

14 Nematodes as Bioindicators of Heavy Metal Contamination and Soil Salinity

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Abstract

Nematodes are the most diverse multicellular animals worldwide. Terrestrial nematodes live in all types of soil, and their response to contaminants and environmental disruptions varies greatly. The community structure, diversity, abundance and metabolic footprint of soil nematode populations are significant indicators of soil health and are closely associated with the constituents of soil and its environment. Several species belonging to Enopliida, Chromadorida and Monhysterida in marine environments have been shown to be influenced by heavy metal pollution. For instance, species of *Araelaimus* and *Hypodontolaimus* have been found to be sensitive to mercury and zinc. Plant-parasitic nematodes may also be affected by metal contamination in the soil. However, bacterivores may serve to be the most reliable indicators of heavy metals. Studies have shown that populations of predators and omnivore nematodes form strong correlations with heavy metal contamination. The abundance of *Achromadora*, *Heterocephalobus*, *Ironus*, *Prismatolaimus* and *Monhystera* has been found to be strongly correlated with mercury levels. Salinity is another important abiotic stress in agricultural fields but it has a low effect on nematodes and is, therefore, not useful as a bioindicator. However, *Scottinema lindsayae* and *Plectus antarcticus* were found to be abundantly present in soils rich in nitrate. Meta-analysis has shown that the maturity index of a nematode community may serve as the best indicator of heavy metals. This chapter presents a critical analysis of the scope and use of nematodes as bioindicators of heavy metal contamination and salinity in the soil, and offers up-to-date relevant information.

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Introduction

Bioindicators, by definition, are living organisms or biological responses that serve as a reliable means of detecting the presence of environmental contaminants. Generally, bioindicators are sensitive to a contaminant and develop typical symptom(s) or measurable response(s), such as changes in growth or behavior. By monitoring bioindicators, researchers and environmentalists can obtain valuable insights into the health of ecosystems and the impact of human activity on the natural world (Mothersill and Seymour, 2016). Soil ecosystems harbor a diverse range of biological organisms, including microbes and microfauna and mesofauna such as fungi, bacteria, algae, protozoa, arthropods and nematodes. These organisms perform important functions in nutrient cycling, organic matter decomposition, soil structure formation, etc., which ultimately contribute to soil health. Since these organisms are very small in size they are highly sensitive to abnormal fluctuations in the environmental matrices, hence they can be used as bioindicators. However, the selection of a bioindicator requires a more comprehensive approach that considers multiple factors. These include the organism's sensitivity to environmental changes, ease of detection and quantification, ability to link its presence or absence to specific environmental stressors, and ecological relevance. A thorough assessment of these factors is necessary to ensure that a good bioindicator provides accurate and relevant information about the health of the soil or an ecosystem (Neher, 2001). Soil health refers to the ability of soil to function as a living system, sustaining the biological activities, maintaining the quality of air and water, and promoting the health of plants, including humans and animals (Doran *et al.*, 1996). Soil is home to a wide variety of organisms, and it can be challenging to identify which ones are the best bioindicators. A recent study has indicated that protozoa are more sensitive than nematodes to heavy metals, and that nematodes are more affected directly by soil physicochemical properties than heavy metals (Wang *et al.*, 2023). However, among the soil organisms, nematodes stand out as bioindicators due to their unique morphological

characteristics and adaptability. These tiny roundworms are easily visible under a microscope, making them ideal candidates for studying soil samples. Further, they can be easily extracted from soil, which makes them highly practical for fieldwork. Additionally, nematodes are highly sensitive to changes in soil status, such as pollution, ecological imbalances and climate change. Therefore, they are a valuable tool for measuring the impact of various factors on the soil environment (Geetanjali *et al.*, 2002). In addition, lower concentrations of heavy metals may promote nematode pathogenesis, leading to greater damage to the plant (Bisessar *et al.*, 1983; Khan *et al.*, 1988, 1996a). The marine and soil nematodes are ecological distinct groups, hence their responses to heavy metals and salinity are discussed separately below.

Reaction of Marine Nematodes to Heavy Metals

Water bodies including oceans, rivers and other aquatic environments have been increasingly facing the threat of heavy metal contamination worldwide (Khan *et al.*, 1996b, 2006). Metal contamination causes serious adverse effects on the ecosystem and poses a significant risk to the health of entire flora and fauna (Okerefor *et al.*, 2020). The issue of metal contamination is wide in prevalence and its detection methods are cumbersome and laboratory based. Hence, it becomes crucial to find reliable bioindicators that could make us aware about soil or water quality with regard to heavy metal contamination. By doing so, we can take proactive steps to save the environment and conserve it for future generations.

Certain types of nematodes, specifically *Daptonema*, *Sabatieria*, *Terschellingia*, *Theristus* and *Paradontophora*, have been found to indicate heavy metals in water bodies by their presence or population fluctuations (Gyedu-Ababio *et al.*, 1999; Moreno *et al.*, 2008). Higher populations of *Daptonema* sp. were found at sites with contaminations of diesel fuel. This species of nematode is categorized as “opportunistic” and plays a crucial role of biomonitoring the marine environments. Therefore, the presence of *Daptonema* sp. in an area suggests that there may be diesel contamination but also indicates minimal levels of metal pollution

(Somerfield *et al.*, 1994). *Pomponema* sp. had a negative correlation with diesel contamination and its population may be very low under diesel contamination. Consequently, this nematode is classified as a diesel-sensitive species, indicating its vulnerability to petroleum contamination (Ng *et al.*, 2022). A research study conducted in Mozambique has shown a strong positive correlation of *Terschellingia* sp. with a range of heavy metals, including cadmium, cobalt, chromium, copper, iron, manganese, nickel, vanadium and aluminium (Soko and Gyedu-Ababio, 2019). These findings indicate the potential of using *Terschellingia* sp. as an indicator of metal-contaminated environments. Further, this nematode also accumulates substantial concentrations of these metals in its body.

The results of nematode diversity analysis conducted on three rivers in Germany have revealed some interesting insights (Heininger *et al.*, 2007). The study found that the sites heavily contaminated with high levels of aluminium and cadmium were mostly colonized by omnivorous nematodes such as *Tobrilus* and predatory nematodes such as *Mononchus*, with relatively high abundance. On the other hand, the sites with low to medium levels of contaminants experienced dominance of bacterivorous nematodes such as *Monhystera* and *Daptonema* or omnivorous nematodes such as *Dorylaimus* (Heininger *et al.*, 2007). Free-living nematodes in aquatic systems can play a vital role in indicating water contamination due to their sensitivity to pollution, especially heavy metals (Balsamo *et al.*, 2012; Semprucci *et al.*, 2015). Ridall and Ingels (2021) noted several marine nematodes which have been affected by heavy metals (Table 14.1). Members of the order Chromadorida, followed by Enoplida, Desmodorida, Monhysterida and Araeolaimida, were the most dominantly detected nematodes in the marine system. The results of a study carried out in Taiwan revealed that the presence of four specific types of nematodes in the aquatic environment, namely *Daptonema*, *Innocuonema*, *Axonolaimus* and *Pomponema*, can be indicative of potential contamination by metals, diesel and other organic substances (Ng *et al.*, 2022). These contaminants are associated

with various human activities in the area, such as the discharge of domestic waste, the use of diesel-powered machinery or vehicles, and the application of antifouling coatings on boats and other marine structures. The study has suggested that these nematodes could serve as a helpful tool for monitoring environmental contamination and identifying potential sources of pollution in aquatic ecosystems (Ng *et al.*, 2022).

<Table 1 here>

Kang *et al.* (2023) demonstrated that higher levels of mercury, chromium, copper, zinc, arsenic, lead, vanadium, manganese and nickel can influence nematode diversity in the Xihe river in China. A positive correlation was recorded between Enoplida and mercury, Triplonchida and manganese, and Plectida and chromium, whereas Mononchida showed a negative correlation to mercury (Kang *et al.*, 2023). It was further discovered that the presence of mercury had an adverse impact on the development of *Caenorhabditis elegans*. In contrast, copper and zinc proved to have a beneficial effect on the reproductive capacity of *C. elegans*. Nevertheless, the presence of vanadium and zinc seemed to have a detrimental impact on the behavior of *C. elegans* (Kang *et al.*, 2023). This suggests that contamination levels in the rivers may directly affect the types of nematodes that inhabit there. A *Propanagrolaimus* species was recorded in Molepo dam, situated in Limpopo Province, South Africa; the species has been found to be intriguingly uncorrelated with any of the water parameters (Shokoohi, unpublished observations, 2024).

Reaction of Soil Nematodes to Heavy Metals

Heavy metal contamination may otiosely affect soil health and its function. Park *et al.* (2011) detected significant differences in pH, electrical conductivity (EC), available phosphorus, and cadmium, lead, zinc and nickel in soil contaminated with mine drainage from an abandoned mine over non-contaminated soil. The density of long-living nematodes was low in the contaminated versus non-contaminated soil, and their abundance, maturity, richness, diversity

and structural indices were also low in the contaminated soil. However, the enrichment index was not significantly affected. This suggests that the heavy metals disturbed the soil ecosystem and suppressed the biological activity. To identify nematode genera, trophic groups and functional guilds as useful heavy metal indicators in soil, the nematode community was investigated at five alluvial meadows receiving water from the Litavka river in the Czech Republic contaminated with lead, zinc and arsenic from a smelter (Renčo *et al.*, 2022). The abundance of bacterivore, predatory, omnivore, plant-parasitic and fungivore nematodes was significantly decreased with the metal levels at four sites, being lowest at the highest polluted site.

Zeng *et al.* (2020) investigated the response of the soil nematode community to multiple metal mining activities containing 13.60, 7.69, 8.56, 2.35, 2.96 and 3.11 times average levels of copper and zinc, and the total metals. They noticed that heavy metals stimulated microbial biomass and nematode biomass consumption but the nematode group community was negatively affected. In another study, examining the impact of distance from a metal source, the soil population of free-living nematodes was affected most at the site nearest to the source, having a relatively low population density and domination of insensitive taxa, and this effect gradually decreased with the increase in distance from the source (Šalamún *et al.*, 2012). Zhang *et al.* (2007) also examined the effect of copper and zinc emanating from a smelter on soil nematode communities with regard to distance from the source. Nematode abundance significantly increased with an increase in distance from the source. *Paratylenchus* was the dominant genus among 20 nematode genera recorded at the sampling sites. They observed negative correlations for nematode channel ratio with total copper and zinc and structure index with total copper.

Soil application of sewage sludge containing high concentrations of copper, zinc + copper and zinc resulted in a decline in free-living nematodes with regard to genus/family richness

and maturity indices (MI and MI2–5) (Georgieva *et al.*, 2002). Suppression or elimination was recorded for persisters (*K*-strategists) in favor of colonizers (*r*-strategists), omnivorous nematodes (Dorylaimida: Dorylaimina) and predatory nematodes (Dorylaimida, Mononchida). The abundance of plant nematodes was increased in the zinc-treated plots, apparently due to the suppressive effects of the metals on some nematode antagonists and predators. The abundance of some bacterivore nematodes was also promoted. In another study, structure footprint, community footprint, abundance of plant feeders, bacteria feeders and omnivores/predators, and taxonomic richness were decreased with the elevated level of metal contamination (Chauvin *et al.*, 2020). Overall, higher abundance of the fungal-feeding nematodes was recorded at the very high contamination level, showing their relatively lower sensitivity to heavy metals.

Several investigations have been performed using the nematode community or specific species dominance as a bioindicator of various environments (Wasilewska, 1997; Neher, 2001; Yeates, 2003; Renčo *et al.*, 2022; Shokoohi, 2023; Young and Unc, 2023). Pollution caused by heavy metals such as cadmium, lead, arsenic, mercury, zinc, chromium, etc., can harm crop cultivation in agricultural lands (Bisessar *et al.*, 1983; Khan *et al.*, 1987). These toxic metals can accumulate in the soil, water and air, thereby contaminating crops and making them unfit for human consumption, besides inflicting a significant decline in quantity and quality of the produce (Khan *et al.*, 1994; Khan and Khan, 1995, 2010).

Heavy metal contamination may affect the maturity index of soil nematodes (Neher, 2001). The most sensitive nematodes to chemicals or soil nutrients belong to the order Dorylaimida, especially *Aporcelaimellus*, *Aporcelaimus*, *Discolaimus* and *Mesodorylaimus* (Fiscus and Neher, 2002). However, the reaction of soil nematodes to heavy metals could be different. A study in New Zealand showed that lands contaminated with sewage had more Cephalobidae than Rhabditidae or Plectidae (Yeates, 2003). In fact, in those lands, copper, nickel and zinc

were detected, among which nickel had suppressed the nematode populations most. The same result was obtained in another study where soil contaminated with a mixture of copper, chromium and arsenic revealed higher numbers of Cephalobidae than other free-living bacterivore nematodes. In addition, lands contaminated with copper in Wageningen in the Netherlands also showed a higher density of Cephalobidae than other free-living bacterivore nematodes. Cephalobidae members were found to be most resistant to heavy metals (Yeates, 2003). A study in industrial regions contaminated with heavy metals in Bulgaria showed that all feeding groups of nematodes had a negative relationship with cadmium, zinc, lead and copper (Mohamedova and Lecheva, 2016). However, the most sensitive groups were predatory and omnivorous nematodes (Table 14.2). The result showed that herbivores were affected mainly by cadmium, fungivores by zinc and omnivores/predators by cadmium, whereas bacterivores were slightly affected by cadmium, showing their resistance to the metal (Mohamedova and Lecheva, 2016). As a concluding remark of the study, populations of individual nematodes increased in the absence of heavy metals or at a low concentration. The maturity index, which is used to show the stability of an ecosystem, had a negative correlation with heavy metals, and therefore the number of beneficial nematodes decreased. Consequently, nitrogen fixation and decomposition of the materials in the soil decreased, leading to a lower uptake of nutrients by the plants. Additionally, heavy metals such as cadmium, zinc, lead and copper caused a decrease in the value of the structure index, affecting predatory and omnivorous nematodes in particular. Finally, a lower structure index value caused the shortening of the food web of the soil (Mohamedova and Lecheva, 2016).

<Table 2 here>

Effect of Heavy Metals on Plant-Parasitic Nematodes

Mining and refining of metals are major sources of heavy metal pollution worldwide (Bisessar *et al.*, 1983; Khan *et al.*, 1987, 1988). Mercury is one of the heavy metals leaking through the

mine activities to the soil. Studies have shown that mercury is highly toxic to free-living and plant nematodes, and can decrease their abundance (Khan *et al.*, 1986a; Martinez *et al.*, 2019). Mercury contamination decreased the density and abundance of *Rotylenchulus*, *Rotylenchus*, *Helicotylenchus* and *Tylenchorhynchus* (Martinez *et al.*, 2019). Mercury and cobalt above 50 ppm decreased the hatching, root penetration, galling, reproduction and soil population of *Meloidogyne incognita* in tomato (Khan *et al.*, 1986a, 1994). A similar response of root-knot nematode was also observed with nickel (Khan *et al.*, 1996a, 1996b, 2006). However, free-living nematodes, including *Achromadora*, *Heterocephalobus*, *Ironus*, *Prismatolaimus* and *Monhystera*, were affected more than the plant nematodes by mercury contamination (Martinez *et al.*, 2019), indicating their greater sensitivity (except *Pratylenchus*).

Martinez *et al.* (2019) indicated that mercury did not affect the nematode assemblage on the field. Soil physicochemical properties such as organic matter, pH, clay contents, etc., may influence the effect of mercury on nematodes (Martinez *et al.*, 2019). Recently, another study conducted in China also revealed that mercury has no effect on nematode diversity, except for *Bursaphelenchus* (Huo *et al.*, 2024). This means that most nematodes have a resistance strategy against mercury contamination. Besides, *Filenchus* and *Acrobeloides* were the only nematodes positively affected by chromium. *Aporcelaimellus*, *Mononchoides*, *Basiria*, *Ditylenchus* and *Alaimus* were affected by cadmium (Huo *et al.*, 2024). At the same time, arsenic affected the density of *Paraphelenchus*, *Nygolaimus*, *Aglenchus* and Rhabditidae members. Interestingly, none of the heavy metals affected the numbers of *Psilenchus* and *Mesodorylaimus*. The result showed that bacterivores, followed by fungivores, have a potential to be used as heavy metal bioindicators (Huo *et al.*, 2024).

A meta-analysis showed that nematodes react to increased levels of contamination, with a significant effect on their different parameters (Chauvin *et al.*, 2020). However, various feeding groups react differently to heavy metal pollution. A greater population of fungivore nematodes

was found in soil experiencing higher levels of contamination (Chauvin *et al.*, 2020), but the population of bacterivore nematodes decreased with increasing levels of heavy metals (Fig. 14.1). The effect of copper, zinc and nickel on plant-parasitic nematodes (*M. incognita*) and entomopathogenic nematodes (*Steinernema carpocapsae* and *Heterorhabditis bacteriophora*) was examined by exposing the nematodes to different concentrations for 24 h (Sun *et al.*, 2016). The treatment caused mortality to infective juveniles of entomopathogenic nematodes and affected the virulence of *H. bacteriophora* against *Galleria mellonella*. In a pot experiment on cucumber, copper, zinc or chromium treatments enhanced *M. incognita* juvenile penetration into the roots.

<Fig. 1 here>

Interestingly, the density of predators and omnivores was higher in low contamination than in higher levels of heavy metals. Overall, total nematode density decreases due to the excess amount of heavy metals. Based on meta-analysis results, bacterivore nematodes can be used as bioindicators of polluted areas with higher concentrations of heavy metals. In addition, the lack of herbivores, omnivores and predators may also be considered as an indicator of high levels of metal contamination. The meta-analysis also revealed that the enrichment index does not correspond to the increasing level of heavy metals, in which the enrichment index was without significant change due to low, medium and high contamination levels. The same scenario was applied to the maturity index and channel ratio. However, the Shannon diversity index and species richness were affected by the level of contamination, in which the highest contamination level decreased the aforementioned indices compared with zero contamination (Chauvin *et al.*, 2020). In addition, heavy metal contamination strongly affected the nematode community footprint, in which herbivores were significantly disturbed compared with other trophic groups. All nematode community indices, except the channel ratio, reacted to the heavy metal contamination. Therefore, the channel ratio is not a suitable index for use as a

bioindicator. In contrast, other indices, despite their considerable sensitivity, also did not show reliability to be used as bioindicator. However, the Shannon diversity index is reliable as it showed a significant trend towards increasing levels of heavy metal contamination. However, Georgieva *et al.* (2002) and Sánchez-Moreno and Navas (2007) have shown no effect of heavy metals, including copper, lead or zinc, on bacterivore nematodes of the *c-p2* group. Hence, their evaluation should be done with caution. Among the feeding trophic groups, omnivores and predators were reported to be the most sensitive to soil heavy metal contamination (Zhang *et al.*, 2007). The community and structure footprint of Mononchida (predators) and Dorylaimida (omnivores), based on nematode biomass, were decreased due to increased levels of heavy metals, and therefore can be used as indicators of soil contamination (Chauvin *et al.*, 2020).

Renčo *et al.* (2022) studied the relationship of arsenic, cadmium, chromium, copper, nickel, lead and zinc with nematodes in the Litavka river in the Czech Republic. The results showed that only lead and zinc significantly decreased the abundance of nematodes. Furthermore, cadmium did not produce any discernible impact on any trophic groups under observation (Renčo *et al.*, 2022). The same observation was achieved with chromium with no effect, except for some herbivore nematodes. Overall, herbivore nematodes did not react to the heavy metals, indicating that they cannot be used as heavy metal bioindicators. The study on the impact of heavy metals on nematodes showed that zinc and lead had a significant impact on the maturity index. Other diversity indices were found to be unaffected. Furthermore, it was observed that the structure index was affected only by lead, indicating that this particular index is more suitable for use as a bioindicator. Overall, these findings suggest that only a few diversity indices are essential for accurately assessing environmental quality and the impact of heavy metals on soil ecosystems. A study conducted by Shokoohi (2023) in South Africa showed that there is a significant correlation between *Psilenchus* ($r = 0.705$) and *Rotylenchus* ($r = 0.637$)

and copper. In a further study, the analysis of nematodes in tomato fields indicated that there is a significant correlation between copper and *Aphelenchoides* ($r = -0.817$), *Rotylenchus* ($r = 0.963$), *Pratylenchus* ($r = 0.762$) and *Rotylenchulus* ($r = -0.926$) (Shokoohi *et al.*, 2023).

Waste water is another crucial source of heavy metal contamination in agricultural lands when used for irrigation. A recent study showed that the abundance of *Panagrolaimus*, *Prismatolaimus*, *Filenchus*, *Mesorhabditis*, *Rhabditis*, *Zeldia* and *Monhystera* was increased with increasing levels of cadmium, zinc, copper, manganese, lead and potassium. However, populations of *Xiphinema*, *Thornenema*, *Nygolaimus*, *Discolaimus* and *Pratylenchus* were decreased with the above metal levels (Ghanem *et al.*, 2024). The findings of this research have revealed that heavy metal contamination in soil may negatively impact the abundance of herbivore and predatory nematodes. Metal pollution may also lead to a decrease in the sigma maturity index. However, the abundance and diversity of other types of nematodes, such as bacterivore and fungivore nematodes, responded positively to metal contamination (Ghanem *et al.*, 2024).

Reaction of Entomopathogenic Nematodes to Heavy Metals

Sun *et al.* (2016) reported the effects of toxic components from excessive fertilizer and manure applications on entomopathogenic and plant-parasitic nematode populations. They found that exposure to high levels of copper (1.5 mg/l), zinc (50 mg/l) or chromium (7.5 mg/l) for 24 h led to increased mortality in *S. carpocapsae* and *H. bacteriophora*, while decreasing *H. bacteriophora* against *G. mellonella*. Notably, *H. bacteriophora* was more affected by these metals than *M. incognita*. In potted plant experiments, metal pretreatment decreased the biocontrol efficacy of *H. bacteriophora* by increasing *M. incognita* root penetration, although low concentrations of zinc and chromium sometimes improved the biocontrol efficacy in *S. carpocapsae*. Overall, heavy metals negatively impact nematodes directly and indirectly by

reducing their biocontrol effectiveness, potentially altering nematode community structures in greenhouse systems.

Cadmium, copper, lead, zinc, manganese and magnesium impact the entomopathogenic nematode *Steinernema feltiae* under laboratory conditions. Cadmium, copper, lead and zinc were found to increase nematode larval mortality, reduce pathogenicity to *G. mellonella* and decrease reproduction. In contrast, manganese at a concentration of 400 mg/dm³ improved nematode survival, pathogenicity and reproduction compared to distilled water (Jaworska and Gorczyca, 2002). Jarmuł and Kamionek (2001) reported the effect of lead nitrate and lead acetate on mortality and invasive properties of the infective juveniles of *S. feltiae* larvae, used in the biopreparation “Ovinema.” Nematodes were exposed to lead ion concentrations of 0.40, 100, 500, 1000 and 5000 ppm over 120 h (Jaworska *et al.*, 1997). Results showed reduced insect mortality and lower invasiveness in lead-exposed nematodes compared to controls, with minimal impact from anionic groups. The effects of lead at concentrations of 40, 100 and 500 ppm, as well as cadmium nitrate at 1, 2 and 3 ppm, were studied on the entomopathogenic nematode *S. feltiae* and *G. mellonella* larvae from a laboratory culture. The study examined the sexual structure of the nematodes developing inside the body cavity of the host as well as the survival rate of infective juveniles before leaving the insect and after migration to the external environment (Kamionek *et al.*, 2011).

Mechanism of Heavy Metal Sensitivity

Cuticle permeability is the primary factor affecting the sensitivity or resistance of nematodes to chemical uptake, including heavy metals. The cuticle is an essential layer for nematodes as it acts as a barrier between them and their environment (Hurley, 1986). However, not all nematodes have a cuticle in their mature stage, such as the *Fergusobia* nematode. Moreover, the cuticle can vary among different genera and species, and even between males and females, different stages of life and distinct areas of the body (Decraemer *et al.*, 2003). The cuticle of

nematodes comprises four layers: epicuticle, cortical zone, median layer and basal zone. In addition, the epicuticle, which serves as a protective barrier against the external environment, comprises three ultra-thin layers primarily made up of a combination of lipids and proteins. These layers work together to provide mechanical strength, flexibility and permeability to the epicuticle, allowing it to regulate the exchange of nutrients and waste between the nematode and its surroundings (Decraemer *et al.*, 2003).

The layers in Mononchida (predators), Dorylaimida (omnivores), Rhabditina (bacterivores) and Tylenchida (herbivores) are different, allowing them to be tolerant or vulnerable to heavy metals in different soil types (Khan, 2008). *C. elegans*, a tiny nematode, possesses a remarkable natural defense mechanism that shields it from harmful chemicals, toxins and pathogens. This mechanism is attributed to a specific type of collagen that forms a protective barrier around the nematode cuticle. The collagen acts as a shield, preventing the entry of harmful substances through the cuticle and safeguarding the organism's internal systems. This remarkable adaptation is a testament to the amazing resilience of nature and the complex mechanisms that govern the behavior of even the smallest living organisms (Sandhu *et al.*, 2021; Njume *et al.*, 2022). That is why bacterivores might be suitable bioindicators of heavy metal contamination in the soil.

In *C. elegans* (a bacterivore species), cuticle permeability is a crucial characteristic that enables the absorption of chemicals. However, this feature also puts nematodes at risk of absorbing harmful molecules that can negatively affect their physiology and behavior (Moens *et al.*, 2013). In addition, heavy metals bind proteins of the epicuticle of various groups of nematodes, causing a change in cuticle functionality (Chauvin *et al.*, 2020). It should be mentioned that concentration and exposure time also affect the permeability of the nematode cuticle. For instance, cadmium up to 160 mg/kg soil did not affect the nematode community. Experimental studies have shown that exposure to nickel, copper and zinc for a period of 1–

2 weeks can significantly reduce the soil populations of omnivores and predators. This decrease occurs at a concentration of 100 mg/kg soil (Korthals *et al.*, 1996). The cuticles of two soil-dwelling nematode groups, Mononchida and Dorylaimida, have not been extensively investigated. As a result, we currently lack knowledge about the composition and structure of the collagens, as well as any natural barrier functions that the cuticle may possess. Further research in this area could provide valuable insights into the unique adaptations of these organisms to their environments.

Effect of Salinity on Nematodes

Reaction of marine nematodes to salinity

Salinity in aquatic systems refers to the amount of salt dissolved in water. Several nematodes have been studied in relationship with salinity of the water, including *Oxystomina* (Enoplida) in the oligohaline zone (0.5–5 ppt salts), *Terschellingia* (Monhysterida) and *Sabatieria* (Araeolaimida) in the mesohaline zone (5–18 ppt salts), and *Paracomesoma* (Araeolaimida), *Synonchiella* and *Odontophora* (Araeolaimida) in the polyhaline zone (18–30 ppt salts) (Adão *et al.*, 2009; Montagna *et al.*, 2013). Another study revealed that *Litoditis marina* (Rhabditidae) and *Diplolaimelloides meylli* (Monhysteridae) isolated from aquatic environments were not affected by salinity. However, juvenile development was strongly affected (Moens and Vincx, 2000). A survey in South Africa revealed that *Propanagrolaimus* (Panagrolaimidae), isolated inside the Molepo dam in Limpopo Province, had no correlation with water salinity (Shokoohi, unpublished observations, 2024). Overall, the findings indicate that salinity cannot significantly affect nematode development.

Reaction of soil nematodes to salinity

Soil salinity refers to the amount of minerals and salts dissolved in the soil, and is an essential measure of soil quality for growing crops and plants. This measure is crucial for crop

cultivation because excessive salt levels in soil can lead to reduced crop yields, soil degradation and even desertification. In most cases, soil water extract contains a range of mineral ions, with some being more important than others. These minerals, such as, Na^+ , Cl^- , Ca^{2+} , SO_4^{2-} , HCO_3^- , K^+ , Mg^{2+} and NO_3^- , are typically listed in order of importance, as they can have varying effects on plant growth and soil fertility. The salinity of the soil is measured as EC (in dS/m): salinity <4 dS/m is considered as non-saline (normal soil), whereas 4–8, 8–16 and >16 dS/m are considered as light, moderate and high salinity, respectively (Brusseau *et al.*, 2019).

The ecology and sometimes the biology of plant-parasitic nematodes are significantly influenced by salinity, depending on the salinity-affected habitats (Khan *et al.*, 1986b; Zaki *et al.*, 2012). A negative relationship was observed between salinity and soil populations of *Helicotylenchus indicus*, *Hoplolaimus indicus*, *Longidorus* sp., *Tylenchorhynchus brevilineatus*, *Tylenchus* sp. and *Trichodorus* sp. (Malhotra and Chaubey, 1993). Verma *et al.* (2007) examined the effect of soil salinity due to sodium sulfate, calcium chloride and magnesium chloride (4:1:3) at 2.8, 5.6, 8.4 and 11.2 dS/m on *M. incognita* infection in cotton. The growth parameters of cotton plants from 2.2 dS/m onwards as well as the nematode galls, egg masses, fecundity, etc., from 5.6 dS/m onwards decreased with increasing EC levels over control (non-saline, 1.15 dS/m). In another experiment, Al-Sayed *et al.* (2014) examined the effect of NaCl at 500, 1000, 2000 and 3000 ppm in the irrigation water on *Meloidogyne* and *Rotylenchulus* spp. infecting eggplant, cowpea and squash. Increasing salt concentrations and the resulting high soil EC led to significant promotion in galling, egg mass production and fecundity of *M. incognita* on eggplant roots with the highest impact at 3000 ppm NaCl. However, negative correlation was recorded on cowpea roots. The population of *Rotylenchulus reniformis* increased on cowpea, but decreased on eggplant and squash with increasing salinity. In another experiment, higher galling caused by *Meloidogyne* sp. was recorded due to irrigating eggplants with 0.1% NaCl solution (Sumera *et al.*, 2015).

In a greenhouse experiment, Hamdy *et al.* (2019) observed significant decline in the soil population of *M. incognita* juveniles, galls and egg masses on okra roots with increasing salinity levels from 0.1% to 0.3% NaCl over control. The salinity levels also decreased peroxidase and catalase activities in the exposed plants. Abdelkader *et al.* (2022) investigated the effect of varying levels of soil EC, nitrogen and moisture on reproduction and infestation of cyst nematode *Heterodera* sp. on durum wheat and *Globodera* sp. on potato in three areas in Algeria. The data showed significant correlations of the above three soil characteristics with both cereal and potato cyst nematodes. The soil EC negatively affected the frequency and infestation level of *Globodera* (77%) and *Heterodera* (65%), but soil moisture and total nitrogen showed positive effects on both nematode species.

Soil salinity is crucial in agriculture because this factor affects not only nematode development but also crop production. Salinity can affect plant-parasitic nematodes by impacting various stages of their development and pathogenesis. Salinity has been verified to have an effect on *M. javanica* in tomato production (Maggenti and Hardan, 1973). In a study conducted by Riddle and Bird (1985), it was observed that *R. reniformis*, *Anguina agrostis* and *M. javanica* displayed an affinity towards some chemical elements. Out of the various salts tested, ammonium sulfate and magnesium sulfate were found to be the least attractive, while magnesium chloride was found to be the most attractive salt for the above nematodes. Khan *et al.* (1997) examined the impact of NaCl and NaHCO₃ on root penetration and development of *M. incognita* race 2 in okra and cucumber and on plant growth. Salts at 5.0 mmhos/cm suppressed root penetration of the nematode and delayed development to adults. The number of galls, egg masses and fecundity had negative correlations with the salt concentrations. However, salinity also caused a suppressive effect on plant growth (Khan *et al.*, 1997).

Salinity is also reported to induce changes in nematode behavior. Studies have shown that *M. enterolobii* and *Pratylenchus coffeae* responded negatively to the salinity levels of soil

(Silva *et al.*, 2022). Increased levels of NaCl, CaCl₂ and MgCl₂ decreased the number of *P. coffeae*. The movement of *P. coffeae* was considerably slow under soil salinity. The experiments conducted by Hixson *et al.* (2005a, 2005b) revealed that seawater or highly saline water greatly suppressed the population of *Pratylenchus* and *Belonolaimus* species. The above information is particularly useful for turfgrass managers encountering nematode issues while maintaining hilograss, *Paspalum conjugatum*. The researchers found that saline irrigation may significantly suppress the above nematodes infesting the grasses and pastures. When analyzing the root length of hilograss, it was found that under low salinity levels (0–10 dS/m), *B. longicaudatus* damaged the roots and caused stunted growth, while at 15–25 dS/m the roots were not affected. This revealed that the ability of nematode larvae in parasitizing the roots was negatively affected by increasing salinity levels (>15 dS/m). The above studies highlight the scope of exploitation of saline irrigation for the management of nematodes in turfgrass, which may also be seen as an effective and environmentally friendly solution to the nematode problem.

Reaction of entomopathogenic nematodes and free-living nematodes to salinity

Soil salinity may also affect entomopathogenic and free-living nematodes. The host-finding ability of *Steinernema glaseri* and *H. bacteriophora* when exposed to soil salts was altered with salinity levels (Thurston *et al.*, 1994). Nielsen *et al.* (2011) examined the impact of salinity ranging from 0 to 50 dS/m on the survival and infectivity of *S. riobrave*. They found that the salinity levels used did not have a significant effect on either the survival or infectivity of the symbiotic bacterium present in the nematode, indicating that *S. riobrave* may be adaptable to a wide range of salinity conditions. An investigation on the impact of NaCl, MgSO₄, KNO₃ and NaCl + MgSO₄ on two free-living bacterivores nematodes, i.e. *Scottinema lindsayae* (Cephalobidae) and *Plectus antarcticus* (Plectidae), showed that these nematodes are highly

sensitive to salinity and did not survive exposure to different concentrations of NO₃ (Nkem *et al.*, 2006).

Besides salinity, soil moisture is also critical for the survival of free-living nematodes. *S. lindsayae* can survive at low soil moisture, and that is a reason for tolerance to soil salinity, as in low moisture, dissolved salts are lower than at high moisture conditions. Salts present in soil water affect osmoregulation of the body through cuticle permeability (Khan, 2008). Poage *et al.* (2008) showed that *S. lindsayae* survival was negatively correlated with soil salinity, such as nitrate. A strong positive correlation was recorded with soil EC and mortality of *S. lindsayae*. Pen-Mouratov *et al.* (2010) also observed a positive correlation of bacterivore nematodes, including *Wilsonema*, *Acrobeles*, *Chiloplacus*, *Cervidellus* and *Cephalobus*, with total soluble nitrogen of the soil. It has been found in China that soil EC generally induces a positive effect on the distribution and dynamics of bacterivore and fungivore nematodes in reclaimed farmland (Yang *et al.*, 2021). However, a significant negative correlation between *Trypilina* ($r = -0.862$), *Prismatolaimus* ($r = -0.728$), *Nanidorus* ($r = -0.864$), *Xiphinema* ($r = -0.862$), *Pratylenchus* ($r = -0.888$) and *Rotylenchus* ($r = -0.765$) and a positive correlation with *Rotylenchulus* ($r = -0.826$) in tomato fields in South Africa was observed (Shokoohi, 2023). A negative correlation ($r = -0.688$) between *Acrobeloides* and nitrate in the soil was also observed in the study. *Tylencholaimus mirabilis*, which was only detected in a forest soil, showed a positive significant relationship ($r = 0.646$) with soil phosphate (Shokoohi *et al.*, 2023).

Several genes have been found to interfere during the interaction of nematodes with salts (Xie and Zhang, 2022). *L. marina*, which is euryhaline nematode (living in a wide range of salinity), possesses a special mechanism which has demonstrated the survival of free-living bacterivore nematodes under salinity stress. When nematodes are exposed to a saline environment, it creates a stressful condition that can paralyze them. However, nematodes have developed a mechanism of osmotic regulation that enables them to resist the stress of the saline

environment. To do so, many genes, such as osmolyte biosynthesis genes, transthyretin-like family genes, V-type H⁺-transporting ATPase and potassium channel genes, are expressed in hypoosmotic and hyperosmotic conditions, which help nematodes adapt to the salty environment. All these genes work together to combat the salinity stress response as well as long-term acclimation processes in *L. marina* (Xie and Zhang, 2022). Although these genes have not been thoroughly examined in other nematodes, it should be noted that bacterivorous nematodes, regardless of their specific trophic group, display an exceptional ability to function as bioindicators for salinity levels. This makes them an invaluable tool for monitoring and assessing changes in the salinity of aquatic environments.

Conclusion and Future Prospects

Nematode taxa, especially those that feed on bacteria, fungi and other organisms, can serve as excellent bioindicators due to their high tolerance to environmental pollution. These tiny creatures, which range in size from 0.1 to 2 mm, are found in almost every ecosystem on Earth, including soil, freshwater and marine environments. Further, the nematodes that use an *r*-strategy, characterized by a short lifespan and high reproductive rates, have been observed to be more vulnerable to harsh environmental conditions that can disrupt their behavior. By utilizing character-based indices, the structure of the nematode community can be studied and the impact of environmental disturbances can be evaluated. This classification system is a valuable tool for understanding the ecology and dynamics of soil nematodes, making it possible to use them as bioindicators. The research suggests that certain families of nematodes, such as Chromadorida, Enoplida and Monhysterida in aquatic environments, and Cephalobidae and Rhabditidae in soil environments, can be used as reliable bioindicators to evaluate the extent of pollution in these environments. Furthermore, the maturity index is a highly effective tool for measuring heavy metal pollution in various soil types. However, nematode populations in soil are also affected by salinity. The bacterivore nematodes, including *Scottinema* and *Plectus*,

have been found to be highly sensitive to salinity (nitrate levels) in soil. As a result, they are regarded as excellent bioindicators for NO₃. On the other hand, herbivore nematodes are known to be impacted by both heavy metals and salinity, but they are not suitable for use as bioindicators due to their life cycle fluctuations. However, it is essential to ascertain their relationship with soil and plants carefully as a considerable number of nematodes species, especially from Tylenchidae and Anguinidae, may feed on roots instead of fungi. However, the use of nematodes as bioindicators requires extensive research and careful consideration of the environment in which they are studied. For example, aquatic environments pose unique challenges due to the constantly changing substrates and the swift water flow, which make it challenging to trace heavy metals. The nematodes can move quickly with the water flow, making it even more difficult to study their behavior. On the other hand, soil environments provide a more stable substrate for nematodes to thrive, and tracking their relationship with heavy metals is comparatively more straightforward. Finally, the use of nematodes as bioindicators is a promising approach in environmental studies, but it requires careful consideration of the environment, and long-term studies are required before making a conclusion.

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FIGURE CAPTIONS

Fig. 14.1. Nematode density (per 100 g soil) of various trophic groups exposed to different levels of heavy metal contamination (0 = no contamination; 1 = low contamination; 2 = medium contamination; 3 = high contamination). Different letters show significant differences at the 0.05 level (amended from Chauvin *et al.*, 2020). [Figure used with permission under Open Access license CC BY 4.0.]

TABLES

Table 14.1. Some marine nematodes and their reaction to heavy metals.

Order	Family	Genus	Cu	Zn	Co	Pb	Hg	Cr	Ni	Cr, Cu, Cu, Pb Ni	Co, Zn	
Araeolaimida	Axonolaimidae	<i>Axonolaimus</i>	R	—	—	—	—	—	—	—	R	—
		<i>Odontophora</i>	—	—	—	—	—	—	R	—	—	—
Chromadorida	Diplopeltidae	<i>Araeolaimus</i>	—	—	—	—	S	—	—	—	—	—
	Chromadoridae	<i>Hypodontolaimus</i>	—	S	—	—	—	—	—	—	—	—
		<i>Ptycholaimellus</i>	R	—	—	—	—	—	—	—	—	—
		<i>Prochromadorella</i>	—	—	—	—	R	—	—	—	—	—
		<i>Neochromadora</i>	—	—	S	—	—	—	R	—	—	—
	Cyatholaimidae	<i>Paralongicyatholaimus</i>	S	—	—	—	—	—	—	—	—	—
<i>Marylynnia</i>		—	—	R	—	R	—	—	—	—	R	
Desmodorida	Selachinematidae	<i>Gammanema</i>	—	—	—	—	—	—	—	S	—	—
	Desmodoridae	<i>Leptonemella</i>	—	—	—	—	—	S	S	S	—	—
		<i>Spirinia</i>	—	—	R	—	—	—	—	—	—	—
Enoplida	Microlaimidae	<i>Microlaimus</i>	S	—	—	—	—	—	—	—	—	—
	Monoposthiidae	<i>Monoposthia</i>	S	—	—	—	—	—	—	—	—	—
	Enchelidiidae	<i>Ditlevsenella</i>	—	—	—	—	—	—	—	S	—	—
	Oncholaimidae	<i>Oncholaimellus</i>	—	—	S	—	—	—	—	R	—	—
<i>Pontonema</i>		—	—	—	—	—	—	—	S	—	—	
<i>Viscosia</i>		—	R	R	—	—	—	—	S	—	R	
<i>Oncholaimus</i>		—	R	S	R	—	—	R	—	—	—	
Tripyloididae	<i>Bathylaimus</i>	—	—	—	—	—	R	R	—	—	—	
	<i>Tripyloides</i>	R	—	—	—	—	—	—	—	—	—	
	<i>Eleutherolaimus</i>	R	—	—	—	—	—	—	—	—	—	
Monhysterida	Linhomoeidae	<i>Terschellingia</i>	R	—	—	—	—	—	—	—	—	
		<i>Xyala</i>	—	R	—	—	—	—	S	—	—	
Xyalidae	<i>Promonhystera</i>	—	—	R	—	—	—	—	—	—	—	
	<i>Theristus</i>	R	—	—	—	—	—	—	S	—	—	
	<i>Daptonema</i>	R	—	—	—	—	R	R	—	—	—	

R: resistant; S: susceptible; –: not studied. [Amended from Ridall and Ingels (2021) and used with permission under Open Access license CC BY 4.0.]

Table 14.2. Correlation between soil nematodes and heavy metals.

Index	Cu	Zn	Pb	Cd
Herbivores	–0.587*	–0.613**	–0.201	–0.626**
Fungivores	–0.424*	–0.583**	–0.191	–0.414**
Bacterivores	–0.198	–0.164	0.022	–0.297
Omnivores/predator	–0.603**	–0.526*	–0.493*	–0.637**
Richness (S)	–0.218	–0.191	–0.138	–0.513*
Maturity index (MI _{2–5})	–0.462*	–0.501*	–0.423*	–0.704**

Structure index (SI)	−0.402*	−0.322	−0.208	−0.501*
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* $P \leq 0.05$; ** $P \leq 0.01$. [Amended from Mohamedova and Lecheva (2016) and used with permission under Open Access license CC BY 4.0.]