Chapter

Nematodes as Biological Indicators of Soil Quality in the Agroecosystems

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Abstract

Soil nematodes have advantages as bio-indicators, because they have beneficial role in the food web. Nematodes associated with bacteria are probably the most studied biological indicators of soil fertility. Saprophytic nematodes act as bio-indicators of soil health because they have different beneficial ways to increase in soil functions such as in management of ecosystem; enhancement of nitrogen in soil by ingestion of nitrogen and secrete extra nitrogen as NH4, that is easily absorbable; putrefaction and by dispersion of bacteria and fungi to recently available organic residues. Therefore, nematode are beneficial in increasing soil health or plant growth by providing the nutrient through associated bacteria. So it can be evaluated that the nematodes use as biological indicators of soil fertility because of remarkable diversity and nematode contribution in many functions of the soil fertility.

Keywords: Soil health, indicator, microorganism, nematodes, diversity, food web, nitrogen mineralization

1. Introduction

Biological indicators have association with different functions of soil and have quality to monitor the soil functions and enhance the health of soil [1, 2]. These indicators play a dynamic role to increase the soil properties, in decomposition and chemical contaminants. Many scientists [3] and Pankhurst *et al*., [4] Haitova and Bileva [5] preferred that biological indicator, indicates ecosystem processes; physical, chemical changes and biological properties and processes [6, 7]. Soil health is the quality of a soil to function within ecosystem's limits to sustain in biotic activity [8], to keep environmental quality for the promotion of plant and animal health. A healthy soil will be needed to help in life processing such as plant production and support soil food web and maintain microbial diversity Vads *et al*., [9]. In agrarian countries the use of chemical fertilizers make independent in food production but it makes polluted atmosphere and cause hazardous impacts on anima and humans. Due to inadequate uptake of these chemical based fertilizer by plants, they absorb into water bodies through rainy water, by which water bodies enriched with nutrients and minerals and effect on biotic fauna flora and also the growth living microorganism. The extra uses of chemical fertilizers in agrarian fauna are more expensive and also have several antagonistic effects on soils as reduction of water holding capacity, soil fertility and disparity in soil nutrients.

Soil nematodes have been recognized as the part of agrarian fauna as they have a significant role in the ecosystem [10–12]; Bileva and Arnaudova, [13]. Nematodes as bio-indicators have been played a key roles in the decomposition of soil organic matter, food chain cycling, degradation of soil pollution, and the formation of healthy soil structure [14]. They have the capability to make differences in their areas, such as stress due to deficiency and contaminants. Biological indicators may reproduce the overall population, category, and activity of microorganisms and the diversity of the living organisms in soil [15].

The presence of plant growth promoting rhizobacteria (PGPR) in soil, is also biological indicator because the microorganisms rebuild the nutrient cycle and maintain the organic matter in the soil. Through the use of PGPR, vital plants can be grown in the soil. Since they play several roles, a preferred scientific term for such beneficial bacteria such as *B. cereus, B. subtilis* [16] and some species of *Serratia* provide "eco-friendly" organic agro-input. Soil nematode of the family Rhabditidae are associated with different bacteria and the secondary metabolites produced by these bacteria have the ability to fix nitrogen in the soil. *Oscheius* is an excellent laboratory model to study internal gene transfer because these microbial worms are bacterial feeders, have vector viable bacteria *B. cereus, B. megaterium, B. subtilis* and *Pseudomonas aeruginosa*. They have the ability to absorb PO4. Insoluble PO4 is generally un-absorbable to the plant. The root system simply cannot absorb it. Soil microorganisms, such as *B. subtilis*, are beneficial to plant health and plant growth.

Besides saprophytic nematodes, free-living marine nematodes use as pollution indicators coastal areas. The use of benthic flora as bio-indicators of different water source like, ocean, river and lake quality can be examined in terms of population density and diversity, test morphology - including size, prolocular morphology, ultrastructure, abnormality, and the chemistry of the test. The study of pollution effects on benthic flora and their use as substitutions began in the 1960s [17–19], and has been increasingly developed in recent decades as a result of environmental research (for reviews, see [20–25]).

Soko, and Gyedu-Ababio [26] reported the relationship between different environmental factors and with free-living marine nematodes. They found some metals such Cadmium, Colbat, Chromium, Copper, Iron, Manganese, Nickel, Vadium, Zinc and Aluminum affected the diversity and density of marine nematodes. Shannon-Wiener Diversity, Maturity Index and colonize-persisters percentage (c% - p%) were also found to be good tools for use as pollution indicators Chander & Brookes, [27]. Nematode genera such as *Terschellingia*, *Theristus* and *Halalaimus* were found during that study to be dominant at a site strongly impacted by both metals concentration and organic matters. The three genera are believed to be good indicators of pollution in the Incomati River Estuary.

2. Materials and methods

2.1 Soil sampling and nematode isolation

Surveys were conducted to check the soil fertility and nematode presence. The experiment was laid out in randomized complete block design (RCBD). The trial was conducted and was repeated to evaluate the nitrogen mineralization and presence of saprophytic nematodes associated with bacteria. In this experiment 5 soils samples (each field) were collected from two different types of vegetation, one from healthy plantations and another from infected plants.

Soil samples consisting of 1.6 cm diam., x 10 cm deep cores were taken from each plot. The samples of the same plot were mixed thoroughly to form a composite sample. 100 g soil samples taken from each composite sample were processed by

Cobb sieving [28] followed by modified Baermann funnel method [29]. Nematodes were collected after 48 h. The population of saprophytic nematodes was increased in the presence of symbiotic bacteria. Plant growth was also increased so it indicates that nematodes use as bio-indicator of plant and soil health.

2.2 Soil analysis

In the present experiment sandy loam soil was used, which was collected from Botanical garden of University of University of Karachi. Soil analysis was performed in the Department of Environmental studies.

3. Results and discussion

In this experiment 5 soils samples (each field) were collected from two different types of vegetation, one from healthy plantations and another from infected plants. Saprophytic and parasitic nematodes were the most abundant groups in all samples. There were significant differences in the numbers of saprophytic ($P \leq 0.01$) and plant parasitic nematodes ($P \leq 0.05$) between the healthy and infected plantations but no difference was observed in the numbers of fungal feeding nematodes. The common genera were found in all samples (*Aphelenchoides*, *Aphelenchus*, *Meloidogyne*, *Pratylenchus*, *Trichodorous*, *Helicotylenchus*, *Hoplolaimus*, *Xiphinema*, *Tylenchus* and *Mononchus*. From healthy plantation the bacteria feeding genera, *Acrobeles*, *Rhabditis*, *Cervidellus*, *Eucephalobus*, *Cephalobus*, *Heterocephalobus*, *Plectus* and *Tylocephalus* were found, showed that these nematodes fixed the nitrogen fixing bacteria in the soil for which soil is healthy source for healthy plantation or it can be evaluated that the presence of nematodes indicates the soil health [30, 31].

3.1 Soil analysis

The soil composed of 55% of sand, 27.4% silt and 16.5% clay and contained PO4 2.5 mg/kg, total N: 11.42 mg /kg by TKN method [32] and the pH was 7.1. Fresh soil passed through a 2 mm mesh to remove stones, macro-fauna and discernible. The half of soil was sterilized, which contained PO4 2.4 mg/kg, total N: 103 mg /kg by TKN method (Total Kjeldhal Nitrogen) and the pH was 6.8.

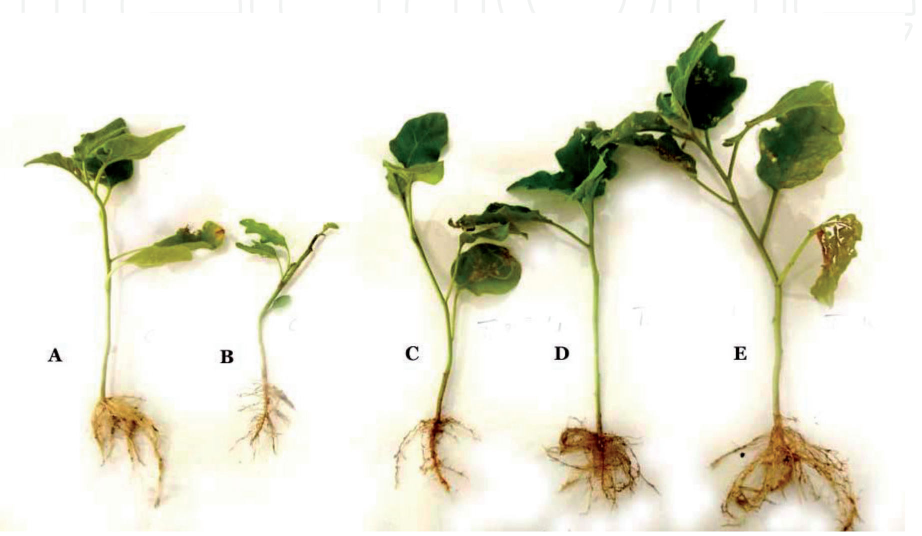

Figure 1.
Healthy plants roots, stem and leaves showed the fertility of soil.

Figure 2.
Infected plants A, B and C and healthy plants D and E.

The saprophytic nematode population significantly increased in healthy soil (RCBD- ANOVA: F = 17; df = 8, 18; P < 0.001) **Figure 1**. Plant parasitic and free-living soil nematodes also differed significantly (RCBD - ANOVA F = 25, df = 2, 18; P < 0.001) and the presence or absence of plant and soil also had marked effect on plant (RCBD- ANOVA: F = 26; df = 16, 18; P < 0.001). No detectable increase in the population of plant parasitic nematodes was observed in healthy soil. The population of *Aphelenchoides sacchari* was significantly decreased in healthy plantation (F = 33.57; df = 2, 6; P < 0.001) as compared infected plants whereas the population of *A. sacchari* (F = 17; df = 2,6; P < 0.001) and *Hemicriconemoides mangiferae* (F = 23; df = 2,6; P < 0.001) were significantly decreased in healthy soil. The population levels of *Rotylenchulus reniformis* and *Helichotylenchus* were also considerably decreased (P < 0.001) in healthy plants. Significant differences were observed between healthy and infected plants on reduction of parasitic nematodes. Overall population of free-living or saprophytic nematodes was high and significantly increased in fertile soil due to which the vegetables and fruits were reproduce more and more vegetables and fruits. The population of *Acrobeles*, *Rhabditis*, and *Cephalobus*, soil nematodes were showed significantly increased in healthy and fertile soil (F = 9; df = 2,6; P < 0.0001); (F = 4; df = 2, 6; P < 0.001); (F = 8; df = 2,6; P < 0.00), respectively **Figure 2**. The overall results showed that the presence of abundant number of saprophytic nematodes indicates that the soil was filled with nitrogen, plant growth promoting rhizobacteria (PGPR) and fertility. Soil nematodes was highly active to fix the nitrogen fixing bacteria in soil. The population of *Acrobeles*, *Rhabditis*, and *Cephalobus*, nematodes species were comparatively more active nematodes for fixing and indicating the health of soil (**Figure 2**).

3.2 Nematode population in soil act as indicator

Rhabditis significantly (P < 0.001) showed the presence of higher level of nitrogen in soil whereas *Acrobeles* (A.) also significantly increased the nitrogen level (**Table 1** and **Figure 3**).

3.3 Plant root and shoot growth

Due to the presence of nematode associated bacteria the root, shoot length and number of forks was significantly (P < 0.01) increased shown in **Figures 1** and **4**.

S. No.	Plant and soil nematodes	Healthy plant	Infected plant
1	*Acrobeles*	75	15
2	*Aphelenchus*	12	45
4	*Aphelnchoides*	15	51
5	*Cephalobus*	59	25
6	*Cervidellus*	62	16
7	*Eucephalobus*	46	03
8	*Hoplolaimus*	19	28
9	*Heterocephalobus*	44	04
10	*Helichotylenchus*	21	55
11	*Meloidogyne*	00	10
12	*Paratylenchus*	09	37
13	*Pratylenchus*	01	18
14	*Plectus*	23	15
15	*Rhabditis*	82	20
16	*Trichodorous*	15	28
17	*Tylenchorhynchus*	24	65
18	*Xiphinema*	10	71

Table 1.
Percentage of plant parasitic and soil nematode population captured from healthy and infected plant.

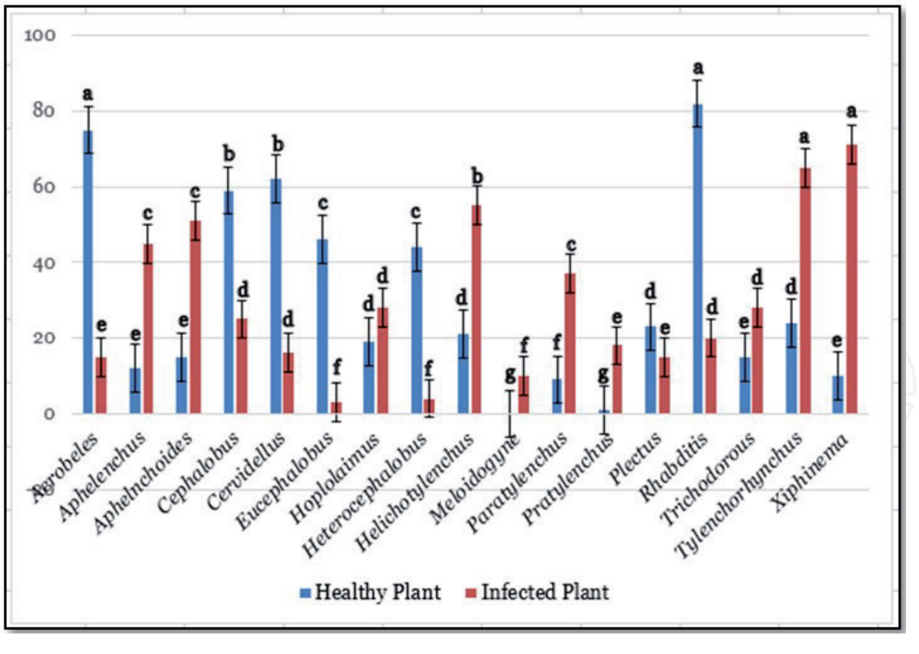

Figure 3.
Population density of plant and soil nematodes found from healthy and infected plants.

The indication of soil fertility and the suppression of plant parasitic nematodes also studies in this study. This observation was already reported by the previous researchers [33–37]. Our findings using saprophytic nematodes associated with bacteria also suppress the population of plant parasitic nematodes on *Cynodon dactylon* grass. Most plant parasitic nematode genera in our experiment was suppressed in

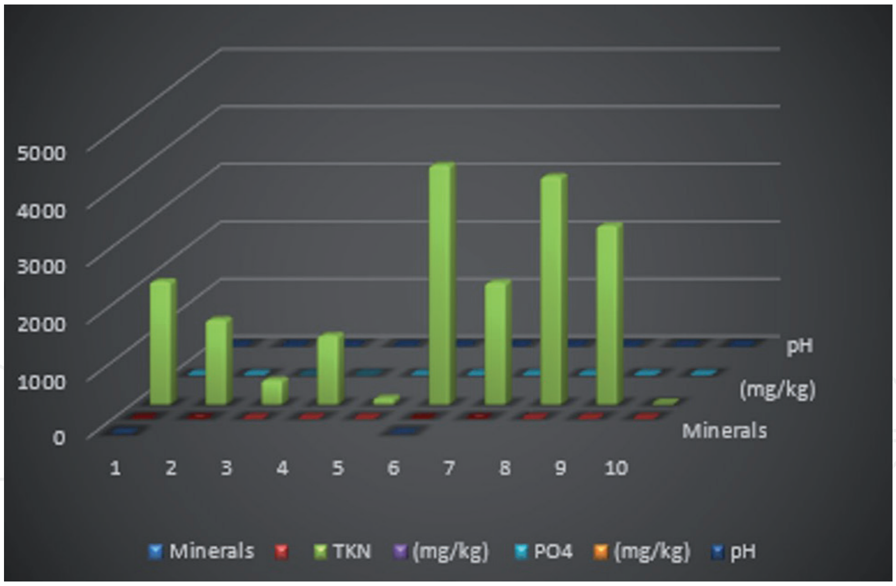

Figure 4.
Enhancement of nitrogen (TKN method) that showed by nematode as bio-indicator.

the abundant presence of free living bacterial feeding nematodes and also entomopathogenic nematodes. So it can evaluated the saprophytic nematodes should be as effective in suppressing the population of plant parasitic nematodes [35, 38].

For this purpose an experiment was conducted in which sterilized and non-sterilized soil by providing heat treatment were used. Four types of treatments with combination of control applied into soil (tomato pots) which has tomato seedlings. Treatments of nematodes culture obtained from the culture lab of NNRC, UoK. Nitrogen enhancement rates were calculated from soil. Different treatments differed in the amount of fixed nitrogen fixed in the soil, *Oscheius* A. treated pot show higher amount of nitrogen as compared to other treatments. The result of that study was conducted in green house condition where different factors were involved. The result of the experiment showed that *Acrobeles* significantly enhanced the soil fertility or nitrogen in soil [39].

The production of nematodes, which is bacterial feeding, nematodes directly associated to the rate of putrefaction of different organic modifications [40]. It is well known that saprophytic nematodes significantly increase soil nutrient absorption and bacterial population [41]. Inorganic nitrogen supports the plant growth initiates mostly from biotic activities in the soil. Thus indication about the richness, multiplicity and activities of different biotic fauna responsible for nitrogen mineralization is of vital position in the health of soil productivity. Soil organic materials could characterize the main source of inorganic nitrogen, even in the presence of fertilizer [42–44].

Nematodes, a diverse group of round worms, exist cosmopolitan in almost all biomes. Saprophytic free-living soil nematodes found as the part of agricultural fauna indicate an important role in the ecosystem. Usually 50 percent nematode fauna present in soil are saprophytic and the ratio reaches 80% at locations for high bacteriological population [40, 45–50] these are useful indicators of soil health because of their remarkable variety and their role in many functions of the soil food web. Many Scientists [51–53] have been studied and proved the evidence of the occurrence of saprophytic nematodes improved the nitrogen mineralization and later on stimulated plant growth experimented by different researchers [50, 54–57] and have indicating properties. They significantly enrolled in C mineralization and nutrient cycling, mainly by feeding on bacteria and fungi. Nematodes are the most abundant metazoans in soil.

Four of every five multi-cellular animals on the planets are nematodes. Normally twenty to fifty percent nematodes are bacterial feeders and the diversity of presence reaches 90–99% at locations of high bacterial activity [41, 46, 58]. These are the free-moving nematodes, not feeding on a particular plant but on the soil and bacteria. They are commonly associated with decaying root galls as probably they feed on decaying plant materials and increase soil fertility.

Different scientists of the world have been given the techniques to measuring the status of soil health by calculating the numbers of nematodes in different families in addition to their variety, they are beneficial indicators because of their population in response to fluctuations in moisture and temperature. Soil saprophytic nematodes preserved the level of plant -absorbable nitrogen in organic farming system. The process of recycling nutrients from organic to inorganic form is termed mineralization, Nematodes involved directly to nitrogen enhancement by their feeding interactions. For example nematodes ingest nitrogen in the form of proteins and other nitrogenous compounds and release extra amount of nitrogen as ammonia which is easily absorb for plant use. When nematodes graze on these microbes they give off CO2 and NH4 and increase soil fertility. Nematodes keep 1/6 of the nitrogen, they process and rest 5/6 is excreted to the soil for plant absorption. Classical management practices along with nematodes as bio-fertilizers are useful to increase soil conditions and crop productivity.

4. Conclusion

Results showed that *Acrobeles* and *Rhabditis* nematodes are significantly enhanced the soil fertility or nitrogen in soil. The increase in nematode numbers, especially bacterial feeding, especially bacterial feeding nematodes is directly associated to the rate of breakdown of different organic materials [40]. It is well recognized that soil nematodes significantly enhance nutrient in soil as well as bacterial populations [41]. The enhancing effect of bacterial-feeding nematodes on microbial population growth in soil microcosm has been reported by Mesfin *et al*., [48], they found that all the treatments having nematodes and bacteria had higher bacterial densities than the treatments without nematodes. Our results supported this conclusion, suggesting that nematodes increased the bacterial densities, and populations of nematodes and bacteria rose simultaneously.

There is need to use the bacterial feeding nematodes as bio-fertilizer for production of healthy plants or crops. Based on the previous studies the practical use of nematodes seems to be more appropriate as they are effective to enhance nitrogen and carbon level in soil. Nematodes use as a bio-fertilizer gave benefits in agriculture to raise productivity. About thirty percent of the total inorganic nitrogen was mineralized in the form of soil organic matter that was for consumption soil micro-organism [48, 59, 60]. Microphagous nematodes, establish an important group that effects on micro-organism activity and are important regulators of decomposition and nutrient release processes [41, 55]. Interactions between nematodes and microbes and have been studied under temperate soil conditions. Nematodes are present in diverse habitat they play the major role in the ecosystem advancement, soil properties, soil microbe's diversity, plant growth and crop production. The agrarian useful nematode fauna increase soil health with fixing the rhizobacteria, Nitrogen fixing cyanobacteria, plant beneficial bacteria and decomposition of microbes [1, 2, 61].

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