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Development and Testing of an Assay for Soil Ecosystem Health Using the Bacterial-Feeding Nematode *Cruznema tripartitum*[1]

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Survival and respiration rates of the bacterial-feeding nematode *Cruznema tripartitum* after incubation in soil for 48 hr provided a useful bioassay of the presence and concentration level of biologically active toxicants. The assay provided an indication of toxicant activity at sublethal levels, and a means of determining when the toxicant had declined to levels not deleterious to physiological function. Assays of soil contaminants based on the community structure of resident soil nematodes were more useful in undisturbed soils than in agricultural soils where the range of taxa was relatively narrow. Assays involving measurement of survival and respiration rates of nematodes after immersion in an aqueous extract of contaminated soils were not useful due to degradation and loss of contaminant during the extraction process.

INTRODUCTION

Biological responses that can be related to a toxicant in the environment are potential candidates for biomarkers. Biomarker responses may be resolved at various levels of biological organization. At the individual level, a biomarker may be the degree of change in an enzyme system, or an effect on development. At the population and community levels, biomarkers include reduced abundance, altered age structure, or altered community structure. At the ecosystem level, biomarkers may be changes in pathways and flows through food webs (Peakall, 1994). The response of biomarkers to toxicants should be well characterized and homogeneous in the test population so that observations can be interpreted. The test should be rapid, inexpensive, and provide consistent results (Giesy and Hoke, 1989). Approaches involving the use of biomarkers at both the physiological level and the community or higher levels are not mutually exclusive. Conceivably multiple levels might be used in a complementary manner to enhance understanding of the nature of environmental stress.

Few metazoan test animals native to soil habitats have well-documented physiological responses to anthropogenic stresses. Earthworms are frequently used, but the tests require extended exposure periods and relatively large soil samples (Callahan *et al.*, 1991; Donkin and Dusenbery, 1993; Greene *et al.*, 1989). The utility of several other taxa has been reviewed and a priority list of measurable criteria proposed for their application as indicators of soil quality (Stork and Eggleton, 1992). All the criteria are related to the presence, abundance, and diversity of species in the soil. There have been several recent uses of the nematode community structure as an assay of soil ecosystem health (Bongers, 1990; Freckman and Ettema, 1993; Stork and Eggleton, 1992; Yeates and van der Meulen, 1996). Generally, predatory nematodes are the most sensitive while bacterial-feeding nematodes are the most resilient to environmental perturbation (Bongers, 1990; Freckman and Ettema, 1993). In severely contaminated soils, bacterial-feeding nematodes may be the only metazoa found (Wieser, 1975).

Existing in water films around soil particles, soil nematodes are a ubiquitous group of multicellular terrestrial animals. In terrestrial ecosystems, nematode populations can reach densities of 30–55 million individuals m^{-2} (Yeates, 1979, 1981). They occur in several trophic classifications and include species that are herbivores, decomposers, and carnivores (Freckman and Ettema, 1993). Many of the bacterial-feeding decomposer species are readily cultured gnotobiotically on bacterial substrates, have short generation times (3 to 4 days), and are tractable test organisms for genetic, development, behavioral, and physiological studies (Nicholas, 1984). They may be useful markers of conditions that will be hazardous to other taxa. Ranking among the LC_{50}s for seven metals in bacterial-feeding nematodes was well correlated with the rankings of LD_{50}s from mammalian tests, and the response level of *Caenorhabditis elegans* was similar to that of rats (Donkin and Dusenbery, 1993; Williams and Dusenbery, 1988).

Organismal health has been defined as the state in which all physiological processes are functioning at or near their maxima (Sorauer, 1922). A similar definition for ecosystem health is proposed: the state of a system in which the collective physi-

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ological processes of the component organisms are able to function at or near their maxima. Clearly it would be difficult to achieve such an assessment for all the organisms in an ecosystem. In these studies the hypothesis that nematode community structure and physiological function can be used as component indicators of toxics in soil ecosystems is tested. Nematode community structure provides an indicator of the effects of the toxicant on organisms of different ecological roles; nematode metabolic rates provide an indicator of the effect of the toxicant on physiological function. The objectives of the studies were to (i) develop bioassays for health or toxicity of the soil ecosystem based on abundance, community structure, metabolic/respiration rates, or combinations thereof and (ii) test the bioassays in laboratory and field settings.

MATERIALS AND METHODS

Soils and Toxicants

Soils selected for laboratory assays in this study were silt loams from agricultural fields at the University of California, Davis, and a neighboring riparian area along Putah Creek (Yolo silt loam, typic xerorthents, 15% H_2O, 1.2% organic C, pH 6.8). Soils were passed through a 4-mm sieve and stored at 4°C. Prior to use, soil was incubated at 25°C for 18–24 hr. Assays were conducted with trichloroethylene (TCE) and toluene, alone and in combination, and with metam-sodium (MS). TCE is a volatile compound of increasing concern as a groundwater contaminant because of its persistence (Fuller *et al.,* 1997; Westrick *et al.,* 1984). While evidence of biodegradation has been reported (Kleopfer and Rittmann, 1982; Parsons and Lage, 1985; Vogel and McCarty, 1985), the products of degradation include equally harmful metabolites, including dichloroethylenes and vinyl chloride. Metam-sodium is a toxicant with fungicidal, nematicidal, and herbicidal actions that result from the release of methyl isothiocyanate.

Abundance and Community Structure Assays

Fifty-gram (dry weight) samples of agricultural or riparian soil were weighed into 250-ml glass bottles (Qorpak Boston Rounds, Fisher Scientific, Fair Lawn, NJ) equipped with 24-mm Mininert caps (Dynatech Precision Sampling Corp., Baton Rouge, LA) and exposed to TCE concentrations of 0, 1, and 30 μg ml^{-1} soil solution. Three or four replications were used in different experiments. Before incubation, and after 28 days of incubation, the resident nematodes were extracted from the soil by a modified Cobb's sieving–centrifugation procedure (Fuller *et al.,* 1997; Jaffee *et al.,* 1988).

Nematodes were counted and classified by family; an index of community structure, the maturity index (MI), was calculated. The MI is based on a grouping of nematode families into five categories, from ''colonizers,'' characterized by a high reproductive rate indicating opportunists or r-strategists, to ''persisters,'' characterized by a low reproductive rate indicating k-strategists. Colonizers (c) are assigned a value of 1 and persisters (p) a value of 5. Families which have characteristics of both types are assigned intermediate values on the c-p scale. The MI value is calculated as the weighted mean of the individual c-p values:

$$MI = \Sigma\, v(\mathrm{i}) \times f(\mathrm{i}), \qquad (1)$$

where v(i) is the c-p value of taxon i and f(i) the proportion of taxon i in a sample (Bongers, 1990). Plant-parasitic nematodes and nematodes in families with the c-p value of 1 were omitted from all MI calculations because their presence reflects substrate availability rather than stress (Bongers and Korthals, 1993, 1994).

Respiration Assay

The first step in developing the assay was to select a bacterial-feeding nematode as the test species. Three species of bacterial feeding nematodes (*Cruznema tripartitum, Cephalobus persegnis,* and *Rhabditis cucumeris*) were placed into concentrations of TCE (0, 1, and 30 μg ml^{-1}) or TCE in combination with toluene (0, 1, and 30 μg ml^{-1} TCE with 20 μg ml^{-1} toluene) in 250-ml bottles. After gentle shaking for 48 hr at 25°C, each sample was aerated for 10 min to remove trace amounts of TCE and toluene. The samples were placed on Baermann funnels. Nematodes that moved across the filter were collected after 48 hr and counted. *Cruznema tripartitum* was selected as the test organism (see justification under Results). Voucher specimens of *C. tripartitum* have been deposited in the University of California, Davis, Nematode Collection (Accession Nos. UCDNC 2910 and 2911).

C. tripartitum was obtained from the field site of a long-term low-input sustainable agriculture experiment at the University of California (Temple *et al.,* 1994). The nematode was grown gnotobiotically on *Escherichia coli* strain OP50 on nematode growth medium (Sulston and Hodgkin, 1988). The growth medium consisted of bacto-peptone (2.5 g liter^{-1}; Difco Laboratories, Detroit, MI), bacto-agar (17 g liter^{-1}; Difco Laboratories, Detroit, MI), cholesterol (1.0 ml of 5 mg ml^{-1} in ethanol; Eastman Fine Chemicals, Rochester, NY), and NaCl (3 g liter^{-1}). This stock solution was autoclaved for 20 min at 250°C. $CaCl_2$ (1 *M,* 1.0 ml), $MgSO_4$ (1 *M,* 1.0 ml), and K_2PO_4 (1 *M,* pH 6.0, 25 ml) were individually prepared, filter-sterilized, and added to the stock solution immediately after autoclaving. Gnotobiotic nematode culturing conditions were used since unknown soil bacteria may transform the toxicants, with a consequent effect on the respiration and mortality rate of *C. tripartitum* (Fuller *et al.,* 1997).

Soil was collected from the agricultural site and 50 g (dry weight) was placed in each of 36 glass bottles with Mininert caps. The toxicant treatment series was 0, 1, and 30 μg ml^{-1} TCE. A suspension of *E. coli* was added to half the bottles at each concentration level to test whether assay results were impacted by the presence of a food source for the nematode. Two methods were tested for assessing the sensitivity of nem-

atode respiration rates to chemical stress: (i) nematodes were incubated in the soil for 48 hr at 25°C and then extracted (Method I) and (ii) nematodes were incubated in a water extract of the contaminated soil (Method II). In Method I, concentrations of the toxicant in the soil were confirmed by gas chromatography after 24 hr. After 48 hr, TCE was removed from the bottles by venting. Approx 50,000 *C. tripartitum* were added to half of the bottles of each TCE concentration/*E. coli* combination. After 48 hr exposure, native and indicator nematodes were extracted from the soil by Cobb's sieving–centrifugation (Jaffee *et al.,* 1988). In Method II, a saturation extract of the soil solution was prepared from each bottle by adding 25 ml distilled water, shaking vigorously, waiting 15 sec for the particles to settle, and withdrawing 10 ml of the supernatant. The supernatant was divided in half, and approx 50,000 *C. tripartitum* were added to one half. The samples were shaken for 48 hr at 25°C. Nematodes from both water extract and soil incubation methods were measured to allow calculation of biomass (Ferris *et al.,* 1995).

After the exposure period in both methods, the nematode suspension was centrifuged and the supernatant removed. The pellets were placed in respiration chambers to measure CO_2 evolution rates using an infrared CO_2 analyzer (LiCor LI-6000, Lincoln, NE). The treatments without *C. tripartitum* allowed assessment of biomass and respiration of the resident nematode population and added *E. coli*. Those values were subtracted from measurements of the equivalent treatments with *C. tripartitum.*

The relationship between nematode respiration rate and body weight is described by

$$R = aW^{0.75} \quad (2)$$

where R is the CO_2 evolution individual^{-1} hr^{-1}, W is the body weight of the individual, and a is the species-specific respiration rate of an individual of unit weight. Standardized respiration rates for *C. tripartitum* (expressed as a values) were calculated from measurements of CO_2 evolution over 30-min periods at 20°C from a known biomass of indicator nematodes (Ferris *et al.,* 1995).

Mortality rates before and after the respiration assay were determined using the Baermann funnel method. *C. tripartitum* from each sample were placed on Baermann funnels for 48 hr. Nematode survival was determined from the number of individuals moving through the filter.

Testing the Assay

In laboratory tests, two concentration ranges of MS (0, 16, and 1600 μg ml^{-1} or 0, 1.56, and 6.25 μg ml^{-1}) were established in 75 g soil in 250-ml glass bottles. In each test, soil in the bottles was inoculated with 15,000–25,000 *C. tripartitum* and the toxicant was vented after incubation for 48 hr at 25°C. To test the effect of a food source for the nematode, *E. coli* was added to soil in half the bottles at each concentration level. The control series consisted of an equal number of bottles set up and treated identically, but without *C. tripartitum.* There were three replications of each treatment combination. After incubation, nematodes were extracted from the soil in each bottle, and respiration rates were measured as previously described. Each test was repeated three times.

To test the utility of the assay in the field, a test site was selected at the Holly Sugar Research Farm in Tracy, California. The site allowed testing of the effects of aldicarb (a carbamate nematicide) alone, and MS and aldicarb in combination. Control and test plots were separated by a 3-m buffer area. Plot size was 30 × 10 m. Soil was collected on eight dates (0, 3, 5, 10, 17, 24, 38, and 52 days after application of MS). Samples consisted of 12 cores at 0–15 cm depth taken randomly from each plot with a 2.5-cm-diam Oakfield sampling tube. A 400-cc subsample was taken from each sample, transferred to a sealed mason jar, placed on ice, and immediately transported to the laboratory. Three samples were taken from each plot at each sampling date. In the laboratory, two 75-g soil samples were removed from each mason jar and placed into 250-ml glass bottles. Soil in one bottle from each jar was inoculated with 15,000–25,000 *C. tripartitum;* bottles were incubated for 48 hr at 25°C. After incubation, nematodes were extracted from the soil in each bottle; respiration and mortality rates of *C. tripartitum* and resident nematodes were measured. Respiration rates of resident nematodes from bottles to which *C. tripartitum* had not been added were subtracted from those to which it had been added.

RESULTS

Abundance and Community Structure Assays

Abundance of the resident nematodes after exposure to TCE was greater in the agricultural soil than in the riparian soil (Fig. 1A). There was no significant effect of TCE on nematode abundance in the riparian soil. In the agricultural soil there was a slight increase in nematode abundance at low TCE levels. The nematode maturity index was somewhat greater in the riparian than the agricultural soil (2.9 to 2.1) with plant parasites and class 1 nematodes eliminated. There was no significant effect of TCE on MI in either riparian or agricultural soils (Fig. 1B). However, there was a strong decreasing trend in MI with increase in toxicant concentration in the riparian soil where the nematode community was more structurally diverse (Fuller *et al.,* 1997).

Respiration Assay

While all three species of bacterial-feeding nematodes were tolerant to lower concentrations of TCE and toluene, *C. tripartitum* was selected as the test organism due to its ability to tolerate higher concentrations of TCE and toluene (Fig. 2). There were no differences in respiration rates of *C. tripartitum* at 20°C after incubation for 48 hr at 25°C in aqueous extracts of soil except at the highest concentration of TCE with no

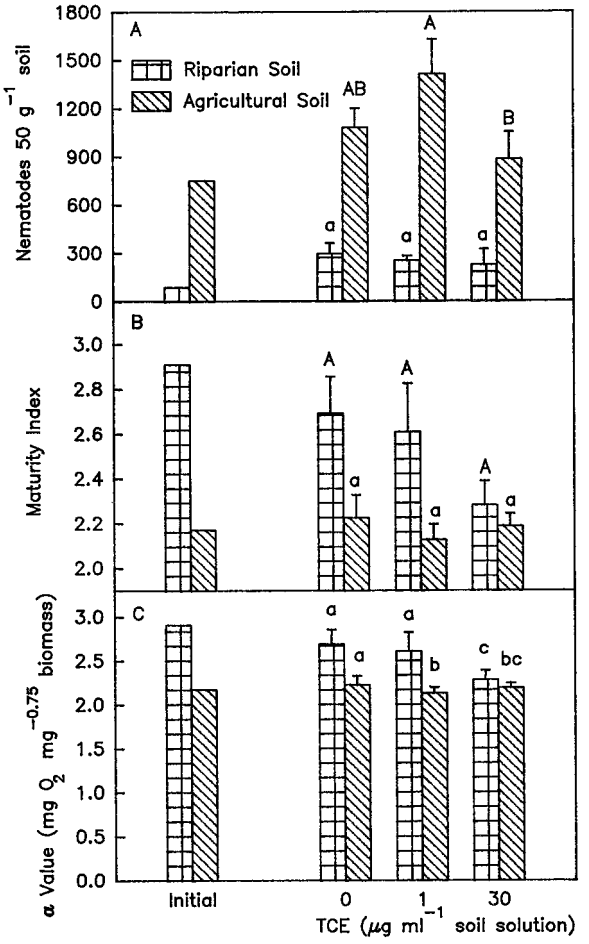

FIG. 1. Assay development with trichloroethylene (TCE). (A) Abundance of resident nematodes in soils from two sites before and after exposure for 28 days to varying concentrations of TCE (from Fuller *et al.,* 1996). (B) Community structure of resident nematodes as measured by the maturity index (Bongers, 1990) in soils from two sites before and after exposure for 28 days to varying concentrations of TCE (from Fuller *et al.,* 1996). (C) Respiration rates of *Cruznema tripartitum* before and after 48 hr exposure to TCE in soil. Values do not differ significantly when bars are marked with the same letter ($\alpha = 0.05$); data from different experiments are marked with letters of different case.

bacterial food substrate added (data not provided). However, following incubation in soil for 48 hr at 25°C in a range of concentrations of TCE, respiration rates of *C. tripartitum* at 20°C declined (Fig. 1C). Expected *a* values were determined from previous research based on the temperature conditions of this assay (Ferris *et al.,* 1995). Most nematodes survived exposure to TCE in the soil.

Testing the Assay

The abundance of all soil nematodes decreased significantly at high concentrations of MS in laboratory tests and no effects were observed at concentrations of 16 μg ml^{-1} or less (Fig. 3A). There was a significant decline in MI in the riparian soil when exposed to MS (Fig. 3B). In tests of the respiration rates of *C. tripartitum* after exposure to MS in soil at 20°C, no assay nematodes survived the highest rate of 1600 μg ml^{-1}; however, all nematodes survived the other dosage rates. At all other dosage rates, the respiration rate declined with increased dosage of toxicant (Fig. 4).

The field study of the impact of multiple stressors (MS and aldicarb) on ecosystem health revealed a 25-day recovery from exposure to aldicarb alone, and a delayed onset of recovery from the combined impact of both stressors (Fig. 5). The assay nematodes did not survive exposure to the multiple-stressed soil until 11 days after treatment.

DISCUSSION

The abundance of organisms, particularly key species, is often proposed as a biomarker of ecosystem health (Peakall, 1994; Stork and Eggleton, 1992). A difficulty with markers of this type is the lack of knowledge of the abundance of individuals present in the system prior to the exposure. There are two solutions to that problem. Nematode abundance in the contaminated area can be compared with that in neighboring noncontaminated areas. That assumes the availability of neighboring noncontaminated areas and that those areas started with the same abundance and community structure as the contaminated area. The second solution is to introduce known numbers of marker nematodes into both contaminated and noncontaminated soils, remove them after a fixed exposure period, and measure survival. The species selected must not already be present in the community. In these studies, the second approach is taken a step further in that physiological activity of the surviving nematodes is measured. Physiological processes inherent in life functions may be differentially impaired in contaminated ecosystems, but measurement of respiration integrates across the effect of toxins on many of those processes. A single-species assay should be more consistent and rigorous than a measurement of general soil respiration.

Several studies suggest that the diversity, species composition, and activity levels of nematode populations may be ex-

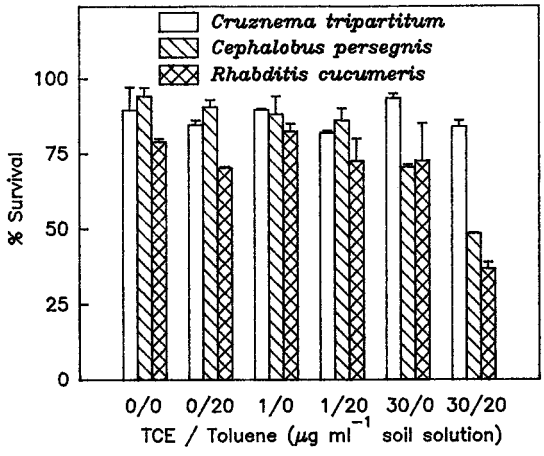

FIG. 2. Survival of three species of bacterial-feeding nematodes after exposure to trichloroethylene–toluene mixtures in water.

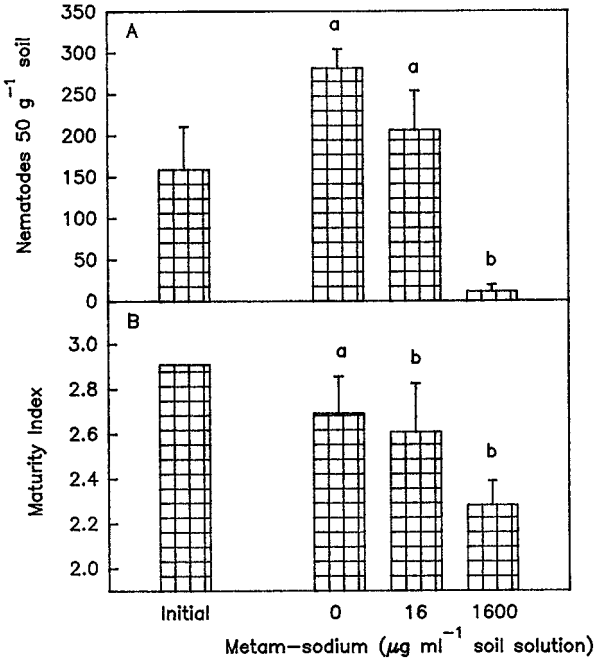

FIG. 3. Assay testing with metam-sodium (MS). (A) Abundance of resident nematodes in soil from a riparian site before and after exposure for 28 days to varying concentrations of MS. (B) Community structure of resident nematodes as measured by the maturity index (Bongers, 1990) in soil from a riparian site before and after exposure for 28 days to varying concentrations of MS. Values do not differ significantly when bars are marked with the same letter ($\alpha = 0.05$).

cellent and readily measured indicators of environmental quality. Nematodes are proving useful indicators of pollution levels in aquatic and soil systems, and in industrial toxicology (Samoiloff, 1990; Van Kessel *et al.*, 1989; Vranken *et al.*, 1991). Since differences in numbers did not correlate with TCE concentrations, nematode abundance was found to be an inconsistent indicator of chemical stress. The Bongers (1990) maturity index, based on composition and abundance of the nematode community, has been tested as a basis for an eco-

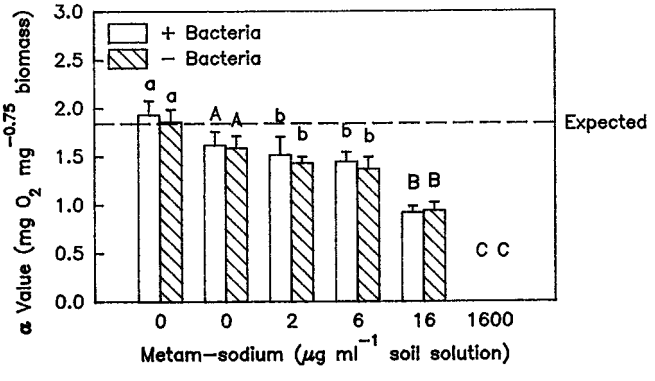

FIG. 4. Assay testing with metam-sodium (MS). Respiration rates of *Cruznema tripartitum* before and after 48 hr exposure to MS in soil. Values do not differ significantly when bars are marked with the same letter ($\alpha = 0.05$); data from different experiments are marked with letters of different case.

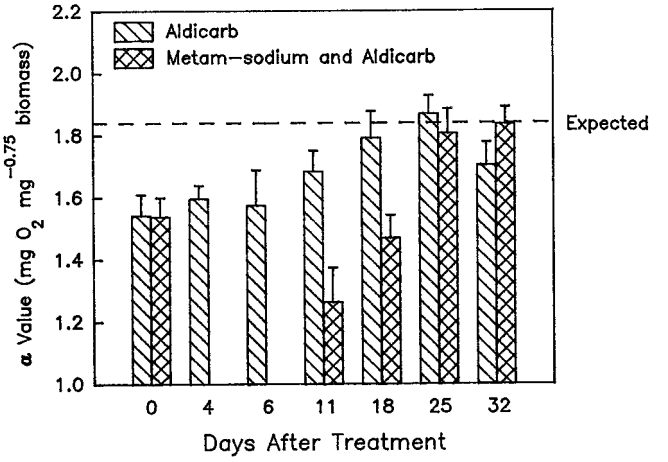

FIG. 5. Respiration rates of *Cruznema tripartitum* after 48 hr exposure in soils at different time intervals after treatment with aldicarb alone or aldicarb and metam-sodium in combination.

logical classification of soil (De Goede and Bongers, 1994). The MI provides a 1–5 scale for ecosystem quality, with 1 being the most contaminated/disturbed and 5 being the most pristine. It is also used as a tool to assess the suitability of reference sites for environmental monitoring (Neher and Campbell, 1994). However, MI was only useful as an assay of toxicant concentration and health of the soil ecosystem when soils contained diverse nematode communities that included significant proportions of species in higher c-p classes. Agricultural soils may be frequently disturbed through tillage practices, cultural operations, and cropping sequences, which may result in relatively low MI values (e.g., see Ferris *et al.*, 1996). In soils where the c-p values of the nematode community are low, the sensitivity of the MI to chemical stress is diminished.

The bacterial-feeding nematode, *C. tripartitum*, proved to be a useful assay organism because it survives in contaminated environments and its respiration rate reflected the degree of contamination. The nematode respiration assay (Figs. 1C and 4) provided a more sensitive and consistent measure of soil health in relation to the chemical stressors MS and TCE than assays based on either nematode mortality (Figs. 1A and 3A) or nematode community structure (Figs. 1B and 3B). Since differences in respiration rates of *C. tripartitum* after exposure to water extract from contaminated soil were not significant, Method II is considered an unreliable indicator of chemical stress. Apparently, the TCE used in these tests volatilized in the soil extract. Since significant differences of respiration rates correlated with degree of chemical stress when nematodes were introduced directly into the contaminated soil, Method I was the most useful assay.

Stress may suppress respiration of nematodes through direct effects on respiratory physiology, or through effects on growth, feeding, and locomotion (Samoiloff, 1980; Vranken *et al.*, 1991). Ecosystem contamination may also result in reduced availability of food for the soil biota, indirectly suppressing

nematode respiration. In the current experiments, the *E. coli* treatments allowed assessment of the contribution of food limitation to respiration suppression. Respiration rates of *C. tripartitum* following the soil exposure method declined with exposure to increasing concentrations of TCE (Fig. 1C). The addition of bacteria during the incubation did not significantly increase respiration rate at any concentration of TCE. Thus, it seems likely that TCE directly affected nematode metabolic processes, rather than indirectly influencing respiration through impacts on food supply.

The Holly Sugar Research Farm provided an opportunity to measure the effects of multiple stressors as the MS was applied to a field already treated with aldicarb. The bioassay proved to be a useful indicator of the impact of the stressors on biological activity in the soil. Recovery of soil health was delayed in the presence of both stressors (Fig. 5). Survival of the assay nematode in the multiple-stressed soil was poor until 11 days after treatment. After that, the rate of recovery from the combined stressors was faster than that for aldicarb alone, probably indicating more rapid bioremediation and/or degradation of the MS than of the aldicarb.

CONCLUSION

Biological indicators of contamination or ecosystem health are useful in that they reflect the integration of a toxicant across the life processes of biological systems. In contrast to chemical analyses, which generally require knowledge of the nature of the contaminant, biological assays indicate the degree of impact of one or many contaminants, even when the nature of those contaminants is unknown. Clearly, such assays are useful in detecting the presence of a contaminant and in monitoring the persistence of that contaminant. Determination of the nature of the contaminant will require appropriate chemical analyses. Population and community level assays with soil nematodes were evaluated and found to be unreliable or ambiguous in interpretation. An assay at the organismal level, based on respiration rates of the bacterial-feeding nematode *Cruznema tripartitum*, could complement existing tests and help fill the need for multicellular organisms in ecotoxicological studies and site assessment (Dowd, 1984; Porcella, 1983).

REFERENCES

Bongers, T. (1990). The maturity index: An ecological measure of environmental disturbance based on nematode species composition. *Oecologia* **83,** 14–19.

Bongers, T., and Korthals, G. (1993). The maturity index, an instrument to monitor change in nematode community structure. *Proc. Int. Symp. Crop Protect.* **45,** 80.

Bongers, T., and Korthals, G. (1994). The behaviour of maturity index and plant parasite index under enriched conditions. *Proc. Int. Nematol. Symp.* **22,** 39.

Callahan, C. A., Menzie, C. A., Burmaster, D. E., Wilborn, D. C., and Ernst, T. (1991). On-site methods for assessing chemical impact on the soil environment using earthworms: A case study at the Baird and McGuire superfund site, Holbrook, Massachusetts. *Environ. Toxicol. Chem.* **10,** 817–826.

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.

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.

Dowd, R. M. (1984). Biological monitoring. *Environ. Sci. Technol.* **18,** 215A.

Ferris, H., Lau, S., and Venette, R. (1995). Population energetics of microbivorous nematodes: Respiration and metabolic rates based on carbon dioxide production. *Soil Biol. Biochem.* **27,** 319–330.

Ferris, H., Venette, R. C., and Lau, S. S. (1996). Dynamics of nematode communities in tomatoes grown in conventional and organic farming systems, and their impact on soil fertility. *Appl. Soil Ecol.,* **3,** 161–175.

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

Fuller, M. E., Scow, K. M., Lau, S., and Ferris, H. (1997). Trichloroethylene (TCE) and toluene effects on the structure and function of the soil community. *Soil Biol. Biochem.,* in press.

Giesy, J. P., and Hoke, R. A. (1989). Freshwater sediment toxicity bioassessment: Rationale for species selection and test design. *J. Great Lakes Res.* **15,** 539–569.

Greene, J. C., Bartels, C. L., Warren-Hicks, W. J., Parkhurst, B. R., Linder, G. L., Peterson, S. A., and Miller, W. E. (1989). *Protocols for Short-Term Toxicity Screening of Hazardous Waste Sites,* EPA 600/3-88/029. U.S. Environmental Protection Agency, Corvallis, OR.

Jaffee, B. A., Gaspard, J. T., Ferris, H., and Muldoon, A. E. (1988). Quantification of parasitism of the soilborne nematode *Criconemella xenoplax* by the nematophagous fungus *Hirsutella rhossiliensis. Soil Biol. Biochem.* **20,** 631–636.

Kleopfer, H., and Rittmann, B. E. (1982). Microbial removal of hazardous organic contaminants in groundwater. *Environ. Sci. Technol.* **19,** 384–392.

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.

Nicholas, W. L. (1984). *The Biology of Free-living Nematodes.* Clarendon, Oxford.

Parsons, F., and Lage, G. B. (1985). Chlorinated organics in simulated groundwater environments. *J. Am. Water Works Assoc.* **77,** 52–59.

Peakall, D. B. (1994). Biomarkers: The way forward in environmental assessment. *Toxicol. Environ. News* **1,** 55–60.

Porcella, D. B. (1983). *Protocol for Bioassessment of Hazardous Waste Sites,* EPA 600/2-83/054. U.S. Environmental Protection Agency, Corvallis, OR.

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

Samoiloff, M. (1990). The nematode toxicity assay using *Panagrellus redivivus. Toxicol. Assess.* **5,** 309–318.

Sorauer, P. (1992). *Manual of Plant Disease,* 3rd ed. Record Press, Wilkes-Barre, PA.

Stork, N. E., and Eggleton, P. (1992). Invertebrates as determinants and indicators of soil quality. *Am. J. Altern. Agric.* **7,** 38–47.

Sulston, J., and Hodgkin, J. (1988). Methods. In *The Nematode Caenorhabditis elegans* (W. B. Wood, Ed.), pp. 587–606. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.

Temple, S. R., Friedman, D. B., Somasco, O., Ferris, H., Scow, K., and Klonsky, K. (1994). An interdisciplinary, experiment station-based participatory comparison of alternative crop management systems for California's Sacramento Valley. *Am. J. Altern. Agric.* **9,** 64–71.

Van Kessel, W. H. M., Brocades Zaalberg, R. W., and Seinen, W. (1989). Testing environmental pollutants on soil organisms: A simple assay to investigate the toxicity of environmental pollutants on soil organisms, using $CdCl_2$ and nematodes. *Ecotoxicol. Environ. Saf.* **18,** 181–190.

Vogel, T. M., and McCarty, P. L. (1985). Biotransformation of tetrachloroethylene to trichloroethylene, dichloroethylene, vinyl chloride, and carbon dioxide under methanogenic conditions. *Appl. Environ. Microbiol.* **49,** 1080–1083.

Vranken, G., Vanderhaeghen, 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.

Westrick, J. J., Mello, J. W., and Thomas, R. F. (1984). The groundwater supply survey. *J. Am. Water Works Assoc.* **76,** 52–59.

Wieser, W. (1975). The mesofauna as a tool in the study of a habitat heterogeneity: Ecosystem aspects. A review. *Cah. Biol. Mar.* **16,** 647–670.

Williams, P. L., and Dusenbery, D. B. (1988). Using *Caenorhabditis elegans* to predict mammalian acute lethality to metallic salts. *Toxicol. Ind. Health* **4,** 469–478.

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

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

Yeates, G. W., and van der Meulen, H. (1996). Recolonization of methylbromide sterilized soils by plant and soil nematodes over 52 months. *Biol. Fertil. Soils* **21,** 1–6.