ECOTOXICOLOGY AND ENVIRONMENTAL SAFETY 18, 181–190 (1989)

59842

LANL Ref

Testing Environmental Pollutants on Soil Organisms: A Simple Assay to Investigate the Toxicity of Environmental Pollutants on Soil Organisms, Using $CdCl_2$ and Nematodes

W. H. M. VAN KESSEL, R. W. BROCADES ZAALBERG, AND W. SEINEN

Environmental Toxicology Section, Department of Veterinary Pharmacology, Pharmacy and Toxicology, University of Utrecht, P.O. Box 80.176, 3508 TD, Utrecht, The Netherlands

Received October 20, 1988

Juvenile stages of *Caenorhabditis elegans* (nematoda) were isolated and grown in an axenic medium containing various concentrations of $CdCl_2$. Growth of the organisms was significantly reduced from a level of 1 μM $CdCl_2$. Reproduction of the nematodes was also reduced from that 1 μM exposure level. At levels of 160 and 320 μM, growth was retarded at the early juvenile stages and the organisms did not reach the adult stage and could therefore not reproduce. The test system turned out to be simple and reproducible and is therefore suitable for the investigation of the toxicity of compounds to soil nematodes. © 1989 Academic Press, Inc.

INTRODUCTION

During the last decade, research into the role of free-living soil-inhabiting nematodes in ecological processes has gained much more attention. Their large numbers in soils and water and their omnipresence ask for this attention. In terrestrial ecosystems, nematode populations can reach densities of 30–55 million individuals per square meter. They can be dominated by one single species, but various species may also be present (Yeats, 1979, 1981). The direct role of nematodes in decomposition processes in land soils and in the aquatic environment is less likely to be important than their indirect role. Nematodes graze on the microflora, which accounts for most of the CO_2 flux in the soil. In this way they maintain its high abundance and stimulate its turnover and activity (Anderson *et al.*, 1981). Nematodes also account for the dispersion of microflora (Abrams and Mitchell, 1980) and stimulate the mineralization of nutrients that would otherwise remain immobilized in stationary-phase microflora (Trofymov and Coleman, 1982). In waste water processes, nematodes may stimulate bacterial activity, especially in the percolating filters (Schiemer, 1975, 1983) and increase the decomposition of sewage sludge (Abrams and Mitchell, 1980).

Research into the effects of toxic compounds on soil-inhabiting organisms is still very limited compared to research of this kind on aquatic organisms. Free-living nematodes are used in toxicological research on environmental pollutants and pesticides by only some research groups (Samoiloff, 1980; Samoiloff *et al.*, 1980; Haight *et al.*, 1982; Mudry *et al.*, 1982; Doelman *et al.*, 1984; Samoiloff and Bogaert, 1984; Coomans and Vanderhaeghen, 1985; Vranken and Heip, 1986; Vranken *et al.*, 1985; Frey, 1971, 1976; Neuschulz and Kämpfe, 1980; Simpkin and Coles, 1981; Ohba and Ishibashi, 1984). Nematodes have already been used in a bioassay to determine the relative toxic effects of sediments (Howell, 1982; Tietjen and Lee, 1984) or fractions of sediments (Samoiloff *et al.*, 1983).

0147-6513/89 $3.00
Copyright © 1989 by Academic Press, Inc.
All rights of reproduction in any form reserved.

9481

TABLE 1

DIFFERENT LIFE-CYCLE STAGES OF *Caenorhabditis elegans* AND THEIR CORRESPONDING LENGTH RANGES (μm)

Stage	Length ranges[a]	Length at molt[b]
Egg		
Juvenile 1 (J-1)	-380	370
Juvenile 2 (J-2)	360–510	480
Juvenile 3 (J-3)	510–650	640
Juvenile 4 (J-4)	620–940	850
Adult (Ad)	>850	

[a] Results obtained from Byerly *et al.* (1976).
[b] Estimation of the length at which the molt occurs.

In order to develop a simple and reproducible assay to investigate the relative toxicity of environmental pollutants on soil-inhabiting free-living nematodes, the effects of cadmium chloride on growth and reproduction of the species *Caenorhabditis elegans* were studied. Cadmium chloride was chosen as a model substance since literature data on this compound exist for nematodes (Feldmesser and Rebois, 1966; Popham and Webster, 1979; Samoiloff *et al.* 1980; Haight *et al.* 1982; Vranken *et al.*, 1985) and numerous other organisms (Canton and Slooff, 1982; Kraync *et al.*, 1987). Axenic culture conditions were chosen since bacteria may alter or decompose the toxic substances themselves, which possibly influences the growth and reproduction of *C. elegans* (De Cuyper and Vanfleteren, 1982).

The bioassay was found to be a promising simple and reproducible system to assay the toxicity of compounds in soil organisms and can be carried out in a short time.

MATERIAL AND METHODS

The Test Organism

C. elegans (Maupas 1900), variety Bristol, is a free-living nematode, originally isolated from the soil in Great Britain (Briggs, 1946). It has been successfully cultured since 1948 (Dougherty and Calhoun, 1948). The populations of this species used in the tests were originally obtained from Dr. Vanfleteren of the State University of Ghent, Belgium.

C. elegans is a hermaphroditic nematode, although sometimes males may occur. The life cycle can be characterized by different developmental stages separated from each other by molts of the cuticula (Byerly *et al.*, 1976).

In Table 1 the different life stages and the ranges of their corresponding lengths are presented. Since observation under an inverted microscope usually cannot determine to what stage a nematode belongs, except for nematodes containing eggs, an estimation of the stages was based on the values obtained by Byerly *et al.* (1976). The life cycle of *C. elegans* lasted 4 to 5 days in the studies reported here.

Culturing of the Nematodes

C. elegans was grown axenically in culture flasks (50 ml, Greiner und Söhne GmbH & Co.KG., Nürtingen, FRG) and placed in an incubator (Inventum, Bilthoven, The

·rlands) in a humidified atmosphere at a temperature of 20 ± 1°C. The growth ım was prepared by solution in aqua bidest of yeast extract (30 g liter^{-1}, Difco ·atories, Detroit, MI), soy peptone (30 g liter^{-1}, Oxoid United, Basingstoke, ɔshire, England), bacto-casitone (10 g liter^{-1}, Difco), and D-glucose (10 g liter^{-1}, ĸ, Darmstadt, FRG) as formulated by Vanfleteren (1976). This stock solution utoclaved for 20 min at 120°C. Five milligrams of bovine hemoglobin (Serva ıiochemica, Heidelberg, FRG) was soluted in 100 ml 0.1 *N* KOH and filter-.zed (FlowPore D, 0.45 μm, Flow Laboratories, Rickmansworth, England), and to use 1 ml was added to 100 ml of the stock solution to prepare the complete :h medium. The hemoglobin solution and the stock solution were stored sepa- at −20°C.

ıronization of Cultures

obtain large quantities of J-1 worms, the method originally described by Findeis (1983) was followed. Cultures in the log-growth phase, containing all deyelop- al stages, were concentrated by filtration over nylon filters (Nybolt, Schweizer- Seidengaze [illegible]rik AG Zurich, Switzerland) with a mesh size of 5 and 10 μm. ·worms in the filtrate, mostly J-1 individuals, were cultured for 4–5 days until ɔroduced offspring. Then the filtering procedure was repeated and the tests were ·d with the worms of this filtrate.

·rimental Procedure

·oups of about 50 J-1 worms were cultured in flat-bottom wells of microtiter s (Greiner and Söhne) in the presence of various amounts of $CdCl_2$ for 24, 48, nd 96 hr. The wells contained 100 μl medium with $CdCl_2$ concentrations ranging 0 to 320 μ*M*. Each concentration and each time interval were tested in triplicate. use reproduction of the nematodes starts after about 100 hr of culture, only a l number of up to 10 worms per well was used for the long-term exposures of ınd 168 hr. The cultures were set up in 10-fold to obtain sufficient reproduction . The plates were sealed and kept at 20 ± 1°C during the desired time interval. :h was operationally defined by the complete lack of movement after tapping on ·late. Then the worms were killed in a water bath at 65°C for 15 min. This treat- ı resulted in a straight, easily measurable carcass. The number of worms and engths of the individual worms were measured for each time interval and each ·entration using an inverted microscope (Diavert, Leitz, FGR) with a calibrated ır micrometer. To overcome doubling in counting and measuring of nematodes, d was scraped on the bottom of the cups.

ɔ study the effects of cadmium on the reproduction of *C. elegans*, the number of ·ring was counted and the mean number of offspring per adult nematode com- ·d. To study the reproducibility of the results, the test was repeated various times etermine the intratest variability.

·istics

ıata have been expressed as means ± standard deviation. When appropriate, ·ysis for significance was performed with the Mann–Whitney *U* test (Sokal and ıif, 1981).

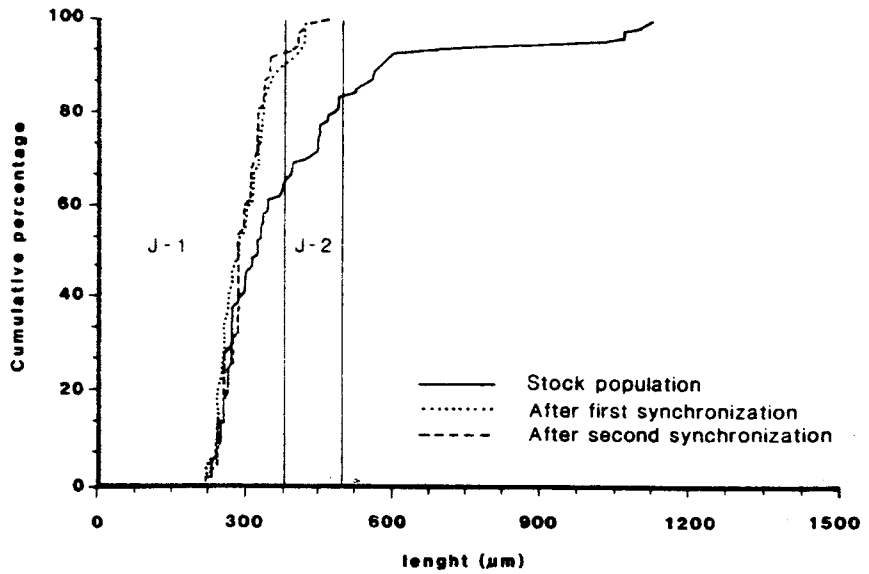


FIG. 1. Length distribution of a stock population of *Caenorhabditis elegans* before synchronization, after the first synchronization, and after the second synchronization. J-1, first juvenile stage; J-2, second juvenile stage.

RESULTS

Cultures of C. elegans

Starting with an inoculum of 2×10^3 nematodes per milliliter medium, the stock cultures reached densities of more than 10^5 nematodes per milliliter within 14 days. Maximum densities observed in the medium exceeded 2×10^5 nematodes per milliliter. This growth of the stock cultures was about the same as that found by Vanfleteren (1976) in an axenic culture.

Growth rate was determined mainly by the nature of the heme in the culture medium. Vanfleteren (1980) found that a precipitated form of heme was much better available to *C. elegans* than a soluble form. In different batches of culture media the authors observed large differences in growth rate. Optimum growth was obtained in batches with a flocculent heme precipitate. In batches of medium without a visible precipitate or with a leaf-shaped precipitate, the growth rate was severely decreased. The factor(s) which cause the hemoglobin fraction to form a flocculent precipitate are not identified. In the toxicity tests, only media with a flocculent heme precipitate were used.

Synchronization

By use of the filter technique of Findeis *et al.* (1983), a homogeneous population of early juvenile stages of nematodes was obtained. In Fig. 1, the length distribution of a stock population, the population after the first synchronization, and the population after the second synchronization, which is used in the toxicity tests, are shown.

After a first synchronization, an average of 89% of the population belonged to the J-1 stage and 11% to the J-2 stage. After the second synchronization, the population

TABLE 2

LENGTH OF *Caenorhabditis elegans* EXPOSED TO DIFFERENT CONCENTRATIONS OF $CdCl_2$ FOR VARIOUS TIME INTERVALS[a]

m	Time of exposure (hr)					
	24	48	72	96	120	168
	365 ± 49	628 ± 94	891 ± 97	1051 ± 66	1200 ± 48	1219 ± 50
	357 ± 52	627 ± 107	884 ± 77	1058 ± 56	1157 ± 60***	1150 ± 61***
	365 ± 60	631 ± 91	892 ± 89	1055 ± 61	1170 ± 59*	1128 ± 98*
	352 ± 52*	626 ± 90	853 ± 112***	1048 ± 58	1133 ± 73***	1147 ± 58***
	347 ± 54***	603 ± 93	840 ± 95***	1040 ± 70	1131 ± 75***	1122 ± 32***
	319 ± 47***	490 ± 94***	596 ± 104***	845 ± 90***	881 ± 102***	867 ± 63***
	297 ± 39***	379 ± 65***	393 ± 60***	484 ± 74***	486 ± 66***	549 ± 112***
	278 ± 35***	318 ± 57***	310 ± 46***	321 ± 41***	327 ± 45***	351 ± 48***

gth of the nematodes is expressed in micrometers. At the onset of the experiment mean length was 8 μm.
< 0.05.
< 0.01.
< 0.001 (Mann–Whitney *U* test).

ted of 94% J-1 and 6% J-2 stages. No J-3, J-4, or adult stages were found after ronization.

s on Growth

legans was cultured in the presence of different concentrations of $CdCl_2$ and at us time intervals the individual lengths of the worms were determined. The length of the nematodes after various times of exposure is given in Table 2. A ct growth retardation is seen at dose levels from 100 μM $CdCl_2$. In the concen- n range 1 to 32 μM the growth is only slightly but significantly retarded, espe- after long-term exposure of 120 and 168 hr. At the highest concentrations of nd 320 μM the growth was almost completely blocked. These organisms did each the adult stage, their movement was shaky or they were immobilized, but of them were still alive after 168 hr of incubation. At levels up to 100 μM, the vior and movement of the nematodes did not appear to change.

s on Reproduction

elegans was cultured in the presence of different $CdCl_2$ concentrations and the ber of offspring in each microtiter well was counted at 96, 120, and 168 hr after eginning of the test. In Table 3 the mean number of juveniles per adult is shown. e highest levels of 160 and 320 μM $CdCl_2$, no reproduction was found at all use these nematodes did not reach the adult stage. At the lower concentrations, e-related decrease in reproduction was found. Up to a level of 10 μM, the repro- ion rate was only slightly diminished. However, the total number of offspring uced during the 168-hr incubation period was lower in all cadmium-exposed ures.

TABLE 3

EFFECTS OF $CdCl_2$ ON THE REPRODUCTION OF *Caenorhabditis elegans*

Time (hr)	Cadmium concentration (μM)					
	0	1	3.2	10	32	100
	Mean number of juveniles per adult					
96	5.8	5.2	4.6	4.4	2.5	0.0
120	34.7	29.6	33.4	27.4	16.0	0.7
168	75.4	64.2	53.1	62.8	48.4	5.8

Reproducibility of the Tests

To study the reproducibility of the results, the test was repeated various times with different concentration ranges, different exposure times, and different batches of medium. The growth of the control nematodes differed significantly among the experiments. Growth and length distribution of the control cultures are shown in Fig. 2. Qualitatively, however, the results are consistent from one experiment to the other (Fig. 3). In all the tests a growth reduction pattern was obtained.

DISCUSSION

This study examined the suitability of a test with the free-living nematode *C. elegans* in an axenic medium to investigate the toxicity of compounds to soil nematodes. Cadmium was used as a reference substance to evaluate the reproducibility of the test and to compare the results with literature data about effects of cadmium on nematodes. With the test system employed, growth and reproduction of nematodes could be measured and dose–effect relationships and no-effect levels could be easily estab-

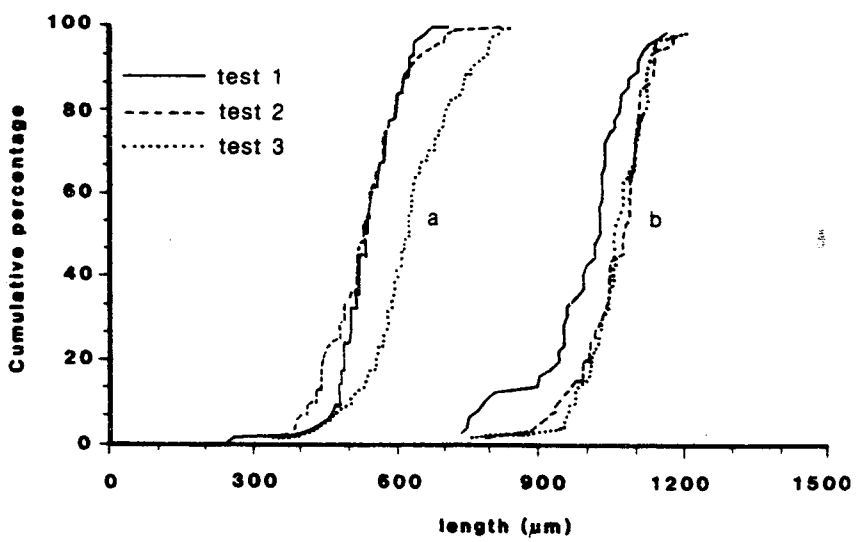
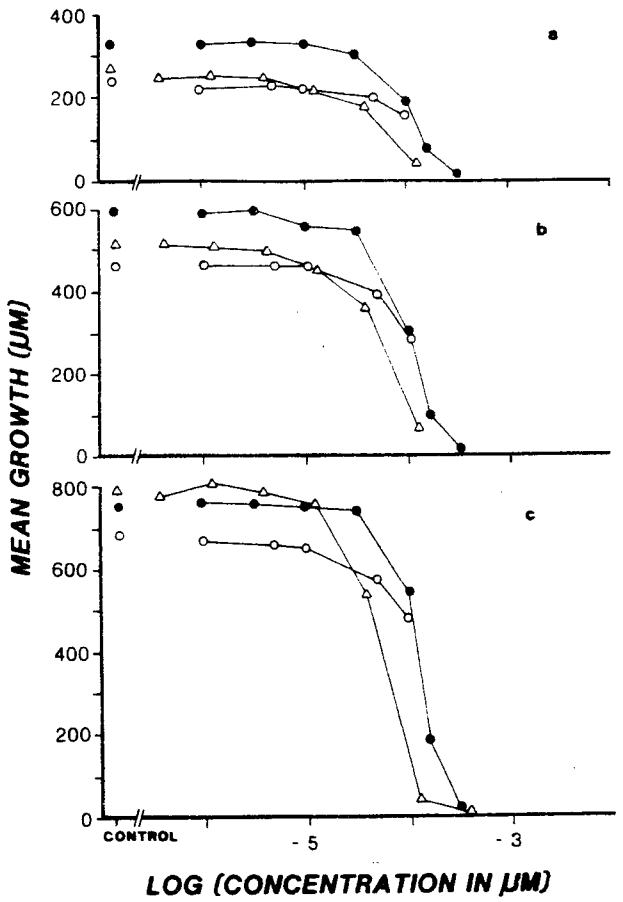


FIG. 2. Length distribution and growth of the control populations of *Caenorhabditis elegans* in three different tests, 48 (a) and 96 (b) hr after the beginning of the tests.



ɜ. 3. Mean growth of populations of *Caenorhabditis elegans* after 48 (a), 72 (b), and 96 (c) hr of sure to various concentrations of $CdCl_2$ in three different tests.

ɜd. Growth and reproduction of the organisms were reduced from a concentration μM $CdCl_2$ in the medium. The results obtained in our test system were highly ·oducible. Dependent on the batch of culture medium, the growth rate of the ıatode population was observed to have some variation, but when the effects of mium concentrations are expressed in relation to their corresponding controls, results are remarkably consistent. The origin of the variation in growth rate is not clear, but there are indications that the growth of the population is determined inly by the nature of the heme in the culture medium (Vanfleteren, 1980).

n these tests, the population growth of *C. elegans* differed by about a factor of 3 m population growth in a monoxenic culture (Klass, 1977). Most results on the :cts of cadmium on nematodes are obtained under xenic or monoxenic test condi- ns. Popham and Webster (1979) observed growth inhibition of *C. elegans* cultured agar with *Escherichia coli* as a food organism, at a concentration of 400 μM cad- um. With *Panagrellis silusiae* Haight *et al.* (1982) found a significant growth inhi- ion of more than 80% at a concentration of 219 μM. At a level of 43.8 μM, the ›wth of *P. silusiae* was not significantly affected. Concentrations of 438 μM were

not lethal per definition, which was also observed with concentrations of 160 and 320 μM cadmium in the tests with *C. elegans*.

In the marine species *Monhystera microphthalma*, *M. disjuncta*, and *Pellioditis marine*, a decreased success in attaining the adult stage was observed at concentrations of 11, 43, and 219 μM $CdCl_2$, respectively. A significant prolongation of developmental time of *M. microphthalma* was observed at 4.4 μM cadmium (Vranken *et al.*, 1985).

In filter-sterilized seawater, Howell (1984) found a concentration-dependent shortening of lifespan. The lifespan of the marine nematode *Enoplus brevis*, at a concentration of 0.44 μM cadmium was severely shortened compared to that of individuals exposed to 0.044 μM cadmium. Similar results were obtained with the same species, isolated from another location, and for a closely related species, *E. communis*.

A very remarkable finding in concentration effect was found for *Panagrellis redivivus* (Samoiloff, 1980; Samoiloff *et al.*, 1980). An exposure to 0.01 μM cadmium resulted in a stage distribution significantly different from that of the untreated nematodes. The tests were carried out in a medium consisting of M-9 buffer to which was added 0.05 mg baker's yeast ml^{-1} and 5 μg cholesterol ml^{-1} with *E. coli* as a food organism. The origin of the great difference in concentration effect between these experiments and the quite similar experiments mentioned earlier is not clear. Probably these differences are due to variability of the experimental procedures followed.

In *Daphnia magna* bioassays with several cadmium compounds, the free cadmium ion appeared to be the predominant toxic agent (Schuytema *et al.*, 1984). Therefore, it is important to consider the binding of cadmium to components of the medium in which the tests are carried out. Ramamoorthy and Kushner (1975) reported a microbial culture medium which contained 0.05% bactopeptone, 0.1% yeast extract, and 0.1% glucose, which bound at least 70% of the free cadmium ion. In that way the free cadmium concentration was depressed dramatically. In the test system reported here binding of cadmium to some components of the medium could have occurred as well. In addition to the medium, the glassware and the plastics in which the tests were carried out are also potential cadmium binding surfaces, which can alter the free cadmium concentration. For tests under monoxenic conditions, complexation of Cd by bacteria is a well-known phenomenon (Beveridge and Koval, 1982).

The influence of bioavailability of cadmium on nematode growth is illustrated by experiments of Haight *et al.* (1982). For *P. silusiae*, they found cadmium dissolved in physiological saline much more toxic than that dissolved in medium containing soy peptone, yeast extract, three sterols, and hemoglobin. The LC_{50} values differed by at least a factor of 1.4 (24 hr), 3.1 (48 hr), and 9.0 (72 hr).

These differences in concentration effect could also be related to the amount of food available for the worms. Although culturing on bacteria usually fulfills the optimal energy requirements better than culturing in an axenic medium, axenic conditions were chosen in the tests to avoid difficulties when testing organic compounds, since bacteria or other organisms might alter or decompose these compounds during the tests.

CONCLUSIONS

The axenic test as described in this article is simple, reproducible, and useful for comparing the effects of environmental pollutants on soil organisms. To exclude the binding capacity of the food compounds in the medium, the nematodes can also be

exposed in simpler media such as buffer solutions. In addition to this test, other test systems and new techniques must be developed to gain more understanding of effects in the natural environment. Exposure in soilwater and direct exposure in soil are under investigation.

REFERENCES

ABRAMS, B. J., AND MITCHELL, M. J. (1980). Role of nematode–bacterial interactions in heterotrophic systems with emphasis on sewage sludge decomposition. *Oikos* **35**, 404–410.

ANDERSON, R. V., COLEMAN, D. C., AND COLE, C. V. (1981). Effects of saprotrophic grazing on net mineralisation. In *Terrestrial nitrogen cycles* (F. E. Clarck and T. Roswall, Eds.). *Ecol. Bull.* **33**, 201–216.

BEVERIDGE, T. J., AND KOVAL, S. F. (1982). Binding of metals to cell envelopes of *Escherichia coli* K-12. *Appl. Environ. Microbiol.* **42**, 325–335.

BRIGGS, M. P. (1946). Culture methods for a free-living nematode. M.A. thesis, Stanford University, Stanford, CA.

BYERLY, L., CASSADA, R. C., AND RUSSELL, R. L. (1976). The lifecycle of the nematode *Caenorhabditis elegans*. I. Wild type, growth and reproduction. *Dev. Biol.* **51**, 23–33.

CANTON, J. H., AND SLOOFF, W. (1982). Toxicity and accumulation studies of cadmium (Cd^{2+}) with freshwater organisms of different trophic levels. *Ecotoxicol. Environ. Saf.* **6**, 113–128.

COOMANS, A., AND VANDERHAEGHEN, R. (1985). *In vitro* testing toxicities. Exercise 29. In *Plant Nematology Laboratory Manual* (B. M. Zuckermann, W. F. Mai, and M. B. Harrison, Eds.), pp. 172–176. University of Massachusetts Agricultural Experiment Station, Amherst, MA.

DE CUYPER, C., AND VANFLETEREN, J. R. (1982). Nutritional alteration of life span in the nematode *Caenorhabditis elegans*. *Age* **5**, 42–45.

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 and fungi. *Bull. Environ. Contam. Toxicol.* **32**, 717–723.

DOUGHERTY, E. C., AND CALHOUN, G. H. (1948). Experiences in culturing *Rhabditis pellio* (Schneider 1866) Butschli, 1873 (Nematoda, Rhabditidae), and related soil nematodes. *Proc. Helminthol. Soc. Wash.* **15**, 55–68.

FELDMESSER, J., AND REBOIS, R. V. (1966). Nematocidal effects of several cadmium compounds. *Nematologica* **12**, 91.

FINDEIS, P. M., BARINAGA, C. J., WILLETT, J. D., AND FARWELL, S. O. (1983). Age-synchronous culture of *Caenorhabditis elegans*: Technique and applications. *Exp. Gerontol.* **18**, 263–275.

FREY, F. (1971). Über die Eignung von *Acrobeloides buetschlii* (Cephalobidae) für nematologische Laboruntersuchungen. *Nematologica* **17**, 474–477.

FREY, F. (1976). Untersuchungen über die Wirkung von im Obstbau verwendeten Herbiziden auf den Testnematoden *Acrobeloides buetschlii* (de Man 1884) Steiner & Buhrer 1933. *Z. Pflanzenkrankh. Pflanzenschutz.* **83**, 434–441.

HAIGHT, M., MUDRY, T., AND PASTERNAK, J. (1982). Toxicity of seven heavy metals on *Panagrellis silusiae*: The efficacy of the free-living nematode as an *in vivo* toxicological bioassay. *Nematologica* **28**, 1–11.

HOWELL, R. (1982). Levels of heavy metal pollutants in two species of marine nematodes. *Mar. Pollut. Bull.* **13**, 396–398.

HOWELL, R. (1984). Acute toxicity of heavy metals to two species of marine nematodes. *Mar. Environ. Res.* **11**, 153–161.

KLASS, M. R. (1977). Aging in the nematode *Caenorhabditis elegans*. Major biological and environmental factors influencing life span. *Mech. Ageing Dev.* **6**, 413–429.

KRAYNC, E. C., VAN GESTEL, C. A. M., MULDER, H. C. M., DE VRIJER, F. L., SINKELDAM, E. J., VINK, G. J., CANTON, J. H., VAN APELDOORN, M. E., JANUS, J. A. (1987). *Integrated Criteria Document Cadmium: Effects*. Appendix to Report 758476002. National Institute of Public Health and Environmental Protection, Bilthoven, The Netherlands.

MUDRY, T., HAIGHT, M., AND PASTERNAK, J. (1982). The effects of some heavy metals on the kinetics of pharyngeal pumping in *Panagrellis silusiae*. *Nematologica* **28**, 12–20.

NEUSCHULZ, N., UND KÄMPFE, L. (1980). Das Reproduktionspotential von *Caenorhabditis briggsae* unter axenischen Bedingungen als Kriterium für unterschiedliche nematizide Einflüsse. *5. Vortr. tag. zu Aktuellen Problemen der Phytonematology, Rostock, GDR, 1980*, S. 46–55.

OHBA, K., AND ISHIBASHI, N. (1984). A nematode *Caenorhabditis elegans* as test organism for nematocide evaluation. Nippon Noyaku Gakkaishi **9**(1), 91–96.

PLATT, M. M., AND WARWICK, R. M. (1980). In *The Shore Environment* (J. H. Price, D. E. G. Irvine, and W. F. Farnham, Eds.) Vol. 2 pp. 729–759. Academic Press, London/New York.

POPHAM, J. D., AND WEBSTER, J. M. (1979). Cadmium toxicity in the free-living nematode, *Caenorhabditis elegans. Environ. Res.* **20**, 183–191.

RAMAMOORTHY, S., AND KUSHNER, D. J. (1975). Binding of mercuric and other heavy metal ions by microbial growth media. *Microb. Ecol.* **2**, 162–176.

SAMOILOFF, M. R. (1980). Action of chemical and physical agents on free-living nematodes. In *Nematodes as Biological Models* (B. M. Zuckermann, Ed.), Vol. 2, pp. 81–98. Academic Press, New York/London.

SAMOILOFF, M. R., AND BOGAERT, T. (1984). The use of nematodes in marine ecotoxicology. In *Ecotoxicological Testing for the Marine Environment* (G. Peroone, E. Jaspers, and Chr. Claus, Eds.) Part I, pp. 407–426. State University of Ghent, Belgium.

SAMOILOFF, M. R., SCHULZ, S., JORDAN, Y., DENICH, K., ARNOTT, E. (1980). A rapid simple long-term toxicity assay for aquatic contaminants using the nematode *Panagrellis redivivus. Canad. J. Fish. Aquat. Sci.* **37**, 1167–1174.

SAMOILOFF, M. R., BELL, J., BIRKHOLZ, D. A., WEBSTER, G. R. B., ARNOTT, E. G., PULAK, R., AND MADRID, A. (1983). Combined bioassay–chemical fractionation scheme for the determination and ranking of toxic chemicals in sediments. *Environ. Sci. Technol.* **17**, 329–334.

SCHIEMER, F. (1975). Nematoda. In *Ecological Aspects of Used Water Treatment* (C. R. Curds and H. A. Hawkes, Eds.), Vol. 1, pp. 269–288. Academic Press, New York/London.

SCHIEMER, F. (1983). Comparative aspects of food dependence and energetics of free-living nematodes. *Oikos* **41**, 32–42.

SCHUYTEMA, G. S., NELSON, P. O., MALUEG, K. W., NEBEKER, A. V., KRAWCZYK, D. F., RATCLIFF, A. K., AND GAKSTATTER, J. H. (1984). Toxicity of cadmium in water and sediment slurries to *Daphnia magna. Environ. Toxicol. Chem.* **13**, 293–308.

SIMPKIN, K. G., AND COLES, G. C. (1981). The use of *Caenorhabditis elegans* for anthelmintic screening. *J. Chem. Technol. Biotechnol.* **31**, 66–69.

SOKAL, R. R., AND ROHLF, F. J. (1981). *Biometry.* Freeman, New York.

TIETJEN, J. H., AND LEE, J. J. (1984). The use of free-living nematodes as a bioassay for estuarine sediments. *Mar. Environ. Res.* **11**, 233–251.

TROFYMOV, J. A., AND COLEMAN, D. C. (1982). The role of bacteriovorous and fungivorous nematodes in cellulose and chitin decomposition in the context of a root rhizosphere soil conceptual model. In *Nematodes in Soil Ecosystems* (D. W. Freckman, Ed.), pp. 117–138. Univ. of Texas Press, Austin.

VANFLETEREN, J. R. (1976). Large scale cultivation of a free-living nematode (*Caenorhabditis elegans*). *Seperatum Experientia* **32**, 1087.

VANFLETEREN, J. (1980). Nematodes as nutritional models. In *Nematodes as Biological Models* (B. M. Zuckermann, Ed.), Vol. 2, pp. 47–49. Academic Press, New York/London.

VRANKEN, G., VAN BRUSSEL, D., VANDERHAEGHEN, R., AND HEIP, C. (1984). Research on the development of a standardized ecotoxicological test on marine nematodes. I. Culturing conditions and criteria for two Monhysterids, *Monhystera disjuncta* and *Monhystera microphthalma.* In *Ecotoxicological testing for the marine environment* (G. Peroone, E. Jaspers, and C. Claus, Eds.), Vol. 2, pp. 159–184. State University of Ghent, Belgium.

VRANKEN, G., AND HEIP, C. (1986). Toxicity of copper, mercury and lead to a marine nematode. *Mar. Pollut. Bull.* **17**(10), 453–457.

VRANKEN, G., VANDERHAEGHEN, R., AND HEIP, C. (1985). Toxicity of cadmium to free-living marine and brackish water nematodes (*Monhystera microphthalma, Monhystera disjuncta, Pellioditis marina*). *Dis. Aquat. Org.*, **1**, 49–58.

WASILEWSKA, L., AND BIENKOWSKI, P. (1985). Experimental study on the occurrence and activity of soil nematodes in decomposition of plant material. *Pedobiologia* **28**, 41–57.

YEATS, G. W. (1979). Soil nematodes in terrestrial ecosystems. *J. Nematol.* **11**(3), 213–227.

YEATS, G. W. (1981). Nematode populations in relation to soil environmental factors: A review. *Pedobiologia* **22**, 312–338.