Pollutant-induced changes in terrestrial nematode communities

Promotor: Dr. L. Brussaard
Hoogleraar in de Bodembiologie

Co-promotor: Dr. Ir. A.M.T. Bongers
Universitair Docent
Vakgroep Nematologie

POLLUTANT-INDUCED CHANGES IN TERRESTRIAL NEMATODE COMMUNITIES

Gerardus Wilhelmus Korthals

Proefschrift

**ter verkrijging van de graad van doctor,
op gezag van de rector magnificus
van de Landbouwuniversiteit te Wageningen,
dr. C.M. Karssen,
in het openbaar te verdedigen
op maandag 26 mei 1997
des namiddags te vier uur in de Aula**

The work presented in this thesis was performed at the Department of Nematology, Wageningen Agricultural University, Binnenhaven 10, 6709 PD, The Netherlands.

CIP-DATA KONINKLIJKE BIBLIOTHEEK, DEN HAAG

Korthals, Gerardus Wilhelmus

Pollutant-induced changes in terrestrial nematode
communities / Gerardus Wilhelmus Korthals.- [S.1. : s.n.]
Thesis Landbouwuniversiteit Wageningen. - With ref. - With summary in Dutch.
ISBN 90-5485-720-x
Subject headings: terrestrial nematode communities / soil pollution/ soil biology

Foto voorkant: H. van Megen

NN08201, 2264

Stellingen

1. Een nematodengemeenschap is indicatief en onderscheidend ten aanzien van vele abiotische en biotische stress-factoren.
   Dit proefschrift.

2. Ecotoxicologisch-onderzoek in afwezigheid van planten is niet representatief voor de effecten van bodemverontreiniging *in situ*.
   Dit proefschrift.

3. Bij de inschaling van nematoden in *cp*-groepen is geen sprake van cirkelredeneringen.
   Van Straalen, N.M. 1997. In: Biological indicators of soil health and sustainable productivity (Ed. Pankhorst, C.E., Doube, B.M. and Gupta, V.V.S.R.), pp. 235-264. CAB international, Wallingford.

4. In onderzoek met betrekking tot de bodemkwaliteit wordt onvoldoende aandacht besteed aan de bodembiologie.

5. Proefdieren in kunstgrond hebben een beperkte relevantie voor de ecotoxicologie.

6. De hypothese dat nematoden een bijdrage leveren aan vegetatieveranderingen in natuurlijke systemen, is in de landbouw reeds lang bekend.
   Putten et al. 1993. Nature 362:53-55.

7. Voor het schrijven van een artikel met meerdere auteurs geldt de economische wet van de afnemende meeropbrengst.

8. Naast een algemene index voor de economie, zoals de Dow-Jones-index, dient er een algemene milieu-index te komen.

9. Natuurontwikkeling op uit produktie genomen landbouwsystemen is wetenschappelijk gezien een braak liggend terrein.

10. 'Jonge' onderzoekers zijn de nieuwe nomaden. Zij kunnen beter een camper kopen en geen partner hebben.

Stellingen behorend bij het proefschrift, getiteld 'Pollutant-induced changes in terrestrial nematode communities' door Gerard Korthals.

Wageningen, 26 mei 1997.

ABSTRACT

This thesis concerns metal-induced changes in terrestrial nematode communities exposed in microcosm-experiments and in a manipulative field experiment. Indigenous nematode communities, present in freshly collected agricultural soil, were exposed to heavy metals applied singly (Cd, Cu, Ni and Zn) or in combination under different test conditions. Depending on abiotic characteristics such as soil pH and biotic characteristics such as the presence of vegetation, the nematode community structure responded very sensitively to increasing metal concentrations.

In general, the effects of the investigated metals were enhanced with increasing exposure time and decreasing soil pH (investigated for Cu only). Furthermore, the presence or absence of vegetation (*Lolium perenne* L.) seems a very important factor in determining the final ecotoxicological effects of metals to nematodes. In soil covered with *L. perenne* the effects of Cu and Zn became apparent only at higher metal concentrations, were less severe and were more often caused in an indirect manner. In an acid sandy soil containing Cu and Zn, it was demonstrated that the dissolved Cu or Zn concentrations measured after equilibrating soil samples with a 0.01 M solution of $CaCl_2$ were not significantly different from single metal additions and that the final effects to the nematode community were all additive or less than additive. Metal-induced reductions in the population size of nematode taxa showed a low intra-taxon variation for the different metals tested. However, there were major differences between the sensitivities of the taxa. For example, some omnivorous and predatory nematodes, known to be "*K*-strategists", were very sensitive and disappeared at Cu and Zn concentrations exceeding 50 mg kg^{-1}. Classifications based on the different life-history and feeding groups both facilitated the interpretation of pollution-induced changes in the nematode community, despite the fact that on a lower level these classifications could not adequately predict the sensitivity of all nematode taxa.

It is concluded that the nematode community structure and some community parameters, such as the Maturity Index, offer excellent perspectives to assess effects of pollutants at the community level. The nematode community can provide an early and sensitive signal of increased Cu, Ni or Zn pollution in the soil. Moreover, it was demonstrated that the nematode structure may also provide opportunities to identify specific types of disturbance, *in casu* pollutants.

Keywords: Nematode community structure, heavy metals, soil pollution, Maturity Index, bioavailability, risk assessment, life-history strategy

CONTENTS

Chapter 1

INTRODUCTION

The ultimate goal in ecotoxicology is to detect, monitor and predict the effects of pollutants on ecosystems (Moriarty, 1983). Since ecotoxicology originates from several disciplines, i.e. toxicology, environmental chemistry, biochemistry and ecology, there is a long tradition in discussing the contributions of each separate discipline (Koeman, 1983; Cairns, 1990; Forbes and Forbes, 1994). Although the number of multidisciplinary studies performed in recent years has greatly increased, several approaches remain for the investigation of the potential risks of pollutants on soil fauna. They can be divided into two extremes and are here referred to as the bottom up and top down approach.

In the much used bottom up approach, pollutant effects are investigated in systems with a low complexity, such as standardized single species tests. These tests are performed on species which meet certain criteria, i.e. ease of culturing, simple food requirements and a high rate of reproduction (Edwards, 1989). Besides the many advantages of these systems, such as the strong relationship between exposure and ecophysiological response of the tested species, there are several disadvantages. One disadvantage is the over-simplification of the actual conditions in the field, or as stated by Van Straalen (1994): 'the action of toxicants cannot be studied without considering all other environmental factors affecting the animal'. This seems to be true, not only for abiotic factors, of which most may be mimicked in test systems, but especially for biotic factors which are often more difficult to manipulate in test systems. To circumvent this over-simplification, ecotoxicologists have started to increase the ecological relevance of the test systems for example by studying effects of pollutants on sublethal parameters, in multi-species systems and under variable abiotic conditions. Nevertheless, it remains difficult to generalize results due to the limited number of species which can be investigated. This can result in a gap in our knowledge of the risks for some species, i.e. with a long iteroparous life history strategy (Laskowski *et al.*, 1996), those depending on sexual reproduction (Siepel, 1994) or those species which are difficult to culture.

At present, the top down approach is gaining more attention. In it, the effects of pollutants are investigated in the field where the highest complexity can be found. These studies realistically reflect the impact of pollutants, since among other things, indirect effects of pollutants and the responses of many taxa which cannot be cultured or kept under laboratory conditions are implicitly accounted for. Despite the realism of studying polluted sites in the field (observational field studies), these studies often have severe drawbacks, such as inadequate reference sites, mixtures of different pollutants and a high natural variability (Edwards *et al.*, 1996), which may complicate the establishment of cause-and-effect relationships and the extrapolation of results to other polluted sites.

A meaningful approach between single-species laboratory tests (bottom up approach) and ecosystem studies (top down approach) seems to either study naturally occurring fauna communities which are intentionally exposed to a pollutant in field experiments or in microcosms or mesocosm tests (Cairns, 1985; Cairns, 1986; Sheppard, 1994). Especially microcosms using freshly collected field soil containing (components of) the indigenous soil organisms seems promising (Kappers and Van Esbroek, 1987; Kappers and Manger, 1990; Parmelee *et al.*, 1993). It is essential for each choice of approach that a critical selection of the organisms is used for micro- or mesocosm experiments (Edwards *et al.*, 1996). In recent years the suitability of nematodes or roundworms as test species in ecotoxicology has been strongly advocated (Vranken and Heip, 1986; Saimoilof, 1987; Freckman, 1988; Bongers, 1990; Bongers *et al.*, 1991; Vranken *et al.*, 1991; De Goede *et al.*, 1993, Yeates, 1994).

For the studies described in this thesis, nematodes were chosen as test species. The terrestrial nematode fauna is characterized by high densities and a species diversity, of which Andrassy (1991) estimated that already more than 11000 free-living species have been described. Nematodes are present in almost every habitat where they play a prominent role, for example in terrestrial food webs (Yeates, 1987; De Ruiter *et al.*, 1995). Nematodes are easy to sample and identify on the genus level and are representative of soil samples in which they are found as a consequence of their low mobility. Although the main interest in nematodes originates from the harmful effects some plant parasitic nematodes can exert on agricultural crops, there are many other nematode species contributing to soil fertility by influencing decomposition and mineralization (Tietjen, 1980; Ingham *et al.*, 1985; Yeates, 1987; Alkemade *et al.*, 1992). In order to evaluate the impact of pollution on the structure of nematode communities, there is often a need for community parameters which can facilitate the interpretation. Besides classical diversity indices such as the Shannon index, specific nematode community parameters have been developed. Some examples are the percentage of dorylaimids, the percentage of omnivorous nematodes and the proportion of "Dauer larvae" within the Rhabditida (Wasilewska, 1974; Sohlenius and Bostrom, 1984; Zullini and Peretti, 1986).

Many of the above mentioned parameters are related to the more classical approaches of classifying organisms on the basis of their life-history strategy, such as the *r-K* concept

(MacArthur and Wilson, 1967) or R-C-S concept (Grime, 1977; Greenslade, 1983). In this line Bongers (1990) proposed the Maturity Index (MI) and the Plant Parasite Index (PPI) for which nematode families were classified on the basis of their ability to colonize new habitats. These indices are based on a tentative colonizer-persister (*c-p*) scale ranging from 1 to 5. Nematode families comprising species that rapidly increase in number during the early stages of succession, were considered as colonizers and received a low *c-p* value. They have similar characteristics to *r*-strategists. The persisters among the nematodes have more characteristics in common with *K*-strategists. For further information of life-history theory and the MI concept see Whittaker (1975), Stearns (1976), Southwood (1977), Grime (1985), Bongers *et al.* (1991), De Goede *et al.* (1993), Yeates (1994), Bongers *et al.* (1995), Korthals *et al.* (1996).

It is often stated that differences between the relative abundance between different life-history strategy groups might reflect not only the successional stage of a habitat, but also the effects of disturbances (Grime, 1977; Odum, 1985). Therefore, the weighed mean of the distribution between the different *c-p* groupings, i.e. the Maturity Index, might also indicate disturbances. Promising results have been obtained with the MI concept in ecological studies. For example, it has been used to differentiate between tillage regimes (Freckman & Ettema, 1993; Yeates & Bird, 1994; Neher & Campbell, 1994), or to study the influence of manuring (Ettema and Bongers, 1993), as well as in ecotoxicological studies to measure xenobiotic-induced stress (Bongers *et al.*, 1991; Weiss and Larink, 1991; Popovici and Korthals, 1995), the effects of acidification and liming (De Goede and Dekker, 1993), organic pollution in estuarine systems (Essink and Romeyn, 1994) and ammonia deposition (Tamis, 1986 cited in Bongers, 1990).

However, most of the studies to date were observational field studies and only few dealt with the influence of persistent pollutants such as heavy metals. The present thesis will report on microcosm-experiments and one field experiment in which heavy metal-induced changes in terrestrial nematode communities were experimentally imposed and the effects studied.

Scope of the thesis

The main objectives of the present thesis are to increase our knowledge on pollutant-induced changes in terrestrial nematode communities and to evaluate the Maturity Index and some other community parameters with respect to biomonitoring and risk assessment of soil pollution. Therefore, the following experiments, in increasing complexity and, hence, ecological relevance will be presented:

In Chapter 2 the short-term effects of cadmium, copper, nickel and zinc on an indigenous nematode community from an agroecosystem are investigated. This 'natural soil method' can provide a valuable tool for the comparison of the effects of different pollutants on nematodes from several feeding and life-history strategy groups. In Chapter 3, the long-term effects of copper and zinc in relation to the presence of vegetation were investigated, in order to examine the influence of vegetation on ecotoxicological effects. Another aspect of heavy metal pollution in the field is the simultaneous presence of several pollutants. Since today's knowledge on these risks is still poorly developed, the interaction between Cu and Zn upon their bioavailability and their final joint toxicity on soil nematodes are described in Chapter 4. Chapter 5 focuses on the long-term effects of copper and pH on the nematode community under the most realistic conditions, i.e. in a field experiment with normal agricultural practices. In Chapter 6 the nematode community structure and parameters such as the Maturity Index are discussed in the light of their potential for future risk assessment of soil pollution. Finally, the conclusion of these chapters and possible implications for future risk assessment of soil pollution are discussed in Chapter 7.

References

Alkemade, R., Wielemaker, A. and Hemminga, M.A. 1992. Stimulation of decomposition of Spartina anglica leaves by the bacterivorous marine nematode *Diplolaimelloides bruciei* (Monhysteridae). J. of Exp. Mar. Biol. Ecol. 159:267-278.

Andrassy, I. 1991. A short census of free-living nematodes. Fund. Appl. Nematol. 15:187-188.

Bongers, T., 1990. The maturity index: an ecological measure of environmental disturbance based on nematode species composition. Oecologia 83:14-19.

Bongers, T., Alkemade, R. and Yeates, G.W., 1991. Interpretation of disturbance-induced maturity decrease in marine nematode assemblages by means of the maturity index. Mar. Ecol. Prog. Ser. 76:135-142.

Bongers, T., De Goede, R.G.M., Korthals, G.W. and Yeates, G.W. 1995. Proposed changes of *c-p* classification for nematodes. Russ. J. of Nematol. 3(1):61-62.

Cairns, J. Jr., 1990. The prediction, validation, monitoring and mitigation of anthropogenic effects upon natural systems. Environ. Aud. 2:19-35.

Cairns, J.Jr. 1985. Multispecies toxicity testing. Pergamon Press, Oxford. 259 pp.

Cairns, J.Jr. 1986. Community toxicity testing. American Soc. for Testing and Materials, ASTM Spec. Tech. Publ. 920, Philadelphia, PA, 345 pp.

De Goede, R.G.M. and Dekker, H.H. 1993. Effects of liming and fertilization on nematode communities in coniferous forest soils. Pedobiologia 37:193-209.

De Goede, R.G.M., Bongers, T. and Ettema, C.H. 1993. Graphical presentation and interpretation of nematode community structure: *c-p* triangles. Mededelingen Faculteit Landbouwkundige en Toegepaste Biologische Wetenschappen, Universiteit Gent 58(2b):743-750.

De Ruiter, P.C., Neutel, A.M. and Moore, J.C. 1995. Energetics, patterns and interaction strengths and stability in real ecosystems. Science 269:1257-1260

Edwards, C.A. 1989. The assessment of the ecological effects of soil pollution by chemicals. Bioindicatores Deteriorisationis Regionis, Czechoslovakia. Proc. Vth Intern. Conf., pp. 93-104.

Edwards, C.A., Subler, S., Chen, S.K. and Bogomolov, D.M. 1996. Essential criteria for selecting bioindicator species, processes, or systems to assess the environmental impact of chemicals on soil ecosystems. In: Van Straalen, N.M. and Krivolutsky, D.A. (Editors), Bioindicator Systems for Soil Pollution. NATO ASI Series 2: Environment. Kluwer, Dordrecht, pp. 67-84.

Essink, K. and Romeyn, K. 1994. Estuarine nematodes as indicators of organic pollution; an example from the Ems estuary. Neth. J. of Aqu. Ecol. 28:213-219.

Ettema, C.H. and Bongers, T. 1993. Characterization of nematode colonization and succession in disturbed soil using the Maturity Index. Biol. Fertil. Soils 16:79-85.

Forbes, V.E. and Forbes, T.L. 1994. Ecotoxicology in theory and practice. Ecotoxicology series 2, Chapman and Hall, London.

Freckman, D.W. 1988. Bacterivorous nematodes and organic-matter decomposition. Agric. Ecosyst. Environ. 24:195-217.

Freckman, D.W. and Ettema, C.H. 1993. Assessing nematode communities in agroecosystems of varying human intervention. Agric. Ecosyst. Environ. 45:239-261.

Greenslade, P.J.M. 1983. Adversity selection and the habitat templet. Am. Nat. 122(3):352-365.

Grime, J.P. 1977. Evidence for the existence of three primary strategies in plants and its relevance to ecological and evolutionary theory. Am. Nat. 111:1169-1194.

Grime, J.P. 1985. Towards a functional description of vegetation. In: White, J. (Editor). The population structure of vegetation. Junk, Dordrecht, pp. 503-514.

Ingham, R.E., Trofymow, J.A., Ingham, E.R. and Coleman, D.C. 1985. Interactions of bacteria, fungi, and their nematode grazers: effects on nutrient cycling and plant growth. Ecol. Monogr. 55:119-140.

Kappers, F.I. and Manger, R. 1990. Population dynamics of free-living nematodes in contaminated soil during the clean-up with a microbiological restoration technique. Nematologica 36:363.

Kappers, F.I. and van Esbroek, M.L.P. 1987. Use of soil organisms in experiments on vulnerability of soil to pollutants. Proceedings and information No. 38, TNO 38:161-164.

Koeman, J.H., 1983. Ecotoxicological evaluation: the eco-side of the problem. Ecotoxicol. Environ. Saf. 6:358-362.

Korthals, G.W., De Goede, R.G.M., Kammenga, J.E. and Bongers, T. 1996. The Maturity Index as an instrument for risk assessment of soil pollution. In: Van Straalen, N.M. and Krivolutsky, D.A. (Editors), *Bioindicator Systems for Soil Pollution*. NATO ASI Series 2: Environment. Kluwer, Dordrecht, pp. 85-94.

Laskowski, R., Maryanski, M., Pyza, E. and Wojtusiak, J. 1996. Sublethal toxicity tests for long-lived iteroparous invertebrates: searching for a solution. In: Van Straalen, N.M. and Krivolutsky, D.A. (Editors), *Bioindicator Systems for Soil Pollution*. NATO ASI Series 2: Environment. Kluwer, Dordrecht, pp 45-53.

MacArthur, R.H. and Wilson, E.O. 1967. *The Theory of Island Biogeography*. Princeton University Press, Princeton, New York.

Moriarty, F. 1983. Ecotoxicology. *The Study of Pollutants in Ecosystems*. Academic Press, London.

Neher, D.A. and Campbell, C.L., 1994. Nematode communities and microbial biomass in soils with annual and perennial crops. Appl. Soil Ecol. 1:17-28.

Odum, E.P. 1985. Trends expected in stressed ecosystems. BioScience 35:419-422.
Parmelee, R.W., Wentsel, R.S. and Phillips, C.T. 1993. Soil microcosm for testing the effects of chemical pollutants on soil fauna communities and trophic structure. Environ. Toxicol. Chem. 12:1477-1486.
Popovici, J. and Korthals, G.W. 1995. Soil nematodes used in the detection of habitat disturbance due to industrial pollution. Stud. Univ. Babes-Bolyai Biol. 38:1-2.
Samoiloff, M.R. 1987. Nematodes as indicators of toxic environmental contaminants. In: Veech, J.A. and Dickson, D.W. (Editors). *Vistas on Nematology.* E.O. Painter Printer Co., DeLeon Springs, Florida, pp. 433-439.
Sheppard, S.C. 1994. Toxicity testing using microcosms. In: Bitton, G., Tarradellas, J. and Rossel, D. (Editors), *Soil Ecotoxicology.* Lewis Publishers.
Siepel, H. 1994. Structure and function of soil microarthropod communities. Thesis, Wageningen.
Sohlenius, B. and Bostrom, S. 1984. Colonization, population development and metabolic activity of nematodes in buried barley straw. Pedobiologia 27:67-78.
Southwood, T.R.E. 1977. Habitat, the templet for ecological strategies. J. Anim. Ecol. 46:337-365.
Stearns, S.C. 1976. Life-history tactics: a review of the ideas. Quart. Rev. Biol. 51(1):3-47.
Tamis, W.L.M. 1986. Nematoden in een ammoniumdepositiegradient in een grove dennenbos. Hydr. Adv. Bur. Klink, Wageningen, Rapp. en Meded. 22.
Tietjen, J.H. 1980. Population structure and species composition of the free-living nematodes inhabiting sands of the New York Bight. Apex. Estuar. Coast. Mar. Sci. 10:61-73.
Van Straalen, N.M. 1994. Biodiversity of ecotoxicological responses in animals. Neth. J. of Zool. 44:112-129.
Vranken, G. and Heip, C. 1986. Toxicity of copper, mercury and lead to a marine nematode. Mar. Pollut. Bull. 17:453-457.
Vranken, G., Vanderhaegen, R. and Heip, C. 1991. Effects of pollutants on life-history parameters of the marine nematode *Monhystera disjuncta*. ICES J. Mar. Sci. 48:325-334.
Wasilewska, L. 1974. Rola wskaznikowa wszystkozernej grupy nicieni glebowych. Wiad. Ekol. 20:385-390.
Weiss, B. and Larink, O. 1991. Influence of sewage sludge and heavy metals on nematodes in an arable soil. Biol. Fertil. Soils 12:5-9.
Whittaker, R.H. 1975. The design and stability of plant communities. In: W.H. van Dobben and R.H. Lowe-McConnell (Editors). *Unifying Concepts in Ecology.* Junk, The Hague, pp. 169-181.
Yeates, G.W. 1987. How plants affects nematodes. Adv. in Ecol. Res. 17:61-113.
Yeates, G.W. 1994. Modification and qualification of the nematode maturity index. Pedobiologia 38:97-101.
Yeates, G.W. and Bird, A.F. 1994. Some observations on the influence of agricultural practices on the nematode faunae of some South Australian soils. Fundam. Appl. Nematol. 17:133-145.
Zullini, A. and Peretti, E. 1986. Lead pollution and moss-inhabiting nematodes of an industrial area. Water Air Soil Poll. 27:403-410.

Chapter 2

SHORT-TERM EFFECTS OF CADMIUM, COPPER, NICKEL AND ZINC ON SOIL NEMATODES FROM DIFFERENT FEEDING AND LIFE-HISTORY STRATEGY GROUPS

Abstract

The effects of cadmium, copper, nickel and zinc on a nematode community were examined with a 'natural soil method'. Changes in the indigenous nematode community structure were studied 1-2 weeks after the addition of these metals (as sulphates) to soil collected from an agroecosystem. The soil was acid and only contained a moderate quantity of organic matter as the main metal-binding constituent. As a result, its metal-binding capacity was rather low. The nematode community was found to be affected by increasing concentrations of Cu, Ni and Zn up to 1600 mg kg^{-1}, but not by Cd up to 160 mg kg^{-1}. EC_{50} values for the reduction in population size of individual taxa showed a low intra-taxon variation for Cu, Ni and Zn. For these heavy metals, uptake and elimination processes as well as their final effect appear similar within the same taxon. Omnivorous and predatory nematodes, known to be *K*-strategist, were among the most sensitive taxa, and were already significantly affected by 100 mg kg^{-1} Cu, Ni or Zn added to the soil. The relative abundance of the different life-history groups and, to a lesser extent, the different feeding groups indicated pollution-induced changes in the soil community. However, neither classification predicts the acute effects of Cu, Ni and Zn on different nematode genera in an adequate way.

Keywords: Nematode community structure; Heavy metals; Life-history strategy; Maturity Index; Bioavailability, Soil pollution

Introduction

It has been stated that ecologically relevant bioindicator systems should be based on information relating to different species and ecological processes (Van Straalen and Krivolutsky, 1996). Studying the effects of pollutants on naturally occurring communities of terrestrial organisms can partially fulfil this requirement.

One of the advantages of community level studies is that indirect effects of pollutants are implicitly accounted for. Food availability, competitive interactions between species or the abiotic environment, may also become influenced by pollutants, and finally affect the community in an indirect way (Clements, 1994). Another advantage is that community studies comprise the responses of many taxa which cannot be cultured or kept under laboratory conditions.

Several studies have shown that nematode communities reflect not only soil characteristics, vegetation and management practices (Yeates, 1981; Bongers, 1989; Wasilewska, 1989; Freckman and Ettema, 1993; De Goede and Bongers, 1994; Neher and

Campbell, 1994), but also the effects of pollutants such as heavy metals (Sturhan, 1986; Weiss and Larink, 1991; Parmelee *et al.*, 1993; Yeates *et al.*, 1994). However, despite the high ecological relevance of such studies, relationships between cause and effects are often complex and difficult to assess.

Several nematode classifications have been developed to facilitate the interpretation of changes in the nematode community structure. One of these classifications is based on the different feeding modes among nematodes (e.g. Freckman and Ettema, 1993; Yeates *et al.*, 1993). Another classification is based on the different life-history strategies among nematode families (Bongers, 1990; Bongers *et al.*, 1991; Bongers *et al.*, 1995). In order to calculate the Maturity Index of a nematode community, all non-plant feeding nematode taxa were placed on a colonizer-persister continuum and subsequently assigned *c-p* values ranging from 1 (colonizer, *r*-strategist, tolerant to disturbances) to 5 (persister, *K*-strategist, sensitive to disturbances).

Both the classification of nematodes in feeding groups and in life-history groups could improve the interpretation of pollution-induced changes in nematode communities as well. Therefore the present study will investigate the short-term effects of Cd, Cu, Ni and Zn on a indigenous nematode community from field collected soil of an agroecosystem. The metal addition rate at which the population size of individual taxa is reduced by 50% (EC_{50}) will be one of the parameters to compare the sensitivities of nematode taxa belonging to different feeding and life-history groups. As nematode exposure to metals strongly depends on soil-metal interactions, we also obtained information on the metal-binding characteristics of the soil.

Materials and methods

Soil characteristics and analysis

In October 1992 soil was collected from the top 10 cm of an arable field on sandy soil located 3 km North North East of Wageningen, the Netherlands. From 1980 onwards the field had been cropped with silage maize, starch potatoes and oats in a 3-year rotation (silage maize in 1992). For more details on site and soil see Korthals *et al.* (1996).

Fresh soil was passed through a 10 mm sieve to remove stones, stubble and coarse roots. After mixing, samples were taken for the determination of soil characteristics and initial metal contents. These samples were dried at 30°C and sieved to pass 2 mm. Soil texture was determined by sieve and pipette, organic carbon by acid dichromate oxidation, and the actual cation exchange capacity by the unbuffered barium chloride method. pH-KCl was measured 2 h after suspending 10 ml of soil in 50 ml 1 M KCl (Houba *et al.*, 1989).

Copper and zinc contents were determined by flame atomic absorption spectroscopy (F-AAS) with Smith-Hieftje background correction after digesting samples with a mixture of concentrated nitric and sulphuric acid (1:1 by volume). Nickel was determined in the same digest by electrothermal atomization AAS (ETA-AAS) with Zeeman background correction. The Cd content was determined by ETA-AAS after extracting samples with 3 M hydrochloric acid for 2 h on a boiling water bath.

Experimental design
The fresh soil was allowed to dry for 2 days by which time its water content had decreased to 10.9% by weight. Portions of soil equivalent to 3 kg dry weight were then treated with solutions of either Cd, Cu, Ni, or Zn sulphate and demineralized water to a total volume of 100 ml. Sulphates were used so as to limit osmotic effects of the added salts. Precipitation, as hydrated calcium sulphate (gypsum), of added sulphate and calcium ions displaced from exchange sites by adsorbing heavy-metal ions, keeps the dissolved salt concentration at a low level. Cd was applied at rates of 0, 10, 20, 40, 80, and 160 mg kg^{-1} dry weight. Cu, Ni and Zn were applied at rates of 0, 100, 200, 400, 800, and 1600 mg kg^{-1}. Differences in sulphate additions were balanced by adding calcium sulphate. There were two controls treated with $CaSO_4$ only: a low $CaSO_4$ treatment (1.4 mmol kg^{-1}) corresponding to the Cd series and a high $CaSO_4$ treatment (25.6 mmol kg^{-1}) corresponding to the other metals series. Each treatment was replicated six times. The treated soil was thoroughly mixed by hand, placed in polythene bags, covered against light and kept at 15°C.

Nematode sampling
Three of the six replicates were sampled after 1 week. The soil was mixed in its own bag and approximately 100 g was taken out to extract nematodes (Oostenbrink, 1960). Sampling of the remaining three replicates was carried out similarly after 2 weeks. The total abundance of nematodes was estimated by counting approximately 10% of the total sample under a dissecting microscope. The abundance of nematodes was expressed per 100 g dry soil after a correction for material left on the top sieve (2 mm) of the Oostenbrink elutriation apparatus (mainly fine gravel). Nematodes were heat-killed and fixed with formalin (4% at 90°C) and placed on a permanent mass-slide. At least 150 nematodes were identified at 400x-1000x according to Bongers (1988) and allocated to feeding groups according to Yeates *et al.* (1993) and *c-p* groups according to Bongers (1990) and Bongers *et al.* (1995).

Metal adsorption experiments
Metal binding characteristics of the soil were studied in a batch experiment. Twenty grams of air-dried soil that passed through a 2 mm sieve were shaken end-over-end in 250 ml centrifuge tubes for up to 2 weeks with 200 ml 0.01 M $CaCl_2$ initially containing 1, 4 or 16 mg l^{-1} Cd or 10, 40 or 160 mg l^{-1} Cu, Ni or Zn. Metals were added as sulphates and calcium sulphate was added to balance the sulphate additions as in the main experiment. After 1, 3, 6, 24, 48, 96, 192 and 384 h pH was measured in the suspension and a sample was taken from the supernatant obtained by centrifugation at 7.000 rev min^{-1} for 5 minutes. Metal concentrations in the supernatant were determined by F-AAS. The amount of metal adsorbed to the soil was calculated from the metal concentration and the total amount of metal added, corrected for the amounts removed by successive samplings.

Data analysis
Data on nematodes were analyzed by analysis of variance (Sokal and Rohlf, 1981). If necessary, logarithmic transformations were applied to meet assumptions of normality and homogeneity of variances. Tukey's multiple range test was employed to test for differences among treatments.

Replicates of week 1 and week 2 were combined in one group with an exposure time of 1-2 weeks. It was not our intention to focus on differences between 1 or 2 weeks of exposure, but because nematode extraction from all 132 samples could not be done at once, we decided to sample three replicates after 1 week and the remaining three in the following week. Furthermore, after analyzing the data with ANOVA we detected no major differences between week 1 and week 2.

Metal addition rates at which the population size of individual taxa was reduced by 50% (EC_{50}) compared with the control soil, were estimated by using a nonlinear regression estimation procedure (Bruce and Versteeg, 1992). In a number of cases the procedure did not converge. For successful cases, only EC_{50} values and 95% confidence intervals that fell within the range of metal addition rates will be discussed. Data on rare taxa (with a relative abundance approximately less than 0.5% of the total number of identified nematodes) will be not presented in detail.

Results

Soil characteristics

The soil characteristics are presented in Table 1. Soil texture and organic matter content are normal for arable land on cover sand, but pH-KCl and actual CEC are rather low. Their values reflect that the soil had not been limed for over a decade. Initial heavy metal contents are within the normal range for Dutch sandy soils and well below the official Dutch reference values that aim to distinguish between uncontaminated and contaminated soils. For this soil the reference values, which depend on clay and organic matter content (De Haan *et al.*, 1990), are 0.50 (Cd), 20 (Cu), 14 (Ni) and 68 (Zn) mg kg^{-1}.

Table 1. Soil characteristics at the beginning of the experiment

Texture (% m/m on the mineral matter)	
clay (<2 µm)	4
silt (2-50 µm)	11
sand (>50 µm)	85
Organic C (% m/m on the dry soil)	1.9
CEC ($cmol_c$ kg^{-1} dry weight)	3.6
pH-KCl	4.1
Metal content (mg kg^{-1} dry weight)	
Cd	0.33
Cu	11
Ni	4.1
Zn	38

% m/m; percentage by mass

Metal adsorption

During the 2 weeks of the metal adsorption experiment, metal concentrations in solution continued to decrease. The pH increased by 0.2 units, irrespective of the metal treatment.

In treatments without heavy metals (only $CaSO_4$ added) pH measured 4.40 after 1 h and 4.59 after 384 h. Metal treatments lowered pH, the size of the effect depending on both the metal and its addition rate (Fig. 1) but not on sampling time. There was a linear relationship between pH and the logarithm of time (*t*, h). Averaged over all treatments, this relationship was described by:

$$pH = 4.29 + 0.079(\pm 0.006)\log t$$

which accounted for 96.6% of the variance due to the time factor. Higher-order terms did not significantly improve the relationship.

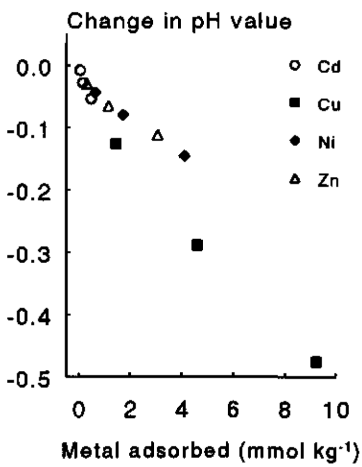


Figure 1. Effects of metal addition on pH at t = 384 h (LSD = 0.02; $P<0.05$)

Table 2. Parameters of the regression model $\log q = \alpha + \beta \cdot \log c + \Gamma \cdot \log t$ (*q* is the amount of metal adsorbed in mg kg^{-1} and *c* is the metal concentration in solution in mg l^{-1})

Metal	α	β	Γ	R^2 [a]	SE [b]
Cd	0.4995	0.7941	0.1468	0.9934	0.0373
Cu	1.7033	0.4082	0.1228	0.9872	0.0392
Ni	0.6364	0.5899	0.1661	0.9698	0.0622
Zn	0.2261	0.8350	0.1435	0.9660	0.0862

[a] Coefficient of determination. [b] Standard error of regression.

In general, data on metal adsorption cannot be described by single-reaction kinetic models (Amacher *et al.*, 1986). In this case, the relationship between pH and time suggested a Freundlich equation, which, extended with a time factor, could serve to summarize the data. The basic equation reads

$$\log q = \alpha + \beta \cdot \log c + \Gamma \cdot \log t$$

where q is the amount of metal adsorbed (mg kg^{-1}) and c is the metal concentration in solution (mg l^{-1}). The results are shown in Table 2.

Table 3. Relative abundances (as a percentage of the total nematode abundance), feeding groups, *c-p* score and total abundances in the different control treatments (mean ± SE; n = 6).

Taxon	*c-p* score	Feeding group[a]	Untreated soil t=0	Low $CaSO_4$ t=1-2 weeks	High $CaSO_4$ t=1-2 weeks	P^b
Filenchus		P	4.1 ± 0.7	6.0 ± 1.2	5.5 ± 1.0	
Tylenchus		P	0.9 ± 0.2	0.8 ± 0.5	0.8 ± 0.5	
Pratylenchus		P	12.7 ± 1.0[a]	20.4 ± 3.2[b]	19.7 ± 1.1[b]	*
Rotylenchus		P	0.7 ± 0.2	1.5 ± 0.7	1.0 ± 0.4	
Tylenchorhynchus		P	9.6 ± 1.1	13.7 ± 1.8	12.1 ± 1.1	
Dauer-larvae only	1	B	15.5 ± 1.6[b]	9.1 ± 4.3[ab]	3.4 ± 1.6[a]	*
Rhabditidae	1	B	42.3 ± 1.8[b]	30.6 ± 3.8[a]	33.9 ± 2.2[ab]	*
Acrobeles	2	B	1.3 ± 0.3	1.8 ± 0.5	1.2 ± 0.2	
Acrobeloides	2	B	7.5 ± 0.6	4.8 ± 1.0	6.2 ± 1.1	
Cephalobus	2	B	0.2 ± 0.1	1.6 ± 0.7	0.8 ± 0.5	
Cervidellus	2	B	0.1 ± 0.1	0.1 ± 0.1	0.3 ± 0.1	
Eucephalobus	2	B	3.1 ± 0.4[b]	0.8 ± 0.5[a]	1.7 ± 0.5[ab]	**
Plectus	2	B	1.5 ± 0.3	1.7 ± 0.2	1.6 ± 0.1	
Alaimus	4	B	0.3 ± 0.1	0.7 ± 0.5	0.5 ± 0.2	
Aphelenchoides	2	H	4.2 ± 0.6	4.4 ± 1.1	5.5 ± 1.3	
Ditylenchus	2	H	1.3 ± 0.3	0.4 ± 0.2	0.3 ± 0.3	
Pseudhalenchus	2	H	2.8 ± 0.6	2.3 ± 0.9	2.6 ± 0.2	
Clarkus	4	C	2.3 ± 0.5	1.9 ± 0.8	1.3 ± 0.4	
Aporcelaimellus	5	O	1.4 ± 0.3	3.0 ± 0.6	1.9 ± 0.4	
Plant feeding			28.6 ± 1.3[a]	42.7 ± 5.0[b]	39.9 ± 2.6[b]	*
Bacterial feeding			58.0 ± 1.7[b]	44.5 ± 3.7[a]	47.2 ± 2.5[a]	**
Hyphal feeding			9.5 ± 1.4	7.9 ± 1.4	9.5 ± 1.3	
Omnivorous/predators			3.9 ± 0.7	4.9 ± 0.7	3.4 ± 0.6	
c-p 1			60.0 ± 2.0	52.7 ± 2.4	56.6 ± 2.5	
c-p 2			32.8 ± 2.2	35.2 ± 1.4	35.7 ± 2.5	
c-p 3			1.3 ± 0.4	1.8 ± 0.6	1.1 ± 0.3	
c-p 4			4.0 ± 0.9	4.7 ± 1.3	3.3 ± 0.8	
c-p 5			2.0 ± 0.4[a]	5.6 ± 1.5[b]	3.3 ± 0.7[ab]	*
Maturity index			1.55 ± 0.03[a]	1.75 ± 0.07[b]	1.61 ± 0.05[ab]	*
Total abundance (per 100 g)			2927 ± 162[b]	1879 ± 143[a]	2140 ± 77[a]	***

[a] P, plant feeding; B, bacterial feeding; H, hyphal feeding; O, omnivorous; C, predators.
[b] * $P<0.05$; ** $P<0.01$; *** $P<0.001$. Significant differences between the three means within rows ($P<0.05$, HSD) are indicated by different letters.

Nematodes: Control soils

The total nematode fauna consisted of 36 genera among 25 families, of which on average 17 different taxa were found in the control samples. Most taxa belonged to the bacterial feeders or plant feeders, of which Rhabditidae, Pratylenchidae, Dolichodoridae, Cephalobidae and Tylenchidae formed approximately 80% of the total nematode fauna (Table 3). Some parameters of the nematode community found at the start of the experiment were significantly different from data obtained for both control soils after 1-2 weeks experimentation. However, no significant differences could be detected as a consequence of the different amounts of $CaSO_4$ added.

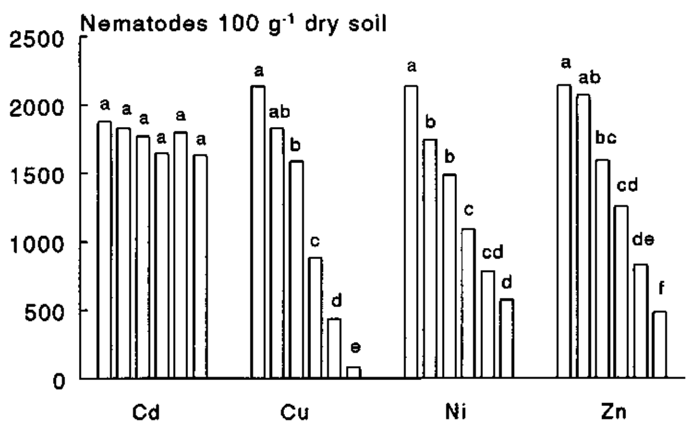


Figure 2. Effects of Cd (0, 10, 20, 40, 80 and 160 mg kg^{-1}) and Cu, Ni and Zn (0, 100, 200, 400, 800 and 1600 mg kg^{-1}) on total nematode abundance after 1-2 weeks exposure. Different letters indicate significant differences between treatments with one metal (HSD test, P<0.05).

Nematodes: Metal addition experiments

1. Total abundance of nematodes and trophic structure

The total abundance of nematodes significantly decreased with higher Cu, Ni and Zn dosages, but were not affected by the investigated range of Cd concentrations (Fig. 2). Omnivorous and predatory nematodes appeared very sensitive and their population size was already significantly smaller in soils with Cu, Ni or Zn additions of 100 mg kg^{-1} (Fig. 3).

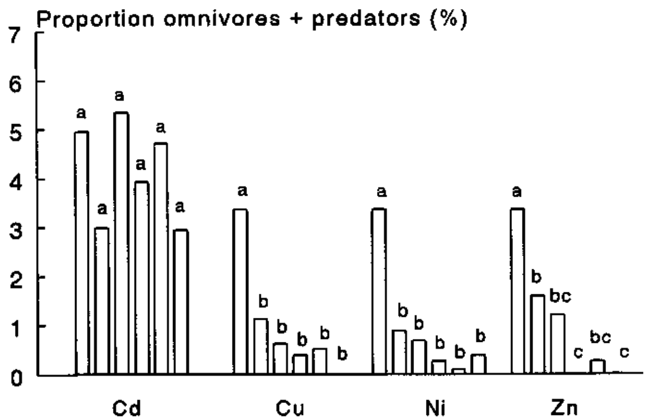


Figure 3. Effects on omnivores and predators (as a percentage of the total nematode community) of exposure to Cd (0, 10, 20, 40, 80 and 160 mg kg^{-1}) and Cu, Ni and Zn (0, 100, 200, 400, 800 and 1600 mg kg^{-1}) for 1-2 weeks. Different letters indicate significant differences between treatments with one metal (HSD test, P<0.5).

The relative abundances of bacterial, plant and hyphal feeding nematodes, were only significantly affected at the highest Cu addition of 1600 mg kg^{-1} (Table 4). The relative abundance of bacterial feeding nematodes was significantly higher, and the relative abundances of the plant and hyphal feeding nematodes were significantly lower than in the control soils (Table 4).

2. Composition of taxa

The addition of Cu, Ni and Zn changed the composition of nematode taxa (Table 4). The relative abundances of most taxa were reduced at the highest metal additions, but for some metals *Pratylenchus* (Zn), Dauer-larvae of the Rhabditidae (Cu), Rhabditidae (Cu) and *Ditylenchus* (Ni and Zn) were found in significantly higher relative abundances than in the control soils. The EC_{50} values for several nematode taxa are presented in Table 5. The differences between the lowest and highest EC_{50} values found for taxa exposed to the same metal differed by a factor 11, 38 or 30 for Cu, Ni and Zn, respectively. Intra-taxon differences in the EC_{50} values were always less than a factor 4.5. In general, *Pseudhalenchus*, Dauer-larvae of the Rhabditidae, *Aphelenchoides*, *Pratylenchus* and

Tylenchorhynchus were the most tolerant to Cu, Ni and Zn. *Plectus*, *Clarkus*, *Aporcelaimellus*, *Prismatolaimus*, *Alaimus* and *Acrobeles* appeared to be the most sensitive taxa.

Table 4. Relative abundances (as a percentage of the total nematode abundance) , feeding groups, *c-p* score and total abundances after 1-2 weeks exposure to the highest metal concentrations (mean ± SE;*n* = 6)

Taxon	*c-p* score	Feeding group[a]	Cadmium (160 mg kg^{-1})	Copper (1600 mg kg^{-1})	Nickel (1600 mg kg^{-1})	Zinc (1600 mg kg^{-1})
Filenchus		P	3.1 ± 0.5	0.4 ± 0.4*	1.3 ± 0.5*	2.0 ± 0.4*
Tylenchus		P	0.7 ± 0.3	0.4 ± 0.4	0.0 ± 0.0	0.5 ± 0.2
Pratylenchus		P	24.7 ± 3.1	17.6 ± 3.0	26.0 ± 3.3	**29.5 ± 1.2***
Rotylenchus		P	1.0 ± 0.5	1.3 ± 1.3	2.3 ± 0.7	1.8 ± 0.6
Tylenchorhynchus		P	13.1 ± 1.2	3.2 ± 1.4*	14.8 ± 1.4	13.3 ± 1.8
Dauer-larvae only	1	B	0.0 ± 0.0	**59.9 ± 5.5***	5.0 ± 2.3	14.1 ± 7.0
Rhabditidae	1	B	30.0 ± 2.4	**73.7 ± 4.5***	43.2 ± 3.0	39.3 ± 2.9
Acrobeles	2	B	0.5 ± 0.4	0.0 ± 0.0*	0.2 ± 0.1*	0.0 ± 0.0*
Acrobeloides	2	B	9.1 ± 1.4	0.0 ± 0.0*	1.7 ± 0.3*	2.0 ± 0.5*
Cephalobus	2	B	0.8 ± 0.4	0.0 ± 0.0	0.6 ± 0.2	0.3 ± 0.2
Cervidellus	2	B	0.3 ± 0.1	0.0 ± 0.0	0.1 ± 0.1	0.0 ± 0.0*
Eucephalobus	2	B	3.4 ± 0.4	0.0 ± 0.0	0.1 ± 0.1*	0.0 ± 0.0
Heterocephalobus	2	B	0.3 ± 0.3	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
Plectus	2	B	0.7 ± 0.4	0.4 ± 0.4*	0.2 ± 0.2*	0.0 ± 0.0*
Prismatolaimus	3	B	1.0 ± 0.1	0.0 ± 0.0	0.0 ± 0.0*	0.1 ± 0.1
Alaimus	4	B	0.6 ± 0.2	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
Aphelenchoides	2	H	2.9 ± 0.6	1.8 ± 0.8*	2.2 ± 0.5	3.2 ± 0.4
Ditylenchus	2	H	1.0 ± 0.5	0.0 ± 0.0	**2.3 ± 0.5***	**2.6 ± 0.7***
Pseudhalenchus	2	H	3.3 ± 0.8	0.7 ± 0.7*	3.0 ± 0.5	4.7 ± 0.9
Clarkus	4	C	1.1 ± 0.3	0.0 ± 0.0*	0.1 ± 0.1*	0.0 ± 0.0*
Aporcelaimellus	5	O	1.7 ± 0.4	0.0 ± 0.0*	0.1 ± 0.1*	0.0 ± 0.0*
Plant feeding			42.7 ± 2.5	23.4 ± 3.2 *	45.2 ± 3.9	47.6 ± 2.1
Bacterial feeding			46.8 ± 2.0	**73.8 ± 3.1 ***	46.1 ± 3.2	41.8 ± 2.4
Hyphal feeding			7.5 ± 0.7	2.9 ± 0.9 *	8.2 ± 1.0	10.5 ± 0.9
c-p 1			52.2 ± 2.3	**95.1 ± 1.6 ***	**79.2 ± 2.1 ***	**74.8 ± 2.6 ***
c-p 2			39.9 ± 2.5	4.3 ± 1.3 *	20.1 ± 1.8 *	25.0 ± 2.5
c-p 3			1.8 ± 0.3	0.6 ± 0.4	0.0 ± 0.0	0.2 ± 0.2
c-p 4			3.0 ± 0.6	0.0 ± 0.0*	0.2 ± 0.2*	0.0 ± 0.0*
c-p 5			3.0 ± 0.7	0.0 ± 0.0*	0.2 ± 0.2*	0.0 ± 0.0*
Total abundance (per 100 g)			1635 ± 65	84 ± 15*	573 ± 77*	479 ± 89 *

[a] P, plant feeding; B, bacterial feeding; H, hyphal feeding; O, omnivorous; C, predators.
Values significantly different from controls at $P<0.05$ are indicated by asterisks (HSD within one metal series).
Values in bold type are significantly higher than the control.

Table 5. Estimates of EC_{50} (mg kg^{-1}) after an exposure period of 1-2 weeks (95% confidence interval in parentheses, n = 36).

Taxon	*c-p* score	Feeding group[a]	Copper		Nickel		Zinc	
Filenchus		P	455	(253-820)	364	(250-478)	141	(45-438)
Pratylenchus		P	508	(322-802)	883	(335-2328)	902	(418-1945)
Tylenchorhynchus		P	175	(91-335)	682	(353-1315)	710	(385-1303)
Dauer-larvae only	1	B	>1600		658	(211-2046)	1538	(1294-1824)
Rhabditidae	1	B	497	(352-697)	321	(69-1496)	444	(155-1268)
Acrobeles	2	B	147	(72-299)	64	(12-355)		
Acrobeloides	2	B	287	(183-451)	386	(222-671)	493	(308-789)
Eucephalobus	2	B	206	(107-394)			300	(135-670)
Plectus	2	B	97	(36-262)	23	(3-167)	52	(20-138)
Prismatolaimus	3	B			62	(10-400)		
Alaimus	4	B	110	(43-281)	<100			
Aphelenchoides	2	H	416	(244-710)	286	(87-942)	527	(318-873)
Pseudhalenchus	2	H	538	(361-800)	836	(245-2838)	>1600	
Clarkus	4	C	65	(20-206)	62	(15-253)	<100	
Aporcelaimellus	5	O	48	(13-172)	<100		145	(69-303)

[a] P, plant feeding; B, bacterial feeding; H, hyphal feeding; O, omnivorous; C, predators.

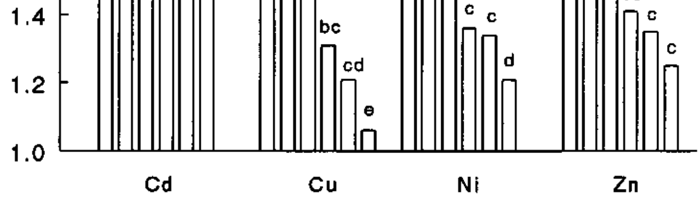


Figure 4. Effects of Cd (0, 10, 20, 40, 80 and 160 mg kg^{-1}) and Cu, Ni and Zn (0, 100, 200, 400, 800 and 1600 mg kg^{-1}) on the maturity index after 1-2 weeks exposure. Different letters indicate significant differences between treatments with one metal (HSD test, P<0.05).

3. Maturity Index

The Maturity Index was significantly lower in soils with higher concentrations of Cu, Ni and Zn (Fig. 4). These differences were driven by relative changes in the *c-p* group distribution among the non-plant feeding nematodes (Table 4), mainly because some taxa with *c-p* values of 1 or 2, e.g. Rhabditidae (including the Dauer-larvae), *Aphelenchoides* and *Pseudhalenchus*, were relatively less sensitive to higher Cu, Ni and Zn dosages than most other taxa.

Discussion

Since the present study was originally started as a range finding experiment focusing on short-term effects, the metal addition rates used were high. For this soil the official Dutch intervention values that separate seriously and not seriously contaminated soils are approximately 8 (Cd), 100 (Cu), 80 (Ni) and 340 (Zn) mg kg^{-1}. Except for Zn, the lowest addition rate already increased the metal content in soil to a level exceeding the intervention value.

Cd additions up to 160 mg kg^{-1} had no acute effects on the nematode community. Several other studies have mentioned the relatively high tolerance of nematodes to Cd (Haight *et al.*, 1982; Williams and Dusenbery, 1990; Kammenga *et al.*, 1994). The results of the adsorption experiment indicate that the absence of Cd effects in this study is not a consequence of a much stronger adsorption of Cd compared to the other metals, but that it is due to the lower addition rates. Cd was added at rates 10 times lower than the other metals because its intervention value is also lower by an order of magnitude. The rationale behind the Dutch intervention values for all 4 metals tested is the existence of 'serious danger' for a soil ecosystem when the survival of 50% of its species is threatened because the NOEC for effects on vital life-history traits like reproduction and growth is exceeded (Denneman and Van Gestel, 1990). The method applied in the derivation of intervention values takes account of toxicological data on all species (Van Straalen and Denneman, 1989). The ten times lower intervention value for Cd compared to Cu can be traced back to Cd being much more toxic to plants, as well as to oribatid mites and springtails (when exposed via food). Effects of Cd and Cu on earthworm fecundity, however, occur at quite similar concentrations in soil and would, when considered separately, not justify such a large difference in intervention values.

The Cu, Ni and Zn additions up to 1600 mg kg^{-1} significantly affected many parameters of the nematode community structure, such as the populations of certain omnivorous and predatory nematodes with a *K*-strategist type of life-history. The populations of several nematode taxa were already significantly affected by the lowest Cu, Ni and Zn addition of 100 mg kg^{-1}. This agrees with other studies on the effects of pollution in the short-term (Parmelee *et al.*, 1993; Kammenga *et al.*, 1994) as well as in the long-term (Zullini and Peretti, 1986; Weiss and Larink, 1991; Popovici and Korthals, 1995), although for some predatory nematode taxa contradicting results have been mentioned (Kappers and Wondergem-van Eijk, 1988; Yeates *et al.*, 1994).

The differences between the lowest and highest EC_{50} for different taxa exposed to the same metal were much larger than between the EC_{50} values for Cu, Ni or Zn within the same taxon. This may indicate that, at least for Cu, Ni and Zn, uptake and elimination processes as well as their final effect do not differ very much within a nematode taxon. Although this may not be true for organic pollutants, such as pentachlorophenol (Kammenga *et al.*, 1994), it emphasizes the importance of investigating which characteristics of nematodes define their sensitivity to these heavy metals.

Our data showed that closely related genera with similar feeding modes and *c-p* values, such as *Acrobeloides* and *Acrobeles* within the family Cephalobidae, can have very different toxicological responses. Furthermore, it was found that some genera with a *c-p* value of 2 (e.g. *Plectus* and *Acrobeles*) were as sensitive as genera with higher *c-p* values. These results indicate that our current knowledge is not sufficient to correctly place all nematode taxa in *c-p* groups or that the relationship between this classification and the sensitivity to short-term effects of these heavy metals may not be as straightforward as presumed.

One of the problems seems to be that present knowledge on feeding behaviour and life-history strategies among nematodes is poorly developed, and that most information is only available at a broad level of taxonomic resolution. Phylogenetic relationships, mainly based on morphological aspects, are used to make assumptions on characteristics for other, less well studied taxa. One of the difficulties with this approach is that the present classification of nematodes does not always reflect monophyletic groups (Bongers *et al.*, 1991) and that differences in traits defining life-history strategies, such as developmental rate and reproductive mode, between nematode families as well as between closely related genera do exist.

The results on the Rhabditidae demonstrate that increasing knowledge on ecological characteristics of nematode taxa can help to understand their ecotoxicological response. In contrast with the Cu-induced decline among the Rhabditidae, the abundance of Dauer-larvae was hardly affected by the treatments. It seems that Dauer-larvae cannot only survive periods of low food availability, but also periods of exposure to pollutants. This capacity is found for the enrichment opportunists (De Goede *et al.*, 1993) among nematodes and was in fact an important additional criterium to classify nematodes in *c-p* group 1 (Bongers *et al.*, 1995).

Although the present paper only studied the short-term effects of fairly high Cd, Cu, Ni and Zn additions in one soil type, we believe that experiments exposing indigenous nematode populations in their natural soil probably lead to less under- or overestimation of the real risks, then experiments carried out in water or artificial soil. The use of natural soil, however, does give rise to some complications.

For example, the soil used in this experiment was acid and only contained a moderate quantity of organic matter as the main metal-binding constituent. As a result, its metal-binding capacity was rather low. After an equilibration period of 14 days, the percentage of Cu retained by the soil ranged from 93.2% at the lowest addition rate to 36.9% at the highest rate. For Zn, the least strongly bound metal, the corresponding figures were only 20.6% and 13.0%, respectively. From the results of the adsorption experiment (Fig. 1, Table 2) it appeared that the binding of Cu and Cd was mainly effected by chemisorption, but the binding of Zn and Ni, at least at the higher addition rates, mainly by cation exchange (McBride, 1994). Had this experiment been done with a soil with a higher binding capacity, the metal concentration in solution would have been lower, metal exposure less intense and EC_{50} values higher than found now. As the relationships between soil composition and pH on the one hand and metal ion adsorption on the other differ quantitatively between heavy metals, it is also conceivable that EC_{50} values for different metals change order when another soil is used.

This also seems true for the local nematode community. Soil characteristics not only affect the bioavailability of pollutants, but they can have a pronounced influence on the nematode community structure itself. The nematode community at the start of the experiment was comparable with data obtained from other arable agroecosystems (Wasilewska, 1989; Weiss and Larink, 1991; Freckman and Ettema, 1993) and in general quite different from nematode communities found in other ecosystems. Although not investigated, it can be assumed that these differences among nematode communities will affect ecotoxicological data such as EC_{50} values for the Maturity Index or percentage bacterivorous nematodes. In the present study there were no major differences in most parameters obtained from the control soil at the start and at the end of the two weeks, which indicated that the influence of mixing the soil and additions of water and $CaSO_4$ probably did not influence the present data to a great extent. However, had this experiment been done with a nematode community from a more mature ecosystem like a forest, then the effect of mixing was probably more pronounced and could have interfered with the final effect of metal additions.

Conclusion

Changes in the relative abundances of nematode life-history groups, reflected by the *c-p* scaling, as well as in the nematode feeding groups, formed an indication of short-term effects of Cu, Ni and Zn on the soil nematode community. However, both characteristics cannot adequately predict differences in sensitivity found among nematode genera. Nevertheless, both classification methods do not only facilitate the interpretation of pollution-induced changes in nematode communities, but can also help to investigate which characteristics within the different groups of nematode taxa are of importance in defining their sensitivity to pollutants. The 'natural soil method' presented in this study seems very promising for this kind of investigations. However, we conclude that unless much effort is put in measuring the bioavailability of pollutants, it seems that the advantages of using this method mainly lie in fundamental studies. Additionally, the 'natural soil method' may be valuable in site-specific risk assessment studies.

References

Amacher, H., Kotuby-Amacher, J., Selim, H.M. and Iskander, I.K. 1986. Retention and release of metals by soils: Evaluation of several models. Geoderma 38:131-154.

Bongers, T. 1988. De nematoden van Nederland. KNNV Bibliotheekuitgave nr. 46, Pirola, Schoorl, The Netherlands, 408 pp.

Bongers, T. 1989. Ecologische typologie van de Nederlandse bodem op basis van de vrijlevende nematodenfauna. Rapport nr. 718602002, RIVM, Bilthoven, The Netherlands.

Bongers, T. 1990. The maturity index: an ecological measure of environmental disturbance based on nematode species composition. Oecologia 83:14-19.

Bongers, T., Alkemade, R. and Yeates, G.W. 1991. Interpretation of disturbance-induced maturity decrease in marine nematode assemblages by means of the maturity index. Mar. Ecol. Prog. Ser. 76:135-142.

Bongers, T., De Goede, R.G.M., Korthals, G.W. and Yeates, G.W. 1995. Proposed changes of *c-p* classification for nematodes. Russ. J. of Nematol. 3:61-62.

Bruce, R.D. and Versteeg, D.J. 1992. A statistical procedure for modelling continuous toxicity data. Environ. Toxicol. Chem. 11:1485-1494.

Clements, W.H. 1994. Assessing contaminant effects at higher levels of biological organization. Environ. Toxicol. Chem. 13:357-359.

De Goede, R.G.M. and Bongers, T. 1994. Nematode community structure in relation to soil and vegetation characteristics. Appl. Soil Ecol. 1:29-44.

De Goede, R.G.M., Bongers, T. and Ettema, C.H. 1993. Graphical presentation and interpretation of nematode community structure: *c-p* triangles. Mededelingen Faculteit Landbouwkundige en Toegepaste Biologische Wetenschappen, Universiteit Gent 58(2b):743-750.

De Haan, F.A.M., Lexmond, Th.M. and van Riemsdijk, W.H. 1990. Soil quality indicators. In: A.G. Colombo and G. Premazzi (Editors), Proceedings Workshop on Indicators and Indices for Environmental Impact Assessment and Risk Analysis, Ispra, EUR 13060EN, Office for Official Publications of the European Communities, Luxembourg, pp. 161-174.

Denneman, C.A.J. and Van Gestel, C.A.M. 1990. Bodemverontreiniging en bodemecosystemen: Voorstel voor C-(toetsings)waarden op basis van ecotoxicologische risico's. Rapport nr. 725201001, RIVM, Bilthoven, The Netherlands.

Freckman, D.W. and Ettema, C.H. 1993. Assessing nematode communities in agroecosystems of varying human intervention. Agric. Ecosystems Environ. 45:239-261.

Haight, M., Mudry, T. and Pasternak, J. 1982. Toxicity of seven heavy metals on *Panagrellus silusiae*: the efficacy of the free-living nematode as an in vivo toxicological bioassay. Nematologica 28:1-11.

Houba, V.J.G., Van der Lee, J.J., Novozamsky, J. and Walinga, I. 1989. Soil and Plant Analysis: Part 5. Soil Analysis Procedures. Department of Soil Science and Plant Nutrition, Wageningen Agricultural University, Wageningen, The Netherlands.

Kammenga, J.E., Van Gestel, C.A.M. and Bakker, J. 1994. Patterns of sensitivity to cadmium and pentachlorophenol among nematode species from different taxonomic and ecological groups. Arch. Environ. Contam. Toxicol. 27:88-94.

Kappers, F.I. and Wondergem-Van Eijk, J.A.A.M. 1988. Effects of chlorophenols on soil mesofauna. In: A.A. Orio (Editor), Environmental Contamination. CEP Consultance Ltd, Edinburgh, pp. 267-269.

Korthals, G.W., Alexiev, A.D., Lexmond, Th.M., Kammenga, J.E. and Bongers, T. 1996. Long-term effects of copper and pH on the nematode community in an agroecosystem. Environ. Toxicol. Chem. 15:979-985.

McBride, M.B. 1994. Environmental chemistry of soils. Oxford University Press, New York, Chapters 3.4 and 4.3.

Neher, D.A. and Campbell, C.L. 1994. Nematode communities and microbial biomass in soils with annual and perennial crops. Appl. Soil Ecology 1:17-28.

Oostenbrink, M. 1960. Estimating nematode populations by some selected methods. In: J.N. Sasser and W.R. Jenkins (Editors), Nematology. The University of North Carolina Press, Chapel Hill, pp. 85-202.

Parmelee, R.W., Wentsel, R.S. and Phillips, C.T. 1993. Soil microcosm for testing the effects of chemical pollutants on soil fauna communities and trophic structure. Environ. Toxicol. Chem. 12:1477-1486.

Popovici, J. and Korthals, G.W. 1995. Soil nematodes used in the detection of habitat disturbance due to industrial pollution. Stud. Univ. Babes-Bolyai, Biol. 30.

Sokal, R.R. and Rohlf, F.J. 1981. Biometry. W.H. Freeman, San Francisco, 300 pp.

Sturhan, D. 1986. Influence of heavy metals and other elements on soil nematodes. Rev. Nemat. 9:311.

Van Straalen, N.M. and Denneman, C.A.J. 1989. Ecotoxicological evaluation of soil quality criteria. Ecotox. Environ. Saf. 18:241-251.

Van Straalen, N.M. and Krivolutsky D.A. 1996. Bioindicator systems for soil pollution. NATO ASI series 16. Kluwer, Dordrecht, The Netherlands. 261 pp.

Wasilewska, L. 1989. Impact of human activities on nematode communities in terrestrial ecosystems. In: M. Clarholm and L. Bergstrom (Editors), Ecology of Arable Land. Kluwer, Dordrecht, pp. 123-132.
Weiss, B. and Larink, O. 1991. Influence of sewage sludge and heavy metals on nematodes in an arable soil. Biol. Fertil. Soils 12:5-9.
Williams, P.L. and Dusenbery, D.B. 1990. Aquatic toxicity testing using the nematode *Ceanorhabditis elegans*. Environ. Toxicol. Chem. 9:1285-1290.
Yeates, G.W. 1981. Nematode populations in relation to soil environmental factors: a review. Pedobiologia 23:312-338.
Yeates, G.W., Bongers, T., De Goede, R.G.M., Freckman, D.W. and Georgieva, S.S. 1993. Feeding habits in soil nematode families and genera - an outline for soil ecologists. J. Nematol. 25:315-331.
Yeates, G.W., Orchard, V.A., Speir, T.W., Hunt, J.L. and Hermans, M.C.C. 1994. Impact of pasture contamination by copper, chromium, arsenic timber preservative on soil biological activity. Biol. Fertil. Soils 18:200-208.
Zullini, A. and Peretti, E. 1986. Lead pollution and moss-inhabiting nematodes of an industrial area. Water Air Soil Pollut. 27:403-410.

Chapter 3

INFLUENCE OF PERENNIAL RYEGRASS ON A COPPER AND ZINC AFFECTED TERRESTRIAL NEMATODE COMMUNITY

Abstract

Effects of copper or zinc (0, 25, 50, 100, 200 and 400 mg kg^{-1}) to soil containing an indigenous nematode community were examined in the presence or absence of ryegrass (*Lolium perenne* L.). Increasing Cu and Zn additions had negative effects on the total abundance, average number of taxa, proportion of plant-feeding nematodes, proportion of omnivorous and carnivorous nematodes, nematode taxa from *c-p* groups 4 and 5 and the Maturity Index, both in the presence and absence of *L. perenne*. At intermediate Cu or Zn concentrations of 25-200 mg kg^{-1}, certain taxa had higher absolute abundances than in the control, which indicated indirect effects. Final effects of Cu or Zn were less extreme and were caused more often in an indirect way in the presence than in the absence of *L. perenne*. These results imply that the presence of vegetation is an important factor in determining the final ecotoxicological effect of pollutants. The risk assessment of pollutants should not only include the response of communities of soil organisms, but this response should be investigated in a realistic way, e.g. in the presence of plants.

Keywords: Nematodes; Zinc; Copper; Community; Soil pollution; Bioavailability

Introduction

Potential risks of pollutants have often been assessed on the basis of single species toxicity tests in the laboratory in combination with pollutant concentrations measured in the field. Apart from the difficulty of measuring relevant concentrations of pollutants in the field, discrepancies between results of laboratory and field studies may also be caused by interactions among organisms and between organisms and their environment (Cairns, 1983). In contrast to direct effects that follow on the action of a toxicant on receptor sites within the organism itself, these indirect effects occur when the pollutant interferes with for example the food availability or predator-prey interactions. It has been suggested that under realistic conditions, indirect effects may be more important for ecosystem functioning than direct effects, although they are more difficult to predict (Yodzis, 1988).

In order to improve our understanding of indirect effects, multispecies micro- and mesocosms tests are currently in use. They enable us to judge whether 'rebuilding nature' is necessary for the risk assessment of pollutants. A step in the direction of increasing ecological complexity is to expose indigenous nematode communities in micro- or mesocosms (Parmelee *et al.*, 1993; Korthals *et al.*, 1996b). Among soil ecotoxicologists these tests are typically done without plants. Plants, however, have a pronounced impact on biotic and abiotic processes in soil. With respect to nematodes, this is most straightforward for plant feeding nematodes, of which some taxa are dependent on a single host species. Some other examples of interactions between vegetation and nematodes are the observations that grass species diversity is correlated with the nematode diversity (Wasilewska, 1995), and that certain plant-feeding nematode species play an essential role in the succession of vegetation (Van der Putten *et al.*, 1993).

With respect to the impact of pollutants, plants may also affect the bioavailability of pollutants, as observed earlier for aquatic ecosystems (Brock *et al.*, 1992), and the toxic effects on plants may in turn influence the nematode community. Hence, it seems obvious that the relationship between vegetation and nematodes can strongly influence the impact of pollutants on the nematode community as well. Therefore, we examined the effects of copper and zinc on nematode communities in bare soil and soil covered with *L. perenne*. This species is the most widely utilized in West European grasslands and is not very sensitive to heavy metals (Dijkshoorn *et al.*, 1979). It is hypothesized that changes in the nematode community structure will depend not only on the metal concentrations in soil, but also on the presence of vegetation.

Materials and methods

Soil and treatments

In October 1992, soil was collected from the top 10 cm of an arable field on sandy soil located 3 km NNE of Wageningen, the Netherlands. From 1980 onwards the field had been cropped with silage maize, starch potatoes and oats in a 3-year rotation (silage maize in 1992). Some soil characteristics are listed in Table 1. For more details on site and soil see Korthals *et al.* (1996a).

After removing stones and organic material of > 1 cm, the fresh soil was mixed and dried to a water content of 10.9% by weight, to allow the addition of metal solutions without exceeding the field capacity. Copper and zinc were added to the soil in two steps in order to prevent the immediate extinction of nematodes, due to the higher bioavailable metal concentrations before an equilibrium between free and adsorbed metals has been reached.

Firstly, both metals (as sulphates) were applied at 0, 100, 200, 400, 800 and 1600 mg kg^{-1} by mixing 0-100 ml stock solutions with 3.3 kg fresh soil (equivalent to 3 kg dry soil). Differences in water and sulphate additions were balanced by adding demineralized water and calcium sulphate. Each treatment was replicated 6 times. Treated soils were placed in plastic bags, covered against light and kept at 15 °C for a period of 2 weeks, by which an equilibrium was assumed to have come about. During this period the short term effects on the nematode community were assessed (Korthals *et al.*, 1996b)

After 2 weeks, portions of treated soil equivalent to 2.5 kg dry weight were thoroughly mixed with portions of fresh and untreated soil equivalent to 7.5 kg dry soil and watered to field capacity (16.5% by weight), resulting in nominal Cu and Zn concentrations of 0, 25, 50, 100, 200 and 400 mg kg^{-1}.

Treated soils were placed in plastic 10 l pots (surface area of 452 cm^2) and *Lolium perenne* L. (10 g seed per pot) was sown on half of the pots; the other half was covered with 400 g fine gravel (heated to 120 °C to kill any nematodes present) to prevent growth of mosses and algae and to diminish evaporation. Pots were placed at random in a greenhouse (± 15 °C) and the soil water content was maintained near field capacity. No artificial light was applied and screens were used to keep the temperature as close to 15 °C as possible during sunny spells. The grass was cut 4 cm above the soil surface each month, dried at 70 °C and weighed. Three or four clippings from each pot were combined for heavy metal analysis.

Table 1. Soil characteristics at the beginning of the experiment.

Texture (% m/m on the mineral matter)	
clay (<2 μm)	4
silt (2-50 μm)	11
sand (>50 μm)	85
Organic C (% m/m on the dry soil)	1.9
CEC ($cmol_c$ kg^{-1} dry weight)	3.6
pH-KCl	4.1
Metal content (mg kg^{-1} dry weight)	
Cd	0.33
Cu	11
Ni	4.1
Zn	38

% m/m; percentage by mass

The grass was fertilized 4 times (on 15 January, 24 March, 15 June and 23 August) with 7.6 mmol KNO_3 and 5.8 mmol $Ca(NO_3)_2$ per pot, equivalent to 60 kg N and 80 kg K_2O per ha. The phosphate status of the soil precluded the need for the addition of P. At the beginning of April, another 3 g of seed was applied to 9 pots to improve the density of the stand, and 200 g gravel was added to all pots without grass to keep the soil well covered. Any emerging weeds were removed during the experiment.

Sampling

After 1 year each pot was sampled by taking 10 cores (diameter 17 mm) from the top 10 cm of the soil. After mixing the subsamples, 100 g was used for chemical analyses and 100 g served to extract nematodes.

Chemical analyses

The grass samples were digested with HF, HNO_3 and H_2O_2 (Novozamsky *et al.*, 1993a). Copper and Zn concentrations in the digest were determined by flame atomic absorption spectroscopy (F-AAS) using D_2 background correction for Zn only.

The water content in the soil samples was determined by drying at 105 °C. Soil samples were also dried at 30 °C, sieved (2 mm) and analyzed for pH and metal concentrations by the $CaCl_2$ method (Novozamsky *et al.*, 1993b). Copper and Zn were determined by F-AAS, but solutions with low Cu concentrations (< 200 μg l^{-1}) were reanalysed using electrothermal AAS.

Nematode analyses

Nematodes were extracted from 100 g soil, using a modified Oostenbrink elutriator (Oostenbrink, 1960). The total number of nematodes was estimated by counting 2 subsamples (± 10 % of the total sample) under a dissecting microscope. Nematode numbers were expressed per 100 g dry soil after a correction for material left on the topsieve (mainly gravel and plant roots). Nematodes were heat-killed and fixed in 4% formalin of 80 °C and included in a permanent mass-slide. At least 150 nematodes were identified at 400x-1000x according to Bongers (1988) and allocated to feeding groups according to Yeates *et al.* (1993). To calculate the Maturity Index, the nematode taxa were assigned a *c-p* value from 1 (colonizer, *r*-strategist, tolerant to disturbances) to 5 (persister, *K*-strategist, sensitive to disturbances), according to Bongers (1990) and Bongers *et al.* (1995).

Data processing
Effects on grass growth were evaluated from the cumulative yield over time. This relationship appeared to be linear, with an average coefficient of determination of 0.983. The metal content in grass was log transformed before being subjected to ANOVA.

Data on nematodes were analyzed by analysis of variance. If necessary, logarithmic transformation was applied to meet assumptions of normality and homogeneity of variances. Tukey's multiple range test was employed to test for differences among treatments.

Results

Soil analysis

Table 2 shows the results of soil analysis at the termination of the experiment. Averaged over all Cu and Zn treatments *L. perenne* increased pH by 0.12 (± 0.02) from 4.24 to 4.36, as a consequence of nitrogen being supplied and taken up as nitrate (Dijkshoorn *et al.*, 1983). *L. perenne* decreased the Cu concentration, but had no consistent effect on the Zn concentration.

Table 2. Average pH and metal concentrations in 0.01 M $CaCl_2$ at the end of the experiment. Cu and Zn (pots without *Lolium perenne*); Cu L and Zn L (pots with *Lolium perenne*).

Metal addition (mg kg^{-1} dry soil)	pH			
	Cu	Cu L	Zn	Zn L
0	4.28	4.39	4.28	4.39
25	4.20	4.38	4.28	4.39
50	4.26	4.38	4.29	4.40
100	4.26	4.29	4.27	4.41
200	4.18	4.32	4.29	4.32
400	4.15	4.25	4.19	4.41
	Cu concentration (mg l^{-1})		Zn concentration (mg l^{-1})	
	Cu	Cu L	Zn	Zn L
0	0.01	0.01	0.73	0.54
25	0.09	0.06	1.99	1.88
50	0.20	0.16	3.39	3.30
100	0.61	0.57	6.07	6.31
200	2.35	1.62	10.60	11.00
400	7.27	6.51	18.20	21.80

Effects on grass growth and metal content

The effects of metal treatments on *L. perenne* are summarized in figures 1 and 2. The rate of grass growth was rather low because conditions in the greenhouse (temperature, light intensity) were aimed at avoiding large fluctuations in soil temperature and water content, rather than maximizing grass growth. Copper reduced grass growth by 50 % at a metal addition of 243 mg kg^{-1} dry soil, whereas Zn reduced grass growth by 50 % at a metal addition of 385 mg kg^{-1} dry soil.

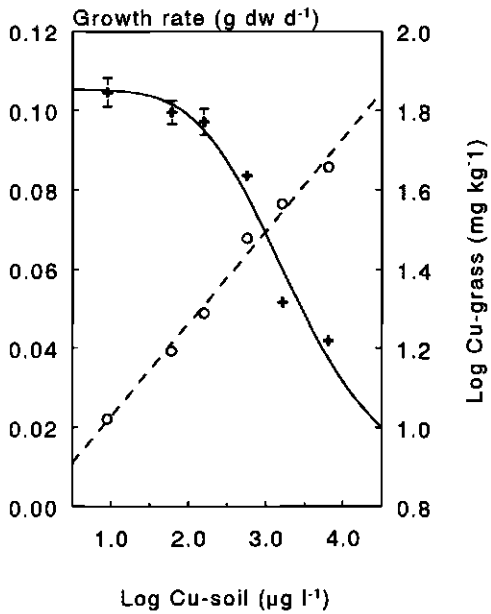


Fig. 1. Effect of Cu in the soil on the growth rate (g dw d^{-1}) and Cu uptake (mg kg^{-1}) of *Lolium perenne*.

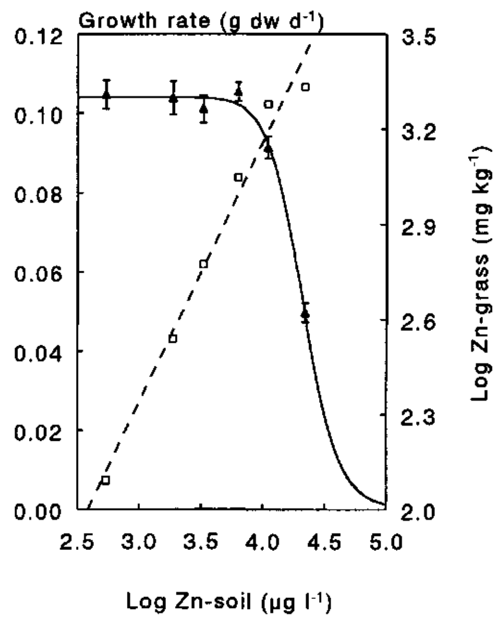


Fig. 2. Effect of Zn in the soil on the growth rate (g dw d^{-1}) and Zn uptake (mg kg^{-1}) of *Lolium perenne*.

Metal contents in *L. perenne* also strongly responded to the treatments. Grass from the control contained 10.5 mg Cu and 124 mg Zn per kg DW. At 50 % reduction in growth rate the Cu content had reached 39 mg kg^{-1} DW and the Zn content 2200 mg kg^{-1} DW. Cumulative Cu uptake reached a maximum of 0.72 mg per pot at a Cu addition rate of 100 mg kg^{-1} soil. Zinc uptake reached a maximum of 51 mg per pot when 200 mg kg^{-1} had been added to the soil. Relative to the amount of metal added, the Cu uptake reached a maximum of 0.055 % (at 25 mg kg^{-1} added). The Zn uptake reached a maximum of 3.1 % when 100 mg kg^{-1} had been added.

ECOLOGICAL EFFECTS

Total nematode abundance and number of taxa

Some data on the nematode community at the start and of the control soils with or without *L. perenne* after one year are listed in Table 3. The total nematode abundance in the control soils increased significantly in the presence of *L. perenne*, but decreased

Table 3. Absolute numbers of nematodes, number of taxa, number of feeding groups, Maturity Indices and absolute numbers of individual taxa in the control treatments at the start of the experiment and after 1 year.

	Control t=0 weeks			Control, Lolium t=52 weeks			Control, bare soil t=52 weeks			
	Mean	SE		Mean	SE		Mean	SE		*P*-value
N (100 g^{-1} DW)	2927	162	b	7278	426	c	1718	257	a	***
Nr of taxa	18.5	0.8		18.0	1.5		14.7	1.5		
% P	28.6	1.3	a	58.3	5.7	b	49.1	5.2	b	***
% B	58.0	1.7	b	32.4	5.3	a	48.8	5.5	b	***
% H	9.5	1.4	b	2.7	1.7	a	1.2	0.6	a	**
% C	2.3	0.5		3.6	2.7		0.8	0.3		
% O	1.6	0.4	ab	3.0	1.0	b	0.0	0.0	a	*
% *c-p* 1	60.0	2.0	b	15.3	4.1	a	9.8	4.2	a	***
% *c-p* 2	32.8	2.2	a	64.5	10.2	b	29.0	2.0	a	***
% *c-p* 3	1.3	0.4	a	1.5	1.0	a	60.0	6.1	b	***
% *c-p* 4	4.0	0.9		12.0	7.6		1.3	0.4		
% *c-p* 5	2.0	0.4	a	6.7	1.8	b	0.0	0.0	a	**
MI	1.55	0.03	a	2.30	0.20	b	2.53	0.10	b	***
MI2-5	2.39	0.07		2.54	0.22		2.69	0.03		
Acrobeles	36	8	a	509	280	b	8	6	a	**
Acrobeloides	217	17	b	618	333	b	52	12	a	***
Alaimus	11	5	a	52	9	b	0	0	a	***
Aphelenchoides	125	24		154	131		13	8		
Aporcelaimellus	41	10	b	211	78	b	0	0	a	***
Cephalobus	7	3		24	14		0	0		
Clarkus	70	15		275	210		12	4		
Ditylenchus	38	10		8	8		0	0		
Eucephalobus	89	11	b	481	72	c	9	2	a	***
Filenchus	115	17	a	371	44	b	249	51	b	***
Monhystera	0	0		33	33		71	40		
Plectus	43	8		96	35		35	14		
Pratylenchus	369	30	b	150	91	a	473	59	b	**
Prismatolaimus	27	9	a	41	30	a	564	185	b	*
Pseudhalenchus	82	22		36	20		4	4		
Rhabditidae	1233	52	c	424	51	b	67	21	a	***
Rotylenchus	22	7	a	669	168	b	11	11	a	**
Tylenchorhynchus	281	35	b	2908	626	c	57	14	a	***

Significant differences between means ($P<0.05$, HSD) are indicated by different letters in the same row. MI=Maturity Index; MI2-5=Maturity Index excluding the *c-p* 1 value. Feeding groups are given as follows: P=plant feeding; B=bacterial feeding; H=hyphal feeding; C=carnivorous; O=omnivorous. *P*-values: *=$0.05>P>0.01$; **=$0.01>P>0.001$; ***=$P<0.001$.

significantly in soils without *L. perenne*. The average number of taxa had not changed in the presence of *L. perenne* (18), but had declined, although not significantly, to 15 in the absence of *L. perenne*. This was mainly due to the disappearance of taxa such as *Alaimus*, *Aporcelaimellus*, *Cephalobus* and *Ditylenchus* in the control with bare soil. However, certain taxa, such as *Monhystera*, were only detected in both controls at the termination of the experiment.

Composition of taxa

In comparison with the initial abundances, the abundances of most taxa were higher in the presence of *L. perenne* and lower in the absence of *L. perenne*. The increase in absolute numbers in the presence of *L. perenne* was most obvious for plant-feeding genera such as *Rotylenchus* and *Tylenchorhynchus*, but also for taxa from other feeding groups such as *Acrobeles*, *Eucephalobus*, *Alaimus*, *Aporcelaimellus* and *Clarkus*. The opposite was detected for the genera *Pratylenchus* and *Prismatolaimus*, which reached the highest abundances in bare soil.

Trophic structure

Compared to the initial proportions it was observed that, irrespective of the presence of *L. perenne*, the relative abundance of plant-feeding nematodes increased, while that of bacterial and hyphal-feeding nematodes decreased. Proportions of the less dominant carnivorous and omnivorous nematodes were, in comparison with the start, higher in the presence of *L. perenne*, but lower in bare soils.

Maturity Indices

The MI and MI2-5 had higher values at the end of the experiment, both in the presence and absence of *L. perenne*. Although the MI values in the controls with or without *L. perenne* were not significantly different, the *c-p* group distribution indicated that the increases in MI values had different causes. The MI increase for soils with *L. perenne* was mainly due to the decrease in *c-p* 1 and increase in *c-p* 2, 4 and 5. The *c-p* group distribution in soil without *L. perenne* indicated that an increase in *c-p* 3 was the main contributor to the final MI increase.

Total nematode abundance and number of taxa

The total nematode abundance declined significantly with increasing Cu or Zn concentrations (Table 4). Increasing metal concentrations lowered the average number of

Table 4. Absolute numbers of nematodes and number of taxa, relative numbers of feeding groups and *c-p* value groups (%) and Maturity Indices after 1 year exposure to Cu and Zn in the soil.

	Copper Lolium (***CuL***)						
Metal addition (mg kg^{-1})	0	25	50	100	200	400	*P*
N (100 g^{-1} DW)	7278 b	7336 b	7005 b	7409 b	3677 ab	1519 a	**
Nr of taxa	18.0	21.3	19.3	16.3	14.3	8.3	
% P	58.3 bc	57.3 bc	65.3 c	44.1 bc	34.6 b	5.7 a	***
% B	32.4 a	36.3 ab	31.5 a	51.5 ab	57.1 b	83.0 c	***
% H	2.7 ab	2.6 ab	1.8 a	1.4 a	6.1 ab	10.5 b	*
% C	3.6	2.7	0.9	3.1	2.2	0.8	
% O	3.0 b	1.1 ab	0.5 a	0.0 a	0.0 a	0.0 a	**
% *c-p* 1	15.3	24.7	19.2	9.6	7.4	26.8	
% *c-p* 2	64.5	62.2	72.8	80.5	92.3	73.0	
% *c-p* 3	1.5	2.9	4.3	6.7	0.3	0.2	
% *c-p* 4	12.0	8.2	2.9	3.3	0.0	0.0	
% *c-p* 5	6.7 c	2.0 b	0.8 ab	0.0 a	0.0 a	0.0 a	***
MI	2.30 b	2.01 ab	1.93 ab	2.04 ab	1.93 ab	1.73 a	*
MI2-5	2.54 b	2.32 ab	2.16 ab	2.15 ab	2.00 a	2.00 a	*
	Zinc Lolium (***ZnL***)						
	0	25	50	100	200	400	*P*
N (100 g^{-1} DW)	7278 ab	8507 b	7520 ab	8909 b	6534 ab	4722 a	*
Nr of taxa	18.0	19.7	14.7	15.3	13.3	9.3	
% P	58.3 b	68.7 b	75.7 b	64.4 b	56.4 b	26.4 a	***
% B	32.4 a	28.0 a	21.9 a	28.4 a	36.8 ab	56.0 b	***
% H	2.7 a	1.9 a	2.3 a	7.0 a	6.7 a	17.6 b	***
% C	3.6 b	0.5 ab	0.0 a	0.1 ab	0.2 ab	0.0 a	*
% O	3.0 b	0.9 ab	0.1 a	0.0 a	0.0 a	0.0 a	**
% *c-p* 1	15.3	23.6	37.1	18.8	27.5	24.6	
% *c-p* 2	64.5	72.1	62.1	79.5	71.9	75.4	
% *c-p* 3	1.5	0.3	0.0	0.5	0.5	0.0	
% *c-p* 4	12.0 b	2.5 ab	0.6 a	1.2 ab	0.0 a	0.0 a	**
% *c-p* 5	6.7 b	1.6 a	0.2 a	0.0 a	0.0 a	0.0 a	***
MI	2.30 b	1.87 ab	1.65 a	1.84 a	1.73 a	1.75 a	**
MI2-5	2.54 b	2.14 ab	2.03 a	2.04 a	2.01 a	2.00 a	**

Significant differences between means ($P<0.05$, HSD) are indicated by different letters in the same row. MI=Maturity Index; MI2-5=Maturity Index excluding the *c-p* 1 value. Feeding groups are given as follows: P=plant feeding; B=bacterial feeding; H=hyphal feeding; C=carnivorous; O=omnivorous. *P*-values: *=$0.05>P>0.01$; **=$0.01>P>0.001$; ***=$P<0.001$.

taxa to a maximum reduction of 54% and 59% for the highest Cu concentration or 48% and 37% for the highest Zn concentration, in soil with or without *L. perenne*, respectively.

Table 4 (extended)

	Copper bare soil (*Cu*)						
Metal addition (mg kg^{-1})	0	25	50	100	200	400	*P*
N (100 g^{-1} DW)	1718 b	1836 b	1690 b	967 ab	412 a	144 a	***
Nr of taxa	14.7	13.3	13.0	13.0	9.0	6.0	
% P	49.1 b	47.3 b	41.7 b	49.6 b	42.0 b	8.6 a	***
% B	48.8 a	50.0 ab	55.0 ab	45.8 a	51.2 ab	68.6 b	*
% H	1.2 a	2.1 ab	2.9 abc	4.4 bc	6.8 c	22.8 d	***
% C	0.8	0.5	0.3	0.2	0.0	0.0	
% O	0.0	0.1	0.0	0.0	0.0	0.0	
% *c-p* 1	9.8	13.4	12.9	18.8	29.0	18.4	
% *c-p* 2	29.0 a	26.5 a	20.1 a	35.8 ab	64.3 bc	81.6 c	***
% *c-p* 3	60.0 c	59.1 c	66.4 c	45.4 bc	6.7 ab	0.0 a	***
% *c-p* 4	1.3	1.0	0.6	0.0	0.0	0.0	
% *c-p* 5	0.0	0.0	0.0	0.0	0.0	0.0	
MI	2.53 b	2.48 b	2.55 b	2.27 ab	1.78 a	1.82 a	**
MI2-5	2.69 c	2.70 c	2.78 c	2.52 bc	2.10 ab	2.00 a	***

	Zinc bare soil (*Zn*)						
	0	25	50	100	200	400	*P*
N (100 g^{-1} DW)	1718 d	1476 cd	1000 bc	658 ab	524 ab	336 a	***
Nr of taxa	14.7	15.7	17.3	13.3	12.7	9.3	
% P	49.1 ab	64.3 bc	73.4 c	66.9 bc	69.4 bc	31.6 a	***
% B	48.8 b	30.5 ab	20.4 a	25.7 a	25.7 a	46.0 b	***
% H	1.2 a	3.8 ab	3.3 ab	7.2 bc	4.7 abc	22.4 c	***
% C	0.8 b	0.4 ab	1.8 c	0.0 a	0.0 a	0.0 a	***
% O	0.0	0.1	0.1	0.0	0.0	0.0	
% *c-p* 1	9.8 a	20.8 ab	23.7 ab	28.2 ab	34.2 b	23.3 ab	*
% *c-p* 2	29.0 a	39.3 ab	43.4 ab	69.8 c	64.3 bc	76.7 c	***
% *c-p* 3	60.0 c	36.5 bc	23.4 ab	1.7 a	0.9 a	0.0 a	***
% *c-p* 4	1.3 a	3.4 a	9.5 b	0.3 a	0.6 a	0.0 a	***
% *c-p* 5	0.0	0.0	0.0	0.0	0.0	0.0	
MI	2.53 d	2.22 cd	2.19 bcd	1.74 ab	1.68 a	1.77 abc	***
MI2-5	2.69 b	2.52 b	2.56 b	2.03 a	2.03 a	2.00 a	***

Table 5. Mean numbers of nematode taxa after 1 year (numbers 100 g^{-1} dry soil).

			Copper Lolium (***CuL***)						
Metal addition (mg kg^{-1})			0	25	50	100	200	400	*P*
Taxon	*c-p*	T							
Acrobeles	2	B	509 b	106 ab	49 a	0 a	0 a	0 a	***
Acrobeloides	2	B	618	589	897	825	1251	879	
Alaimus	4	B	52 b	28 ab	0 a	0 a	0 a	0 a	**
Anaplectus	2	B	41	17	0	0	0	0	
Aphelenchoides	2	H	154	70	72	51	134	133	
Aporcelaimellus	5	O	211 c	29 bc	19 ab	0 a	0 a	0 a	***
Cephalobus	2	B	24 a	7 a	39 a	336 c	219 b	0 a	***
Clarkus	4	C	275	194	68	126	0	0	
Ditylenchus	2	H	8	61	38	0	25	13	
Drilocephalobus	2	B	23	19	26	0	0	0	
Eucephalobus	2	B	481 b	732 b	525 b	1732 b	245 b	6 a	***
Filenchus		P	371	314	405	202	23	18	
Monhystera	2	B	33	76	0	0	0	0	
Plectus	2	B	96 ab	49 ab	26 ab	173 b	91 ab	0 a	*
Pratylenchus		P	150	200	183	315	131	46	
Prismatolaimus	3	B	41	87	125	250	6	2	
Pseudhalenchus	2	F	36	40	21	62	29	29	
Rhabditidae	1	B	424 b	596 b	173 ab	223 ab	57 a	344 ab	*
Rotylenchus		P	669 ab	696 ab	1002 b	1098 b	524 ab	11 a	*
Seinura	2	H	0	7	0	97	68	10	
Trichodorus		P	76	78	0	0	0	0	
Tylenchorhynchus		P	2908 b	2892 b	2868 b	1580 b	745 b	3 a	***
Tylenchus		P	44	40	53	38	0	0	
			Zinc Lolium (***ZnL***)						
			0	25	50	100	200	400	*P*
Acrobeles	2	B	509 b	38 a	0 a	0 a	0 a	0 a	***
Acrobeloides	2	B	618	570	396	1290	1359	1775	
Alaimus	4	B	52 b	5 ab	10 ab	34 ab	0 a	0 a	*
Anaplectus	2	B	41	28	0	0	0	0	
Aphelenchoides	2	H	154	73	29	356	197	756	
Aporcelaimellus	5	O	211 b	56 ab	5 a	0 a	0 a	0 a	**
Cephalobus	2	B	24	28	39	17	4	0	
Clarkus	4	C	275	45	0	0	0	0	
Ditylenchus	2	H	8	67	46	125	49	0	
Drilocephalobus	2	B	23	60	20	11	0	0	
Eucephalobus	2	B	481	815	479	599	225	0	
Filenchus		P	371 b	221 ab	147 ab	143 ab	154 ab	43 a	*
Monhystera	2	B	33	22	4	0	0	0	
Plectus	2	B	96 b	11 a	0 a	0 a	0 a	0 a	**
Pratylenchus		P	150 ab	217 ab	72 a	437 bc	697 c	225 ab	***
Prismatolaimus	3	B	41	11	0	0	8	0	
Pseudhalenchus	2	F	36	33	95	132	187	76	
Rhabditidae	1	B	424	623	591	414	855	566	
Rotylenchus		P	669	448	1277	2072	1800	929	
Seinura	2	H	0	0	0	4	8	0	
Trichodorus		P	76	0	0	0	0	0	
Tylenchorhynchus		P	2908 ab	4806 b	4158 b	2998 ab	825 a	0 a	**
Tylenchus		P	44	0	0	0	0	0	

Significant differences between mean abundances of one taxon within the same experiment are indicated by different letters ($P<0.05$, HSD) in the same row. *c-p* value and T (feeding group) are given. P=plant feeding; B=bacterial feeding; H=hyphal feeding; C=carnivorous; O=omnivorous. Differences between treatment means are given by: *=$0.05>P>0.01$; **=$0.01>P>0.001$; ***=$P<0.001$.

Table 5 (extended)

			Copper bare soil (*Cu*)						
Metal addition (mg kg^{-1})			0	25	50	100	200	400	*P*
Taxon	*c-p*	T							
Acrobeles	2	B	8	0	0	0	0	0	
Acrobeloides	2	B	52	101	86	88	119	69	
Alaimus	4	B	0	0	0	0	0	0	
Anaplectus	2	B	0	0	0	0	0	0	
Aphelenchoides	2	H	13	0	8	10	16	28	
Aporcelaimellus	5	O	0	0	0	0	0	0	
Cephalobus	2	B	0	0	0	1	1	0	
Clarkus	4	C	12	8	6	0	0	0	
Ditylenchus	2	H	0 a	21 b	15 b	19 b	2 a	2 a	***
Drilocephalobus	2	B	56	58	23	0	0	0	
Eucephalobus	2	B	9	4	14	1	0	0	
Filenchus		P	249 abc	364 c	284 bc	97 ab	5 a	0 a	**
Monhystera	2	B	71 c	32 bc	11 b	0 a	0 a	0 a	***
Plectus	2	B	35	16	9	16	0	0	
Pratylenchus		P	473 c	457 c	390 bc	352 bc	165 ab	12 a	***
Prismatolaimus	3	B	564 b	590 b	647 b	264 ab	19 a	0 a	**
Pseudhalenchus	2	F	4	18	21	12	11	3	
Rhabditidae	1	B	67 ab	103 ab	105 b	71 ab	57 ab	28 a	*
Rotylenchus		P	11	5	6	8	2	0	
Seinura	2	H	4	3	0	2	0	0	
Trichodorus		P	0	0	0	0	0	0	
Tylenchorhynchus		P	57 b	31 ab	36 ab	11 ab	2 a	0 a	*
Tylenchus		P	15	0	0	4	0	0	

			Zinc bare soil (*Zn*)						
			0	25	50	100	200	400	*P*
Acrobeles	2	B	8	2	0	0	0	0	
Acrobeloides	2	B	52	69	32	72	72	95	
Alaimus	4	B	0	10	6	0	1	0	
Anaplectus	2	B	0	0	0	0	0	0	
Aphelenchoides	2	H	13	7	4	11	5	71	
Aporcelaimellus	5	O	0	0	0	0	0	0	
Cephalobus	2	B	0	0	0	0	0	0	
Clarkus	4	C	12 ab	6 ab	17 c	0 a	0 a	0 a	**
Ditylenchus	2	H	0 a	20 ab	24 ab	29 b	17 ab	10 ab	*
Drilocephalobus	2	B	56 ab	43 b	39 b	26 b	0 a	0 a	**
Eucephalobus	2	B	9	6	2	0	0	0	
Filenchus		P	249 bc	309 c	248 bc	94 ab	150 abc	23 a	**
Monhystera	2	B	71	0	0	0	0	0	
Plectus	2	B	35 b	23 ab	3 ab	0 a	0 a	0 a	*
Pratylenchus		P	473 cd	568 d	457 cd	315 bc	197 ab	60 a	***
Prismatolaimus	3	B	564 c	188 bc	47 b	1 a	0 a	0 a	***
Pseudhalenchus	2	F	4	16	4	6	1	5	
Rhabditidae	1	B	67	104	59	57	53	51	
Rotylenchus		P	11	13	5	17	7	8	
Seinura	2	H	4	0	1	0	0	0	
Trichodorus		P	0	0	0	0	0	0	
Tylenchorhynchus		P	57 c	62 bc	26 abc	8 abc	3 ab	1 a	**
Tylenchus		P	15	0	0	0	0	0	

Trophic structure

Increasing Cu and Zn concentrations caused a gradual decrease in the proportion of plant-feeding nematodes and an increase in that of the hyphal-feeding and to a lesser extent bacterial-feeding nematodes (Table 4). The decrease in plant-feeding nematodes was most extreme in ***CuL***, whereas the relative increase was greatest for the hyphal-feeding nematodes in ***Cu*** and ***Zn***. Intermediate Zn concentrations (50-200 mg kg^{-1}) tended to increase the proportion of plant feeders and to decrease the proportion of bacterial feeders. In all Cu and Zn treatments, the proportion of omnivorous and carnivorous nematodes declined, that of the omnivorous nematodes the most.

Maturity index

Irrespective of the presence of *L. perenne*, the Maturity Index and MI2-5 declined with increasing Cu and Zn additions (Table 4). Maturity Index values at concentrations of 400 (***CuL***), 200 (***Cu***), 50 (***ZnL***) or 100 (***Zn***) mg kg^{-1} were significantly lower than in the control. The decline in MI values was mainly affected by the increase in the proportion of taxa with *c-p* value 1 or 2 (*i.e. Acrobeloides*, *Aphelenchoides* and Rhabditidae), while that of most other taxa declined. From these taxa, the decline in *Prismatolaimus* in ***Cu*** and ***Zn*** was most obvious and had the largest impact on the final MI value.

Composition of taxa

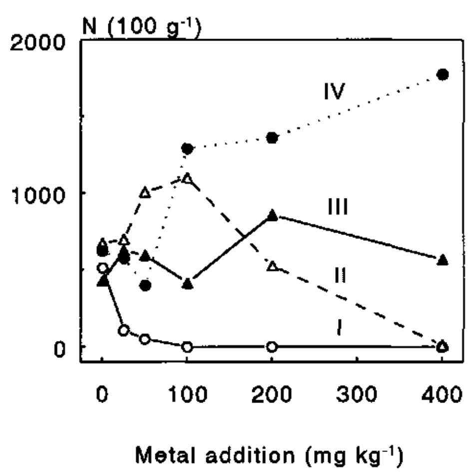


Fig. 3. Change in population numbers of 4 taxa to increasing concentrations of Cu (open symbols) and Zn (closed symbols) in the presence of *Lolium perenne*. (-o-) *Acrobeles*; (-Δ-) *Rotylenchus*; (-▲-) Rhabditidae; (-●-) *Acrobeloides*.

Irrespective of the presence of *L. perenne*, most taxa had declining population numbers with increasing metal concentrations (Table 5). This response (response type I Fig. 3) is most common from a toxicological point of view. However, certain taxa responded differently to the metal additions in one or more of the treatments. The absolute numbers of some taxa were negatively affected by increasing Cu and Zn additions in the absence of *L. perenne*, but were increased at one or more intermediate concentrations in pots with *L. perenne* (response type II Fig. 3). A type II response was found for *Rotylenchus* and *Seinura* in both the **CuL** and **ZnL** experiments, for *Tylenchorhynchus* and *Pratylenchus* in the **ZnL** and for *Cephalobus*, *Prismatolaimus* and *Eucephalobus* in the **CuL**. Some taxa were hardly affected (response type III Fig. 3) or tended to increase at intermediate or high Cu and Zn concentrations (response type IV Fig. 3). Response types III and IV were found for *Pseudhalenchus*, *Ditylenchus*, Rhabditidae, *Acrobeloides* and *Aphelenchoides.*

Discussion

ECOLOGICAL EFFECTS

The nematode community at the start of the study was comparable with that in other arable agroecosystems (Wasilewska, 1989; Weiss and Larink, 1991; Freckman and Ettema, 1993). Based on the high number of bacterivorous nematodes with a *c-p* value 1, observed at the start and the significant decline in both control soils at the termination of the experiment we assume that initially the soil was disturbed, probably because the soil was collected shortly after harvest, and that during the experiment the nematode communities followed trends previously observed after disturbance and subsequent recovery (Ettema and Bongers, 1993; De Goede *et al.*, 1993). However, the presence or absence of *L. perenne*, did influence this process of secondary succession.

Total nematode numbers in control soils with *L. perenne* increased, which was most pronounced for some obligate plant-feeding taxa (*Rotylenchus* and *Tylenchorhynchus*), as well as for a few omnivorous and carnivorous taxa. This follows the observation that the quantity and quality of plants, in combination with the addition of plant nutrients can increase the population size of not only plant-feeding taxa, but also taxa from other feeding groups (Yeates, 1987).

In contrast, the total number of nematodes, the average number of omnivorous and carnivorous nematodes and taxa from *c-p* groups 4 and 5 had declined in soils without *L.*

perenne. These changes indicate unnatural conditions in the bare soils, causing a steady decline in food-availability (indicated by a significant decrease in the number of Rhabditidae). Unnatural conditions may also have been caused by covering bare soil with gravel. Although not quantified, the absence of plant roots in combination with the gravel resulted in a more compact soil with a higher, less variable soil moisture content, all important "driving" factors for nematode communities (*c.f.* Yeates, 1981).

There were very few taxa not affected (*Filenchus*) or which benefitted (*Pratylenchus* and *Prismatolaimus*) from the conditions in the bare control soils. For *Prismatolaimus*, a bacterial feeder preferring wet conditions, this is less surprising than for the other two taxa, which are both plant feeding taxa. No living roots were present in these soils, but it is possible that in comparison to the initial populations, *Pratylenchus* could persist, or in the case of *Filenchus* even increase, since this taxon can probably feed on fungi as well.

ECOTOXICOLOGICAL EFFECTS

The purpose of this experiment was to compare the effects of Cu and Zn on a nematode community in bare soil and soil with *L. perenne*. At the community level, Cu and Zn reduced the total nematode abundance, average number of taxa, proportion of plant feeding nematodes, proportion of omnivorous and carnivorous nematodes and the Maturity Index. In general these findings are in agreement with results obtained earlier in field studies (Zullini and Peretti, 1986; Weiss and Larink, 1991; Popovici and Korthals, 1995; Korthals *et al.*, 1996a).

At the population level, most taxa showed the expected response to both metals, i.e. numbers declined with increasing metal concentrations. However, at intermediate Cu or Zn concentrations (25-200 mg kg^{-1}), certain taxa appeared to be stimulated (response type II, Fig. 3) in absolute abundances. Based on short-term exposure periods, Cu and Zn ions may have stimulated the hatching of nematode eggs (Clarke and Shepherd, 1966). However, since our data were obtained after a period of one year, the results are more likely due to less food competition and/or predation experienced by the tolerant taxa (Hendrix and Parmelee, 1985). This kind of results form an example of indirect effects, which to our opinion may be expected more often when exposing whole communities to pollutants under realistic conditions.

By comparing the experiments done in bare soil with those in the presence of *L. perenne*, it became clear that many of the observed effects occurred at higher metal addition rates, were less extreme and may have been caused in a indirect way. One possible explanation is that *L. perenne* and fertilizers enriched the soil, which caused not only 'ecological effects' but interfered with the ecotoxicological effects of Cu or Zn as well. The observation that the presence of vegetation reduced the 'stress' to the nematode community, agrees with the view that quantity and quality of food can influence the response of individual species as well as whole communities to pollutants (Vanni and Lampert, 1992; Norberg-King and Schmidt, 1993; Barreiro Lozano and Pratt, 1994).

One other possible explanation is that *L. perenne* may have influenced metal bioavailability. Based on available metal concentrations measured with the $CaCl_2$ method, the nematodes were exposed to fairly similar concentrations in the presence or absence of the grass. These concentrations, however, characterized the bulk soil and not the rhizosphere soil, where the grass may have effected a larger increase in soil pH due to excess anion uptake when supplied with nitrate as nitrogen source. The influence of nitrate or ammonium uptake on soil pH and metal bioavailability has been observed earlier (Dijkshoorn *et al.*, 1979). Compared with the experiment by Dijkshoorn *et al.* (1979), in which a very similar soil was used, metal toxicity to *L. perenne* appeared much lower in our experiment. For example, the metal addition rates associated with a 50 % reduction in yield were approximately 2.4 times higher in our case. The slower rate of growth due to less favourable growing conditions is likely to be responsible for this apparent increase in metal tolerance. The demands placed on the nutrient and water uptake function of the roots and, consequently, the effects of root malfunctioning will be stronger in vigorously growing plants than in plants with a limited light supply.

Metal uptake by *L. perenne* could also have contributed to lower metal availability. The total amount of metal removed with the grass clippings was small, but the effect of metal binding to and in the roots was not accounted for. As both the increase in pH and metal uptake may be expected to have their largest effect on bioavailability in the rhizosphere, where many nematode taxa aggregate, they could have affected the present results.

The effects of Cu and Zn on *L. perenne* itself may have influenced the quantity and quality of the food source for several nematode taxa, especially those which are completely dependent on plants. This is not only negative, since, especially with intermediate pollutant concentrations, it may also be beneficial to herbivores or plant-associated organisms, for example due to an increase in N-availability, a higher leakage of root exudates or a break-down of the plant's defence strategy (White, 1984). This may play a role in the increased abundances of *Tylenchorhynchus* at intermediate Zn concentrations in ***ZnL***.

The present study demonstrated that a field collected nematode community changes due to biotic (presence of *L. perenne*) as well as abiotic (heavy metals) factors. It is clear that the presence of vegetation is a very important factor in determining the final ecotoxicological effects of Cu and Zn. In soils covered with *L. perenne* it was found that the effects of Cu and Zn became apparent at higher metal concentrations, were less severe and were more often caused in an indirect way. The present data were based on a monoculture of *L. perenne* during one year. The effects of pollution on nematode communities in soil with a more diverse vegetation can be expected to be even more complex. Therefore, it is recommended that future risk assessment studies are based on experimental methods using increased ecological complexity. To expose natural nematode communities in micro- or mesocosms in the presence of vegetation seems necessary.

References

Barreiro Lozano, R. and Pratt, J.R. 1994. Interaction of toxicants and communities: the role of nutrients. Environ. Toxicol. Chem. 13:361-368.

Bongers, T. 1988. De Nematoden van Nederland. KNNV Bibliotheekuitgave nr. 46, Pirola, Schoorl, 408 pp.

Bongers, T. 1990. The Maturity Index: an ecological measure of environmental disturbance based on nematode species composition. Oecologia 83:14-19.

Bongers, T., De Goede, R.G.M., Korthals, G.W. and Yeates, G.W. 1995. Proposed changes of c-p classification for nematodes. Russ. J. Nematol. 3:61-62.

Brock, T.C.M., Van den Bogaert, M., Bos, A.R., Van Breukelen, S.W.F., Reiche, R., Terwoert, J., Suykerbuyk, R.E.M. and Roijackers, R.M.M. 1992. Fate and effects of the insecticide Dursban[r] 4E in indoor *Elodea*-dominated and macrophyte-free freshwater model ecosystems: II. Secondary effects on community structure. Arch. Environ. Contam. Toxicol. 23:391-409.

Cairns, J.Jr. 1983. Are single species toxicity test alone adequate for estimating environmental hazard? Hydrobiologia 100:47-57.

Clarke, A.J. and Shepherd, A.M. 1966. Inorganic ions and the hatching of *Heterodera* spp.. Ann. Appl. Biol. 58:497-508.

De Goede, R.G.M., Bongers, T. and Ettema, C.H. 1993. Graphical presentation and interpretation of nematode community structure: *c-p* triangles. Med. Fac. Landbouw. Univ. Gent, 58:743-750.

Dijkshoorn, W., Van Broekhoven, L.W. and Lampe, J.E.M. 1979. Phytotoxicity of zinc, nickel, cadmium, lead, copper and chromium in three pasture plant species supplied with graduated amounts from the soil. Neth. J. agric. Sci. 27:241-253.

Dijkshoorn, W., Lampe, J.E.M. and Van Broekhoven, L.W. 1983. The effect of soil pH and chemical form of nitrogen fertilizer on heavy-metal contents in ryegrass. Fert. Res. 4:63-74.

Douben, P.E.T. and Siepel, H. 1993. Extrapolation from laboratory to field and from individual to populations: pitfalls to avoid. Sci. Tot. Environ. (suppl.):1025-1036.

Ettema, C.H. and Bongers, T. 1993. Characterization of nematode colonization and succession in disturbed soil using the Maturity Index. Biol. Fert. Soils 16:79-85.

Freckman, D.W. and Ettema, C.H. 1993. Assessing nematode communities in agroecosystems of varying human intervention. Agr. Ecosyst. Environ. 45:239-261.

Hendrix, P.F. and Parmelee, R.W. 1985. Decomposition, nutrient loss and microarthropod densities in herbicide-treated grass litter in a Georgia piedmont agroecosystem. Soil Biol. Biochem. 17:421-428.

Korthals, G.W., Van de Ende, A., Van Megen, H., Lexmond, Th. M., Kammenga, J.E. and Bongers, T. 1996a. Short-term effects of cadmium, copper, nickel and zinc on nematodes from different feeding and life-history strategy groups. App. Soil Ecology 4:107-117.

Korthals, G.W., Alexiev, A.D., Lexmond, Th. M., Kammenga, J.E. and Bongers, T. 1996b. Long-term effects of copper and pH on the nematode community of an agroecosystem. Environ. Toxicol. Chem. 15:979-985.

Norberg-King, T.J. and Schmidt, S. 1993. Comparison of effluent toxicity results using *Ceriodaphnia dubia* cultured on several diets. Environ. Toxicol. Chem. 12:1945-1955.

Novozamsky, I., Houba, V.J.G., Van der Lee, J.J., Van Eck, R. and Mignorance, M.D. 1993a. A convenient wet digestion procedure for multi-element analysis of plant materials. Commun. Soil Sci. Plant Anal. 24:2595-2605.

Novozamsky, I., Lexmond, Th.M. and Houba, V.J.G. 1993b. A single extraction procedure of soil for evaluation of uptake of some heavy metals by plants. Int. J. Environ. Anal. Chem. 51:47-58.

Oostenbrink, M. 1960. Estimating nematode populations by some selected methods. In: Sasser, J.N. and W.R. Jenkins (eds): Nematology. The University of North Carolina Press, Chapel Hill, pp. 85-102.

Parmelee, R.W., Wentsel, R.S. and Phillips, C.T. 1993. Soil microcosm for testing the effects of chemical pollutants on soil fauna communities and trophic structure. Environ. Toxicol. Chem. 12:1477-1486.

Popovici, J. and Korthals, G.W. 1995. Soil nematodes used in the detection of habitat disturbance due to industrial pollution. Studia Univ. Babes-Bolyai, Biologia, 38:1-2.

Van der Putten, W.H., Van Dijk, C. and Peters, B.A.M. 1993. Plant-specific soil-borne diseases contribute to succession in foredune vegetation. Nature 362:53-55.

Vanni, M.J. and Lampert, W. 1992. Food quality effects on life history traits and fitness in the generalist herbivore *Daphnia.* Oecologia 92:48-57.

Wasilewska, L. 1989. Impact of human activities on nematode communities in terrestrial ecosystems. In: M. Clarholm and L. Bergstrom (eds.), Ecology of arable land, Kluwer Academic Publishers, pp.123-132.

Wasilewska, L. 1995. Differences in development of soil nematode communities in single- and multi-species grass experimental treatments. App. Soil Ecology 2:53-64.

Weiss, B. and Larink, O. 1991. Influence of sewage sludge and heavy metals on nematodes in an arable soil. Biol. Fert. Soils 12:5-9.

White, T.C.R. 1984. The abundance of invertebrate herbivores in relation to the availability of nitrogen in stressed food plants. Oecologia 63:90-105.

Yeates, G.W. 1981. Nematode populations in relation to soil environmental factors: a review. Pedobiologia 22:312-338.

Yeates, G.W. 1987. How plants affects nematodes. Advances in Ecological Research 17:61-113.

Yeates, G.W., Bongers, T., De Goede R.G.M., Freckman, D.W. and Georgieva, S.S. 1993. Feeding habits in soil nematode families and genera - an outline for soil ecologists. J. Nematol. 25:315-331.

Yodzis, P. 1988. The indeterminacy of ecological interactions as perceived through perturbation experiments. Ecology 62:508-515.

Zullini, A. and Peretti, E. 1986. Lead pollution and moss-inhabiting nematodes of an industrial area. Water Air Soil Pollut. 27:403-410.

Chapter 4

JOINT TOXICITY OF COPPER AND ZINC TO A TERRESTRIAL NEMATODE COMMUNITY IN AN ACID SANDY SOIL

Abstract

Heavy metal toxicity to an indigenous nematode community was examined following the addition of Cu and Zn, alone or in combination, to agricultural soil. The dissolved Cu or Zn concentrations measured after equilibrating soil samples with a 0.01 M solution of $CaCl_2$ showed that the metal concentrations found in soils with combined metal additions were not significantly different from those with single metal additions. After an exposure period of six months, many nematode community parameters and individual nematode taxa were significantly affected by increasing concentrations of Cu and Zn up to 200 mg kg^{-1}. Some nematode taxa, such as *Thonus*, *Alaimus* and *Aporcelaimellus* were very sensitive and disappeared at Cu and Zn concentrations exceeding 50 mg kg^{-1}. For several nematode community parameters and nematode taxa, EC_{50} values for single metal exposures were used to calculate TU_{50} values for the joint toxicity of Cu and Zn. Based on these calculations, it is concluded that the effects of a combined exposure to Cu and Zn were additive or less than additive. Before this conclusion can be generalized, however, more data are needed on other types of soil, other pH values and other combinations of pollutants.

Keywords: Nematodes; Copper; Zinc; Joint toxicity; Bioavailability

Introduction

An important aspect in the protection of our environment is the definition of soil quality criteria. There is a strong tradition in the Netherlands to relate these criteria with ecotoxicological risk assessment. For example, the method applied in the derivation of limit values accounts for all available No Observed Effect Concentrations (NOEC) on life-history traits (growth, reproduction, mortality) of different species (Van Straalen and Denneman, 1989; Denneman and Van Gestel, 1990). To date, these limit values have been divided by a safety factor of 100 in order to define target values (Anon., 1989; Anon., 1991).

One of the arguments for the use of this safety factor was the uncertainty of the obtained limit values. Can the often very limited number of NOEC's, obtained for different organisms exposed to a single pollutant, indeed reflect the potential risk of that particular pollutant to an entire ecosystem? An even more important argument for the use of this safety factor is the simultaneous presence of several pollutants, which is the rule rather than the exception in the field. Knowledge on joint toxicity of pollutants, especially with respect to terrestrial animals, is still poor (Eifac, 1987; Hensbergen and Van Gestel, 1995).

There is general agreement that the simultaneous presence of several pollutants, although each may be below their specific limit concentrations, may still imply a risk. In the most simple case, the joint toxicity of two pollutants is additive. Even then, methods to estimate the potential risks of mixtures are only in an early stage of development (Ikeda, 1994). Besides this simple case, there are several studies indicating that the joint toxicity of pollutants can be larger (Sprague and Ramsay, 1965; Babich *et al.*, 1986; Spehar and Fiandt, 1986; Asztalos *et al.*, 1988; Kraak *et al.*, 1994) or smaller (Spehar *et al.*, 1978; Babich *et al.*, 1986; Vranken *et al.*, 1988; Kraak *et al.*, 1993) than predicted on the basis of addition of effects of single exposures. This makes standards based on data obtained from single pollutant dose-response relationships unreliable.

This holds more strongly in soils where pollutants interact with the soil matrix. Pollutants such as heavy metals may compete for binding sites in the soil. Where two or more metals are present in the soil, competition may lead to different concentrations in solution than expected from the binding behaviour of the individual metals (Van Riemsdijk and Hiemstra, 1993).

This article describes the joint toxicity of Cu and Zn to an indigenous nematode community present in agricultural soil. The phylum Nematoda is comprised of many species showing a high diversity of life cycles, feeding types and sensitivities to pollutants. Since terrestrial nematodes live in the soil pore water, they are assumed to be exposed to the pollutant concentration in the soil solution, which offers good perspectives to assess effects of pollutants in relation to their bioavailability in soil. Furthermore, by studying effects on the nematode community, one has the opportunity to examine the joint toxic effects on various parameters simultaneously, from a low (population) to a high (community) level of biological organization.

Materials and methods

Experimental design

In October 1993 soil was collected from the top 10 cm of an arable field on sandy soil located 3 km NNE of Wageningen, the Netherlands. Fresh soil was passed through a 9 mm sieve to remove stones, stubble and coarse roots. After mixing, samples were taken, dried (30°C) and sieved (2 mm) to determine some soil characteristics. The soil used in this experiment was a loamy sand (4% clay, 11% silt, 85% sand) with an organic carbon content of 1.9% by mass. The initial pH, measured in 1 M KCl, was 4.1 and the actual CEC amounted to 3.6 $cmol_c\ kg^{-1}$ (unbuffered $BaCl_2$). Initial Cu and Zn contents, as determined following digestion with a mixture of concentrated nitric and sulphuric acid, were 11 and 38 $mg\ kg^{-1}$, respectively. For more details on site and soil see Korthals *et al.* (1996a).

The fresh soil was allowed to dry to a water content of 10.6% by weight, so as to allow for metal solutions to be added without exceeding the field capacity of the soil. Copper and zinc (sulphates) were added to the soil in two steps, in order to increase the survival chances of nematode species.

First, eight concentrations of Cu (0, 100, 140, 200, 280, 400, 560 and 800 mg kg^{-1} dry weight) were combined with five concentrations of Zn (0, 100, 200, 400 and 800 mg kg^{-1}) by mixing 0-200 ml stock solutions with 3.3 kg moist soil (equivalent to 3 kg dry soil). Each combination was replicated three times. Differences in water and sulphate additions were balanced between the treatments by adding demineralized water and calcium sulphate. The treated soil was thoroughly mixed by hand, placed in polythene bags and kept at 15°C in the dark for a period of 3 weeks, after which an equilibrium between added metals and the soil was assumed.

Following this treatment, portions of treated soil (2 kg dry weight) were thoroughly mixed with portions of untreated fresh soil equivalent to 6 kg dry soil and brought to field capacity (17.7% by weight). Thus, the final metal concentration ranges were lowered by a factor 4. Plastic 7.5 l pots were filled with treated soil and covered with 800 g fine gravel (heated to 120°C to kill any nematodes present). The pots were placed at random in a greenhouse at 15°C, watered to field capacity and kept free of weeds.

Sampling

After 6 months 10 soil cores (diameter 17 mm) were taken from the top 10 cm of the soil from each pot. After mixing the subsamples, 100 g was used for chemical analyses and 100 g served to sample nematodes.

Chemical analyses

The soil samples were dried at 40 °C for 24 h and sieved through a 2 mm mesh size. Soil pH was determined in suspension according to Novozamsky *et al.*, (1993) after shaking soil samples end-over-end for 24 h with 0.01 M $CaCl_2$ in a soil to solution ratio of 1:10. A sample was taken from the supernatant obtained by centrifugation at 5000 rev min^{-1} for 10 min. and acidified by adding 1 % (by volume) of concentrated HNO_3, to prevent the adsorption of heavy metals during storage (Houba *et al.*, 1993). Copper and Zn concentrations were then determined by flame atomic absorption spectroscopy (F-AAS) with Smith-Hieftje background correction. Low Cu concentrations (< 200 mg l^{-1}) were re-analyzed using electrothermal AAS.

Nematode analyses

Nematodes were extracted from 100 g fresh soil, using a modified Oostenbrink elutriator (Oostenbrink, 1960). The total number of nematodes was estimated by counting 2 subsamples (± 10% of the total sample) under a dissecting microscope. Nematode numbers were expressed per 100 g dry soil after a correction for material left on the top sieve (mainly gravel and plant roots). Nematodes were heat-killed and fixed with formalin (90°C, 4%) and placed on a permanent mass-slide. At least 150 nematodes were identified at 400x-1000x according to Bongers (1988) and allocated to feeding groups according to Yeates *et al.* (1993) and allocated to c-p value groups according to Bongers (1990) and Bongers *et al.* (1995) in order to calculate Maturity Index values.

Data processing

Data on nematodes were analyzed by analysis of variance (Sokal and Rohlf, 1981). If necessary, logarithmic transformations were applied to meet assumptions of normality and homogeneity of variances. EC_{50} values for Cu and Zn were estimated by using non-linear regression estimation (Bruce and Versteeg, 1992) of metal concentrations against several nematode community parameters from the single metal exposure data. The concentrations of all Cu and Zn combinations were expressed in Toxic Units (TU) by dividing the sum of the individual Cu or Zn concentrations by their specific EC_{50} obtained for the single metal exposure series (Sprague, 1970; Könemann, 1981). For several nematode parameters, the exposure-response relationship (in Toxic Units) was used to estimate where a parameter was lowered by 50%, hereafter referred as TU_{50} value. TU_{50} values were used to estimate whether the effects of a combined exposure to Cu and Zn were additive (TU_{50} = 1 TU), less than additive (TU_{50} > 1 TU) or more than additive (TU_{50} < 1 TU).

Results

Chemical analysis

After six months, at the end of the experiment, the pH of the soil suspended in 0.01 M $CaCl_2$ was 4.32, with a standard deviation of 0.13. Differences between treatments were not significant. The Cu and Zn concentrations in 0.01 M $CaCl_2$ are shown in Figs. 1 and 2, respectively. Metal concentrations increased with the rate at which they had been added,

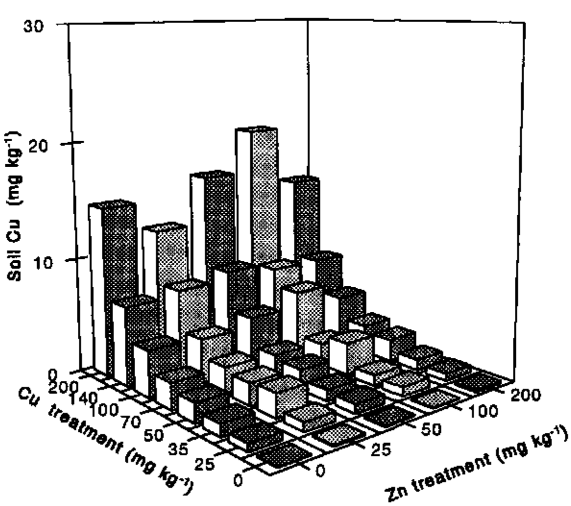


Fig. 1. Measured Cu concentrations (mg kg^{-1}) in 0.01 M $CaCl_2$.

but they were not affected by addition of the other metal. Much more Zn than Cu was extracted, 55.5% and 4.3%, respectively, indicating that Zn was less strongly bound to the soil matrix. The amount of Cu extracted as a percentage of applied Cu, increased with the higher amounts applied, whereas in the case of Zn it was always ca. 55%.

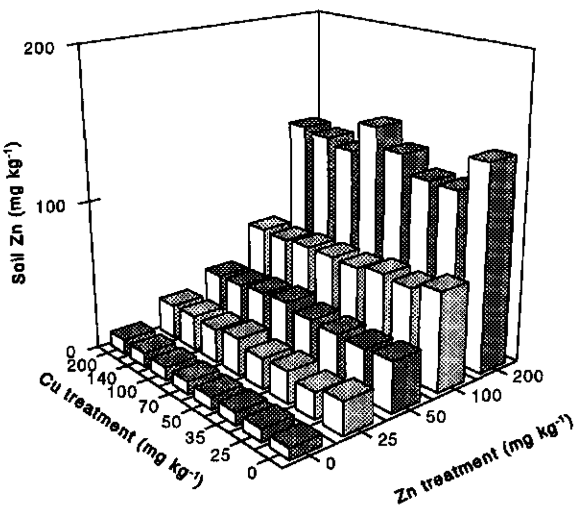


Fig. 2. Measured Zn concentrations (mg kg^{-1}) in 0.01 M $CaCl_2$.

Effects on nematodes

The mean values of several nematode community parameters and nematode taxa (for some selected treatments only), and results of ANOVA (carried out on all treatments) are presented in Tables 1 and 2. Increasing Cu and Zn caused a significant decline in most community structure parameters, with the exception of the total number of nematodes with a *c-p* values of 1 and 3 which were only significantly affected by Zn. Nematode taxa with *c-p* values 3, 4 and 5 (such as *Thonus*, *Alaimus* and *Aporcelaimellus*) were present in low numbers in the control soil and disappeared at Cu and Zn addition rates exceeding 50 mg kg^{-1}. Few nematode taxa were significantly reduced in numbers by Cu only (*Ditylenchus* and *Aphelenchus*) or Zn only (Dauer-larvae and *Prismatolaimus*).

Table 1. Absolute abundances (per 100 g dry soil) for some selected treatments only (means ± SE; n=3). Results of ANOVA are based on all treatments.

Applied Cu ($mg\ kg^{-1}$)			0	200	0	100	200	Main effect		Interaction
Applied Zn ($mg\ kg^{-1}$)			0	0	200	100	200	Cu	Zn	Cu*Zn
Taxon	T	*c-p*						*P*	*P*	*P*
Pratylenchus	P	-	370 ± 68	135 ± 31	205 ± 27	184 ± 32	62 ± 31	***	***	ns
Tylenchorhynchus	P	-	168 ± 51	29 ± 2	76 ± 7	140 ± 29	28 ± 5	***	***	**
Filenchus	P	-	78 ± 51	5 ± 5	0 ± 0	0 ± 0	0 ± 0	***	**	ns
Basiria	P	-	29 ± 29	3 ± 3	10 ± 5	23 ± 12	12 ± 3	ns	ns	ns
Rotylenchus	P	-	19 ± 10	17 ± 10	8 ± 8	20 ± 6	3 ± 3	ns	ns	ns
Diplogasteridae	B	1	22 ± 13	26 ± 11	66 ± 3	51 ± 7	22 ± 14	ns	ns	**
Rhabditidae	B	1	1359 ± 56	996 ± 173	1078 ± 144	984 ± 27	873 ± 6	*	**	ns
Dauer-larvae[1]	B	1	55 ± 45	65 ± 45	13 ± 13	13 ± 7	9 ± 0	ns	*	ns
Drilocephalobus	B	2	61 ± 20	0 ± 0	0 ± 0	0 ± 0	0 ± 0	***	***	***
Eucephalobus	B	2	76 ± 10	0 ± 0	0 ± 0	0 ± 0	0 ± 0	***	***	***
Cervidellus	B	2	0 ± 0	0 ± 0	4 ± 4	0 ± 0	0 ± 0	ns	ns	ns
Acrobeloides	B	2	329 ± 78	141 ± 25	140 ± 35	145 ± 24	16 ± 6	***	***	**
Acrobeles	B	2	53 ± 30	0 ± 0	0 ± 0	0 ± 0	0 ± 0	***	***	*
Prismatolaimus	B	3	53 ± 27	0 ± 0	0 ± 0	0 ± 0	0 ± 0	ns	***	ns
Pseudhalenchus	H	2	0 ± 0	17 ± 5	21 ± 11	13 ± 8	0 ± 0	ns	ns	ns
Aphelenchus	H	2	39 ± 29	0 ± 0	10 ± 5	9 ± 4	0 ± 0	*	ns	ns
Ditylenchus	H	2	22 ± 13	11 ± 7	10 ± 5	4 ± 4	6 ± 6	*	ns	ns
Aphelenchoides	H	2	238 ± 80	86 ± 8	130 ± 24	146 ± 36	47 ± 6	**	***	ns

c-p=*c-p* value; T: feeding group (P plant feeder, B bacterial feeder, H hyphal feeder); ANOVA results based on data from all treatments: ns: $P > 0.05$;
* $0.05 > P > 0.01$; ** $0.01 > P > 0.001$; *** $P < 0.001$; [1] Dauer-larvae of Rhabitidae

Table 2. Absolute abundances (per 100 g dry soil) and Maturity Index values for some selected treatments only (means ± SE; n=3). Results of ANOVA are based on all treatments.

Applied Cu (mg kg^{-1})	0	200	0	100	200	Treatment effects		
Applied Zn (mg kg^{-1})	0	0	200	100	200	Cu	Zn	Cu*Zn
Total abundance	3100 ± 104	1538 ± 164	1789 ± 149	1731 ± 53	1085 ± 48	***	***	ns
Plant feeders	684 ± 82	197 ± 19	310 ± 27	367 ± 39	112 ± 33	***	***	ns
Bacterial feeders	2101 ± 71	1228 ± 187	1310 ± 129	1192 ± 36	920 ± 13	***	***	ns
Hyphal feeders	300 ± 59	114 ± 9	169 ± 31	172 ± 51	53 ± 4	***	***	ns
c-p 1	1443 ± 83	1086 ± 166	1162 ± 129	1047 ± 13	904 ± 15	ns	**	ns
c-p 2	850 ± 103	255 ± 16	318 ± 61	317 ± 41	68 ± 9	***	***	ns
c-p 3	61 ± 31	0 ± 0	0 ± 0	0 ± 0	0 ± 0	ns	***	ns
c-p 4	46 ± 14	0 ± 0	0 ± 0	0 ± 0	0 ± 0	***	***	***
c-p 5	15 ± 15	0 ± 0	0 ± 0	0 ± 0	0 ± 0	ns	ns	ns
Maturity Index	1.47 ± 0.04	1.19 ± 0.02	1.22 ± 0.04	1.23 ± 0.02	1.07 ± 0.01	***	***	ns

ANOVA based on data from all treatments: ns: $p>0.05$; * $0.05>p>0.01$; ** $0.01>p>0.001$; *** $p<0.001$

Data obtained for the single metal treatments allowed estimation of EC_{50} values for added metal concentrations and concentrations measured in $CaCl_2$ (Table 3). It was found that several nematode taxa were too tolerant (e.g. Rhabditidae and Diplogasteridae) or too sensitive (e.g. *Basiria* and *Prismatolaimus*) to estimate an EC_{50}. EC_{50} values based on nominal added Cu concentrations ranged from the 234 mg kg^{-1} for the number of nematode taxa to 32 mg kg^{-1} for *Acrobeles*, corresponding with Cu concentrations in $CaCl_2$ of 32 and 1 mg l^{-1}, respectively. Within the same parameter, EC_{50} values based on nominal added Zn

Table 3. Estimates of EC_{50} (mg kg^{-1}) for Cu and Zn applied singly (95% confidence interval in parentheses). EC_{50} values are based on nominal concentrations and concentrations obtained after extraction with 0.01 M $CaCl_2$.

Parameter	Cu nominal		Cu $CaCl_2$		Zn nominal		Zn $CaCl_2$	
N (100 g^{-1} DW)	234	(126-447)	23	(5.4-100)	295	(224-398)	204	(123-331)
Nr. of Taxa	234	(155-363)	32	(5.6-182)	457	(251-813)	457	(129-1585)
Plant feeding	148	(112-196)	6.6	(3.6-12)	224	(170-295)	126	(85-191)
c-p 2	170	(120-240)	8.5	(4.3-17)	204	(155-269)	112	(76-162)
p-p 2	63	(47-85)	1.3	(0.8-2)	110	(49-240)	44	(16-117)
p-p 3	170	(126-229)	8.7	(4.4-17)	245	(186-324)	141	(93-214)
Pratylenchus	200	(146-269)	13	(5.9-28)	269	(102-708)	166	(47-589)
Tylenchorhynchus	120	(91-162)	4.4	(2.6-7.4)	-		129	(65-257)
Acrobeloides	196	(124-268)	14	(5.3-37)	219	(158-309)	123	(78-195)
Eucephalobus	56	(35-89)	2.2	(1.4-3.5)	-		30	(25-35)
Aphelenchus	43	(16-117)	1.6	(0.58-4.3)	-		-	
Acrobeles	32	(19-54)	1	(0.89-1.1)	60	(25-141)	21	(12-35)

c-p=*c-p* value group; *p-p*=*p-p* value group concentrations were always higher by a factor 1.1 to 2, but ranking the different parameters from low to high EC_{50} values results in a more or less similar order of parameters for both metals. Comparing EC_{50} for metal concentrations in $CaCl_2$ shows larger differences between Cu and Zn.

A significant interaction between Cu and Zn was found for 25% of the investigated parameters (Tables 1 and 2). However, there were no obvious trends, indicating that the combined exposure to Cu and Zn differed from that expected on the basis of single

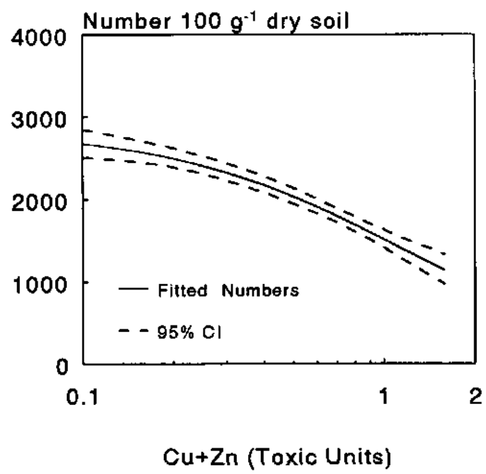


Fig. 3. Influence of the joint toxicity of Cu and Zn (in TU) on the number of nematodes in the soil. The mean relationship is given as well as the 95% confidence intervals.

Table 4. Estimates of TU_{50} for Cu and Zn (95% confidence interval in parentheses). TU_{50} values are based on EC_{50} values for the singel metal exposures expressed in nominal concentrations and concentrations obtained after extraction with 0.01 M $CaCl_2$.

Parameter	TU_{50} nominal		TU_{50} $CaCl_2$	
N (100 g^{-1} DW)	1.11	(0.75-1.46)	0.83	(0.58-1.08)
Plant feeding	1.12	(0.84-1.41)	0.89	(0.48-1.29)
c-p 2	1.26	(1.09-1.43)	1.12	(0.88-1.36)
p-p 2	1.3	(0.91-1.69)	1.27	(0.67-1.88)
p-p 3	1.26	(1.04-1.47)	1.06	(0.72-1.41)
Pratylenchus	1.31	(1.04-1.57)	0.99	(0.57-1.41)
Tylenchorhynchus	1.25	(1.02-1.48)	1.16	(0.78-1.54)
Acrobeloides	1.3	(1.18-1.42)	1.01	(0.88-1.15)
Eucephalobus	1.32	(1.01-1.63)	1.3	(1.03-1.57)
Acrobeles	0.96	(-0.19-2.1)	0.88	(0.46-1.31)

c-p=*c-p* value group; *p-p*=*p-p* value group exposures. For some nematode parameters it was possible to examine the joint toxicity by expressing all metal treatments in Toxic Units. One example of such a dose-response relationship for the joint toxicity of Cu and Zn to the total nematode abundance is shown in Figure 3. For these dose-response relationships TU_{50} values for the joint toxicity of Cu and Zn was estimated (Table 4). Most of the TU_{50} values based on nominal concentrations were above, or close to 1 TU, indicating that the effects of combinations of Cu and Zn to the nematode community of this soil were additive or less than additive. TU_{50} values based on metal concentrations measured in $CaCl_2$ were always lower than those based on nominal concentrations, and with the exception of *Eucephalobus,* all indicate additive effects.

Discussion

Although there is an increasing amount of data on joint toxicity, the present study is still one of the few based on terrestrial invertebrates exposed to pollutants in the soil i.e. in the presence of abiotic binding sites. Heavy metals may compete for binding sites in the soil and where two or more metals are present, this may change the bioavailable concentrations (Calamari and Alabaster, 1980; Christensen, 1987; Van Gestel and Hensbergen, 1997), which in turn might affect biota in a different manner than expected on the basis of exposure to a single metal. However, the present study demonstrated that the Cu or Zn concentrations measured in 0.01 M $CaCl_2$ were not influenced by the applied concentration of the other metal.

A likely explanation for the lack of competition between copper and zinc for binding sites in the soil is that different binding mechanisms were involved. The results of the $CaCl_2$ extraction indicate that Cu was mainly bound by chemisorption, whereas at the low pH of the soil used, Zn was bound by electrostatic adsorption (ion exchange). One would expect chemisorption of Zn to gain importance with increasing pH; therefore competition could also become more important at higher pH.

Although the soil pH between treatments in the present study was not significantly different, a previous study (Korthals *et al.*, 1996b) demonstrated that soil pH may change, depending on the metal and the amount added. Since soil pH influences the availability of heavy metals (Korthals *et al.*, 1996a), this might lead to differences in metal toxicity between soils in which metals are applied singly or in combination, which in turn may cause deviations from additivity, i.e. antagonistic or synergistic effects.

Several authors have argued that the effects at the highest metal concentrations may be enhanced by the high anion levels introduced (Weltje *et al.*, 1995; Van Gestel and Hensbergen, 1997). In the present study this was circumvented by adding the metals as sulphates and balancing differences in sulphates between the treatments by adding calcium sulphate. Precipitation of added sulphate and calcium ions displaced from exchange sites by adsorbing heavy-metal ions (gypsum), probably kept the dissolved salt concentration low and comparable between the treatments. Another advantage of using $CuSO_4$ and $ZnSO_4$ is that the total amount of SO_4^{2-} anions is only half of that when using metal chlorides or nitrates. Another possibility for removing excessive soluble salts is by percolating the treated soil with water prior to the experiments (Van Gestel and Hensbergen, 1997). This seems less advisable, however, when using a 'natural soil' in which the test organisms are already present in the soil before the metals are added.

The present study demonstrated that the effects on a terrestrial nematode community jointly exposed to Cu and Zn were all additive or less than additive. This is in agreement with the conclusions of two reviews (Ikeda, 1994; Hensbergen and Van Gestel, 1995), that in more than 90% of 210 studies, the joint toxicity of pollutant combinations were additive or less than additive. Despite the fact that in the present study Cu and Zn did not compete for binding sites, there are still many factors which could have caused differential effects on the biota. For example the chemical analyses only characterized the bulk soil at the end of the experiment. The nematode community integrates the effects of (changes in) soil conditions during a period of six months, which in fact is one of the major advantages of using organisms for monitoring the quality of the environment in comparison to chemical analyses. Furthermore, interactions between Cu and Zn could have occurred in uptake processes and in binding processes in the target organ(s) of organisms.

For the soil used in the present study, the official Dutch reference values that aim to distinguish between uncontaminated and contaminated soils are 20 (Cu) and 68 (Zn) mg kg^{-1}. These reference values were derived from current metal concentrations in the top soil of rural areas. They depend on the clay and organic matter content of the soil (De Haan *et al.*, 1990). Several nematode taxa were negatively affected by metal concentrations below the reference values, such as *Acrobeles* with an EC_{50} value of 60 mg kg^{-1} for Zn. This result is in agreement with Lexmond and Edelman (1987) who have stated that concentrations above the reference values do not necessarily cause negative effects, whereas concentrations below the reference values may still cause negative effects.

The official Dutch intervention values that separate seriously and not seriously contaminated soils calculated for the soil used in this study are approximately 100 (Cu) and

340 (Zn) mg kg^{-1}. Taking into consideration that at these values approximately 50% of the species are supposed to be endangered, the intervention value for Zn seems to high for this type of soil. More than half of the investigated nematode taxa were already seriously affected by the highest Zn additions of 200 mg kg^{-1}. Other species, especially those species which are not living in the soil pore water such as litter dwelling organisms, could still be protected at these metal concentrations. Nevertheless, it is realistic to assume that the negative effects on this nematode community are illustrative for many species living in the soil pore water. Furthermore, since nematodes play an important role in the functioning of the whole food web, other organisms may also be affected with yet unknown impacts on ecosystem functioning.

References

Anonymous 1989. Omgaan met risico's. Ministerie van Volkhuisvesting, Ruimtelijke Ordening en Milieubeheer. Kamerstukken II, 1988-1989. 21137, nr.5.

Anonymous 1991. Gezondheidsraad. Kwaliteitsparameters voor terrestrische en aquatische bodemecosystemen; een selectie van hanteerbare ecotoxicologische toetsen. Nr1991/17. Den Haag.

Asztalos, B., Nemcsók, J., Benedeczky, I., Gabriel, R. and Szabó, A. 1988. Comparison of effects of paraquat and methidation on enzyme activity and tissue necrosis of carp, following exposure to the pesticides singly or in combination. Environ. Pollut. 55:123-135.

Babich, H., Shopsis, C. and Borenfreund, E. 1986. Cadmium-Nickel toxicity interactions towards a bacterium, filamentous fungi, and a cultured mammalian cell line. Bull. Environ. Contam. Toxicol. 37:550-557.

Bongers, T. 1988. De Nematoden van Nederland. KNNV Bibliotheekuitgave nr. 46, Pirola, Schoorl, 408 pp.

Bongers, T., 1990. The Maturity Index: an ecological measure of environmental disturbance based on nematode species composition. Oecologia 83:14-19.

Bongers, T., De Goede, R.G.M., Korthals, G.W. and Yeates, G.W. 1995. Proposed changes of c-p classification for nematodes. Russ. J. Nematol. 3:61-62.

Bruce, R.D. and Versteeg, D.J. 1992. A statistical procedure for modelling continuous toxicity data. Environ. Toxicol. Chem. 11:1485-1494.

Calamari, D. and Alabaster, J.S. 1980. An approach to theoretical models in evaluating the effects of mixtures of toxicants in the aquatic environment. Chemosphere 9:533-538.

Christensen, T.H. 1987. Cadmium soil sorption at low concentrations: VI. A model for zinc competition. Water Air Soil Pollut. 34:305-314.

De Haan, F.A.M., Lexmond, Th.M. and Van Riemsdijk, W.H. 1990. Soil quality indicators. In: *Indicators and Indices for Environmental Impact Assessment and Risk Analysis.* Workshop Proceedings. A.G. Colombo and B. Premazzi (eds). ISPRA, EUR 13060EN, Office for Official Publications of the Europena Communities, Luxembourg, pp. 161-174.

Denneman, C.A.J. and Van Gestel, C.A.M. 1990. Bodemverontreiniging en bodemecosystemen: Voorstel voor C-(toetsings)waarden op basis van ecotoxicologische risico's. Rapport nr. 725201001, RIVM, Bilthoven, The Netherlands.

Eifac, 1987. Water quality criteria for European freshwater fish. Revised report on combined effects on freshwater fish and aquatic life of mixtures of toxicants in water. Rome.
Hensbergen, P.J. and Van Gestel, C.A.M. 1995. Combinatie-toxiciteit in het terrestrische milieu. TCB R04, Den Haag.
Houba, V.J.G., Novozamsky, I. and Temminghoff, E. 1993. Soil And Plant Analysis: Part 5A. Extraction With 0.01 M $CaCl_2$. Department of Soil Science and Plant Nutrition, Wageningen Agricultural University, Wageningen, the Netherlands.
Ikeda, M. 1994. Complex exposures: potentials for assessing integrated exposures. Clin. Chem. 40:1444-1447.
Könemann, H. 1981. Fish toxicity tests with mixtures of more than two chemicals: a proposal for a quantitative approach and experimental results. Toxicol. 19:229-238.
Korthals, G.W., Alexiev, A.D., Lexmond, Th.M., Kammenga, J.E. and Bongers, T. 1996a. Long-term effects of copper and pH on the nematode community of an agroecosystem. Environ. Toxicol. Chem. 15:979-985.
Korthals, G.W., Van de Ende, A., Van Megen, H., Lexmond, Th.M., Kammenga, J.E. and Bongers, T. 1996b. Short-term effects of cadmium, copper, nickel and zinc on nematodes from different feeding and life-history strategy groups. App. Soil Ecol. 4:107-117.
Kraak, M.H.S., Lavy, D., Schoon, H., Toussaint, M., Peeters, W.H.M. and Van Straalen, N.M. 1994. Ecotoxicity of mixtures of metals to the zebra mussel *Dreissena polymorpha*. Environ. Toxicol. Chem. 13:109-114.
Kraak, M.H.S., Schoon, H., Peeters, W.H.M. and Van Straalen, N.M. 1993. Chronic ecotoxicity of mixtures of Cu, Zn and Cd to the zebra mussel *Dreissena polymorpha*. Ecotox. Environ. Saf. 25:315-327.
Lexmond, Th.M. and Edelman, Th. 1987. Huidige achtergrondwaarden van het gehalte aan een aantal zware metalen en arseen in de grond. Handboek voor milieubeheer, Deel IV Bodembescherming. Samson, Alphen aan de Rijn.
Novozamsky, I., Houba, V.J.G., Lee, J.J. van der, Eck, R. van and Mignorance, M.D. 1993. A convenient wet digestion procedure for multi-element analysis of plant materials. Commun. Soil Sci. Plant Anal. 24:2595-2605.
Oostenbrink, M. 1960. Estimating nematode populations by some selected methods. In: Sasser, J.N. and W.R. Jenkins (Editors): Nematology. The University of North Carolina Press, Chapel Hill, pp. 85-102.
Sokal, R.R. and Rohlf, F.J. 1981. Biometry. W.H. Freeman, San Francisco, 300 pp.
Spehar, J.B. and Fiandt, J.T. 1986. Acute and chronic effects of water quality criteria-based metal mixtures on three aquatic species. Environ. Toxicol. Chem. 5:917-931.
Spehar, R.L., Leonard, E.N. and Defoe, D.L. 1978. Chronic effects of cadmium and zinc mixtures on flagfish (*Jordanella floridae*). Trans. Am. Fish. Soc. 107:354-360.
Sprague, J.B. 1970. Measurement of pollutant toxicity to fish. II. Utilizing and applying bioassay results. Water Res. 4:3-32.
Sprague, J.B. and Ramsay, B.A. 1965. Lethal levels of mixed copper-zinc solutions for juvenile Salmon. J. Fish. Res. Bd. Can. 22:425-432.
Van Gestel, C.A.M. and Hensbergen, P.J. 1997. Interaction of Cd and Zn toxicity for *Folsomia candida* (Collembola: Isotomidae) in relation to bioavailability in soil. Environ. Toxicol. Chem. In press.
Van Riemsdijk, W.H. and Hiemstra, T. 1993. Adsorption to heterogeneous surfaces. In: Allen, H.E., Perdue, E.M. and Brown, D.S. (Editors). Metals in groundwater. Lewis Publ., Boca Raton, Florida, USA, pp. 1-36.
Van Straalen, N.M. and Denneman, C.A.J. 1989. Ecotoxicological evaluation of soil quality criteria. Ecotox. Environ. Saf. 18:241-251.

Vranken, G., Tiré, C. and Heip, C. 1988. The toxicity of paired metal mixtures to the nematode *Monhystera disjuncta* (Bastian, 1865). Mar. Environ. Res. 26:161-179.

Weltje, L., Posthuma, L., Mogo, F.C., Dirven van Breemen, E.M. and Van Veen, R.P.M. 1995. Toxische effecten van combinaties van cadmium, zink en koper op terrestrische oligochaeten in relatie tot bodem-chemische interacties. Rapport nr. 719102043, RIVM, Bilthoven, The Netherlands.

Yeates, G.W., Bongers, T., De Goede, R.G.M., Freckman, D.W. and Georgieva, S.S. 1993. Feeding habits in soil nematode families and genera - an outline for soil ecologists. J. Nematol. 25:315-331.

Chapter 5

LONG-TERM EFFECTS OF COPPER AND pH ON THE NEMATODE COMMUNITY IN AN AGROECOSYSTEM

Abstract

Four copper (0, 250, 500 and 750 kg Cu ha^{-1}) and pH (4.0, 4.7, 5.4 and 6.1 in 1M KCl) treatments were applied to an arable agroecosystem. Effects on the nematode community were assessed after 10 years of exposure under field conditions. Both copper and pH had major influences on nematodes. The effect of copper was generally enhanced with decreasing soil pH. The lowest copper application rate which had a significant negative effect on the total number of nematodes was 250 kg ha^{-1} at pH 4.0, which is equivalent to a copper concentration of 0.32 mg l^{-1} in 0.01 M calcium chloride (Cu-$CaCl_2$) in 1992. Species composition and the abundance of trophic groups were more sensitive than the total number of nematodes. Combinations of high copper and low pH significantly reduced the number of bacterial feeding nematodes, whereas the number of hyphal feeding nematodes increased. Omnivorous and predacious nematodes showed the most sensitive response, becoming extinct when Cu-$CaCl_2$ was 0.8-1.4 mg l^{-1}. Plant feeding nematodes showed the largest differences in abundance and appeared to reflect the effects of Cu and pH on primary production. The results suggest that the nematode community was also affected indirectly by copper and pH via other components of the soil food web. It is concluded that nematodes offer excellent perspectives to assess effects of pollutants at the community level.

Keywords: Nematodes; Copper; Community; pH; Soil

Introduction

The ultimate goal of ecotoxicology is to predict the effects of pollutants on ecosystems. Experiments on the long-term effects of pollutants on whole fauna communities are important to reach this objective. The advantage of these studies is that they include mechanisms in which the pollutant indirectly affects the community, by changing the food availability and the interactions between species. Several authors (Underwood and Peterson, 1988; Clements, 1994) suggested that the importance of indirect effects increases in complex soil systems and that they are of major concern for the interpretation of ecotoxicological effects.

Studying fauna communities of polluted sites in the field (observational field studies) is a suitable approach, but mainly because of inadequate reference sites, mixtures of different pollutants and natural variability, the interpretation of results and establishment of cause-and-effect relationships are very complicated. To circumvent these problems, studies in which naturally occurring fauna communities are intentionally exposed to a pollutant (field experiments) probably provide better possibilities for documenting a causal relationship between contaminants and their effects.

Nematodes offer perspectives for the study of pollutant effects at the community level. The phylum Nematoda is comprised of many species showing a high diversity of life cycles, feeding types and sensitivities to pollutants. Based on the food source, nematodes have been assigned to trophic groups which reflect the trophic structure of the soil food web. Furthermore, they are abundant and important for key processes such as nutrient cycling (Freckman, 1988). Finally, terrestrial nematodes live in the soil pore water and are therefore assumed to be in close contact with the bioavailable concentration of pollutants (Houx and Aben, 1993).

It has been demonstrated that terrestrial nematode communities can indicate disturbances caused by manipulating soil pH (Ruess and Funke, 1992; de Goede and Dekker, 1993), tillage (Freckman and Ettema, 1993; Yeates, 1990) or manuring (Ettema and Bongers, 1993). Information on effects of heavy metals on terrestrial nematodes relates mainly to short-term single-species laboratory studies, although there are some observational field studies in which the structure of a nematode community was investigated (Zullini and Peretti, 1986; Yeates *et al.*, 1994; Popovici and Korthals, 1995). However, field experiments in which the long-term effects of an intentional heavy metal pollution on nematodes have been studied are rare.

The objective of the present study was to examine the long-term effects of copper and pH on the nematode community of an agroecosystem. It was hypothesized that the species composition and abundance of trophic groups will be sensitive to changes in the agroecosystem. In a previously unpolluted arable field copper concentrations and soil pH were manipulated. Copper was chosen because of the ongoing accumulation of this heavy metal in the top soil layer of many agroecosystems, mainly as a result of adding pig manure and sewage sludge (van Driel and Smilde, 1990). The pH was selected as a main variable because both the binding of copper to the soil and its phytotoxicity are pH dependent (Lexmond, 1980). After 10 years of normal arable land use, the effects of copper and pH were assessed by studying the composition of the nematode fauna.

Materials and methods

Study site

The experimental site is located on the eastern side of the Gelderse Vallei, ca. 3 km NNE of Wageningen, The Netherlands. It belongs to an area known as the Bovenbuurt pastures (Buringh, 1951). The soil parent material is cover sand, consisting of slightly loamy, moderately fine sand (de Bakker, 1979). The dominant soil type is a fimic anthrosol (FAO, 1988) with an A-horizon between 50 and 60 cm thick. The subdominant soil type can be classified as a dystric gleysol. The presence of a fimic A-horizon indicates that the field had been used as arable land in the past, which is confirmed by historical records.

Experimental design

In 1978 the experimental field and two adjacent areas, which had been used as permanent pasture for at least 30 years, were ploughed and the surface smoothed. As a result, the A-horizon was reduced to 30-40 cm. Silage maize was grown for three successive years, followed by starch potatoes in 1981. During these four years the crops were fertilized with both liquid cattle manure and mineral fertilizers. After 1981, only mineral fertilizers were applied and no organic materials other than crop residues entered the soil. In autumn 1981, the ploughed layer was sampled on 42 10*10 m plots, randomly distributed over the field, to measure the variability of some important soil characteristics. The organic carbon content was 2.1 ± 0.3 % by mass, pH-KCl 4.7 ± 0.4 and copper extractable with dilute nitric acid (Novozamsky *et al.*, 1993) 3.9 ± 0.4 mg kg^{-1}. The soil had a texture of 3 % clay, 10 % silt and 87 % sand and a CEC of 5.6 $cmol_c$ per kg (NH_4acetate, pH 7). In 1982 oats were grown on ninety 3*10 m plots with a mean grain yield of 5730 kg dry matter ha^{-1} (coefficient of variation 4.3 %). The field thus appeared to be sufficiently uniform for experimentation.

In the autumn of 1982 the experimental field (48*176 m) was divided into 128 plots of 6*11 m each. The plots were arranged in 8 blocks of 22*48 m each. Four copper (Cu) levels were introduced by applying $CuSO_4.5H_2O$ at rates of 0, 250, 500 and 750 kg Cu ha^{-1}. The pH was adjusted to pH-KCl 4.0, 4.7, 5.4 or 6.1 by adding flower of sulphur or ground calcitic limestone at rates of -1310, 510, 2330 and 4150 kg CaO-equivalents ha^{-1} respectively. Half of the Cu, lime or sulphur required were applied in September 1982 and were worked in with a rotary tiller. In October 1982, the field was ploughed and the remaining half of the chemicals was applied. Each Cu and pH treatment was represented and distributed randomly once in each of the 8 blocks.

From 1983 onwards, the crop rotation of silage maize, starch potatoes and oat started in 1980 was continued. During this period yields of maize and potatoes were determined. Mineral fertilizers applied to maize, potatoes and oat averaged 210, 230 and 60 kg N, 120, 110 and 30 kg P_2O_5 and 170, 210 and 110 kg K_2O_2, ha^{-1} yr^{-1}, respectively. In 1988 pH levels were readjusted to their nominal values by application of ground dolomitic limestone at rates of 0, 1440, 2280 and 2930 kg CaO-equivalents ha^{-1} for pH-KCl 4.0, 4.7, 5.4 or 6.1 respectively.

Sampling

Soil samples were taken in March 1992. In the centre of each plot (4 m x 9 m) 30 cores (diameter 17 mm) from the top 10 cm were taken in a regular pattern, mixed and divided into two portions.

One portion was dried at room temperature and sieved (mesh-size 2 mm). pH-KCl was measured after 10 ml soil was suspended in 50 ml 1 M KCl. To characterize the copper status of the soil, soil samples were extracted with 0.43 M HNO_3 or 0.01 M $CaCl_2$, so as to estimate the quantity (Cu-HNO_3) or the intensity (Cu-$CaCl_2$) of available copper respectively (Westerhoff, 1955). Copper (Cu-HNO_3, in mg kg^{-1} dry soil) was extracted from 10 g soil by shaking for 2 h with 100 ml 0.43 M HNO_3 (Novozamsky *et al.*, 1993). Another 10 g dry soil was suspended in 100 ml $CaCl_2$ (0.01 M) for 20 h. The suspension was centrifuged to determine the copper concentration in solution (Cu-$CaCl_2$ in mg l^{-1}). Metal analyses were performed by atomic absorption spectrometry (Houba *et al.*, 1989; Houba *et al.*, 1993). In addition, the vertical distribution of Cu was investigated by sampling the soil profile in 10 cm layers to a depth of 60 cm. Corresponding layers from each of the eight treatment plots were combined and analyzed for Cu-HNO_3.

Nematodes were extracted from the other portion of the soil sample using a modified Oostenbrink elutriator (Oostenbrink, 1960). The total number of nematodes was estimated by counting 2 subsamples (approx. 10 % of the total sample) under a dissecting microscope. Nematode numbers were expressed per 100 g dry soil after a correction for material left on the topsieve (mainly stones). Nematodes were heat-killed, fixed in 4% formalin and brought on a permanent mass-slide of which at least 150 nematodes were identified at 400x-1000x according to Bongers (1988). Nematodes were allocated to feeding groups according to Yeates *et al.* (1993).

Statistical analysis

A two factorial design with 8 completely randomized blocks was used. Data were analyzed by analysis of variance (ANOVA) to test the main effects of copper and pH, and their interaction. If necessary logarithmic transformations were applied to meet assumptions of normality and homogeneity of variances (Sokal and Rohlf, 1981). Tukey's multiple range test was employed to test for differences among treatments. All statistical analyses were performed with the software program Statgraphics 2.6 (Manugistics, 1986).

Results

Soil analysis

The copper and pH levels found after 10 years of experimentation are presented in Table 1. Cu-HNO_3 increased linearly with the level of applied copper. Within the copper treatments, Cu-HNO_3 tended to decrease with decreasing nominal pH, which is probably due to leaching into deeper soil layers, as is shown for the two most obvious treatments (Fig. 1). Cu-$CaCl_2$ increased with increasing copper addition and decreasing pH level (Fig. 2). Differences between the actual pH-values were smaller than between the nominal pH-values.

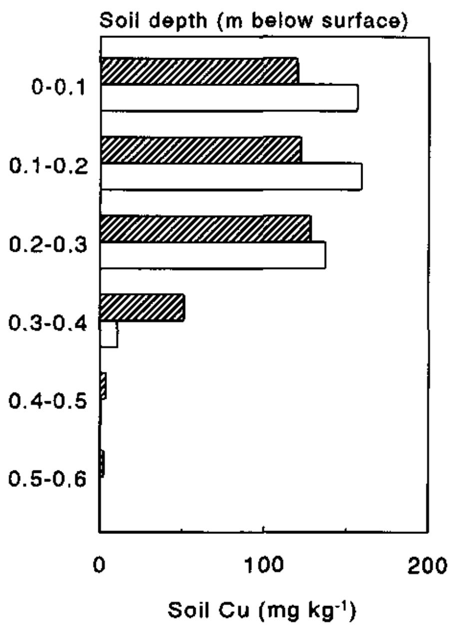
Crop yield

Maize and potatoes showed strong treatment effects (Table 1). The effects of Cu, pH and their interaction were all highly significant ($p < 0.01$) in every single year.

Table 1. Actual pH and Cu values in 1992 (n = 8; Means ± SE) and crop yield during 1983-1993.

Treatment		Soil data in 1992			Crop Yield	
pH-KCl	kg Cu ha^{-1}	pH-KCl	Cu-HNO_3 (mg kg^{-1})	Cu-$CaCl_2$ (mg l^{-1})	Maize[a]	Potatoes[b]
4.0	0	3.88 ± 0.03	25 ± 3	0.10 ± 0.02	9.5	36.2
4.0	250	3.96 ± 0.03	65 ± 3	0.32 ± 0.04	8.7	41.0
4.0	500	3.90 ± 0.03	100 ± 4	0.77 ± 0.05	2.7	28.0
4.0	750	3.82 ± 0.04	134 ± 6	1.40 ± 0.07	0.9	8.8
4.7	0	4.30 ± 0.04	27 ± 3	0.04 ± 0.01	14.6	50.3
4.7	250	4.29 ± 0.05	78 ± 4	0.18 ± 0.01	12.5	48.4
4.7	500	4.32 ± 0.07	104 ± 5	0.36 ± 0.07	6.6	41.4
4.7	750	4.27 ± 0.07	151 ± 6	0.71 ± 1.05	2.1	21.4
5.4	0	5.05 ± 0.12	27 ± 3	0.03 ± 0.00	14.1	48.6
5.4	250	4.97 ± 0.08	74 ± 3	0.08 ± 0.01	14.6	48.7
5.4	500	4.74 ± 0.07	108 ± 7	0.18 ± 0.01	11.2	47.0
5.4	750	4.75 ± 0.08	160 ± 9	0.25 ± 0.02	5.2	34.6
6.1	0	5.65 ± 0.07	29 ± 5	0.02 ± 0.00	15.0	45.9
6.1	250	5.38 ± 0.08	65 ± 4	0.06 ± 0.00	15.2	47.3
6.1	500	5.45 ± 0.05	119 ± 4	0.10 ± 0.00	13.5	46.6
6.1	750	5.37 ± 0.07	168 ± 6	0.14 ± 0.00	10.5	42.0

[a]: mean dry matter yield in ton ha^{-1} in 1983, 1986, 1989 and 1992
[b]: mean tuber yield in ton ha^{-1} in 1984, 1987, 1990 and 1993



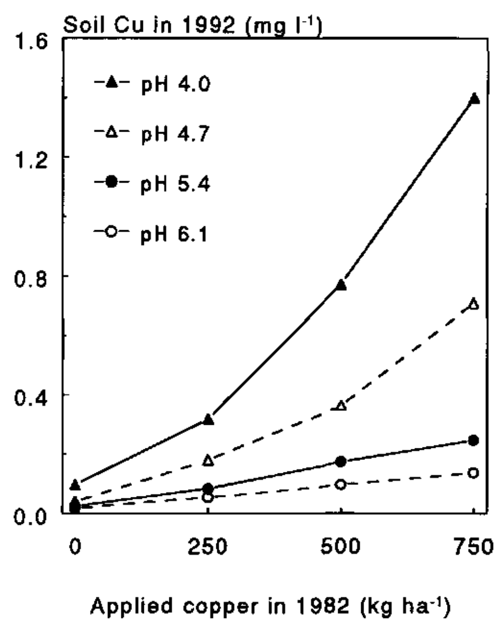


Fig. 1. Mean Cu concentrations (mg kg^{-1}) at different depths in 1992 for the applications of 750 kg ha^{-1} at pH-KCl 4.0 (hatched bars) and 6.1 (open bars) (n = 8).

Fig. 2. Mean Cu-$CaCl_2$ concentrations (mg l^{-1}) 10 years after copper and pH treatments in 1982, (n = 8).

Table 2. Abundance of nematode taxa (N 100 g^{-1}) in 1992

Treatment								
pH-KCl	4.0				4.7			
kg Cu ha^{-1}	0	250	500	750	0	250	500	750
Total number	3379 bc	3379 bc	2366 ab	1027 a	4094 cd	4114 cd	3297 bc	2125 ab
Plant-feeding								
Trichodorus	0 a	4 ab	0 a	0 a	66 cd	15 abc	0 a	0 a
Filenchus	116	89	34	12	77	101	41	32
Basiria	5 abc	15 abcd	0 a	0 a	172 e	125 de	83 cde	3 ab
Merlinius	14 a	58 abc	10 a	0 a	62 abc	88 abc	59 ab	9 a
Bitylenchus	91	51	11	2	409	204	47	6
Pratylenchus	934	1023	354	111	1153	1630	1270	319
Bacterial-feeding								
Acrobeloides	678 bcd	389 bcd	327 bc	118 a	561 bcd	380 bcd	300 bc	253 ab
Chiloplacus	142 abcd	282 bcd	368 d	150 abcd	154 abcd	335 cd	270 cd	228 abcd
Plectus	163	223	82	12	187	205	165	114
Rhabditis	95	118	115	58	145	100	97	150
Eucephalobus	85	85	74	59	70	63	80	68
Protorhabditis	86	79	75	82	82	45	82	149
Panagrolaimus	50	79	35	11	112	102	77	70
Cephalobus	12	28	20	5	29	57	46	54
Acrobeles	6 a	0 a	0 a	0 a	70 bc	8 a	0 a	2 a
Mesorhabditis	3 ab	8 abcd	0 a	0 a	48 cde	25 abcde	10 abcd	5 abc
Cervidellus	65 bc	34 abc	0 a	1 a	45 abc	30 abc	12 ab	0 a
Pristionchus	53 abc	12 abc	0 a	3 ab	17 abc	18 abc	16 abc	9 ab
Drilocephalobus	48	30	13	3	21	77	48	13
Hyphal-feeding								
Aphelenchoides	496 b	510 b	626 b	344 ab	272 ab	169 ab	370 ab	426 b
Ditylenchus	65 abc	31 ab	75 abc	11 a	71 abc	99 bc	86 bc	85 bc
Pseudhalenchus	108	185	124	33	49	91	75	59
Diptherophora	0 a	0 a	0 a	1 a	44 bcd	14 ab	10 ab	0 a
Nothotylenchus	9	4	9	2	25	14	12	5
Aphelenchus	0 a	3 a	0 a	1 a	6 a	5 a	11 ab	7 a

[a] Average abundances (n = 8) within one row followed by different letters differed significantly (P<0.05)
[b] Asterisks indicate significant treatment effects: * 0.05>P≥0.01, ** 0.01>P≥0.001, *** P<0.001

Table 3. Abundance of trophic groups (N 100 g^{-1}) in 1992

Treatment								
pH-KCl	4.0				4.7			
kg Cu ha^{-1}	0	250	500	750	0	250	500	750
Bacterial-feeding	1503 bcdef	1381 bcde	1110 ab	506 a	1587 bcdef	1489 bcdef	1212 abcd	1126 abc
Hyphal-feeding	684 abc	739 abc	836 abc	394 abc	477 abc	394 abc	564 abc`	582 abc
Plant-feeding	1180	1252	420	127	1978	2216	1500	407
Omnivores	10 ab	6 ab	0 a	0 a	33 bc	2 ab	13 ab	3 ab
Carnivores	3	2	0	0	19	13	9	8

[a] Average abundances (n = 8) within one row followed by different letters differed significantly (P<0.05)
[b] Asterisks indicate significant treatment effects: * 0.05>P≥0.01, ** 0.01>P≥0.001, *** P<0.001

Table 2. Extended

5.4				6.1				Treatment effect		
0	250	500	750	0	250	500	750	Cu	pH	Cu * pH
5022 d	4555 cd	4468 cd	4080 cd	4401 cd	3923 cd	4898 d	4585 cd	***	***	***
82 bcd	68 d	5 ab	0 a	79 bcd	50 abcd	55 abcd	12 abc	***	***	***
91	115	144	107	180	60	195	144			
265 e	295 e	67 bcde	97 abcde	168 e	190 e	158 de	141 de	***	***	
447 c	309 bc	125 bc	116 abc	368 bc	270 bc	317 c	237 bcd	***	***	
131	138	122	96	245	381	109	120		*	
1255	1054	1219	866	1100	961	1209	1076			
849 d	669 cd	393 bcd	359 b	732 cd	552 bcd	725 cd	909 d	***	***	
165 abcd	151 abcd	250 abcd	319 bcd	20 a	87 ab	115 abc	242 abcd	**		
156	138	212	210	94	94	100	66			
48	43	161	151	55	80	91	85			
106	119	175	129	100	95	147	186		***	
40	58	142	140	55	78	55	97			
110	135	186	115	92	87	184	107			
129	118	111	213	46	63	93	172		***	
247 c	94 c	15 ab	6 a	206 c	159 c	87 bc	9 a	***	***	**
104 e	116 e	24 abcde	58 bcde	82 cde	57 bcde	69 cde	65 de		***	
95 c	47 abc	11 ab	12 ab	124 c	66 bc	61 bc	11 ab	***	***	
36 abc	25 abc	47 bc	105 bc	18 abc	40 bc	84 abc	159 c		***	**
64	36	41	24	38	29	72	29			**
168 ab	265 ab	373 ab	529 b	74 a	105 a	161 ab	264 ab		***	*
159 bc	203 c	263 c	214 c	100 bc	149 bc	222 c	148 bc		**	
28	28	119	67	16	13	34	29		***	
39 bcd	74 cd	35 bcd	17 abc	67 cd	72 d	69 cd	24 abcd	**	***	*
6	36	103	29	11	10	90	84		**	
34 abc	20 abc	13 ab	22 ab	64 c	10 ab	35 abc	54 bc		***	

Table 3. Extended

5.4				6.1				Treatment effect		
0	250	500	750	0	250	500	750	Cu	pH	Cu * pH
2197 f	1860 cdef	1833 bcdef	1889 def	1739 bcdef	1522 bcdef	2004 ef	2211 f	*	***	***
477 abc	641 abc	908 c	877 bc	351 a	366 ab	631 abc	602 abc	**	**	*
2348	2031	1708	1304	2202	1963	2242	1754			
16 abc	11 ab	11 abc	0 a	100 c	39 bc	14 abc	10 ab	***	***	
14	12	8	10	9	34	7	8		**	

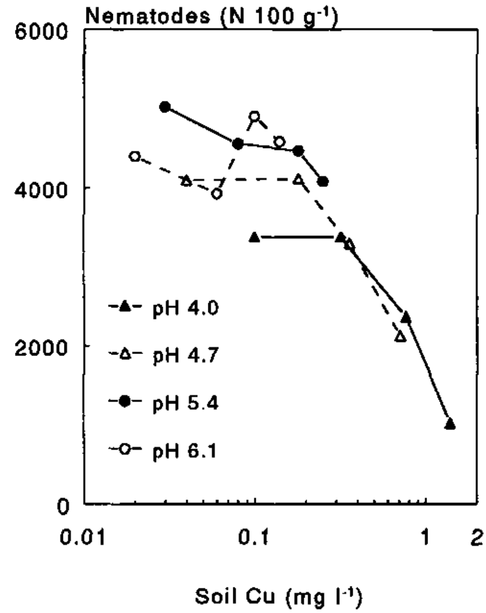


Fig. 3. Mean total number of nematodes found in 1992 for each treatment combination, (n = 8).

The coefficient of variation between years amounted to 15% for maize and 24% for potatoes. Since the yield of oats was not determined, no quantitative information can be provided on crop yields in the growing season preceding sampling.

Density and number of nematode taxa

The total number of nematodes was significantly lower in soils with pH adjusted to 4.7 or 4.0 and loaded with more than 250 kg Cu ha^{-1} (Fig. 3, Table 2). These copper and pH combinations were equivalent to $CaCl_2$ extracted Cu concentrations of above 0.32 mg l^{-1}.

In total 74 different taxa were identified. In plots where no copper was added an average of 22 taxa was counted and this number declined to 18 taxa in plots with the highest copper concentrations. Soil pH caused a shift in the mean number of taxa from 15 to 25 for pH 4.0 and pH 6.1 respectively. Pratylenchidae, Cephalobidae, Plectidae, Rhabditidae and Aphelenchoidae were the most dominant genera.

Composition of the nematode fauna

Taxa with an overall abundance of more than 1 % are summarized in Table 2. Copper significantly reduced numbers of *Trichodorus, Basiria, Merlinius, Acrobeloides, Acrobeles, Cervidellus* and *Diphterophora*. However, the number of *Chiloplacus* showed an opposite response. An increase in soil pH had a significant positive effect upon the numbers of most of the genera included in Table 2, except for *Aphelenchoides* and *Pseudhalenchus*, which were significantly reduced in numbers at pH 6.1. For *Trichodorus, Acrobeloides, Acrobeles, Pristionchus, Drilocephalobus* and *Diptherophora* a significant interaction of copper and pH on nematode numbers occurred with negative effects being most apparent at high copper concentrations and low pH. However numbers of *Aphelenchoides* were found to increase in the presence of copper in combination with a lower pH, except for plots where 750 kg Cu ha^{-1} was added and the nominal pH was adjusted to 4.0.

Trophic groups

Increasing copper significantly reduced the total number of bacterial feeding nematodes and had a significant positive effect on the number of hyphal feeding nematodes with highest densities in combinations where 500 kg Cu ha^{-1} was added (Table 3). Although omnivorous nematodes were not very abundant, they showed the most sensitive response to copper, decreasing significantly by more than 90 % in the treatment combinations with the highest copper additions.

Increasing pH had a significantly positive effect on the total number of bacterial feeding nematodes. Conversely, an increase in soil pH led to significant reductions of the total number of hyphal feeders, although the highest densities were found in plots where the pH was adjusted to 5.4. With respect to pH as well as to copper, omnivorous and predacious nematodes were the most sensitive trophic groups, totally disappearing when Cu-$CaCl_2$ concentrations were 0.8-1.4 mg l^{-1}. A significant interaction between copper and pH was found for bacterial and hyphal feeding nematodes.

Discussion

The present study provides an assessment of the effect of copper and pH treatments on the nematode community of an agroecosystem after an exposure period exceeding 10 years. The composition of the nematode fauna and abundance of trophic groups were sensitive measures for the direct and indirect effects of Cu and pH.

A significant interaction between copper and pH was found for many nematode genera, with negative effects being most apparent in plots treated with a high copper dose and a low pH. This reflects that pH affects heavy metal adsorption to the soil, as best described by Cu-$CaCl_2$. Although it has been found that increasing pH also affects the relationship between exposure and final effect (toxicity) (Nederlof *et al.*, 1993), the present study indicates that a lower pH enhances the toxicity of copper, which has also been observed for other organisms and heavy metals (Ma, 1982; van Gestel and van Dis, 1988).

The present study showed that the total number of nematodes did respond to an increase in Cu-$CaCl_2$, although earlier reports suggested that this is not a sensitive indicator of heavy metal pollution (Bisessar, 1982; Sturhan, 1989). The most likely reason for this discrepancy is the difference in bioavailability, which emphasizes the advantage of the $CaCl_2$ extraction method. The fact that changes at the genus or family level were better detectable than changes in the total number of nematodes is in agreement with many other studies (Bisessar, 1982; Zullini and Peretti, 1986; Sturhan, 1989; Yeates *et al.*, 1994; Popovici and Korthals, 1995).

There are several possible ways in which copper might have had a direct effect on individual nematodes and thus on the nematode community in this study. One way is via ingestion of contaminated food (Donkin and Dusenbery, 1993; Doelman *et al.*, 1984). Since it can be expected that a change in soil pH has a more or less similar effect on the binding of Cu to the soil as to biotic surfaces (Nederlof *et al.*, 1993), this ingestion can not explain the Cu and pH interaction on nematodes found in the present study.

Another way of copper uptake is via the cuticle. It is possible that in the present study Cu^{2+} passed through the cuticle and accumulated to toxic concentrations, which has also been observed for cadmium (Popham and Webster, 1979). It has been suggested that differences in cuticle characteristics contribute to the large variations in sensitivity to the acute effects of pollutants among nematode species (Kammenga *et al.*, 1994).

Changes in soil pH itself could have led to direct effects on the nematode community. In order to regulate their osmotic pressure, nematodes exchange several ions through their cuticle (Castro and Thomason, 1971). It has been suggested that soil acidification can lead to increasing ion concentrations in the soil pore water to such an extent that nematodes might experience problems in regulating their water status (Bååth *et al.*, 1980). The fact that a lower pH leads to enhanced copper bioavailability and to more ion exchange through the nematode cuticle, is in agreement with the observed interaction between the influence of copper and pH on nematodes.

Copper and pH could have also indirectly affected the nematode community, i.e. by influencing food availability, by interfering with the competitive interactions between species or by affecting the abiotic environment. Although it was not the objective of this study to investigate differences between direct and indirect effects of copper and pH, there are indications that indirect effects did influence the nematode community.

One indication that indirect effects did occur is found among the trophic group comprising of plant feeding nematodes. This trophic group showed the largest differences at the population level and seemed to reflect the effects of Cu and pH on primary production. Although, due to their more aggregated distribution, the present data appeared to be not significant, other studies have indicated that primary production influences densities of plant feeding nematodes (Ingham *et al.*, 1985; Yeates, 1987). Furthermore, it can be expected that in the present study copper reduced the root biomass (Marschner, 1986), which is the food source of plant feeding nematodes. Another indication for indirect effects is the shift from bacterial feeding nematodes to fungal feeding nematodes in plots with high Cu-$CaCl_2$. Several other studies have shown that a decrease in soil pH reduces the biomass of bacteria in favour of that of fungi and that such changes are reflected by the trophic composition of the nematode community (Ruess and Funke, 1992; de Goede and Dekker, 1993; Bassus, 1960; Heungens, 1981; Moore and De Ruiter, 1993).

Aphelenchoides species have been found to be tolerant to direct effects of pH (Schouten and Van der Brugge, 1989) and heavy metals (Pitcher and McNamara, 1972). Hence, the absolute increase in abundance with low pH and high copper concentrations found in this study has to be an indirect effect, probably caused by increased fungal biomass, reduced food competition with other organisms, reduced predation pressure or combinations of these factors.

The observation that omnivorous and predacious nematodes were the most sensitive trophic groups agrees with earlier observations on direct effects of pollutants (Kammenga *et al.*, 1994), as well as with long-term investigations in which populations could have been affected indirectly (Ferris and Ferris, 1974; Wasilewska, 1979). Feeding behaviour and life histories of most of the species belonging to these trophic groups are not well understood, but the fact that they generally have a rather long generation time and a more permeable cuticle seems important. Research on the relationship between these characteristics and the effects of pollutants seems to be of major importance to understand why these species are more affected by changes in their environment.

Whereas some earlier studies found an increased abundance or proportion of predacious nematodes in soil contaminated with heavy metals (Yeates *et al.*, 1994; Weiss and Larink, 1991), the present study showed a decrease. This discrepancy may be explained by differences in food availability or bioavailability of the metals, since in the present study metals were applied as salts whereas the earlier studies added metals in combination with organic materials.

While the relative importance of direct and indirect effects of metals on communities remain to be assessed, it is clear that soil nematodes are sensitive indicators of environmental stress induced by copper and pH. Studying trophic groups and species composition of soil nematode communities can provide a sensitive measure of soil quality in the field.

References

Bååth, E., Berg, B., Lohm, U., Lundgren, B., Lundkvist, H., Rosswall, T., Söderström, B. and Wiren, A. 1980. Effects of experimental acidification and liming on soil organisms and decomposition in a Scots pine forest. Pedobiologia 20:85-100.

Bassus, W. 1960. Die Nematodenfauna des Fichtenrohhumus unter dem Einfluss der Kalkdüngung. Nematologica 5:86-91.

Bisessar, S. 1982. Effects of heavy metals on microorganisms in soils near a secondary lead smelter. Water Air Soil Pollut. 17:305-308.

Bongers, T. 1988. De Nematoden van Nederland. Pirola, Schoorl, The Netherlands.

Buringh, P. 1951. Over de bodemgesteldheid rondom Wageningen. Versl. Landbouwk. Onderz. 57.4:1-131.

Castro, C.E. and Thomason, I.J. 1971. Mode of action of nematicides. In B.M. Zuckerman, W.F. Mai and R.A. Rohde, eds., Plant Parasitic Nematodes, Vol. 2. Academic Press, NY, pp. 289-296.
Clements, W.H. 1994. Assessing contaminant effects at higher levels of biological organization. Environ. Toxicol. Chem. 13:357-359.
De Bakker, H. 1979. Major Soils And Soil Regions In The Netherlands. Junk, The Hague and Pudoc, Wageningen.
De Goede, R.G.M. and Dekker, H.H. 1993. Effects of liming and fertilization on nematode communities in coniferous forest soils. Pedobiologia 37:193-209.
Doelman, P., Nieboer, G. Schrooten, J. and Visser, M. 1984. Antagonistic and synergistic toxic effects of Pb and Cd in a simple foodchain: Nematodes feeding on bacteria or fungi. Bull. Environ. Contam. Toxicol. 32:717-723.
Donkin, S.G. and Dusenbery, D.B. 1993. A soil toxicity test using the nematode *Caenorhabditis elegans* and an effective method of recovery. Arch. Environ. Contam. Toxicol. 25:145-151.
Ettema, C.H and Bongers, T. 1993. Characterization of nematode colonization and succession in disturbed soil using the Maturity Index. Biol. Fertil. Soils 16:79-85.
Ferris, V.R. and Ferris, J.M. 1974. Inter-relationship between nematode and plant communities in agricultural ecosystems. Agro-Ecosystems 1:275-299.
Food and Agricultural Organization (FAO-UNESCO). 1988. Soil map of the world: Revised legend. FAO, Rome.
Freckman, D.W and Ettema C.H. 1993. Assessing nematode communities in agroecosystems of varying human intervention. Agric. Ecosyst. Environ. 45:239-261.
Freckman, D.W. 1988. Bacterivorous nematodes and organic-matter decomposition. Agric. Ecosyst. Environ. 24:195-217.
Heungens, A. 1981. Nematode population fluctuations in pine litter after treatment with pH changing compounds. Mededelingen Faculteit Landbouwwetenschappen Rijksuniversiteit Gent 46:1267-1281.
Houba, V.J.G., Novozamsky, I. and Temminghoff, E. 1993. Soil And Plant Analysis: Part 5A. Extraction With 0.01 M $CaCl_2$. Department of Soil Science and Plant Nutrition, Wageningen Agricultural University, Wageningen, the Netherlands.
Houba, V.J.G., Van der Lee, J.J., Novozamsky J. and Walinga, I. 1989. Soil an Plant Analysis: Part 5. Soil Analysis Procedures. Department of Soil Science and Plant Nutrition, Wageningen Agricultural University, Wageningen, the Netherlands.
Houx, N.W.H. and Aben, W.J.M. 1993. Bioavailability of pollutants to soil organisms via the soil solution. Sci. Total Environ. Suppl. 1:387-395.
Ingham, R.E., Trofymow, J.A., Ingham, E.R. and Coleman, D.C. 1985. Interactions of bacteria, fungi, and their nematode grazers: effects on nutrient cycling and plant growth. Ecol. Monogr. 55:119-140.
Kammenga, J.E., Van Gestel, C.A.M. and Bakker, J. 1994. Patterns of sensitivity to cadmium and pentachlorophenol among nematode species from different taxonomic and ecological groups. Arch. Environ. Contam. Toxicol. 27:88-94.
Lexmond, T.M. 1980. The effect of soil pH on copper toxicity to forage maize grown under field conditions. Neth. J. Agric. Sci. 28:164-184.
Ma, W. 1982. The influence of soil properties and worm-related factors on the concentration of heavy metals in earthworms. Pedobiologia 24:109-119.
Marschner, H. 1986. Mineral Nutrition of Higher Plants. Academic Press, London.
Moore, J.C. and De Ruiter, P.C. 1993. Assessment of disturbance on soil ecosystems. Vet. Parasitol. 48:75-85.

Nederlof, M.M., Van Riemsdijk, W.H. and De Haan, F.A.M. 1993. Effect of pH on the bioavailability of metals in soils. In H.J.P. Eijsackers and T. Hamers, eds., Integrated Soil and Sediment Research: A Basis for Proper Protection. Kluwer Academic Publishers, Dordrecht, the Netherlands, pp. 215-219.
Novozamsky, I., Lexmond, Th.M. and Houba, V.J.G. 1993. A single extraction procedure of soil for evaluation of uptake of some heavy metals by plants. Intern. J. Environ. Anal. Chem. 51:47-58.
Oostenbrink, M. 1960. Estimating nematode populations by some selected methods. In: J.N. Sasser and W.R. Jenkins, eds., Nematology. University of North Carolina Press, Chapel Hill, NC, pp. 85-102.
Pitcher, R.S. and McNamara, D.G. 1972. The toxicity of low concentrations of silver and cupric ions to three species of plant-parasitic nematodes. Nematologica 18:385-390.
Popham, J.D. and Webster, J.M. 1979. Cadmium toxicity in the free-living nematode, *Caenorhabditis elegans*. Environ. Res. 20:183-191.
Popovici, J. and Korthals, G.W. 1995. Soil nematodes used in the detection of habitat disturbance due to industrial pollution. Stud. Univ. Babes-Bolyai Biol. 38:1-2.
Ruess, L. and Funke, W. 1992. Effects of experimental acidification on nematode populations in soil cultures. Pedobiologia 36:231-239.
Schouten, A.J. and Van der Brugge, I.R. 1989. Acute toxiciteit van aluminium en H^+ ionen voor bodemnematoden uit een zuur en kalkrijk dennenbos. I. Ontwikkeling en toepassing van een toets in waterig medium. Rapport 718603001. National Institute of Public Health and Environmental Protection, Bilthoven, The Netherlands.
Sokal, R.R. and Rohlf, F.J. 1981. Biometry. W.H. Freeman, San Francisco, CA.
Statgraphics 2.6. 1986. Manugistics Inc., Rockville, Maryland.
Sturhan, D. 1989. Nematodes as potential indicators of heavy metals in natural systems. In A.F. Wal and R.G.M. de Goede, eds., Nematodes in Natural Systems: a Status Report. Department of Nematology, Agricultural University Wageningen, Wageningen. 41.
Underwood, A.J. and Peterson, C.H. 1988. Towards an ecological framework for investigating pollution. Mar. Ecol. Prog. Ser. 46:227-234.
Van Driel, W., and Smilde, K.W. 1990. Micronutrients and heavy metals in Dutch agriculture. Fertilizer Research 25:115-126.
Van Gestel, C.A.M. and Van Dis, W.A. 1988. The influence of soil characteristics on the acute toxicity of four chemicals to the earthworm *Eisenia andrei* (Oligochaeta). Biol. Fertil. Soils 6:262-265.
Wasilewska, L. 1979. The structure and function of soil nematode communities in natural ecosystems and agrocenoses. Ekol. Pol. 27:97-146.
Weiss, B. and Larink, O. 1991. Influence of sewage sludge and heavy metals on nematodes in an arable soil. Biol. Fertil. Soils 12:5-9.
Westerhoff, H. 1955. Beiträge zur Kupferbestimmung im Boden. Landwirtsch. Forsch. 7:190-192.
Yeates, G.W, Bongers, T., De Goede, R.G.M., Freckman, D.W. and Georgieva, S.S. 1993. Feeding habits in soil nematode families and genera- an outline for soil ecologists. J. Nematol. 25:315-331.
Yeates, G.W. 1987. How plants affects nematodes. Adv. Ecol. Res. 17:61-113.
Yeates, G.W. 1990. Effect of three tillage regimes on plant and soil nematodes in oats/maize rotation. Pedobiologia 34:379-387.

Yeates, G.W., Orchard, V.A., Speir, T.W., Hunt, J.L. and Hermans, M.C.C. 1994. Impact of pasture contamination by copper, chromium, arsenic timber preservative on soil biological activity. Biol. Fertil. Soils 18:200-208

Zullini, A. and Peretti, E. 1986. Lead pollution and moss-inhabiting nematodes of an industrial area. Water Air Soil Pollut. 27:403-410.

Chapter 6

THE MATURITY INDEX AS AN INSTRUMENT FOR RISK ASSESSMENT OF SOIL POLLUTION

Abstract

It is widely acknowledged that the impact of contaminants on ecosystems is difficult to predict from single-species laboratory tests. Moreover there is growing evidence that the ultimate effect of a toxicant can be mediated through indirect effects. Studies carried out at the community level provide a meaningful step between single-species laboratory test and ecosystem studies. In order to interpret complex changes in community structure, there is an urgent need to develop new approaches to monitor soil pollution.

The present paper reports on methods to detect various kinds of disturbances by studying changes in the composition of natural occurring nematode communities. We examined wether the Maturity Index (MI), originally developed to monitor colonization and subsequent development of the nematode fauna, can also provide an instrument to measure the effects of soil pollution. It was found that within the Maturity Index concept, most taxa originally scaled in *c-p* 1 indicate food-rich conditions. Withholding these taxa from the calculation of the MI results in an index (MI2-5) to indicate disturbances resulting from chronic soil pollution, such as heavy metals.

Keywords: Soil pollution; Nematode community; Maturity Index; Biomonitoring

Introduction

The ultimate goal of ecotoxicology is to detect, predict and monitor the effects of pollutants on ecosystems (Moriarty, 1983). To overcome the existing gap between our knowledge of the effects that pollutants have on single species or on ecosystems, there is an urgent need to develop new bioindicator systems with more environmental realism (Cairns, 1992). Studying pollution-induced changes in the structure of natural occurring faunal communities can be an important step to bridge this gap. The advantage of such studies is that they also include mechanisms in which the pollutant indirectly affects the community, like for example via changes in the food availability. This can lead to a better understanding of the role ecological interactions play in toxicological responses of more complex systems (Moore and De Ruiter, 1993; Clements, 1994).

The objective of the present paper is to demonstrate new approaches in the development of bioindicator systems based on changes in the structure of nematode communities. Therefore, first a general overview on the successional stages in the development of faunal communities in general and nematode communities in particular will be given. Next we will test the hypothesis that effects of disturbances can be described as a retrogression in the succession of the nematode community. To test this hypothesis the effects of several kinds of disturbances on the nematode community are studied in terms of changes in the proportion of colonizers and persisters, i.e. *r*- and *K*-strategists, respectively. Finally, the prospects of the nematode Maturity Index (Bongers, 1990) an index based on the relative abundance of colonizers and persisters, as an instrument to discriminate between several types of disturbances of the soil ecosystem, will be discussed.

Changes in the structure of communities during succession

Faunal communities within an ecosystem are in a continuous process of change. It has been suggested that certain trends can be distinguished during successional changes of ecosystems (Odum, 1969). Newly formed habitats are invaded by colonizers (I), which results in an initial dominance of rapidly reproducing species (II). Subsequently, more organisms invade the system and the species diversity and structural and functional complexity increases (III). Finally, the system matures and becomes less controlled by external disturbances but more by biotic interactions (IV). In this stage there is a strong selection for species with a high competitive ability (V).

Although it can be debated whether succession is an unidirectional process and comprise predictable changes, there is a long tradition among ecologists to unravel the biotic and abiotic properties which determine the ecological range of the species within a successional sequence. The theory of *r*- and *K*-strategies showed that the degree of crowding can be one of the explaining factors. Although the meaning of the *r*-*K* concept has broadened, originally it was suggested that *r*-selected species are characterized by a high population growth in uncrowded populations, whereas selected species exhibit a high competitive ability in crowded populations (MacArthur and Wilson, 1967). The *r*-strategists are the successful colonizers. They have a high rate of population increase and a high dispersal ability. In uncrowded environments, like early stages of succession, these species are in favour. *K*-strategists show opposite characteristics and are found in more stable habitats, in which they maximize their carrying capacity (Pianka, 1978; Brown and Southwood, 1987).

Theoretically all species can be placed on a *r-K* continuum of strategies, of which some important characteristics are listed below:

Type of strategy	*r*-strategy	*K*-strategy
Community attributes		
Diversity	Low	High
Interspecific competition	Occasional	Frequent
Degree of specialization	Low	High
Species attributes		
Colonization ability	High	Low
Distribution	Wide	Restricted
Length of life	Short	Long
Maturity	Early	Late
Rate of development	Rapid	Slow
Fecundity	High	Low
Population density	Variable	Constant
Capacity for dormancy	Variable	Low

The *r-K* theory has been debated thoroughly. Most of the criticism was based on the fact that other factors than crowding can explain the diversity in life-history strategies as well (Wilbur *et al.*, 1974; Parry, 1981; Southwood, 1977), or that certain species exhibit a dynamic strategy involving shifts between relative *r-K* positions along the *r-K* continuum (Nichols *et al.*, 1976), or the fact that some pioneer species do have certain characteristics which are not in line with the *r-K* concept (Bengtsson and Baur, 1993). Despite this criticism there is much empirical support for the *r-K* concept, which warrants an analyses of the application of this ecological concept in studying changes in the structure of faunal communities within ecotoxicological studies.

Changes in the structure of nematode communities during succession

A helpful tool in studying changes in the structure of nematode communities during natural succession is the nematode Maturity Index (Bongers, 1990). Based on their ability to colonize new habitats, nematode families were classified on a tentative colonizer-persister (*c-p*) scale ranging from 1 to 5. Nematode families comprising species that rapidly increase in number in early stages of succession, were considered as colonizers and received a low *c-p* value. They have similar characteristics as *r*-strategists. In general colonizers live in unstable habitats. *Species of the families* Rhabditidae, *Panagrolaimidae and*

Diplogasteridae represent typical colonizers. The persisters among the nematodes are comparable with *K*-strategists and they generally live in habitats with a long durational stability. The most extreme persisters are found among the families Nygolaimidae, Thornematidae, Belondiridae, Actinolaimidae and Discolaimidae. During the last couple of years, further information on this MI-concept has become available (Bongers *et al.*, 1991; Bongers *et al.*, 1995; De Goede and Bongers, 1997).

The MI is calculated as the weighted mean of the *c-p* values assigned to the constituent nematode families (and genera and species they contain), such that:

$$\mathrm{MI} = \frac{\sum_{i=1}^{n}\left(v(i)\,.\,a(i)\right)}{\sum_{i=1}^{n}a(i)}$$

where v(i) is the *c-p* value assigned to taxon i and a(i) is the abundance of taxon i in a sample. If, for example, a community exists of 10 Rhabditidae (*c-p* value=1), 10 Diplogasteridae (*c-p* value=1), 30 Cephalobidae (*c-p* value=2) and 50 Dorylaimidae (*c-p* value=5) the MI is {(10+10)*1 + (30)*2 + (50)*5}/(10+10+30+50) = (330/100) = 3.3.

Following this classification of nematode taxa into *c-p* groups it is expected that early stages in the succession of a habitat will be characterized by relatively low MI values. The first colonizers comprise species with the characteristics of *r*-strategists and thus with low *c-p* values. Subsequently, during further development of the habitat the MI will increase, because of increasing numbers of *K*-strategists belonging to the higher *c-p* values. Such pattern of nematode fauna development was observed in several studies comprising natural successional series (De Goede *et al.*, 1993).

Changes in the structure of nematode communities and MI after disturbances

Natural succession of habitats coincides with patterns of nematode community development, generally resulting in an increasing value of the Maturity Index. However, the process of succession can be influenced by external disturbances, resulting in a retrogression or an arrestment of the succession of habitats (Regier and Cowell, 1972; Whittaker, 1975; Odum, 1985). If environmental disturbances also lead to a retrogression or arrestment of the development of nematode communities, then this should result in decreased MI values.

In terms of the *c-p* group classification within the MI-concept, two kinds of changes in the composition of the nematode fauna after a disturbance were suggested (Bongers, 1990; De Goede *et al.*, 1993). A type I response is found when disturbances result in increased numbers of taxa with low *c-p* values (1-2), whereas taxa from the higher *c-p* groups hardly respond (see e.g. Ettema and Bongers, 1993). A second type of response (response type II), is found when disturbances result in an absolute decrease among most taxa, but in particular those with high *c-p* values (Ruess *et al.*, 1993; Korthals *et al.*, 1996).

Despite essential differences between these types of response of the nematode community after a disturbance, both response types can be seen as a retrogression in the succession of the nematode community; that is, from the perspective of the *r*- and *K*-concept and in terms of the *c-p* classification. Therefore, the MI is not only an index to monitor natural succession, but can also be applied to detect disturbances and to monitor any subsequent recovery of the ecosystem. A review of applications of the MI in environmental studies comprising among others effects of water pollution, heavy metals, eutrophication, oil spill, liming, acidification, physical stresses and tillage regimes is given by De Goede and Bongers, (1997). These studies showed that in general, disturbances are followed by a decrease in MI, whereas recovery or natural succession coincided with an increase in MI.

Bioindicators should give opportunities to distinguish between different stress factors (Bongers and Schouten, 1991). De Goede *et al.* (1993) indicated that the MI-concept offers possibilities to discriminate between effects of eutrophication and pollution, which correspond to changes in the composition of the nematode fauna following the earlier mentioned response types I and II, respectively. Examples of studies demonstrating response type I involve effects of eutrophication, fertilization, oil spill, liming and several physical stresses (De Goede and Bongers, 1997). These disturbances cause such an increase in nutrient availability that, as a result of the (temporal) increased microbial activity, *c-p* 1 taxa ('enrichment opportunists' *sensu* Ettema & Bongers, 1993) can increase in number. When the nematode food supply decreases the densities of the *c-p* 1 taxa will decrease, while often at the same time the densities of *c-p* 2 taxa can increase. The higher scaled taxa (*c-p* 3-5) hardly react numerically to these changes in food supply (Ettema and Bongers, 1993).

At present, examples of the type II response are less often documented. Type II responses were found for soil acidification (Ruess *et al.*, 1993) and for the chronic effects of heavy metals (Korthals *et al.*, 1996; Popovici and Korthals, 1995). Although further confirmation is required, it seems that a type II response can be expected for long-term effects of disturbances where toxic stress is accompanied with conditions of low food availability. Under such conditions most taxa, but especially those belonging to higher *c-p* groups, decrease in number and the food availability is to low to have high populations of *c-p* 1 taxa. Certain taxa from the *c-p* 2 group (the 'general opportunists' *sensu* Ettema & Bongers, 1993) are the most tolerant to survive these severe conditions, or even benefit of it and increase in number (Korthals *et al.*, 1996).

Table 1. Correlation coefficients (r) for heavy metal content versus MI and MI2-5 for 20 agricultural soils in The Netherlands with mixed pollutants (Bongers, 1992).

	MI	MI2-5
Copper	-0.300	-0.496
Zinc	-0.318	-0.502
Cadmium	-0.178	-0.466
Lead	-0.154	-0.461

The response types I and II both result in a decrease of the MI. As a rule of thumb a decrease of the MI below 2 as a consequence of an absolute increase found among *c-p* 1 taxa, usually indicates a type I response (eutrophication), whereas samples characterized by MI values close to 2, and a lowered species diversity consisting mainly of taxa with *c-p* value 2, indicates a type II response (pollution). However, it is possible that in habitats with pollution, organic inputs can lead to a period in which *c-p* 1 taxa predominate the nematode

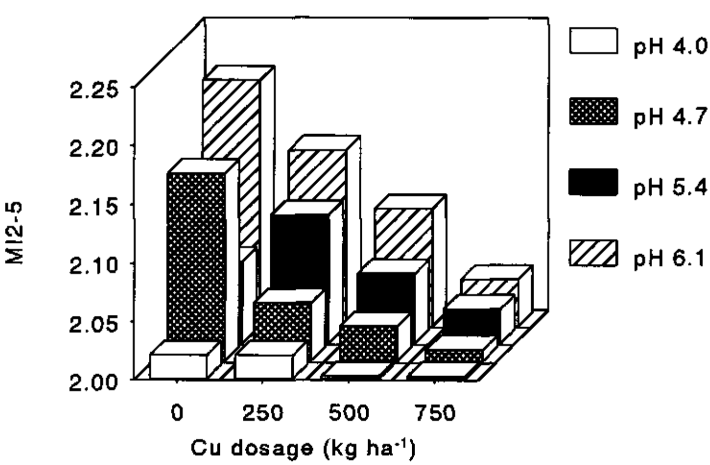


Fig. 1. Applications of MI2-5.

fauna such that the MI is below 2. Under these conditions the MI is less effective to identify pollution (type II response). To reduce this effect of enrichment in measuring pollution induced stress, it might have advantages to omit taxa scaled in *c-p* 1 from the calculation of the MI. We propose to express this new index as the MI2-5. Compared to the MI, the MI2-5 has proved to be more sensitive to indicate pollution as demonstrated in Table 1. Moreover, comparison of the scores of both indices can help to discriminate between pollution induced stress or eutrophication. First applications of this MI2-5 can be found in Figure 1., Korthals *et al.* (1993) and in Popovici (1994).

Discussion

The classification of nematode taxa into *c-p* groups reflects a first attempt to categorize nematodes in ecological groups with similar life-history characteristics. Although promising results were obtained when using this concept, further calibration of the *c-p* scaling might be necessary. Moreover, substantial more knowledge is needed on the relationship

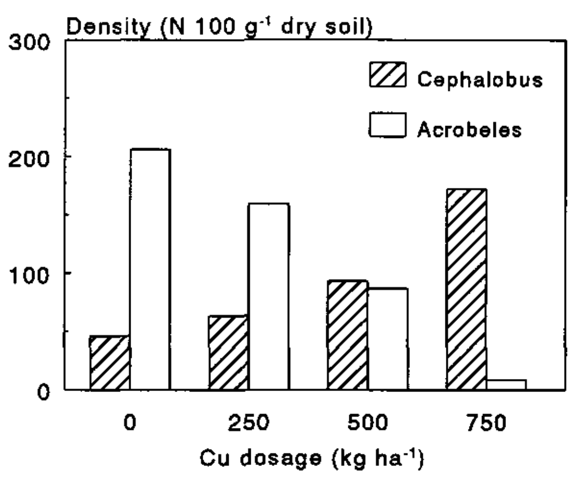


Fig. 2. Relationship between life-history and soil pollution.

between the life-history of certain species and their sensitivity towards soil pollution. Figure 2., for example, shows the effects of Cu on two closely related genera within the Cephalobidae. Although both genera have the same *c-p* value (*c-p* 2), their sensitivity towards Cu differs greatly. When it becomes possible to unravel these relationships, any improvements of *c-p* scaling can result in an increased sensitivity of the MI and MI2-5.

In addition to the MI and MI2-5, examination of the changes within each of the *c-p* groups on which the indices are based, can provide additional information on the nature of the underlying changes in the habitat. De Goede *et al.* (1993) presented a *c-p* triangle in which they schematically indicated the main directions along which changes in the nematode fauna composition may occur. Since this method makes it possible to visualize the changes in *c-p* 1, 2 and 3-5 distribution, it can also be used to identify type I and type II responses within the nematode community. Moreover, examination of *c-p* groups can also be helpful to detect simultaneous increase or decrease of opportunists and persisters which may remain unnoticed when using only the MI.

As was mentioned in the recommendations of the NATO Advanced Research Workshop (Van Straalen and Krivolutsky, 1996) ecological relevant bioindicator systems should, among others, be based on information obtained from different species and processes. Studies carried out at the community level provide opportunities in this direction. However, in order to interpret complex changes in the structure of whole communities, there is an urgent need to develop new methods which can be used by decision makers as well. The MI-concept can become one of these tools, especially because the changes in MI not only indicate the disturbance acting on a nematode community, but also provide opportunities to identify specific types of disturbance. Therefore, it seems worthwhile to investigate the usefulness of this MI-concept also for other groups of organisms, especially for those organisms that, in comparison to nematodes, have a different route of exposure.

References

Bengtsson, J. and Baur, B. 1993. Do pioneers have *r*-selected traits? Life history patterns among colonizing terrestrial gastropods. Oecologia 94:17-22.

Bongers, T. 1990. The maturity index: an ecological measure of environmental disturbance based on nematode species composition. Oecologia 83:14-19.

Bongers, T. 1992. De nematodenfauna als bio-indicator voor de bodemkwaliteit. Internal report.

Bongers, T. and Schouten, T. 1991. Nematodengemeenschappen als potentieel diagnostisch instrument voor chemische verontreinigingen. In G.P. Hekstra and F.J.M. van Linden (eds.). Flora en fauna chemisch onder druk. Pudoc, Wageningen, pp. 175-186.

Bongers, T., Alkemade, R. and Yeates, G.W. 1991. Interpretation of disturbance-induced maturity decrease in marine nematode assemblages by means of the maturity index. Mar. Ecol. Progress Series 76:135-142.

Bongers, T., De Goede, R.G.M., Korthals, G.W., and Yeates, G.W. 1995. Proposed changes of c-p classification for nematodes. Russian J. of Nematol. 3:61-62.

Brown, V. and Southwood, T.R.E. 1987. Secondary succession: patterns and strategies. In A.J. Gray, M.J. Crawley and P.J. Edwards (eds.). Colonization, succession and stability. Blackwell, Oxford, pp. 315-337.

Cairns, J. 1992. The threshold problem in ecotoxicology, Ecotoxicology, 1:3-16.

Clements, W.H. 1994. Assessing contaminant effects at higher levels of biological organization. Environ. Toxicol. Chem. 13:357-359.

De Goede, R.G.M. and Bongers, T. 1997. The nematode Maturity Index. Manual of methods for research on the ecology of nematodes, Techniques in nematode ecology. Soc. Of Nematologists (in press).

De Goede, R.G.M., Bongers, T. and Ettema, C. 1993. Graphical presentation and interpretation of nematode community structure: C-P triangles. Meded. Fac. Landbouw. Univers. Gent 58:743-750.

De Goede, R.G.M., Georgieva, S.S., Verschoor, B.C. and Kamerman, J.W. 1993. Changes in nematode community structure in a primary succession of blown-out areas in a drift sand landscape. Fund. Appl. Nematol. 16:501-513.

Ettema, C.H. and Bongers, T. 1993. Characterization of nematode colonization and *succession in disturbed soil using the Maturity Index. Biol. and Fertil. of Soils 16:79-85.*

Korthals, G.W., Alexiev, A.D., Lexmond, Th.M., Kammenga, J.E. and Bongers, T. 1995. Long-term effects of copper and pH on the nematode community in an agroecosystem. Envir. Tox. and Chem. 15:979-985.

Korthals, G.W., Bongers, T., Alexiev, A.D., and Lexmond Th.M. 1993. Influence of copper and pH on a terrestrial nematode community. Poster presented at SETAC World Conference, March 1993, Portugal.

MacArthur, R.H. and Wilson, E.O. 1967. The theory of island biogeography. Princeton University press, Princeton.

Moore, J.C. and De Ruiter, P.C. 1993. Assessment of disturbance on soil ecosystems, Veterinary Parasitology 48:75-85.

Moriarty, F. 1983. Ecotoxicology. The study of pollutants in ecosystems, Academic Press, London.

Nichols, J.D., Conley, W., Batt, B. and Tipton, A.R. 1976. Temporally dynamic reproductive strategies and the concept of r- and K-selection. Am. Nat. 110:995-1005.

Odum, E.P. 1969. The strategy of ecosystem development. Science 164:262-270.

Odum, E.P. 1985. Trends expected in stressed ecosystems. BioScience 35:419-422.

Parry, G.D. 1981. The meanings of *r*- and *K*-selection. Oecologia 48:260-264.

Pianka, E.R. 1978. Evolutionary ecology. Harper & Row, New York.

Popovici, I. 1994. Nematodes as indicators of ecosystem disturbance due to pollution. Stud. Univ. Babes-Bolyai, Biol. 37:15-27.

Popovici, I. and Korthals, G.W. 1995. Soil nematodes used in the detection of habitat disturbance due to industrial pollution. Stud. Univ. Babes-Bolyai, Biol., XXXVIII:1-2.

Regier, H.A. and Cowell, E.B. 1972. Applications of ecosystem theory, succession, diversity, stability, stress, and conservation. Biol. conserv. 4:83-88.

Ruess, L., Funke, W. and Breunig, A. 1993. Influence of experimental acidification on nematodes, bacteria and fungi: soil microcosms and field experiments. Zool. J. Syst. 120:189-199.

Southwood, T.R.E. 1977. Habitat, the template for ecological strategies. J. Anim. Ecol. 46:337-365.

Van Straalen, N.M. and Krivolutsky D.A. 1996. Bioindicator systems for soil pollution. NATO ASI series 16. Kluwer, Dordrecht, The Netherlands. 261 pp.

Whittaker, R.H. 1975. Communities and ecosystems. MacMillan, New York.

Wilbur, H.M., Tinkle, D.W. and Collins, J.P. 1974. Environmental certainty, trophic level, and resource availability in life history evolution. Am. Nat. 108:805-817.

Chapter 7

GENERAL DISCUSSION

The largest problem ecotoxicologists face is how to deal with the high degree of variation between field sites. One possibility is to 'remove' much of the heterogeneity by using a well-defined artificial soil (Edwards, 1983; Edwards, 1984). Since it seems impossible to 'rebuild' whole faunal communities in an artificial soil, the applicability of this kind of standardized toxicity testing will be limited to an initial screening of new pollutants, which should always be succeeded by investigations with a higher field relevance.

Another possibility is to focus on the chemical aspects. Increasing knowledge of the pollutant and the most important factors which control its bioavailability can lead to the incorporation of these factors in standard settings, as has been done earlier for clay and organic matter content in the official Dutch reference values (Lexmond and Edelman, 1987; De Haan *et al.*, 1993). That soil pH is another important factor which should be incorporated in soil standard settings was clearly demonstrated in Chapter 5, where a lower pH increased the bioavailability and enhanced the toxicity of copper to nematodes. This has also been observed for other organisms and heavy metals (Ma, 1982; Van Gestel and Van Dis, 1988). Therefore, the influence of other soil properties and abiotic environmental factors in relation to the bioavailability of pollutants should be investigated in the future.

In this line, increasing efforts in developing methods to measure bioavailable pollutant concentrations should be mentioned. To characterize the metal status in the different experiments of the present thesis, soil samples were extracted with 0.43 M HNO_3 or 0.01 M $CaCl_2$, in order to estimate the total quantity or the presumed bioavailability of the metals, respectively (Novozamky *et al.* 1993). Both estimates are important with respect to risk assessment, but based on the present thesis, it seems that for comparing the actual risks for nematodes at different sites, the last method seems most suitable.

Standard settings improved with our increasing knowledge of pollutant bioavailability are a useful tool for evaluating the degree of contamination. However, they cannot be used for soil quality assessment in relation to soil functioning (De Haan *et al.*, 1993), unless the (cor)relations with the effects on biota are incorporated. A first attempt to incorporate more ecotoxicology in risk analysis methods is shown by Van Straalen and Denneman (1989), in which No Observed Effect Concentrations (NOEC) for several organisms are incorporated. But in this approach as well, it is necessary that the ecotoxicological tests have a higher relevance for real soil.

Studying changes in nematode communities by exposing field-collected soil to pollutants seems to be a method with a higher degree of realism. Most nematode taxa showed declining numbers with increasing metal concentrations, some of them being very sensitive (*Plectus*, *Clarkus*, *Aporcelaimellus*, *Prismatolaimus*, *Alaimus* and *Acrobeles*) and others being very tolerant (*Pseudhalenchus*, Dauer-larvae of the Rhabditidae, *Aphelenchoides*, *Acrobeloides*, *Pratylenchus* and *Tylenchorhynchus*). Several experiments indicated that indirect effects did occur, such as the absolute increase in abundance of *Aphelenchoides* in soils with low pH and high copper concentrations (Chapter 5). This type of indirect effect was probably caused by increased fungal biomass, reduced food competition by less tolerant organisms, a reduced predation pressure by sensitive predators, or combinations of these factors. Evidence was obtained that these factors are more likely to occur in community-level micro- or mesocosm experiments with 'natural soil', which may cause less, under-, or overestimation of the actual risks, than do results based on soil organisms exposed to pollution in water or artificial soil.

In Chapter 3 it was demonstrated that a field-collected nematode community changed not only due to an abiotic factor (heavy metals), but also due to a biotic factor (presence of *Lolium perenne*). Compared to bare soils, the effects of Cu and Zn in soil covered with *L. perenne* only became apparent at higher metal concentrations, were less severe and were more often caused indirectly. This result was based only on experiments with a monoculture of *L. perenne*, so the final effect of pollutants in a soil covered by a more diverse vegetation may differ to some extent. Therefore, it is recommended that future ecotoxicological experiments with nematode communities should be carried out in the presence of a more natural (diverse) vegetation.

One other important aspect in exposing whole nematode communities to pollutants is that certain soil and vegetation characteristics not only influence the bioavailability of a pollutant, but also influence the structure of the original (unexposed) nematode community. The conclusions of the present thesis were based on a nematode community collected from one agroecosystem which was dominated by some bacterial feeders and plant feeders, of which Rhabditidae, Pratylenchidae, Dolichodoridae, Cephalobidae and Tylenchidae constituted approximately 80% of the total nematode community. This type of community may be quite different from nematode communities found in other ecosystems (De Goede and Bongers, 1994) and, although not investigated, it can be assumed that these differences will affect ecotoxicological results based on community parameters.

Ecotoxicological effects on the community level are the final outcome of the effects on the constituting species. To increase our knowledge on pollutant-induced changes in terrestrial nematode communities we have attempted to discern general patterns in the sensitivities among different nematode groupings, based on feeding strategies or life-history strategies. An example of classifying life-history strategies among nematodes has formed the basis for the Maturity Index (Bongers, 1990) and has been used to facilitate comparisons of the metal-induced changes in nematode communities in the present thesis. It was hypothesized that external disturbances might retrogress or arrest the succession of faunal communities (Regier and Cowell, 1972; Whittaker, 1975; Odum, 1985). Since the Maturity Index was originally developed as an ecological measure of the state of succession, disturbances should also be reflected in changed MI values.

It was demonstrated that, depending on the disturbance, two kinds of change in the composition of the nematode fauna may occur. Some disturbances, such as eutrophication, fertilization, oil spills, liming and several physical stresses (De Goede and Bongers, 1997), result in increased numbers of taxa with low *c-p* values (1-2), whereas taxa from the higher *c-p* groups hardly respond (e.g. Ettema and Bongers, 1993). A second type of disturbance results in an absolute decrease in most taxa, in particular those with high *c-p* values. Although further confirmation is required for other pollutants than the heavy metals investigated in this thesis, it seems that the second type of response can be expected for long-term effects of pollutants, especially those which are accompanied with conditions of low food availability. To improve the discrimination between "pollution stress" and "eutrophication stress", both of which result in a decreased MI, we proposed to omit taxa scaled in *c-p* 1 from the calculation of the MI and to express this new index as the MI2-5 (Chapter 6). In addition to the MI2-5, the so-called *c-p* triangles, in which it is possible to visualize the changes in *c-p* 1, 2 and 3-5 distribution, can also be used to detect the simultaneous increase or decrease of opportunists and persisters (De Goede *et al.*, 1993). These changes may otherwise remain unnoticed when using only the MI.

The classification of nematode taxa in *c-p* groups, reflects a first attempt to categorize nematodes in ecological groups with similar life-history characteristics. Although promising results were obtained when using this concept, further calibration of the *c-p* scaling seems necessary. The present thesis showed that closely related genera with similar feeding modes and *c-p* values, such as *Acrobeloides*, *Acrobeles*, *Cephalobus* and *Chiloplacus*, within the family Cephalobidae, can have very different toxicological responses. Furthermore, it was found that some genera with a *c-p* value of 2 (*e.g. Plectus* and *Acrobeles*) were as sensitive as genera with higher *c-p* values. These results indicate that substantially more knowledge on the ecology and life-history characteristics of nematodes on the genus- or species-level in relation to soil pollution is needed. Improvements of *c-p* scaling are likely to result in an increased indicator value of the MI or related indices.

Indices such as the MI are relative measures; their usage in field monitoring is limited to a signal function. To increase their usability, there is a strong need for a reference system, i.e. an estimate of what kind of nematode community might be expected on a certain site, under natural vegetation and unpolluted conditions. For The Netherlands (Bongers *et al.*, 1989; De Goede and Bongers, 1994; Alkemade and Van Esbroek, 1994), this type of knowledge is gradually increasing. Studying the taxa lists also remains useful in biomonitoring. The number of species, the trophic structure, absolute densities and the dominance of certain indicator species all help to differentiate between sites and to identify the most dominant stress factor.

Since nematodes feeding on higher plants were excluded from the calculation of the MI, a separate Plant Parasite Index (PPI) was proposed (Bongers, 1990). More recently, Yeates (1994) and Wasilewska (1994) proposed the inclusion of plant feeders in the MI. However, the present thesis demonstrated that under certain circumstances, the inclusion of plant parasites can lead to an index which is less sensitive to disturbances than the Maturity Index. Therefore, it is proposed to maintain them separately or to use the ratio between Maturity Index and Plant Parasite Index (Bongers *et al.*, 1997). In the future, the advantages of a set of indices for every trophic level above the MI calculated for all nematodes should be investigated. These separate MI's may provide more detailed information regarding shifts within trophic groups, which is more closely related to changes in the functioning of ecosystems.

To further increase our knowledge on potential risks of pollutants at the community or ecosystem level, an integration of pollutant effects on different taxonomic groups in relation to the functioning of the soil seems important. The advantages of such an approach have been illustrated by Edwards *et al.* (1996). We hope that the work of many nematologists and the present thesis have demonstrated that nematodes should be included in such investigations.

References

Alkemade, J.R.M. and Van Esbroek, M.L.P. 1994. Naar een effectenvoorspellingsmodel voor de bodemfauna: BOEF. RIVM-rapport 712901001. RIVM, Bilthoven, The Netherlands.

Bongers, T., 1990. The Maturity Index: an ecological measure of environmental disturbance based on nematode species composition. Oecologia, 83:14-19.

Bongers, T., De Goede, R.G.M., Kappers, F.I. and Manger, R. 1989. Ecologische typologie van de Nederlandse bodem op basis van de vrijlevende nematodenfauna. RIVM-rapport nr. 718602002. RIVM, Bilthoven, The Netherlands.

Bongers, T., Van der Meulen H. and Korthals, G.W. 1997. Relation between the nematode Maturity Index and Plant Parasite Index under enriched conditions. Appl. Soil Ecol. In press.

De Goede, R.G.M. and Bongers, T. 1994. Nematode community structure in relation to soil and vegetation characteristics. Appl. Soil Ecol. 1:29-44.

De Goede, R.G.M. and Bongers, T. 1997. The nematode Maturity Index. In: *Manual of Methods for Research on the Ecology of Nematodes, Techniques in Nematode Ecology.* Soc. Of Nematologists. In press.

De Goede, R.G.M., Bongers, T. and Ettema, C.H. 1993. Graphical presentation and interpretation of nematode community structure: *c-p* triangles. Meded. Fac. Landbouw. Univers. Gent 58:743-750.

De Haan, F.A.M., Van Riemsdijk, W.H. and Van der Zee, S.E.A.T.M. 1993. General concepts of soil quality. In: *Integrated Soil and Sediment Research: A Basis for Proper Protection.* Eijsackers, J.P. and Hamers, T. (Eds). Kluwer, Dordrecht, The Netherlands.

Edwards, C.A. 1983. Development of a standardized laboratory method for assessing the toxicity of chemical substances to earthworms. Report EUR 8714 En. Environment and Quality of Life. Commission of the European Communities, 141 pp.

Edwards, C.A. 1984. Report of the second stage in development of a standardized laboratory method for assessing the toxicity of chemical substances to earthworms. Report EUR 9360 EN. Environment and Quality of Life. Commision of the European Communities. 99 pp.

Edwards, C.A., Subler, S., Chen, S.K. and Bogomolov, D.M. 1996. Essential criteria for selecting bioindicator species, processes, or systems to assess the environmental impact of chemicals on soil ecosystems. In: *Bioindicator Systems for Soil Pollution.* Van Straalen, N.M. and Krivolutsky, D.A. (Eds.) NATO ASI Series 2: Environment. Kluwer, Dordrecht, The Netherlands. p 67-84.

Ettema, C.H. and Bongers, T. 1993. Characterization of nematode colonization and succession in disturbed soil using the Maturity Index. Biol. Fertil. Soils 16:79-85.

Lexmond, Th.M. and Edelman, Th. 1987. Current background values of the contents of some heavy metals and arsenic in soil. In: *Handboek voor milieubeheer*, deel Bodembescherming, Samson, Alphen a.d. Rijn, The Netherlands.

Ma, W. 1982. The influence of soil properties and worm-related factors on the concentration of heavy metals in earthworms. Pedobiologia 24:109-119.

Novozamsky, I. Lexmond, Th. M. and Houba, V.J.G. 1993. A single extraction procedure of soil for evaluation of uptake of some heavy metals by plants. Intern. J. Environ. Anal. Chem. 51:47-58.

Odum, E.P. 1985. Trends expected in stressed ecosystems. BioScience 35:419-422.

Regier, H.A. and Cowell, E.B. 1972. Applications of ecosystem theory, succession, diversity, stability, stress, and conservation. Biol. Conserv. 4:83-88.

Van Gestel, C.A.M. and Van Dis, W.A. 1988. The influence of soil characteristics on the acute toxicity of four chemicals to the earthworm *Eisenia andrei* (Oligochaeta). Biol. Fertil. Soils 6:262-265.

Van Straalen, N.M. and Denneman, C.A.J., 1989. Ecotoxicological evaluation of soil quality criteria. Ecotox. Environ. Saf. 18:241-251.

Wasilewska, L. 1994. The effect of age of meadows on succession and diversity in soil nematode communities. Pedobiologia 38:1-11.

Whittaker, R.H. 1975. *Communities and Ecosystems.* MacMillan, New York.

Yeates, G.W. 1994. Modification and qualification of the nematode Maturity Index. Pedobiologia 38:97-101.

SUMMARY

One way to assess the quality of our environment is by comparing chemical data against existing soil quality standards. Preferably these standards are based on effect concentrations obtained for different organisms. Unfortunately there is only a limited number of test species, the test conditions are often very unrealistic and it is very difficult to measure bioavailable pollutant concentrations.

One other approach is to compare biological data collected from the site of which the quality must be assessed with that of a reference site, i.e. those having a good quality. Main difficulties of this approach are the limited knowledge of reference sites and the complexity of interpreting the data.

Within both approaches there is a strong need for more knowledge on the effects of pollutants on whole communities. Therefore the research presented in this thesis mainly investigated heavy metal induced changes in terrestrial nematode communities exposed in microcosms tests or in a field experiment. Nematodes were chosen as test species since they are present in almost every habitat and their communities are normally characterized by high densities and a high species diversity. Nematodes play a prominent role in terrestrial food webs, are easy to sample and are representative of the soil being sampled. Furthermore, some existing functional groupings, based on for example feeding strategy or on life-history strategies, might facilitate the interpretation of pollutant induced changes in nematode communities.

In several experiments whole nematode communities, present in freshly collected agricultural soil, were exposed to cadmium, copper, nickel or zinc. Our main objective was to increase the complexity of the successive experiments, in order to get a better understanding of the effects of some heavy metals upon terrestrial nematode communities in the real world.

Therefore, in Chapter 2 the nematode community structure was studied one to two weeks after the addition of cadmium (Cd), copper (Cu), nickel (Ni) or zinc (Zn) to soil collected from an agroecosystem. The nematode community was found to be affected by increasing concentrations of Cu, Ni and Zn up to 1600 mg kg^{-1}, but not by Cd up to 160 mg kg^{-1}. EC_{50} values for the reduction in population size of individual taxa showed a low intra-taxon variation for Cu, Ni and Zn, which seemed to indicate that for these metals, uptake and elimination processes as well as their final effect appeared similar within the same taxon. However, major differences between the sensitivities of different nematode taxa were detected, with for example some omnivorous and predatory nematodes, known to be

'*K*-strategist', already significantly affected by 100 mg kg^{-1} Cu, Ni or Zn added to the soil. The relative abundance of the different life-history groups and, to a lesser extent, the different feeding groups indicated pollution-induced changes in the soil community. However, these classifications couldn't predict the sensitivity of different nematode genera in an adequate way.

In Chapter 3 the same 'natural soil method' was used to study the long-term effects of copper and zinc (0, 25, 50, 100, 200 and 400 mg kg^{-1}) in the presence or absence of ryegrass (*Lolium perenne* L.). It was demonstrated that the presence of vegetation is a very important factor in determining the final ecotoxicological effects of Cu and Zn. In soils covered with *L. perenne* it was found that the effects of Cu and Zn became only apparent at higher metal concentrations, were less severe and were more often caused in an indirect way. Therefore it was recommended that future risk assessment based on results obtained from micro- or mesocosms should include vegetation.

In Chapter 4 the possible consequences of another realistic aspect of pollution in the real world, namely the simultaneously presence of several pollutants, were studied. After an exposure period of a half year, it was found that many nematode community parameters were affected by increasing concentrations of Cu and Zn up to 200 mg kg^{-1}. However the bioavailable Cu or Zn concentrations measured in soils with combined additions of Cu and Zn were not significant different from single metal additions, indicating that in the present study Cu and Zn did not affect each other's bioavailability. After evaluating changes in the nematode community with the Toxic Unit model, it was concluded that the potential risks of a combined exposure to Cu and Zn can be judged by assuming additiveness or less than additiveness.

Chapter 5 focused on the long-term effects of copper and pH on a nematode community under the most realistic conditions, that is in an agroecosystem. Both copper and pH had major influences on nematodes. The effect of copper was generally enhanced with decreasing soil pH. The lowest copper application rate which had a significant negative effect on the total number of nematodes was a copper concentration of 0.32 mg l^{-1}. Species composition and the abundance of trophic groups were even more sensitive than the total number of nematodes. Combinations of high copper and low pH significantly reduced the number of bacterial-feeding nematodes, whereas the number of hyphal-feeding nematodes increased. Omnivorous and predacious nematodes showed the most sensitive response, becoming extinct when the Cu concentrations were between 0.8-1.4 mg l^{-1}.

In Chapters 6 and 7 the nematode community structure and some community parameters, such as the Maturity Index, are discussed in the light of their potential for future risk assessment of soil pollution.

Based on the present results, it is concluded that nematodes offer excellent perspectives to assess effects of pollutants at the community level. Nematode community parameters, such as the Maturity Index and the distribution between different trophic groups, gave an early and sensitive signal of increased Cu, Ni or Zn pollution in the soil. Moreover, it was demonstrated that the nematode structure may also provide opportunities to identify specific types of disturbance, i.e. pollutants.

nematodengemeenschap veranderde bij toenemende concentraties Cu, Ni en Zn, maar niet bij Cd waarvan de hoogste onderzochte concentratie 160 mg kg^{-1} was. EC_{50} waarden voor een 50% reductie in de populatie van individuele nematodentaxa gaven kleine intra-taxon verschillen voor Cu, Ni and Zn te zien, wat aantoont dat voor deze metalen het uiteindelijke effect vergelijkbaar is binnen eenzelfde geslacht. Er werden echter wel grote verschillen gevonden tussen de gevoeligheid van verschillende taxa. In een aantal experimenten bleek bijvoorbeeld dat de populatie omnivoren en carnivoren, die kenmerken bezitten van *K*-strategen, al significant verlaagd waren bij concentraties Cu, Ni en Zn van 100 mg kg^{-1}. Relatieve verschuivingen binnen de levensstrategiegroepen, en in mindere mate ook binnen de voedselgroepen, waren indicatief voor metaal-geïnduceerde veranderingen in de bodem. Deze classificaties waren echter niet geschikt genoeg om de gevoeligheid van alle nematodensoorten te voorspellen.

In hoofdstuk 3 werd ook gebruik gemaakt van de 'natuurlijke grond methode' om de lange-termijn effecten van koper en zink (0, 25, 50, 100, 200 and 400 mg kg^{-1}) in relatie tot de aan- of afwezigheid van engels raaigras (*Lolium perenne* L.) te bestuderen. Na 1 jaar bleek de aanwezigheid van vegetatie een belangrijke factor in het uiteindelijke ecotoxicologische effect van Cu en Zn. In grond begroeid met *L. perenne* werden de effecten van Cu en Zn pas duidelijk bij hogere metaalconcentraties, waren minder sterk en kwamen vaker op een indirecte manier tot stand. Daarom is het raadzaam om in de toekomst de ecotoxicologische effecten van verontreinigende stoffen op nematoden in de aanwezigheid van vegetatie te onderzoeken.

In hoofdstuk 4 werd een ander belangrijk aspect van verontreinigingen onderzocht, namelijk het gelijktijdig voorkomen van meerdere toxische stoffen. De biologisch beschikbare concentraties Cu en Zn, gemeten nadat de grond was geëxtraheerd met 0.01 M $CaCl_2$, toonden aan dat de metaalconcentraties in grond met Cu en Zn niet significant verschillend waren ten opzichte van grond waar slechts één metaal aanwezig was. Na een half jaar waren veel gemeenschapsparameters en nematodenpopulaties significant beïnvloed door toenemende concentraties Cu en Zn tot maximaal 200 mg kg^{-1}. De effecten op nematoden die gelijktijdig aan Cu en Zn waren blootgesteld waren echter altijd additief of antagonistisch.

In hoofdstuk 5 werden de lange-termijn effecten van Cu en pH op nematoden onderzocht. Dit vond plaats onder de meest realistische omstandigheden, namelijk in een akker. Zowel Cu als pH hadden een grote invloed op nematoden. In het algemeen was het effect van Cu groter bij afnemende bodem-pH. De laagste Cu-concentratie, waarbij een significant negatief effect op het totaal aantal nematoden werd gevonden, was 0.32 mg l^{-1}.

De soortsamenstelling en verdeling tussen de verschillende voedselgroepen waren zelfs gevoeliger dan het totaal aantal nematoden. Combinaties van een hoge Cu concentratie en lage pH gaven een significante verlaging van het aantal bacterie-eters, terwijl het aantal schimmel-eters toenam. Omnivoren en carnivoren waren het meest gevoelig en verdwenen bij Cu-concentraties tussen de 0.8-1.4 mg l^{-1}.

In hoofdstuk 6 en 7 worden de structuur van een nematodengemeenschap en sommige gemeenschapsparameters, zoals de Maturity Index, besproken met betrekking tot hun geschiktheid om verontreinigde bodems te beoordelen. Gebaseerd op de huidige resultaten luidt de conclusie dat nematoden zeer geschikt zijn om de effecten van verontreinigingen op het niveau van de levensgemeenschap te beoordelen. Parameters zoals de Maturity Index en de verdeling tussen de verschillende voedselgroepen, gaven een snel en gevoelig signaal bij verhoogde concentraties Cu, Ni en Zn in de bodem. Daarnaast werd er aangetoond dat de structuur van een nematodengemeenschap ook mogelijkheden biedt om verschillen tussen stress-factoren, waaronder giftige stoffen, op te sporen.

Dankwoord

Na ten minste 150 000 km reizen, 50 000 sigaretten en 18 000 koppen koffie is het eind van mijn AIO-periode in zicht! Voor de afronding van dit proefschrift was dit alles misschien niet noodzakelijk, een aantal mensen waren dat in ieder geval wel! Daarom wil ik een aantal van hen persoonlijk bedanken.

Tom Bongers, voor het vertrouwen dat jij de hele periode in mij hebt gehad. Ik bewonder jouw enthousiasme en ben nog steeds erg gecharmeerd van de Maturity Index en de impact van die MI voor ons vakgebied!

Lijbert Brussaard, voor de stimulerende discussies, het bespreken van manuscripten en de puntjes op de i(i's). Ik ben heel blij dat jij in een cruciale fase bereid bent geweest om als promotor op te treden. Ik heb het gevoel dat ik nu al veel van jou heb geleerd en hoop ook in mijn nieuwe werk nog veel contact met je te hebben.

Tom Dueck, bedankt voor al jouw hulp. Ik hoop dat ik je al duidelijk heb kunnen maken hoe belangrijk jouw bijdrage is geweest. Met name jouw interesse en bijna blinde vertrouwen dat jij vanaf het begin in mij hebt gehad zijn uitzonderlijk. Ik zal jouw opmerking dat je mijn engels zo goed vond ook nooit vergeten! De baan als paranimf heb je dubbel en dwars verdiend.

Frank van der Laan, bedankt voor de lange en intense vriendschap die wij reeds hebben. Praten, zeilen, vissen, vakantie, bellen en sinds kort mailen, het is allemaal even belangrijk voor mij, en ik hoop dat dit altijd zo zal blijven. Theo Lexmond, bedankt voor de intense samenwerking. Naast de 'vliegende start' op jouw proefveld, zouden de andere experimenten zonder jouw inbreng zeker anders zijn geweest. Ook al konden wij de interactie tussen Cu en Zn niet hard maken, ik hoop dat de interactie tussen ons wel duidelijk naar voren komt in dit boekje en alle artikelen.

Jan Kammenga, bedankt voor alle discussies en je interesse in mijn vorderingen. Ik denk met genoegen terug aan onder andere het opzetten van de milieucursus en de 'tox-groep' samen met Paul van Koert, Joost Riksen en Wendy Oude Breuil.

Hanny van Megen, bedankt voor jouw grote hulpvaardigheid en inzet en ik denk dat we een heel goed team zijn geweest.

Ron de Goede, bedank ik voor het 'voorwerk', zijn grote interesse in de nematologie (en mijn bescheiden bijdrage daaraan) en de vele keren Nematoden-Overleg. Tevens dank ik de deelnemers van het N-O, met Ton Schouten in het bijzonder, voor alle presentaties en discussies.

Jan van Bezooien bedankt voor de vele praktische kennis die ik van jou heb opgestoken, maar ook voor alle bakkies koffie (met een snufje zout en een beetje Buisman).

Daarnaast een woord van dank aan alle studenten die ik in mijn AIO-periode heb mogen begeleiden; Harriet de Ruiter, Erik Clijssen, Johan Maats, Hans van der Meulen, Dirk Jaap van de Veer, Jürgen Wolthuis, Rose-Marie Lambregts, Marina Bongers, Antje Fokkema, Jan Arisen, Linda Kaster en Tycho Schol. Ik heb een heleboel van en met jullie geleerd, en ik hoop dat jullie er met net zoveel genoegen op terug kijken als ik!

Furthermore I would like to thank Alexey Alexiev, Juliana Popovici and Ilian Iliev for the help you gave. I had a very nice time with you and learned a lot more about nematodes as well as about your beautiful countries.

Ook wil ik mijn nieuwe collega's op het N.I.O.O., en dan met name Wim van der Putten, Cees van Dijk en Jan Woldendorp, bedanken voor de vrijheid die jullie mij boden om mijn proefschrift af te ronden en de fijne werksfeer op het instituut. Daarnaast een speciaal woord van dank voor de mede-AIO'ers, Ineke van der Stoel, John Lenssen, Ronald Kester, Paul Bodelier, Atie Stienstra en Joke Nijburg voor het oprechte 'meevoelen' en ik wens ook jullie succes met de afronding van je proefschrift.

Tevens dank ik de leden van de leescommissie en opponenten, Nico van Straalen, F.A.M. de Haan, C.H.R. Heip en Jan Woldendorp, voor de bereidwilligheid om aan de promotie deel te nemen. Nico, dankzij jouw ben ik achtereenvolgens ecoloog, ecotoxicoloog en nematoloog 'geworden'. Als ik jouw de afgelopen jaren sprak was het in verre oorden (Maleisië, Portugal, Moskou) en onder het genot van een peukie en neutje, dus ik hoop je nog vaak te spreken. Ik bewonder jouw werkwijze en om dat te benadrukken wil ik niet onvermeld laten dat jij de eerste bent geweest die mij om een overdrukje vroeg!
Daarnaast heb ik (nog) geen rijbewijs en hebben de carpool-maatjes Marry Schreurs, Tom Dueck, Paul van Koert en George Kowalchuk, het mij mogelijk gemaakt om die 150 000 km op de teller te krijgen. Ook al is reizen 'grijze tijd', door jullie was het in ieder geval minder grijs.
Alleen werken is ook niet alles, dus ik dank al mijn vrienden en dan met name de 'kwakboy's', Kees van Vliet, Winfried Scheulderman, Rien van der Post, Martin van Wijk, Frank van der Laan, Marcel Baartman en Mark Vervloet voor de vele mooie momenten die we met zijn allen hebben gehad en nog zullen krijgen.
Dit geldt ook voor de VU-BIO's van het jaar ± 1984. Ik vind het heel leuk dat wij nog contact met elkaar hebben, en ik beloof jullie dat ik deze zomer iets leuks zal regelen!
Daarnaast een speciaal woord van dank voor mijn naaste familie; Rem, Vera, Karin en Remmert Korthals en Marjolijn de Waal. Jullie hebben mij altijd de vrijheid en ondersteuning gegeven om dit alles tot stand te brengen. Zonder jullie was ik er niet.

Nogmaals allemaal bedankt en hopelijk tot ziens op 26 mei!

Gerard Korthals
17 april 1997

Publications

Bongers, T. and Korthals, G.W. 1992. De nematodenfauna als instrument voor het beoordelen van waterbodems. In: Waterbodems te vies om op te pakken? KNCV-sectie milieuchemie, Den Haag.

Burghouts, T, Ernsting, G., Korthals, G. and De Vries, T. 1992. Litterfall, leaf litter decomposition and litter invertebrates in primary and selectively logged dipterocarp forest in Sabah, Malaysia. Phil. Trans. R. Soc. Lond. B 335:407-416.

Van Hattum, B., Korthals, G.W., Van Straalen, N.M., Govers, H.A.J. and Joosse, E.N.G. 1993. Accumulation patterns of trace metals in freshwater isopods in sediment bioassays - Influence of substrate characteristics, temperature and pH. Water Res. 27:669-684.

Bongers, T. and Korthals, G.W. 1995. The behaviour of Maturity Index and Plant Parasite Index under enriched conditions. Nematol. 41:286.

Popovici, J. and Korthals, G.W. 1995. Soil nematodes used in the detection of habitat disturbance due to industrial pollution. Studia Univ Babes-bolyai, Biologia, XXXVIII:1-2.

Bongers, T., De Goede, R.G.M., Korthals, G.W. and Yeates, G.W. 1995. Proposed changes of *c-p* classification for nematodes. Russ. J. Nemat. 3:61-62.

Kammenga, J.E., Van Koert, P.H.G., Riksen, J.A.G., Korthals, G.W. and Bakker, J. 1996. A toxicity test in artificial soil based on the life-history strategy of the nematode *Plectus acuminatus*. Environ. Toxicol. Chem. 15:722-727.

Korthals, G.W., Alexiev, A.D., Lexmond, Th.M., Kammenga, J.E. and Bongers, T. 1996. Long-term effects of copper and pH on the nematode community in an agroecosystem. Eviron. Toxicol. Chem. 15:979-985.

Korthals, G.W., De Goede, R.G.M., Kammenga, J.E. and Bongers, T. 1996. The Maturity Index as an instrument for risk assessment of soil pollution. In Van Straalen N.M. and D.A. Krivolutsky (eds). *Bioindicator Systems for Soil Pollution*. Kluwer Academic Publishers, Dordrecht, The Netherlands, pp. 85-94.

Korthals, G.W., Van de Ende, A., Van Megen, H., Lexmond, Th.M., Kammenga, J.E. and Bongers, T. 1996. Short-term effects of cadmium, copper, nickel and zinc on soil nematodes from different feeding and life-history strategy groups. Appl. Soil Ecol. 4:107-117.

Kammenga, J.E., Korthals, G.W., Bongers, A.M.T. and Bakker, J. Reaction norms for life-history traits as the basis for the evaluation of critical effect levels of toxicants. In: *Ecological Principles for Risk Assessment of Contaminants in Soil.* Van Straalen, N.M. and Lokke, H. (eds.) in press.

Bongers, T. Van der Meulen, H. and Korthals, G.W. 1997. Inverse relationship between the nematode Maturity Index and Plant Parasite Index under enriched nutrient conditions. Appl. Soil Ecol., in press.

Korthals, G.W., Popovici, J., Iliev, I. and Lexmond, Th.M. Effects of copper and zinc on a terrestrial nematode community affected by the presence of perennial ryegrass. Appl. Soil Ecol., submitted.

Curriculum Vitae

Naam:	Korthals, Gerardus Wilhelmus (Gerard)
Adres:	Blauwe Zegge 9, 3648JK, Wilnis tel. werk 026-4791308 tel. privé 0297-241263
Geboortedatum:	29 januari 1965 te De Kwakel
* 1984	Eindexamen VWO, Alkwin College te Uithoorn
* 1990	Doctoraalexamen Biologie, Vrije Universiteit te Amsterdam -Hoofdvak: dieroecologie en ecotoxicologie (VU) -Bijvakken: ecotoxicologie (IVM) en (tropische) oecologie (VU)
* 1990-1991	Wetenschappelijk medewerker bij het Instituut Voor Milieuvraagstukken (IVM) te Amsterdam
* 1991-1995	Assistent In Opleiding bij de vakgroep Nematologie, Landbouwuniversiteit Wageningen
* 1996-heden	PostDoc onderzoeker bij het Nederlands Instituut Oecologisch Onderzoek (NIOO) te Heteren