While in case of the Nagyhörcsök soil Na-selenate had clearly a more toxic effect, in case of the soils from Kompolt and Karcag the effects of the two element forms equalised, in some cases Na-selenite became more toxic.
4. Examining the behaviours of the different selenium forms (Na-selenite, Na-selenate) during the 6-week period it was found that in case the added agent was in the form of Na-selenate, the ratio of the measured quantity of the total and of the absorbable element content both at the beginning and the breakdown of the experiment practically remained unchanged (only in case of the soil from Karcag was a decrease of 8-15% detected.)

In case of the agent of Na-selenite, beside the 9-12% decrease in the total element content we experienced a 35-75% decrease in the absorbable element content by the end of the six-week experiment series.

5. We introduced a new, in Hungary less well-known and unused ecotoxicological test method – shorter than the tests carried out with earthworms – practically at the same time with the Ring test of the method. With the introduction of methodological changes in the methodology (isolation according to the O'Connor's "cone execution") the process became safer and more accurate. The advantage of

the methodological change is that compared to the alcoholic devastation and staining, it does not apply further chemicals. At the exploitation of the animals it bases on their active movement and their escape from the light and heat, thus only the actually, by the end of the experiment survived animals are present in the statistical evaluation

CONCLUSIONS AND RECOMMENDATIONS

Effect investigation of micro elements

It is difficult to tell or even to estimate with approximate accuracy the effect of materials incorporated into the ecological systems. This is due to the fact that the applied substances – considering the specific example of our analysis, the compounds containing different metal ions and micro elements - are affected by more than one factor at the same time. The effect of these factors in vast majority of the cases is not of the same size, is not constant in time and often lacks of periodicity. Due to this it is absolutely necessary to carry out such examinations that test the same factors in different circumstances with the help of various indicators.

An explanation for the result obtained at the examination of selenium may be that it may become very mobile on calcareous soils, as a result of which it becomes very easy to be absorbed by both the plants and the animals connected to these plants due to their diet at some level.

We examined the effect of the heavy metals in the two highest applied concentration levels (270mg/kg, 90mg/kg). Following seven years of the application none of the concentrations influenced the changes in the number of mature (adult) animals and mortality in a verifiable way. When examining the change in the reproductive ability, at the highest load level all heavy metals resulted in a significant effect, the number of individuals of the young animals decreased. At the lower original load level, in comparison with the results of the control, in this concentration only zinc (Zn) had a significant effect.

When comparing the results of the experiments performed on the soil samples of Nagyhörcsök with the test results of other, heavy metal and micro element toxicity researches with potworm, I verify the general thesis that states that most elements may become toxic to the animals of the soil if it exceeds the natural amount and is present in sufficiently high concentration.

The most often examined – and on the basis of which qualified as toxic - metals at the investigations, such as cadmium (Cd), copper (Cu), mercury (Hg), chromium (Cr), zinc (Zn) and lead (Pb) were also included in our investigations. Similarly to the processed researches, the effect on reproduction was the stronger in our case as well. In case of cadmium and zinc several researchers proved the existence of some sort of biological mechanism, in which the animals were able to select faster the toxic elements for themselves or inhibit their uptake. This effect was not observed in case of the zinc. The zinc in the studied potworm was accumulated in its body at an appropriate environmental concentration level. This phenomenon may be the reason behind that in case of the examined heavy metals zinc proves to be the most toxic. It would lead to interesting results and would give more accurate explanations if we would continue the investigations in case of further elements (Hg, Cr, Pb) for the determination of the inhibited uptake or accelerated selection.

Comparing the obtained results with the results of other organisations' toxicity tests at the department we also have to highlight zinc. In the open field toxicity test carried out by the *Collembola* with this element it was not possible to demonstrate the effect influencing the reproductive rate even at the largest concentration level, while in case of the potworm (*Enchytraeus albidus*) this already significantly occurred at the third level. It is further interesting that while in case of most of the heavy metals (Cd, Cr, Cu, Pb) the *Collembola* responded to the highest concentration level with the decrease of the number of individuals (in some cases not even with that), the potworm reacted with the decrease of the reproductive ability in case of all heavy metals (Cd, Cr, Cu, Hg, Pb, Zn) without exception.

Furthermore, it was found that of the examined elements selenium had the longest-lasting negative effect by far, somewhat contradictory to that information that in the appropriate environment with optimal pH value it becomes easily mobile and washes into the deeper layers of the soil or evaporates.

It is important to highlight that the experiment series confirmed the suitability and high degree of sensitivity of the potworm in the soil ecotoxicological tests, at the time when the test method to be developed with these animals, the Enchytraeid Reproduction Test (ERT) was only in its trial phase and the description of the method only existed in a draft version.

All these information together laid down the foundation for further investigations and made it evident that with the examination of selenium and their different forms of prevalence we may take possession of further important knowledge.

Acute effect investigation of the different selenium forms

The aim of our experiments was to bring to light the effect of the different selenium forms absorbable by plants on the potworm in different soil types. Those investigations started in the spring of 2004 in which we analysed three different soil types taking into account that the parameters of the soil types should still meet the ecological need of the test animal but substantially differ in structure – based on literature data - in order to influence the absorbable capability of selenium

Unfortunately, although it is of great importance, it has an extensive research in a wide range of agricultural uses that is more justified, the effect of selenium on the animals of the soil hardly been studied. Very limited data are available about the effect of selenium on those animals that gnaw them through the soil – thus they get in contact with the soil both via their surface of the body and their gastrointestinal -. Is the pH-dependent toxicity generally accepted in case of plants valid for these animals as well? According to the biochemical degradation, does selenite have the most toxic effect on these animals too? Is there any other non-specific regularity that applies neither to the plants nor the animals?

The absorption of selenite and selenate (both are water soluble selenium forms) may be influenced by numerous factors in the soil. Comparative analytical studies carried out on similar soils show that the absorption of selenite into the solid phases of the soils is greater than of the selenate within similar conditions. According to the gradually increasing pH value of the soils, the absorption capability of selenium decreases. The main parameters of the soils were similar in our investigation as well – influencing the absorption of selenium – thus we assumed that there will be no significant transformation among the selenium forms during the time period of the investigation. In contrast, we could demonstrate the strongest selenium absorption in relation to selenite in the soil form Karcag characterised with the highest level of pH value, the explanation of which may be searched in the higher microbiological activity.

Examining either mortality or reproductive ability it could be concluded that either selenium form was minimum an order of magnitude toxic than the heavy metals or even the Phenmedipham pesticide tested earlier on these animals. On the basis of comparing the different literature data it could be stated that the toxic effect of selenium on *E. albidus* is stronger than on other animals of the soil, like *Eisenia fetida*, *Lumbricus terrestris* or *Megaselia scalaris*.

Our results indicate that in respect with the examined two elements (Na-selenite, Na-selenate) considering the total element content, under the experimental circumstances used by us, there is a significant difference in the measured toxic threshold values. Na-selenate proved to be significantly more toxic in case of all three soil types than Na-selenite. However, the fact that the difference in toxicity disappears in case we analyse not the total but the absorbable element content infers that the mode of absorption of selenite and selenate may differ in case of our test animal. The uptake of selenium (either selenite or selenate) may occur via the surface of the body or via the alimentary canal. It may be achieved merely via the pore water, but, beside the pore water, with the help of the digestive system even via the soil particles and through the diet as well. Analysing the results we can also assume that only via the pore water does the soluble element content of the selenium get into the animals. This may also explain, that analysing the absorbable element contents the applied concentrations converge. We can also assume however that the enzymes of the digestive system and their microbial processes also participate in the absorption of selenium, since gnawing through the soil these animals get in contact with the total selenium content in the soil. In this case we must live with the hypothesis that selenite more strongly bounds to the soil particles, thus is harder for the intestinal flora to reach than selenate, since it became toxic to the same extent in larger quantities. Both hypotheses should be considered for further investigation in the future.

PUBLICATIONS RELATED TO THE DISSERTATION THESIS

Published in scientific journals (accepted for publication), proof-read, full-text scientific papers

In foreign language impact factor journals:

Z. SOMOGYI, I. KISS, I. KÁDÁR, G. BAKONYI (2007): Toxicity of selenate and selenite to the potworm *Enchytraeus albidus* (Annelida: Enchytraeidae): a laboratory test, *Ecotoxicology, Springer Netherlands* 16 379-384 p. (IF: 2,405)

Z. SOMOGYI, I. KÁDÁR, I. KISS, T. JURÍKOVÁ, L. SZEKERES, S. BALLA, P. NAGY, G. BAKONYI (2012): Comparative toxicity of the selenate and selenite to the potworm *Enchytraeus albidus* (Annelida: Enchytraeidae) under laboratory conditions, *European Journal of Soil Biology*, 50 159-164 p. (IF: 1,578)

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SOMOGYI Z., BAKONYI G., CSATHÓ P. (2004): A nátrium-szelenit hatása a közönséges televényféregre (*Enchytraeus albidus*), Állattani Közlemények 89 (1) 67-71 p.

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