ECOTOXICOLOGICAL EFFECTS OF A MICROELEMENT SOIL POLLUTION ON FREE-LIVING NEMATODES

PH. D. THESIS

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Contents

1. Background

Among many functions of soils there are some of particular importance, such as „Ecological" functions: biotopes (biological habitats), biomass producing site, regulatory (buffering) substrate and

Functions connected to human activities: physical substrate (from building sites to recreation areas), source of natural resources and carrier of paleontological information.

The above mentioned variability of functions is seriously threatened by a number of (principally human) disturbances. These lead the Council of Europe to compile principles of European Soil Chart in 1972. This document declares soils to be one of the most valuable treasure of mankind that is limited in availability and easy to destroy. Therefore it should be protected from erosion, pollution and other destructive human impacts. The related Dutch legislation introduced the concept of soil multifunctionality in the 1980-ies. The above societal-legislatory expectations strengthened research activities on soil biological effects of soil pollutions. „Environmental nematology", i. e. use of free-living terrestrial nematodes as bioindicators became a remarkable segment of this research.

2. Literature studies

Free-living nematodes are wide spread, diverse both in terms of feeding types and reproductive strategies, and are numerous in most aquatic or terrestrial habitats. These characters make them useful organisms in bioindication studies (Bongers and Yeates, 1988; Samoiloff, 1987; Zullini, 1976). Nematode densities and genus numbers, together with calculating Maturity Indices and analyzing feeding types are useful tools to detect effect of soil disturbances that are reflected in changes of nematode community structure (Bongers 1990, Neher et al., 1995; Freckman and Ettema, 1993).

Regarding more specific publications dealing with microelement effects on soil nematode assemblages, there is a relative scarcity of comparable studies. This is due to the different experimental approaches and the great variety of the experimental background conditions.

Sturhan (1986) carried out a microplot experiment to investigate the effects of 12 elements on nematode fauna. Regarding the elements common in his study and the present experiment, he found that nematode abundance was not markedly reduced in the polluted soils, and that Hg and Pb did obviously not affect the structure of the community. On the other hand, some taxa were missing from soil with high concentrations of Cd, Cr, Ni and Se. There is no numeric value given for concentrations. Densities of fungal feeding nematodes as Aphelenchus avenae and Aphelenchoides spp. showed no remarkable decrease in any of the contaminated plots. More recently, Korthals et al. (1996a) have carried out an experiment on the toxic effects of Cd, Cu, Ni and Zn on the nematode assemblage of an acid sandy soil collected from a cultivated field. Some of the more important conclusions were: increasing heavy metal concentrations resulted in a significant decrease in total abundance, Maturity Index values and especially in the proportion of omnivorous and predatory nematodes compared to the total number of nematodes. These effects were in most of the cases significant at the concentration level of 200 ppm, which is comparable to the maximum concentrations used originally in present experiment. Cd being applied in a concentration of only one tenth of the other pollutants had no significant effects on the target parameters.

In another study on application of sewage sludge contaminated with various heavy metals Georgieva et al. (2002) found Cu and Zn to have a negative effect on various parameters of a nematode assemblage on a sandy loam in England. Cu, Zn and Zn+Cu decreased taxon richness and MI, affected c-p group and feeding group distribution.

3. Objectives

Heavy metal pollution in soils constitutes an acute problem threatening various groups of living organisms, including man. Since each of the major compartments of such a food-chain system reacts to the provocation according to its own regulatory mechanisms, a thorough study on this type of environmental disturbance should involve several trophic levels of an actual food chain. It is especially important nowadays when both environmental legislation and public interest requires reliable data on the biological effects of different environmental pollutants. The optimal construction to obtain results fulfilling these needs is a kind of controlled field experiment, which gives more information on actual (in vivo) processes in a complex system than an over-simplified laboratory experiment (Korthals, 1997), without bearing all the uncertainties and contingency of an observational field study on a contamination event (Korthals, 1997). In addition, it is presumable that results from the above mentioned exploratory studies can not be generalised to global level, i.e. these experiments should be possibly set up under different local conditions (Kádár, 1995). In this case the advantages of extending our knowledge are expected to compensate for the obvious contamination of a given area. Such a complex food-chain experiment is being carried out after artificially contaminating small parts of agrobiotopes at different experimental fields of Research Institute for Soil Science and Agricultural Chemistry (RISSAC) of the Hungarian Academy of Sciences.

In my thesis research I studied possibilities to apply free living terrestrial nematodes as indicators of soil microelement pollution effects.

The main objective of the work was to study how microelement contamination of various concentrations affects terrestrial nematode assemblages. The experimental approaches were as follows:

1. Exploring medium-term effects of 13 microelements on nematode assemblages, 5.5-6 years after application. It was studied whether these effects could be exclusively negative and whether it is possible to find differences in intensity of the effects.

2. Study of dose-dependent effects. A concentration gradient of the microelements still affective after 6 years (Cd, Cr, Cu, Se, Zn) was analysed for nematological effects.

3. Analysis of long-term (up to 10 years) effects of elements shown to be effective earlier (Cd, Cr, Se, Zn). It was also studied how much phytotoxic and nematode-depressive effects coincide.

4. Materials and methods

Sampling location was an experimental field of Research Institute for Soil Science and Agricultural Chemistry (RISSAC) of the Hungarian Academy of Sciences at Nagyhörcsök, Hungary. This area is located on the Western edge of the Hungarian Great Plane (UTM-code: CT 00). Soil samples were collected once yearly between 1996 and 2001. Soil type of the site is calcareous loamy chernozem with medium to deep humus layer.

The soil contamination experiment was commenced in April, 1991. Initial nominal element concentrations were set to 30 $mg \cdot kg^{-1}$, 90 $mg \cdot kg^{-1}$, and 270 $mg \cdot kg^{-1}$ („total" values). Nematode samples were taken in November 1996 and June in the subsequent years.

Following sample extraction with Cobb's decanting and sieving method (modified according to s'Jacob and van Bezooijen 1984). Subsequently nematodes were counted (typically approx. 20% of the obtained suspension) then after fixing with 80-90 °C hot formalin (cc. 8%), stored until further processing.

Methods for data processing included the following steps: calculating Maturity Index (Bongers, 1990), Structure Index (Ferris et al., 2001), performing ANOVA for nematode densities, evaluation of Maturity Index data using the non parametric Kruskal-Wallis ANOVA by ranks in order to compare effects of heavy metal treatments (Statistica for Windows 5.00 software package).

5. Results

The results displayed by the 24 tables and 5 graphs of the dissertation are condensed in the following paragraphs:

1. During „screening" the 270 $mg \cdot kg^{-1}$ load of the 13 microelements (Al, As, Ba, Cd, Cr, Cu, Hg, Mo, Ni, Pb, Se, Sr és Zn) nematode densities, taxon numbers and nematological indices were negatively affected by Cr and Se, while Zn treatment resulted in a stimulating effect. Cd and Cu also showed some effects, however these required a more thorough analysis. All the other elements were proved to be ineffective under the given circumstances. The latter finding could be attributed to the favourable local (soil) conditions and the considerable decrease in the available concentrations.

2. Studies on dose-dependent effects of Cd, Cr, Cu, Se and Zn revealed that the concentration gradient (originally 30, 90 and 270 mg•kg^{-1}) of certain elements had a considerable impact on nematode assemblages. Namely, 90 and 270 mg•kg^{-1} Se, significantly decreased nematode density and number of taxa. Density was also decreased by the 270 mg•kg^{-1}Cr treatment. Regarding Maturity Index, MI (2-5), i.e. the index calculated with the exclusion of the most typical colonizer c-p 1 group, consistently decreased along the increasing Cr and Se load. Similarly, the Structure Index decreased markedly in the Cr-, Se- and to a much lesser extent in the Zn plots as well. The c-p group distribution was significantly affected by the increasing Cr and Se doses. Reactions showed by feeding type distribution patterns were similar to the above mentioned nematological parameters: Cr and Se caused a uniformisation of this parameter. The proportion of the most sensitive predatory and omnivorous taxa significantly decreased in these treatments. Zn treatment slightly decreased, while Cd increased their proportion. Nematode diversity profiles showed a significant decrease along the gradients of Cr and Se, while increasing Cu and Zn doses did not cause any obvious effect.

3. Results of the long-term studies on Cd, Cr, Se and Zn pollution effects: Cd that decreased crop biomass to a significant extent 8 years after pollution hardly caused any notable effect on nematodes. Cr, however, though it lost its phytotoxic character after the first years, decreased *Aporcelaimellus* density and Maturity Index but increased *Pratylenchus* density and ratio of bacterial feeding nematodes compared to fungal feeders. Furthermore, it altered the proportion of other feeding groups and c-p group distribution as well. Se proved to be highly toxic in the measured available concentration of 11 mg•kg^{-1}. In the course of the long-term experiment, Zn has apparently lost its favourable character measured in the first year of our study. There appeared a considerable fluctuation in the composition of the nematode assemblage in between the studied years.

5.1. New observations

1.) Documentation regarding various microelements of previously unknown nematological effects. Al, As, Ba, Hg, Mo, Ni, Pb and Sr given in a nominal total concentration of 270 mg•kg^{-1} 6 years prior to sampling caused no effect on nematode assemblages of the calcareous loamy chernozem site. The current „available" concentrations to which the above statement refers were: Al: 66,4 mg•kg^{-1}, As: 41,8 mg•kg^{-1}, Ba: 56,2 mg•kg^{-1}, Hg: 22,8mg•kg^{-1} Mo: 8,1 mg•kg^{-1}, Ni: 51,9 mg•kg^{-1}, Pb: 188,5 mg•kg^{-1}, Sr: 132,5 mg•kg^{-1}. Similarly, Cd in a dose of 190 mg•kg^{-1} and Cu in a dose of 133 mg•kg^{-1} did not cause any obviously negative nematological reaction, though these elements affected certain parameters in the beginning of the experiment.

2.) Articulated demonstration of the dose-dependent harmful effects caused by the depressive elements (Cr and Se) for the whole nematode fauna as well as for certain taxa and functional groups. Accordingly, it was shown that nematodes are more sensitive indicators of the Cr-pollution than several crop plants. Especially large predatory and omnivorous Dorylaimid nematodes were sensitive to Cr-effects even in the medium- and the long-term experiments. Among plant feeding nematodes members of the genus *Pratylenchus* and (although to an insignificant extent) family Tylenchorchynchidae decreased in dominance, while the genus *Paratylenchus* reacted to this treatment with a slight increase.

3.) In spite of the fact that Se given in a high dosis generally supressed nematode taxa, certain Cephalobid bacterial feeders (as *Chiloplacus* and *Acrobeloides*) survived even this stress. Therefore the Se-tolerance of these nematodes can be reasonably declared.

4.) Zn in an available concentration of 143 mg•kg^{-1} had a clear stimulating effect on various nematological parameters up to six years after the contamination. This proves that nematode assemblages are able to indicate positive microelement effects as well. This finding underlines the excellent bioindicative character of free-living nematodes.

5.) Parameters of partly similar background, as Maturity Index and Structure Index, as well as the diversity profiles, which stand on a completely different theoretic basis and have never been applied in environmental nematology studies, led to largely similar conclusions regarding the effects of the studied elements. Thus these parameters together offer a promising tool for the use of nematodes in bioindication studies. Therefore it is advisable to apply these techniques simultaneously in order to improve the robustness of the results.

Further specific results:
Tolerance of *Aphelenchoides* genus to As, Hg and Sr: available concentrations of As above 40 mg•kg^{-1}, Hg above 20 mg•kg^{-1} and Sr above 130 mg•kg^{-1} caused an increase in their % dominance values under field conditions. This can be interpreted as a sign of tolerance for the given microelement doses.

Sensitivity of nematodes belonging to *Aphelenchus* genus to Zn mentioned by other authors earlier was underlined based on a more detailed experimental background.

6. Conclusions, proposals

As an overall consequence it can be drawn that although various pollutants have different ways of action in the soil-crop-soil biota system, most of these has not affected terrestrial nematode assemblage structure drastically (similarly to crop biomass of the experimental plots). This may most likely be attributed to the favourable soil conditions and the time elapsed since the contamination. Moreover, the moderate doses in case of certain microelements may also explain the not detectable effects of such microelements as Al, As, Ba, Hg, Mo, Ni, Pb and Sr. Further elements, as Cd and Cu affected nematode assemblage structure to a negligible extent. However, it became obvious that the pollutants of the most outstanding phytotoxic character, Cr and Se, are also harmful for the nematodes, even in the long-term. On the other hand, local nematode fauna gave favourable reaction to Zn treatment that can be termed a „microelement-completion” from agrochemical point of view.

Based on the above findings it can be stated that assemblages of terrestrial nematodes, these creatures of outstanding importance in sustaining soil productivity, give similar reactions to several microelements as important crop parameters. On the other hand, terrestrial nematodes proved to be more sensitive to long-term effects of Cr, than the experimental crops. Therefore it can be concluded, that free-living nematodes offer a useful tool for bioindication studies on microelements pollution effects.

7. Publications related to the topic of the dissertation

Articles in scientific journals:

NAGY P. (1999): Effects of an artificial metal pollution on nematode assemblage of a calcareous loamy chernozem soil. Plant and Soil 212: 35-43.

BAKONYI, G., NAGY, P., KÁDÁR I. (2003): Long term effects of heavy metals and microelements on nematode assemblage. Toxicology Letters 140-141: 391-401.

NAGY, P., BAKONYI, G., BONGERS, T., KÁDÁR I., FÁBIÁN M., KISS, I. (2003): Effects of microelements on soil nematode assemblages seven years after contaminating an agricultural field. The Science of the Total Environment, 320:131-143.

KOVÁTS, N., REICHEL, A., SZALAY, T., BAKONYI, G., NAGY, P. (2004): Assessment of soil contamination using ToxAlert test. Journal of Hungarian Geomathematics. http\\:www. sci.u-szeged.hu/foldtan/geomatematikai_szakosztaly/JHG/default.htm

Conference abstracts:

NAGY, P. (1998): Medium-term effects of an artificial heavy-metal pollution on soil nematode fauna. Abstracts of the 24th International Symposium of the European Society of Nematologists.

EKSCHMITT, K., BAKONYI, G., BONGERS, M., BONGERS, T., BOSTRÖM, S., DOGAN, H., HARRISON, A., NAGY, P., O'DONNELL, A.G., SOHLENIUS, B., STAMOU, G.P. and WOLTERS, V. (2000): Soil biodiversity as a driving force for and an indicator of soil function. Abstracts of the 13th International Congress on Soil Zoology, Ceske Budejovice, p. 27.

NAGY, P., BAKONYI, G., FÁBIÁN, M., and KISS, I. (2000): Are heavy metals applied in high concentrations still toxic over 5 years after contaminating an agricultural field? Abstracts of the 13th International Congress on Soil Zoology, Ceske Budejovice, p. 207.

LEFLER K., KOMÁROMI J., NAGY P.: Mikroelemek akut toxicitásának vizsgálata szabadon élő fonálférgeken. A VI. Magyar Ökológus Kongresszus előadásainak és posztereinek összefoglalói, p. 169.

NAGY P., BAKONYI G., KÁDÁR I.: Terepi mikroelemszennyezés hatásai a szabadon élő talajlakó fonálférgekre eltérő talajtípusokon. A VI. Magyar Ökológus Kongresszus előadásainak és posztereinek összefoglalói, p. 200.