

## Review Article

# Entomopathogenic nematodes as bioindicator of soil health

### Abstract:

Soil health or soil fertility is an ecological characteristic of a living and dynamic system whose functions are mediated by a diversity of living organisms and promote plant growth. Soil health assessment requires a comprehensive combination of chemical, physical and biological indicators. As soil is a natural habitat for the development of many organisms, biological indicators are important key for monitoring soil quality. Soils are increasingly subject to deterioration in physico-chemical properties along with reduced biodiversity that leads to low productivity. Invertebrates present in soil remain directly in contact with soil and can serve as biological indicators. Nematode is a major mesofaunal member that have been considered for use as biological indicator. An important group of nematodes are entomopathogenic nematodes (EPNs), which are widely used in biological plant protection. There are many specific biological characteristics of EPNs which makes them useful candidates as soil quality indicators. This review illustrates the impact of land management practices on the biological parameters of EPNs and their role as bioindicator of soil health.

**Key words:** Soil health, bioindicator, Entomopathogenic nematodes (EPNs), soil pollution, food productivity.

### 1. Introduction

Soil health or soil fertility is the living and dynamic system and mainly an ecological characteristic that provide all essential plant nutrients in available forms and in a suitable balance. Healthy or fertile soil maintains the quality of air and water environments, and promotes plant health. Soil fertility shows the status of plant nutrients in the soil whereas the soil productivity is the resultant of various factors influencing crop production (Doran and Zeiss, 2000). Competition of different plant types for the nutrients, methods of cultivation, use of pesticides and fertilizers are factors which indirectly affecting soil productivity. In farming, soil fertility may be lost through human activities or through natural process. Soil fertility can be lost through leaching of water soluble especially nitrogenous

nutrients, inadequate moisture content, wind and water mediated soil erosion, exhaustion of nutrients due to monocropping, increase in salinity or salt content due to insufficient availability of water and which get enhanced due to high evaporation rate, change in soil pH due to inappropriate use of pesticides and fertilizers.

Soils are increasingly subject to degradation, pollution, deterioration in physico-chemical properties, impacting the terrestrial environment and thereby lowering food production. Established soil quality determination considers many parameters, and soil health assessment requires a comprehensive combination of chemical, physical and biological indicators. Soil organisms are an integral part of overall soil productivity and health. Therefore, biological indicators are essential for monitoring soil health (Anderson 2003). Among the useful biological indicators, soil nematodes are an excellent model group to investigate soil health (Bonger and Ferris, 1999; Neher, 2001). An important group of nematodes are entomopathogenic nematodes (EPNs), which are widely used in biological plant protection. Specific biological characteristics of nematodes make them useful candidates as soil quality indicators. The presence of permeable cuticle, responsible for restorative capacity of soil ecosystems, presence of heat shock proteins that are highly conserved (Hashmi et al., 1997) is another important feature that can be utilized in assessing toxic elements in soil. Expression of these proteins is enhanced when exposed to stresses such as heat, metal ions, or organic toxins. Now-a-days these proteins are able to serve as biomarkers for ecotoxicological assessment of soils (Kammenga et al., 2000).

## 2. Entomopathogenic nematodes (EPNs):

Entomopathogenic nematodes (EPNs) in the genera *Steinernema* and *Heterorhabditis* (order: Rhabditida) are well-known biological control agents. EPNs occur in both natural and anthropogenic ecosystems, where they shape biological

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diversity. The occurrence of certain nematodes species depends on their nutritional preferences and consequently, on the presence of specific host insect species in these locations. EPNs are beneficial soil fauna that participate in the food chain. Natural populations of these EPNs keep insect numbers at a certain level and provide food for other groups of invertebrates. The third stage infective juveniles (IJs) are the resistant stage naturally occurring in soil, they have the ability to actively locate their host by recognizing different signals that reveal their presence (vibrations, significant increase in the concentration of CO<sub>2</sub>, volatile specific produced by plants damaged by herbivore, etc.) (Griffin, 2015). Infective juveniles react to the presence of a host by moving towards an increasing concentration of chemical signals (chemotaxis) emitted by it, such as CO<sub>2</sub>, components of insect feces and other specific compounds. In addition to chemical signals, IJs also respond to insect movements in the soil. After penetrating through their natural orifices (mouth, anus, spiracles), they reach the hemocele and release the symbiont bacteria that they carry inside, *Xenorhabdus* in the case of *Steinernema* and *Photorhabdus* for the *Heterorhabditis* species (Stock, 2015). The joint action of both organisms makes it possible to avoid the host's immune response and kill it by septicemia within 24-48 h post-infection. Both the bacteria and the nematode reproduce within the dead host for 7-15 days, depending on the species and environmental conditions, until a new generation of IJs massively emerge to start the cycle again. Many EPN species are naturally present in soil ecosystem. However, their populations can be boosted through controlled releases in areas with pest infestations.

### 3. EPNs can be used to evaluate soil health:

The characteristics of the soil environment in which EPNs occur are influenced by human activities. These include chemical or physical changes to the soil caused by land management practices. Recent studies are providing evidence that the natural

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presence of EPN can be an indicator of the impact of soil management (Campos-Herrera et al.,2008;Blanco-Perez et al.,2020).

### 3.1.Impact of different agricultural practices on EPNS:

Changes in EPN population structure can reflect shifts in soil conditions, such as pollution,compaction,or changes in cultural practices. Certain EPN species are more sensitive to contaminants or disturbances, and their presence or absence can indicate the impact of pollutants or disturbances on soil health.Detecting EPN presence and activity and their relation with other soil organisms associated with them can help us to understand the impact of different agricultural practices on crop management.Reduced tillage or organic farming practices can lead to increased EPN abundance indicating healthier soils.The soil is a complex, species-rich environment and thus, various organisms have the potential to influence the survival and reproduction of EPNs. The higher soil organic matter contents in the field favor the development of sustainable hosts for *S. feltiae* to allow their long-term persistence.Unhealthy soils are associated with the excessive use of chemical fertilizers and pesticides, especially in long-term monocropping systems.Commonly used fertilizers and chemical pesticides reduce the pathogenicity of EPNs and negatively affect their reproduction, which reduces the population size.In an environment in which herbicides were present, EPN reproduction was limited.Excessive use of inorganic fertilizers contributes to increase soil salinity and accumulation of heavy metals and nitrates.In saline soil, EPN movement is hindered and their ability to find and identify hosts is limited(Nielsen et al.,2011).Long-term exposure to high concentrations of inorganic fertilizers inhibited the infectivity and reproduction of EPNs. Moreover,organic fertilizer increased *S.feltiae* density, whereas NPK fertilizer decreased nematode density (Bednarek and Gaugler,1997).

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**Comment [MA5]:** How the change is population can be inferred please provide the reference. How the EPN can be extracted from soil and identified or baiting techniques is used. And how the change can be inferred by look at just the EPN community. Please explain.

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**Comment [MA7]:** Rewrite the sentences with proper structure. It seems you are telling the effects of chemicals present in soil changes the EPN abundance. So vice versa can happen by looking the abundance of EPN community we can infer the soil health. Provide more information of such studies and if possible provide a graph or statistics information of population/community abundance that indicates soil type or health

The survival of both naturally occurring IJs and those augmented for biocontrol action, is affected by biotic as much as abiotic factors (Lewis et al., 2015). Moreover, introduced EPNs may alter the naturally occurring microbiota in the soil (Duncan et al., 2007, Ishibashi and Kondo, 1986, Lewis et al., 2015). The availability of hosts, and the presence of competitive or predatory organisms and their ecological associations in the soil are important aspects in potential use of EPNs in agro-ecosystems (Stuart et al., 2015).

The occurrence of EPN populations depends on many environmental parameters, such as temperature, moisture, soil texture, and pH. Extreme temperatures result in a decrease in the survival rate and infectivity of EPNs. Both high humidity and acidic soil pH reduce populations and disrupt the biological activity of EPNs. The structure and texture of the soil also affects the infectivity and survival of EPNs. Lighter soils are better aerated and are more easily permeable to lower layers. Heavier soils have a greater water-carrying capacity, but air circulation is poorer. Individual species of EPNs differ in their requirements in terms of air-water relations in the soil environment. Soil structures influence, among other things, the movement, infectivity, and survival of nematodes. Depending on the size of the soil fraction, the rate of movement and the frequency of undulating movements changes. In heavier soil, infectivity and movement are hindered, whereas in light loam soil with similar percentages of silt and clay, they are facilitated. In sandy soil with a mean grain diameter of 0.2-0.5  $\mu\text{m}$ , IJs covered a distance of 2 cm (87%) within 48 h; 0.5% of the IJs covered a distance of 12-14 cm from the site of release (Moyle and Kaya, 1981). Research into vertical migration showed that it decreases in response to the increase in clay and silt content, with sandy soil making it easier for nematodes to move in this direction (Georgis and Poinar, 1983). *S. carpocapsae* prefer to migrate downward from the site of application of the infective juveniles, they move deep into the soil, where they

effectively infest host insects(Sandner,1986), whereas *H.bacteriophora* infests insects above the EPN application site.Juveniles have been shown to penetrate hosts more quickly and willingly in sandy and sandy loam soils(Lankin et al.,2020).The highest infestation levels were obtained in clay-sandy soils with low moisture and in sand with high relative moisture, close to saturation.*S.carpocapsae* and *S.glaseri* infective juveniles placed on sterile sandy,sandy loam, or loam soil for 16 weeks showed different degrees of survival.The lowest survival rate was associated with the loam soil because the low porosity and poor aeration of this type of soil whereas highest survival rate was recorded in sandy loam and sandy soil.Heavy moist soils are conducive to anaerobic conditions, which, in most cases are harmful to nematodes.Factors that are negatively related to the oxygen content of the soil environment include the contents of clay and organic matter.Despite oxygen being present in soil clods, nematodes only use the oxygen dissolved in the water surrounding the clods.Low oxygen concentration interfered with pathogenicity and led to the death of IJs.The survival rate of *S.carpocapsae* and *S.glaseri* IJs in sandy loam soils decreased in response to a decrease in oxygen content from 20% to 1%(Kung et al.,1991).IJs use elevated CO<sub>2</sub> concentrations as signals with which to locate a potential host(Kaya,1990).EPNs especially cruiser burrowing and movement activities create channels and pores in the soil,enhancing the soil structure.In degraded soils, nematodes can help alleviate compaction and improve water infiltration, aeration and root penetration.The level of soil moisture affects IJs survival rate and movement within the soil pore.Soil moisture level 25-40%,IJs have the most favorable conditions for invasion of the host.Soil moisture also affects nematodes living inside a host insect's body. In dry soil, a small number of juveniles were observed leaving the host body.At low moisture, *S.glaseri* and *S.carpocapsae* IJs leave the body of the host insect the earliest , whereas *S.riobrave* IJs leave the insect body the latest.Soil pH is a key element that

effects many biological and physicochemical processes in soils. The degree of soil acidification largely determines the mobility and bioavailability of heavy metals, as well as ionic organic pollutants. With an increase in acidification of the soil environment, the bioavailability of heavy metals increases. The optimum pH range of soil for the growth and reproduction of most plant species and soil organisms is considered to be between 5.5-7.2. Acidic soil pH level cause decreases in juvenile infectivity and survival. The survival of *S. carpocapsae* and *S. glaseri* was reported to be similar at pH 4,6,8 for the first 4 weeks. *S. feltiae* and *S. bacteriophora* showed greater mobility, activity, and pathogenicity at pH 6.8 and 8.

### **3.2. Impact of soil pollutants on EPNs:**

Nematodes are exposed to anthropogenic agents, such as heavy metals, oil, gasoline, and even essential oils. These limit their ability to move in the soil, thereby reducing their chances of successfully finding a host. Oil derivatives primarily aromatic hydrocarbons, cause substantial changes in soil environment affecting all living organisms. EPNs were used to evaluate the effect of soil pollution with heavy metals (Ropek and Gondek, 2002; Ropek and Nicia, 2005). Petroleum derivatives found in agricultural soil destabilize the biological properties of soils, as well as the biotic community, and finally affect the quality and fertility of the soil. Petroleum substances that may adversely affect the development and function of EPNs are mainly crude oil, diesel oil, engine oil, and gasoline (petrol). All these substances reduce the pathogenicity and reproductive capacity of EPNs. The longer the contaminant is active, the higher the mortality of the nematodes. EPNs could not be isolated from soil contaminated with petroleum derivatives (Ropek and Gospodarek, 2013). In addition, these compounds inhibit the ability of EPNs to find a host and reduce their infectivity and reproduction. Petrol revealed the most toxic effect on *S. feltiae*. *S. feltiae* revealed the greatest sensitivity to soil contamination with petrol at the dose of 8000 mg per kg

soil d.m. The applied oil derivatives had an adverse effect on the female/male ratio of nematodes infesting host insect. Application of the biopreparation accelerated bioremediation process and reduced a negative effect of soil contamination with oil derivatives on the occurrence of EPNs.

Heavy metals are soil pollutants; persist in the soil environment for a long time, limiting the suitability of the soil for agriculture. The toxicity of heavy metals and their high bioavailability depend on factors like amount of contamination and the chemical form as well as on the properties of the soil environment: temperature, pH, presence of other metals, and oxidation-reduction potential. In the soil environment IJs will be directly exposed to any heavy metals found there. These compounds affect the occurrence, survival, infectivity, and reproductive capacity of nematodes (Ropek, 2003). Natural concentrations of heavy metals in the habitats of nematodes are not the cause of their mortality. EPNs do not respond directly by higher mortality but by a decrease in their pathogenic abilities, which adversely affects the success in searching and eliminating pests (Ropek, Gondek, 2002). Laboratory studies on the effect of heavy metals on EPNs have shown that heavy metal ions can adversely affect the pathogenicity of EPNs. It was found that magnesium interaction with heavy metal ions positively affects the pathogenic activity of nematodes (Jaworska et al., 1999; Jaworska and Gospodarek, 2009). In soils with a higher concentration of heavy metal ions, lower infectivity, increased mortality of nematodes inside the body of host insect, and increased mortality. The most adverse effects were observed with nickel (Ni(II)) and lead (Pb(II)), Cd and Pb ions. In such a case, the mortality of infective juveniles was affected both by the time of contact of nematodes with pollutants (*S. carpocapsae*) (Jaworska et al., 1999) and their concentration. Effects of combinations of several metals on *S. carpocapsae* showed that these combinations reduced the pathogenicity of EPNs. Heavy metals also hinder the movement of nematodes. Studies on the effects

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of metal salts showed that the presence of these compounds in soil prevented nematodes from moving, finding and infecting hosts. Chloride salts have been found to be particularly toxic. Instead of being toxic to nematodes, magnesium ion can act synergically, by protecting them against the unfavourable impact of other heavy metals (Jaworska et al. 1999; Jaworska, 2014). Insect nutrients contaminated with Pb, Cd, or Cu ions also had an adverse effect on infectivity, nematode development in the host, and the viability of juveniles migrating from the host into the environment. These ions accumulate in the tissues of the insect, which indirectly affects the nematodes. Heavy metals affect not only EPNs, but also the associated bacteria present. It was shown that copper, manganese, and nickel ions were most harmful to these microorganisms. In contrast, lead was not toxic to nematode-associated bacteria.

#### 4. Conclusion and future perspectives:

By investigating the EPN soil food web, it is possible to implement agricultural practices that can contribute to the maintenance of soil health as an indirect indicator of the resilience of the agricultural fields (Blanco-Perez et al., 2020). Monitoring EPNs during soil health restoration efforts can indicate the success of restoration activities. By reducing pest population, EPNs contribute to the overall health of plants and agricultural systems. EPNs can be integrated with other pest management practices, such as cultural practices, other biological control agents, and insect resistant plant varieties, to create a comprehensive and sustainable pest management strategy that will ultimately improve soil health and food productivity.

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**Comment [MA9]:** It seems you discussing the effects of pollutants on EPN. Please explain how bioindication is reflected

**Comment [MA10]:** Give some information of EPN soil food web or you want to say nematode food web indices. Please provide relevant information.

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