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RESEARCH ARTICLE

NEMATODES AS ENVIRONMENTAL INDICATOR

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ABSTRACT

The use of bioindicators in monitoring programmes is helpful to detect environmental status or to evaluate the efficacy of measures taken to improve environmental quality and to anticipate emerging problems. Nematodes are highly diversified group of animals on earth and excellent indicator organism for determination of environmental status at the place under investigation. This review draws the importance of nematode as biological indicator of environmental status.

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INTRODUCTION

Various environmental problems like global climate change, stratospheric ozone depletion, habitat destruction, and species extinction have threatened the world. Environmental scientists are trying to adequately assess ecological status and to detect trends and changes in environmental condition. Bioindication is scientific analysis of ecological information to make interferences about the quality of the environment at that area. Therefore indicator is used to describe and evaluate ecological conditions and trends, to anticipate emerging problems and address national and international monitoring for policy, legislation and administrative purpose. The use of bioindicator is an innovative approach for assessing various types of environmental mismanagement, including pollution, high input farming, inappropriate disposal of wastes, contamination, etc. This approach uses biological organisms and biodiversity as tools to assess ongoing situations in the environment. Biologically, soil ecosystems maintain a diversity of microbes (fungi, bacteria, and algae), microfauna (protozoa), and mesofauna (arthropods and nematodes).The prevalence of organisms reflect the nature and quality of the environment. Ecologists have argued about diversity, biological richness, and animal and plant abundance as measure of environmental quality.

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Any indicator should reflect the structure and function of ecological processes and respond to changes in soil condition that result from land-management practices. According to Stenberg (1999), the use of microbial community of the soil as a quality indicator is due to the dynamic nature of soil microorganisms. The microorganisms play essential functions in the soil structuring processes, humus formation, nitrogen biological fixation, mycorrhizal associations, nutrients solubilization for the plants, pests and pathogen reduction, persistent compounds degradation applied to the soil and other changes in the soil properties that affect the plant growth (Kennedy and Papendick, 1995; Kennedy and Smitth, 1995). Knowledge on the diversity of these organisms in natural soils and soils in use under different production systems can be a very useful indicator tool to determine a sustainable agriculture. Soil fauna have advantages over soil microbes as bioindicator. Because they serve as integrators of physical, chemical and biological properties related with their food resources and their generation time is longer than metabolically active microbes, making them more stable temporally and not simply fluctuating with ephemeral nutrient flushes (Nannipieri *et al*., 1990).

Qualities Of Indicator Species: It is essential to choose a minimum set of indicators that have the qualities such as simplicity, ease of evaluation, use of the largest possible number of habitat, highly sensitive to environmental changes, to soil and climate management and be measured by quantitative and/or qualitative methods (Saviozzi *et al*., 2001).

Nematode as Indicator species: Nematodes (Bongers, 1990), Collembola (Frampton, 1997), and mites (Ruf, 1998) are three groups of mesofauna that have been considered for use as biological indicators. Of these three groups, nematodes have been evaluated most often for their use as indicator. Nematodes belong to the animal kingdom, phylum Nematoda. They are usually microscopic organisms (Ekschmitt *et al.*, 2001), highly diversified group of animals on earth. Nematode constitutes nearly 90% of all Metazoa in number (Hugot *et al.*, 2001). Nematodes are found in almost every kind of habitat i.e. terrestrial, rivers, lakes, marine, freshwater, ice land etc. Nematodes are representing a central position in the soil food web. Nematodes occur in all soil and aquatic systems: in acidified forests soil, in heavily polluted soil, on heavy clay, in deep sea sediments, in rotting plant material, in compost and in any habitat in which organic material is decomposed. They are sensitive to environmental factors and changes in their activity and distribution. The use of nematode as indicators in the detection of environmental status is currently attracting wide interest among biologists. Such an indicator is needed since the number of potential environmental contaminants increases yearly, primarily because of an inability to predict toxicity. The important early work on the nematode indicator was done by E.C Dougherty, V Nigon and their respective colleagues between 1945 and 1965. Nematodes have been recognized as good soil health bioindicator since the1970s in both Europe (Prejs, 1970; Wasilewska, 1970; Sohlenius, 1973; Zullini, 1976; Sudhaus, 1981) and NewZealand (Yeates, 1979), and since the 1980s in the U.S.(Stinner and Crossley, 1982; Yeates and Coleman, 1982; Ingham *et al.*, 1985; Freckman, 1988).

Nematodes may be the most useful group for community indicator analysis because more information exists on their taxonomy and feeding roles (Gupta and Yeates, 1997) than does for other mesofauna. The development of nematodes as bioindicator required determination of appropriate ways to assess and quantify their contributions to ecological processes, and the validation of their utility as indicators of environmental condition. Several unique characteristics of nematodes facilitated those developments (Bongers, 1990; Cairns *et al.*, 1993; Yeates *et al.*, 1993). Small size, nearly ubiquitous distribution, present in large number, wide diversity of nematode species. Nematodes are transparent, and can be identified without biochemical procedures. They have relatively short generation time, thus allowing rapid response to environmental changes. Sampling for nematodes creates minimal habitat disturbance and is virtually non-destructive. They must be able to be readily cultured in the laboratory under defined and controlled conditions and be capable of being handled by available technique.

- Nematodes have a permeable cuticle, which allows them to respond with a range of reactions to pollutants and correspond with the restorative capacity of soil ecosystems
- Some nematodes have resistant stages such as cryptobiosis or cysts that allow them to survive inactively during environmental conditions unfavorable for growth and development. However, some nematode taxa such as Dorylaimidae have no resistant stages, which may make them more sensitive to environmental change.
- They do not rapidly migrate from stressful conditions and many species survive dehydration, freezing or oxygen stress.
- Nematodes have heat shock proteins that are highly conserved. Expression of these proteins is enhanced when exposed to stresses such as heat, metal ions, or organic toxins. Perhaps these proteins could serve as biomarkers for ecotoxicological assessment of soils.

The nematode, especially the free-living nematodes has several features that make them excellent indicator organism for determination of the presence of toxic contaminants in aquatic, marine and terrestrial environment. M R Samiloff, a zoologist in 1980s carried out important studies by utilizing *Panagrellus redivivus* as an indicator species. This species is the indicator organism of choice because of wide applicability, post-embryonic development readily entrained to external stimuli, synchronized, culture in axenic or monogenic media, and effect of a known concentration of contaminants to the parameters of post-embryonic development. Application of bacterial-feeding nematode, *Caenorhabditis elegans* model to address ecological questions is also interesting. Several toxicity tests with this nematode species have been developed to determine the risk of chemicals to biota. Studies have been primarily concerned with finding lethal endpoints of metals and organic compounds in single species cultures, aqueous solution, soil and sediment dose bioassays (Sochova *et al.*, 2006).

Nematodes have been used as environmental bioindicator in relation to diverse ecosystem services, soil health, plant diseases, management of parasites in grazing mammals, human health, or insect control, etc. Their fauna composition, together with its ecological indices, has emerged as a useful monitor of environmental conditions and soil ecosystem function (Neher, 2001). Since nematode live in the soil pore water, they are assumed to be exposed to the contaminant concentration in the solution, which offers good perspectives for assessing the effects of contaminants as well as soil status (Bongers and Ferris, 1999; Cortet *et al.*, 1999; Achazi, 2002). Although, nematodes represent a relatively small amount of biomass in the soil, their occurrence across multiple trophic levels is vitally important in the soil environment. There are differences in feeding behavior, and predominantly, omnivore and predators have great sensitivity to disturbances (Yeats *et al.*, 1993). There is a clear relationship between structure and function, and nematodes respond rapidly to disturbance and enrichment: increasing microbial activity leads to changes in the proportion of bacterial feeders in a community. Different nematode taxa exhibit specificities of food sources and changes in the food web are mirrored in shifts among feeding groups. Since their feeding habits are clearly related to oral structure, their trophic roles are readily inferred. Both terrestrial and aquatic nematodes are used to infer about conditions of food web status and function in managed and natural systems (Danovaro *et al.*, 2009; Nagy, 2009; Neher, 2010). Many families within the Tylenchina feed exclusively on the roots of higher plants but never on bacteria. Cephalobidae and Plectidae feed on bacteria but not on higher plants or fungi. Mononchidae and Anatonchidae are specialist predators of other nematodes.

MONITORING: Monitoring is done by chemical analysis and biomonitoring.

Chemical analysis: Most monitoring programme focus on the presence and concentration of individual chemical rather than on the net toxic effect in a particular environment. Usually monitoring is carried by chemical analysis; and determining whether these chemicals individually occur at concentration greater than some previously established safe-level.

Biomonitoring: Biomonitoring is an approach that focuses on the combination effects of environmental contaminants by the application of one or more biological system as indicator. It can be (a) field based analysis that lack strict experimental control, (b) laboratory based analysis (bioassay), using appropriate indicator organism. Biomonitoring and habitat assessment are two tools that river ecologists use to assess the water quality. Sample analysis of *in situ* nematode faunae at family level provides abundant information on benthic ecosystems.

TECHNIQUES USED IN BIOASSAY

Microcosms: Microcosms are defined as a controlled, reproducible laboratory system which attempts to stimulate the situation (i.e. processes and interaction of components) in a portion of the real world. The researcher can permit the system to be open, semi-closed or closed to fluxes of air, water and biota to environmental conditions (light, temperature, humidity) and to the content of soil or other compounds, as befits the knowledge and assumptions about the environment simulated, the experiment being executed. Microcosms can be very incisive, cost-effective and practical. The rational in design and operation of microcosm places them between single spp. laboratory toxicity tests and chemical laboratory bench tests, where there is a high degree of investigator control and great freedom in simple variation of experimental conditions and components one at a time, and the field, where investigator have very limited control over components or conditions.

Pollution-Induced Community Tolerance (PICT): Toxic chemicals in the environment exert selective pressure on biota, eliminating sensitive organisms. The restructured community will be more tolerant to the toxicant than the original community. This tolerance increase is the basis for the new ecotoxicological tool (PICT) developed by Blanck *et al*., (1988). PICT may be used to detect minor changes occurring in contaminated ecosystem, estimate the size of the influenced area and find the (group of) compounds(s) causing the impact. This is achieved by combining ecological and physiological approach. PICT can be used to discriminate between primary and secondary effects of the toxicant. Finally, if co-tolerance patterns are identified, these will give an indication of the physiological mode of action of the toxicant and also a rough idea of possible tolerance mechanisms.

INDICES

A variety of graphical and statistical technique or indices have been used to describe environmental change using nematodes.
Ecological indices are derived from nematode faunal analysis; provide useful bioindicator for disturbance of the soil environment and condition of the soil food web. Nematode fauna composition, together with its ecological indices, has emerged as a useful monitor of environmental conditions and soil ecosystem function as well as benthic ecosystems. Interpretation of soil health condition by using nematode community (trophic structure, sex structure, and taxa composition) analysis required a comprehensive analysis that included different nematode trophic groups, fungal to bacterial-feeding nematode ratio, richness, diversity, dominance, maturity index(MI) , Enrichment index (EI) , structure index (SI), channel index (CI) and basal index (BI). Nematodes are found to correlate with concentration of soil pollutants: maturity index, diversity index, similarity index, key species, N/C ratio, physiological index, Plant parasite index (PPI), Sigma MI (including all the soil nematodes), Diversity index (H''), and Wasilewska index (WI) (Bongers, 1990; Neher, *et al*., 1995; Neher and Campbell, 1996; Bongers *et al*., 1997; Ferris *et al*., 2001; 2004). Nematode population correlations can established nitrite-nitrogen levels, sulphur dioxide, heavy metal concentration in terrestrial, marine and soil, petroleum fraction in soil, acid and alkaline emissions in soil (Callahan *et al*., 1979; Kathman *et al*., 1984).

Generic richness (S): as indicated by the number of genera, reflects biodiversity of soil habitat. However, it has been suggested that diversity indices are too insensitive to measure the effects of pollution .This is because the majority of the indices and statistical techniques used are purely mathematical functions and do not take account of the biology of the organism being studied. Furthermore, as these indices ignore the ecological requirements of the species being studied, diversity may increase in situations where it would be expected to decrease.

Maturity Index (MI): This index is used as a measure of both terrestrial and marine disturbance. Bongers (1990) suggested the Maturity Index (MI) which reflects aspects of nematode biology. Nematode (excluding PP) are categorized depending upon their reproductive rate and are assigned a value ranging from 1 (colonizers), usually r strategies to 5 (persisters) generally considers k strategies. C-p classifications of nematodes lead to the formation of the maturity index (MI), which is a weighted mean frequency of c-p scaling across the entire nematode community and provides the information of the likely condition of the soil environment. The development of MI represented a significant advancement in interpreting the relationships between the ecology of nematode communities and functions of the soil. The Maturity and modified Maturity Indices, reflecting the degree of disturbance of the soil ecosystem, are the most sensitive indices. Nematode genera were assigned to trophic groups according to Yeates *et al*., (1993), and assigned to functional guilds. *Bax*, Fu*x*, Ca*x*, Om*x*, H*x* (where *x* = 1-5) represent the functional guilds of nematodes that are bacterivores (Ba), fungivores (Fu), carnivores (Ca), omnivores (Om) or herbivores (H), where *x* represents its position along the Coloniser-Persister (CP) scale (1-5). Nematodes in the same functional guilds respond similarly to food web enrichment and to environmental perturbation. The enrichment opportunists (c-p 1) respond positively to disturbances that result in enrichment of the food web. This concept leads to the development of food web indices or ecological indices.

Nematodes with c-p value equal to 1 are short lived, have high fecundity; feed on enriched media, e.g. *C. elegans* and *Panagrellus redivivus.* Most abundant nematode taxa under stressed conditions are those in c-p 2. (e.g., *Plectus acuminatus*). Those of c-p value 5 have large body size, longer life span, and low fecundity, susceptible to disturbance and are predominantly omnivores and predators. Ordination of nematodes into c-p groups has proven useful to predict sensitivity of taxa to stressors. For example, the survival of nematodes of high c-p groups in soil is reduced by chemical stressors, including nitrogen fertilizers , swine slurry , metals , soil acidification , and nematicides. An extension of such tests for acute toxicity assays involves subjecting nematode faunae extracted from soil to various stressor levels in solution.

Enrichment index (EI) is based on the expected responsiveness of the opportunistic guilds (bacteriovore nematodes with c-p value equals to one) to organic resources enrichment. Therefore, EI describes whether the soil environment is nutrient enriched (high EI) or depleted (low EI).

Structure index (SI) represents an aggregation of functional guilds with c-p values ranging from 3-5 and describes whether the soil ecosystem is structured with greater trophic links (high SI) or degraded (low SI) with fewer trophic links. Plotting of EI and SI provide a model framework of nematode faunal analysis as an indicator of the likely conditions of the soil food web.

Channel index (CI), which is a percentage of fungivores among the total fungivores and c-p one opportunists bacterivores. CI indicates predominant decomposition channels in the soil food web, a high CI (> 50 %) indicates fungal decomposition channels whereas low CI (< 50 %) suggests bacterial decomposition channels.

The ratio of fungivorous to bactiverous nematodes calculated as:

FF: BF = 100 × fungivores / (fungivores + bacteriovores).

Nematode Channel Ratio (NCR)

NCR = 100 × [B / (B+F)], where B and F are the proportions of the nematode fauna allocated to bacterivorous and fungivorous groups. Disturbance to the soil environment was more severe when MI, Sigma MI, and H' values are lower.

Nematodes as indicator of toxic pollutant: It has been shown that nematodes respond differentially to xenobiotic substances (Saly and Ragala, 1984; Wasilewska, 1979, 1989; Bongers *et al*., 2001; De Nardo and Grewal, 2003; Jonker *et al*., 2004). With rapid urbanization in many parts of the world, pollution in the terrestrial environment has become widespread in a global context. Increasing heavy metal pollution from vehicular emissions, incinerators, industrial wastes and other activities has severely disturbed soil ecosystems and has continuous effects on the soil food web. Nematodes have variable responses to stress factors; some species are extremely sensitive to pollutants and others extremely tolerant (Tenuta and Ferris, 2004).As the very first and immediate reaction on sensing a toxic substance, nematodes can cease pharyngeal pumping and thereby avoid intake of the toxicant (Jones and Candido, 1999). Nematodes have elaborate sensorial equipment, including receptors for cadmium and copper ions (Sambongi *et al*., 1999), which enables them to avoid intake of a broad spectrum of substances by means of the same behavioral response mechanism. Once a toxicant has passed into the body, molecular decontamination mechanism is induced (Downs *et al*., 2001). Superoxide dismutases accumulate in response to oxidative stress and are one of the main anti-oxidant defense pathways (Fridovich, 1995). Cytochrome P450 has both physiologically relevant oxidative and reductive reactions and catalyzes many xenobiotic-based substrates (Menzel *et al*., 2001). Glutathiones and glutathione S-transferases are involved in the detoxification of organic xenobiotics and in the discharge of metal ions from the cell (Eaton and Bammler, 1999; Sies, 1999). Metallothioneins can act as scavengers for radicals, and mainly they protect against metal toxicity by sequestering Zn, Cu, Cd, and Hg (Klaassen *et al*., 1999). Phyto-chelatins also sequester Cd, as well as As, Ag, and Cu (Clemens *et al*., 2001; Vatamaniuk *et al*., 2001). In this way, metal ions are neutralized within the nematode body if they cannot be excreted (Vijver *et al*., 2004). Storage of Pb can reach levels where visible lead particulates are formed in the oesophageal region of *Panagrolaimus superbus* (Williams and Seraphin, 1998). Similarly, organic pollutants can accumulate in the tissue if metabolization and excretion do not keep pace with intake rates. Haitzer *et al*., (2000) observed accumulation of pyrene in lipid-rich body regions in *Caenorhabditis elegans*. The processes of avoidance, detoxification, and sequestration of pollutants are accompanied by a range of more general mechanisms for protein survey and repair (Kammenga *et al*., 1998; Guven *et al*., 1994, 1999; Kammenga *et al*., 2000).

Climate change effects on nematode: climate change and loss of biodiversity are addressed in a simulation model for C and N transfers among grassland plants and soil biota (Hunt and Wall, 2002). A generalization regarding plant responses to elevated CO_2 is increasing N limitation as plant growth potential from increased CO_2 might outstrip soil N supply. Soil food web (i.e., increases in fungal and faunal biomass) increased N availability to offset potential N limitation. Bacterial-feeding nematodes in the model accounted for 60% of faunal mineralization, yet if bacterial-feeding nematodes were deleted from the model (i.e., extreme biodiversity loss) the changes within the food web (i.e. more bacteria, fewer fungi, and increases in other bacterial-feeding fauna and reductions in nematode predators were such that plant growth (net primary production) was unchanged.

When combining the effects of elevated CO_2 and UV-B radiation in a forest system, there were negative effects on fungal-feeding and omnivore nematodes (Kuijper *et al*., 2005). Elevated levels of CO_2 decreased nematode abundances in deciduous and coniferous forest soils (Neher *et al*., 2004), while Li *et al*., (2007) observed an interaction between elevated CO_2 and levels of N fertilization in a wheat system. They observed increases in omnivores and predatory nematodes and changes to several ecological indices (i.e., MI, SI, NCR). Papatheodorou *et al*., (2004) noted no synchronization between nematodes and their food resources generally, but the nematode response to altered soil temperature and moisture was taxa dependant. Similarly, Bakonyi *et al*., (2007) noted that *Cephalobus* and *Plectus* were associated with dried plots, while *Cervidellus*, *Ditylenchus*, *Eudorylaimus*, *Seinura* and *Thonus* were favoured in experimentally warmed plots. Drying and warming effects on the soil nematode community were most pronounced in bare soil, less so in soil under poplar, while no significant effect was found in soil under Fescue. Sonnermann and Wolters (2005) saw an effect in semi natural temperate grassland, on root-hair feeders and predators, which increased and then decreased over the three years of the study. The effect of elevated CO_2 increased the abundances of the root-feeder *Longidorus elongatus* over a nine-year study in a sheep-grazed pasture on sand but other root-feeders were unaffected and other nematode tropic groups and taxa not or marginally affected (Yeates *et al*., 2003; Yeates and Newton, 2009). Ayres *et al*., (2008) reported a neutral response of herbivores in three grassland systems, despite a large increase in root production, which they attributed to simultaneous antagonistic mechanisms.

Conclusion

There are opportunities for further studies on the use of nematodes as indicators for other ecosystem functions, such as the status of re-establishment in semi-natural ecosystems and the capacity of soils to sustain diverse plant communities. However, indicators must be modified for each problem and environment, with the response variable(s) and indicator being situation-specific. Bioindicator-based studies rely extensively on field assessment of a few or limited number of taxa than laboratory work. Sampling, species identification (there must be sufficient taxonomic knowledge to identify organisms accurately and efficiently) and statistics form a large part of these studies, and must be supported by knowledge of the basic biological and ecological features of the organisms and site. Evaluations of new genetically engineered crops must consider biodiversity as a value and bioindicator as tools that can help in reaching decisions about their environmental impact. Bioindicator-based studies have the potential to make a major contribution to optimizing different farming systems, input practices, new crops, rotation, etc., and to influence political policies governing landscape management, landscape reclamation and transformation, urban and industrial areas. In particular, laws aimed at reducing environmental contamination and at remediating high input farming must take into consideration environmental benefits that can be assessed using bioindicator.

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