

Review

Nematodes as soil stress indicators for polycyclic aromatic hydrocarbons: A review

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Summary

Polycyclic aromatic hydrocarbons (PAHs) are an important group of organic pollutants present in all parts of the environment, affecting ecosystems and human health. PAHs, which have a strong affinity for organic carbon, are found in large quantities in soil, which is one of the most important sinks for these contaminants. Their impact on the soil biotic compartments depends on a number of different factors in combination with PAH behaviour and can be assessed using soil monitoring. Soil fauna have already shown excellent properties for biomonitoring of contaminants with most promising indicator frameworks based on nematodes, which are involved in essential processes in this environment. Nematodes respond to PAHs at multiple levels, including molecular, individual and community levels. At the molecular level, this is associated with activation of metabolic pathways for xenobiotics and increased demand for energy and resources. At the individual level, this is reflected in the slowing down of various physiological processes, which has consequences at the individual and community level for sensitive taxa. In this review, the toxicity and the direct and indirect effects of PAHs on soil nematode communities are discussed. It also considers the perspectives and challenges in assessing the toxicity of PAHs and their indication using soil nematodes.

Keywords: PAHs; soil pollution; Nematoda; monitoring; environment

Introduction

Ecological systems are exposed to various types of environmental stresses of different origin and duration (Bengtsson, 2002). Throughout evolution, organisms have developed effective strategies to survive and recolonize environments affected by such events (Egres *et al.*, 2019). However, with increasing industrialization, human society introduced new types of disturbances to which native fauna and flora were not yet able to adapt (Kraft *et al.*, 2015). The overall impact of the perturbation on the local fauna depends on the intensity, character or recurrence of the event, as well as on the actual resilience and stability of the local system and

its subsystems. In terms of the recurrence of environmental disturbances, the effect could have an acute character, with a relatively short-term duration, or a chronic character, with more prolonged effects on the local ecosystem. Acute disturbances are usually a part of the system as a natural force for maintaining diversity by creating heterogeneity and new niches (Bengtsson, 2002). Chronic disturbances, on the other hand, are the result of long-term stress on ecosystem structures and are usually of anthropogenic origin, e.g. agricultural practices, climate change, introduction of various pollutants, etc. (Bengtsson, 2002; Höss *et al.*, 2009; Šalamún *et al.*, 2014; Šalamún *et al.*, 2017).

Another important attribute is the rate of degradation/persistence

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of stressors in the environment. For example, pollutants, such as toxic heavy metals that have no natural degradation pathways, can cause stress for a relatively long time and alter the original structure and functions of the ecosystem (Alexander, 2000; Šalamún *et al.*, 2017). In contrast, organic pollutants are degradable and therefore, at first glance, may not pose much of risk to native soil communities (Alexander, 2000). However, the half-life of degradation of these pollutants can vary from days to decades depending on their structure (molecular structure, functional groups present, etc.) and the general conditions in the soil (Navarro *et al.*, 2007). Persistent organic pollutants (POPs), including polycyclic aromatic hydrocarbons (PAHs), are compounds that are relatively difficult to degrade and pose a high risk to soil fauna. PAHs are suspected carcinogens and are produced by incomplete combustion of organic compounds, industry, the seepage of crude oil and volcanic activity. (Wang *et al.*, 2007; Net *et al.*, 2015). Their metabolism in soil carried out by living organisms is not always successful due to their resistance, and the transition and biomagnification of these xenobiotics towards the top of the food pyramid can be usually observed (Fig. 1). Therefore, a detailed knowledge of the toxic effects of PAHs at different levels of the food web is necessary to better understand their impact on soil processes and the proper functioning of the soil ecosystem (Langenbach, 2013). This article provides a comprehensive overview of the different aspects of polycyclic aromatic hydrocarbons (PAHs) as hazardous pollutants with a particular focus on their impact on soil nematode communities, as they represent one of the most important groups of soil fauna and are involved in various soil processes (organic matter degradation, mineralization, pathogens regulation, etc.) and occupy various places in soil food webs (Ferris *et al.*, 2001). The fate, behaviour and toxicity of PAHs in contaminated soils are discussed with particular attention to their direct and indirect ef-

fects on these invertebrates and their regulatory functions in soil processes. The relatively small number of publications and the lack of other sources of information focusing on PAHs in relation to soil nematodes suggest that much more attention should be paid to this problem. This article will serve as a valuable source for researchers in the environmental sciences.

Ethical Approval and/or Informed Consent

This article does not contain any studies with human participants or animals by any of the authors.

Nematodes in the Environment

Of the various features of soil, its ecology is one of the most vulnerable to pollutants and other forms of disturbances (Bongers, 1990). The actual condition of the soil can be described by the current conditions of soil flora and fauna, which can change under stress. Consequently, native soil communities are carefully studied by scientists seeking an effective tool to indicate soil disturbance. The spatial and temporal heterogeneity of soil provides myriads of habitats for a wide diversity of organisms that depend on each other through their involvement in the nutrient and energy cycling (Bongers & Ferris, 1999). Hence, understanding the structure and function of below-ground food webs in relation to the presence and abundance of their components is a basic requirement for deeper insight into the soil ecosystem. To condense the information and facilitate the interpretation of soil health in relation to the actual state of the soil food web, it is necessary to include as many food web links or functional groups as possible. Since a functional group is not restricted to a phylogenetic unit, representatives of any taxonomic group could be involved in the different function-

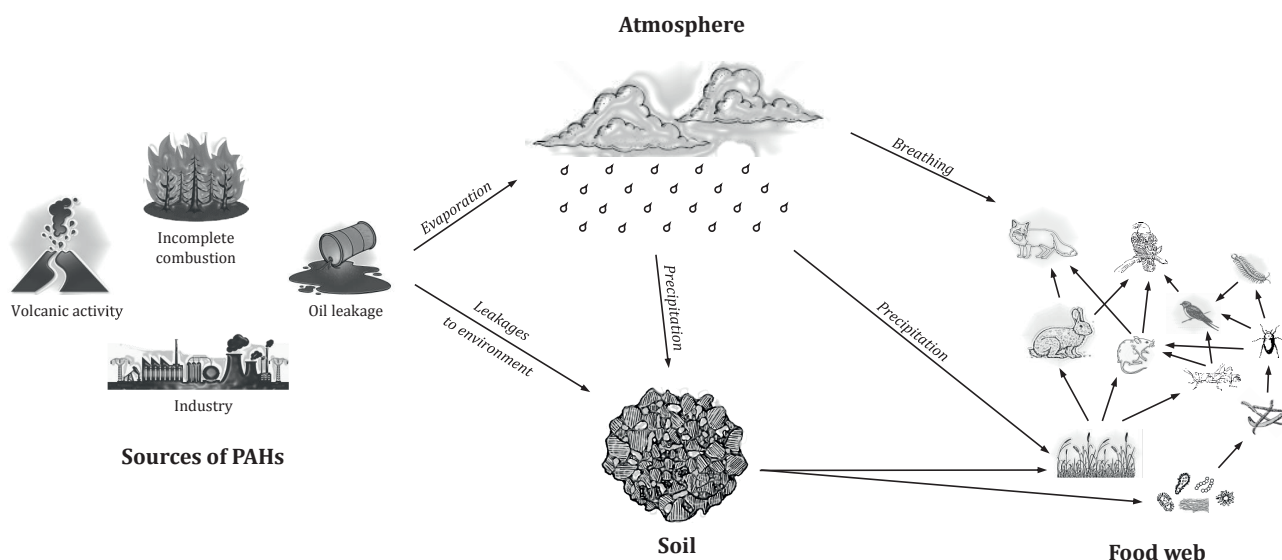


Fig. 1. The schematic PAHs pathways in the environment and trophic food webs.

al groups responsible for various soil processes and functions. Nematodes fall into this category; with a wide variety of trophic preferences, life strategies and occupation of important aspects of the food web, their activity affects primary production, decomposition, energy flows and nutrient cycling, especially nitrogen (Bongers, 1990; Van den Hoogen *et al.*, 2020). Furthermore, their high abundance, omnipresence in almost all aquatic and terrestrial ecosystems and good adaptation to a wide range of environmental conditions make them perfect model organisms for environmental assessment (Ferris *et al.*, 2001).

The use of nematodes as soil condition transmitters initially benefited greatly from the mass of available information for plant-feeding nematodes as important agricultural pests (Bongers & Bongers, 1998). After gaining a deeper insight into nematode communities, scientists found that nematodes are one of the most important taxa in the soil ecosystem. Their distribution across different levels of the food web secure a relatively fast and stable response to a new food resources and changing environmental conditions, making them a suitable tool for the evaluation of soil conditions (Bongers & Bongers, 1998). Functional indices have been developed as a practical and effective tool not only for assessing the nematode community structure, but also for indirectly assessing the stability and health of the whole soil ecosystem. Using these new indication tools, soil scientists are able to look more closely at the responses of soil ecosystems and predict the possible pathways of their future development under the effect of compaction, acidification and the decline in soil fertility caused by toxic substances and erosion (Ferris *et al.*, 2001; Hlava *et al.*, 2017).

The main focus of indication capabilities in relation to soil nematodes has been on the effects of heavy metals (Georgieva *et al.*, 2002; Šalamún *et al.*, 2012), even though organic pollutants have also been introduced into the soil in large amounts. Although they are probably more of an acute disturbance to soil fauna and flora, their long-term introduction into the soil could pose a serious threat to the diversity and further development of the soil environment.

Polycyclic aromatic hydrocarbons (PAHs) in the soil

Polycyclic aromatic hydrocarbons (PAHs) enter the environment either from natural sources, e.g. plants, termites or early stages of diagenesis (Wilcke *et al.*, 2000), or from anthropogenic sources and accidental spills. Production from more recent sources, such as combustion, transportation, oil or the wood processing industry is more important for the amount and variability of PAHs released. These contaminants are widespread in all components of the environment, including air, water, sediments and soil (Höss *et al.*, 2007; Höss *et al.*, 2009), and have been detected around the world from tropical to polar regions, even at sites far from industrial activities (Kuppusamy *et al.*, 2017). PAHs are an important group of organic pollutants containing two or more unsubstituted benzene rings fused together when a pair of carbon atoms is shared between them (Duan *et al.*, 2015). There are several hundred variants of

PAHs, but only 24 compounds (shown in Fig. 2) have been preferentially monitored (Lerda, 2011). Depending on their molecular weight, PAHs are split into light molecular weight PAHs (LMW), which have 2 – 3 benzene rings, and high molecular weight PAHs (HMW) having 4 – 7 rings. LMW PAHs, such as naphthalene, fluorene or anthracene, have a shorter persistence in soil compared to the more recalcitrant and carcinogenic HMW PAHs, mainly due to their higher solubility, volatility and lower hydrophobicity (Duan *et al.*, 2015; Kuppusamy *et al.*, 2017). The higher hydrophobicity of HMW PAHs results in their higher tendency to be absorbed by the soil's organic matter. Therefore, HMW PAHs, due to higher possible toxicity, low bioavailability and recalcitrance, represent 80 – 90 % of weathered PAHs in soils globally (Okere & Semple, 2011; Kuppusamy *et al.*, 2017).

Soil appears to be the final deposition site for PAHs (> 90 % of total PAHs in the environment), with atmospheric deposition being the major pathway of entry (Agarwal *et al.*, 2009). Depending on the nature of the PAHs, they can be eliminated from the soil by a number of physico-chemical and biological processes or leached into deeper soil layers including groundwater (Okere & Semple, 2011). The rate of PAHs degradation depends on numerous factors, e.g. soil properties (the redox-potential, organic matter and mineral content, temperature, moisture), individual PAHs properties (biodegradation half-life, toxicity, bioavailability) and the presence and activity of degrading soil organisms (Reid *et al.*, 2000). The pace and extent of PAH degradation decreases over time. This is especially true for clay soils and soils with high organic matter content, where PAHs may be unreachable for biodegradation through sequestering into organic matter or diffusion into micropores (Okere & Semple, 2011). Thus, the bioavailability of PAHs to microorganisms represents a crucial factor for soil restoration and, at the same time, the most important way to remove PAHs from soils (Kuppusamy *et al.*, 2017).

Effects of PAHs on soil nematodes

The interstitial life strategy (among soil particles) of nematodes gives them a unique ability to reflect direct and indirect influence of toxic organic compounds on the soil environment.

Direct effects of PAHs on nematodes

The most important factor for the degradation of PAHs in freshly contaminated soils is their hydrophobicity. The more soluble nature of LMW PAHs and their often higher concentrations in soil solutions compared to HMW PAHs influences the interstitial mesofauna directly through oral or transcuticular nutrient intake (Kammenga *et al.*, 1994). Nematodes that are sensitive to toxicants (e.g. predators or omnivores) and have a relatively permeable cuticle are exposed to this risk immediately after the introduction of PAHs into the soil ecosystem. On the other hand, representatives of stress-resistant nematode genera (e.g. mostly bacterivores and fungivores) may benefit from the introduction of PAHs through,

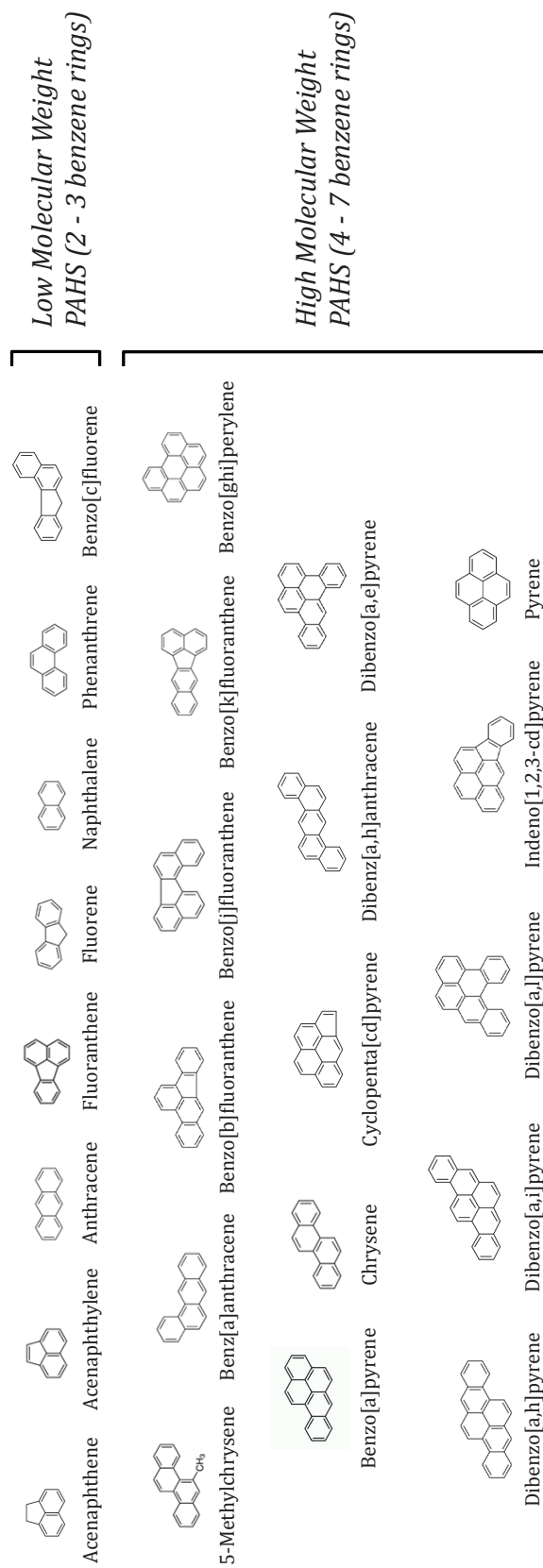


Fig 2. Names and structures of Polycyclic Aromatic Hydrocarbons (PAHs) frequently monitored according to recommendations by the EU Scientific Committee for Food (SCF), The European Union (EU), and the US Environmental Protection Agency (EPA).

for example, a decrease in predation pressure or an increase in available food resources. The shift towards stress-resistant species in the nematode community structure has been well documented by Moreno *et al.* (2009) and Soto *et al.* (2017) in localities heavily polluted with PAHs, where nematode genera well-adapted to disturbed conditions significantly dominated. The replacing of sensitive nematode species by resistant ones can affect the food web from both bottom-up (food availability, overpopulation, etc.) and top-down effects (predation, pests' control, etc.) and ultimately result in a lower ecosystem biomass despite constant population densities (Soto *et al.*, 2017). Chen *et al.* (2009) also observed the decline of the environmental maturity level and simplification of the soil food web under the higher pressure of PAHs contaminants in the soil.

The toxic effect of contaminants on nematodes, which have a relatively permeable body surface may be reinforced by ingestion of food containing risky substances. Except omnivores and nematodes that consume the soil substrate itself, such as some diplogasterids, or *Daptonema* spp. (Yeates *et al.*, 1993), which are under the direct risk of digesting soil organic matter particles, the pollutants may also act as a bottom-up limiting factor to the food web. Li *et al.* (2005) described experimentally various physiological responses and behavioural changes in *Aphelenchus* and *Acrobeloides* (fungivorous and bacterivorous nematodes, respectively) exposed to benzo(a)pyrene and phenanthrene. Even though the trophic preferences of these genera are different, they were similarly sensitive to PAHs. The development of both taxa was delayed after the addition of benzo(a)pyrene, despite the fact they are classified as genera with well-developed physiological and behavioural adaptations to stress conditions. Aside from the direct, lethal effect on organisms, PAHs can also act as narcotics in lower dosages and affect essential physiological processes, including the growth, reproduction and development of organisms (Menzel *et al.*, 2005). The development delay may be the result of interference between the PAHs and nutrient uptake and metabolism ultimately leading to the organism's "physiological starvation" (Postma & Davids, 1995). Reproduction processes seem to be even more sensitive to PAHs than survival and development processes of nematodes. Benzo(a)pyrene significantly affected the egg size and hatch rate in representatives of both *Acrobeloides* and *Aphelenchus* genus (Li *et al.*, 2005). Swain *et al.* (2010) suggest that organisms exposed to PAHs may have to expend a greater amount of their available energy for survival, which is then reflected in lower fertility and offspring production. In this study, the exposure of nematodes to fluoranthene probably caused a switch in the energy metabolism from carbohydrates to proteins, resulting in a high amount of free amino acids (as degradation products) found in nematode cells. Similar responses have been observed in other organisms subjected to PAHs, where re-synthesis of degraded key structural proteins during growth, development and egg production went hand in hand with a considerable energetic cost to the organism and the delaying or disrupting of these processes (Jones *et al.*, 2008;

Swain *et al.*, 2010). A decline in the populations of key organisms responsible for various important processes (nutrition and energy flow, pathogens control, etc.) in the soil ecosystem can gradually lead to the overall decrease of ecosystem production and loss of its functions. This negative trend could subsequently mean the beginning of a negative spiral heading to the lower maturity of the ecosystem.

Caenorhabditis elegans is the nematode most commonly used as a model in ecotoxicological studies. Individuals of this species exposed to PAH substances at concentrations between 2.7 and 5.2 mg.L⁻¹ in the soil showed acute mortality rates from 56 to 99 percent (Cofield *et al.*, 2008). Lower doses of PAHs used in experiments did not appear to have a lethal effect, but they caused numerous developmental defects, such as inhibition of growth, fertility and reproduction in this species (Höss *et al.*, 2009). Similar results (inhibition of reproduction in *C. elegans*) were observed under field conditions with a similar level of soluble PAHs contamination in freshwater sediments (Höss *et al.*, 2007). The level of the PAHs contamination seems to be the most important value in assessing the toxic effects of these contaminants on nematodes, and it is relatively independent of other environmental factors, such as the physico-chemical properties of the substrate. However, as nematodes correlate strongly with the labile dissolved PAHs fraction (Cofield *et al.*, 2008), the differences in substrate-binding capacity and composition of PAHs applied in the studies may decrease/increase their original concentration to comparable levels of the soluble PAH fraction to which the nematodes were exposed in both studies.

On the molecular level, PAHs induce multiple detoxification responses in nematodes, including the expression of cytochrome P450 genes responsible for the detoxification of xenobiotics. A concentration-dependent relationship was found between the intensity of expression of these genes and the benzo(a)pyrene added to the soil (Menzel *et al.*, 2005). An even stronger induction of the P450 genes family was found in the use of fluoranthene (Menzel *et al.*, 2001). Saint-Denis *et al.* (1999) pointed out that benzo(a)pyrene may also be activated by cytochrome P450-independent metabolic pathways. Alternative metabolic pathways of PAHs may include the generation of free radicals or the formation of reactive oxygen species as metabolic by-products, leading to oxidative stress (Penning, 2014). Wu *et al.* (2015) came to a similar conclusion in their study on oxidative stress in *C. elegans* exposed to benzo(a)pyrene. Laboratory studies provide important information on the potential direct effect of PAHs on living organisms, and even though they do not accurately simulate the influence of PAHs under field conditions, they provide important insights for future studies.

Indirect PAHs effects on nematodes

The introduction of PAHs into an ecosystem can indirectly cause both positive and negative responses in nematode communities. The positive effects can be channeled through the ability of bacte-

ria and fungi (decomposers, primary food sources for nematodes) to utilize various PAH compounds, including the most common PAHs – naphthalene, phenanthrene and pyrene – as sole carbon sources (Duan *et al.*, 2015). This process is primarily controlled by the bioavailability of these compounds to microorganisms, even without the need for PAHs to be present in the soil solution (Alexander, 2000; Zhang *et al.*, 2012). Depending on the nature of the PAHs introduced into the soil ecosystem and the decomposer channels used for their breakdown, soil communities respond distinctively by changes in the internal structure of their community. Bacteria can use LMW PAHs as a direct source of energy (Sack *et al.*, 1997), but only fungi are able to degrade HMW PAHs, despite their recalcitrant and hydrophobic nature (Cerniglia, 1992). The increase of decomposer populations (bacteria and fungi) in the soil may act as a *positive* stimulus for other trophic groups of microorganisms, such as fungivorous or bacterivorous nematodes, and later for higher levels of the food web. Blakely *et al.* (2002) observed the delayed response to a prospering decomposers community under sufficient food availability. Both bacterial biomass and bacterivorous nematodes flourished in soils contaminated by LMW PAHs, while the fungi population in the system was attenuated, which was illustrated by the continual presence of fungivores in the soil (Blakely *et al.*, 2002). Furthermore, the increase of bacterivorous nematodes grazing on PAH-degrading bacteria could even accelerate the dissipation of these organic pollutants (Sun *et al.*, 2017). According to Zhou *et al.* (2013), the reason for this contradiction between increasing pollutant degradation and predation could be the more intense in activity of native bacteria that can degrade PAHs. The trigger action of such enhancement could be the direct selective pressure of contaminants and predators or increased nutrient mineralization and nutrient cycling provided by bacterivorous nematodes (Sun *et al.*, 2017). In the latter case, the addition of a key nutrient to the system may significantly enhance the degradation, especially in a contaminated environment with limited nutrient resources (Yu *et al.* 2005).

Apart from this, the grazing of bacterivores keeps the population of soil bacteria at a reasonable level, thus preventing the inhibition of their growth caused by substrate shortage or the accumulation of toxic metabolites originating from the degradation. On the other hand, Näslund *et al.* (2010) found in marine sediments that, in the case of higher mesofaunal densities, naphthalene mineralization, as well as, the number of naphthalene-degrading bacteria, decreased. However, the authors pointed out that this phenomenon could be due to the higher predation pressure during the experiment in combination with significantly different bacterial diversity in treatments with different mesofaunal abundances.

The negative effects of PAH contamination on nematodes may be reflected, for example, in the availability of suitable microhabitat conditions. Given that the distribution of nematodes in the soil is largely dependent on their feeding habits and body size (Blakely *et al.*, 2002), the intra-aggregate pore space could be an important determining factor for nematodes distribution. Therefore, increas-

ing the bulk density of the soil by tightly bind PAHs to soil particles could significantly alter the actual conditions of microhabitats. This change may be a significant barrier to large-sized nematodes (e.g. Dorylaimida, or other omnivores and predators) using the intra-aggregate space as a refuge from other predators, or as a preferential place for reproduction and development (Briar *et al.*, 2011). The decrease in soil pores diameter also means less oxygen and nutrient transportation through the soil microhabitats (Blakely *et al.*, 2002). A lack of nutrition flow and suitable habitats for larger nematodes could lead to their decrease in the soil community. As larger nematodes represent mainly omnivores and carnivores, their decrease may significantly influence the top-bottom pressure in the habitat (Blakely *et al.*, 2002; Sun *et al.*, 2017). Nevertheless, Snow-Ashbrook and Erstfeld (1998) reported high abundance of omnivores/carnivores accompanied by a slight stimulatory effect of PAHs on the overall invertebrate community. Even though the genuine reason for the increased occurrence of sensitive nematodes in the most contaminated plots could not be explained from the study, Erstfeld and Snow-Ashbrook (1999) hypothesized that bottom-up effect regulation by the stimulated microflora, together with physico-chemical characteristics (organic carbon, soil moisture, pH and grain size) at the study sites, may play the key role.

Future perspective and challenges in using soil nematodes for PAH indication

As a result of rapidly growing urban land use, traditional agriculture or industrial activities, the environment is exposed to a variety of pollution discharges. With such pollution, environmental managers and decision-makers need a tool to better understand and manage the acute and chronic impacts on the local environment to protect, clean-up and restore it. The realization of the need to protect and restore affected ecosystems has led to a search for suitable environmental indicators. One possible option to distinguish affected soil ecosystems is the use of native soil communities that are able to reflect trends following contamination by various pollutants, including PAHs. Based on the literature collected, we have identified several research fields that could improve soil monitoring using native soil nematode communities in the future.

Indication of pollution at the individual and community levels

In the toxicological data collection and assessments of the impact of soil contamination, toxicological tests predominantly rely on a single-species laboratory test. For nematodes, the *Caenorhabditis elegans* toxicity test is the most commonly used (Wu *et al.*, 2015). The adaptation of nematodes to life in the soil has resulted in a great diversity of their life strategies and different nematode traits, including morphology, physiology, food preferences and behaviour. Therefore, even though single-species tests are important for characterizing potential acute and subacute impacts of contaminants under controlled conditions (Bejarano & Michel, 2016), they are not able to provide enough insight into the ecosystem from an

ecotoxicological perspective and describe all interactions that may occur among nematodes, contaminants and other components of the food web. Preferring information obtained on the community level, the monitoring framework might gain robustness, higher resolution and additional knowledge about the ecosystem and the processes running within it (organic matter degradation, nutrient and energy flow, availability of food resources, etc.).

Acute and chronic effects of pollution

As shown above, the outcomes of experimental studies can sometimes contradict each other, which means that no clear and relatively reasonable conclusions can be drawn about the impact of pollutants on nematodes. To obtain clear results under laboratory conditions, concentrations of pollutants beyond compare with real field conditions are often used. High levels of contamination indeed have an acute impact on nematode community structure, but this represents only a relatively short and partial effect of contaminants on the soil ecosystem, while contaminants availability in the system is still relatively high. On the other hand, the chronic phase lasting much longer (superseding the acute phase, when the availability of contaminants drops to a certain level) has possibly a stronger effect on the nematode community structure. In this phase, contamination does not shape the community directly but rather indirectly influences various physiological and behavioural aspects of nematode life.

Another problem closely associated with the chronic effects of contamination is the duration of exposure. Under controlled conditions, nematodes are exposed to pollutants for days or weeks, usually capturing the reaction of mostly a few following generations. The subacute exposure to foreign compounds leads to a transgenerational changes in nematode fitness (Yu & Liao, 2016). The induced multigenerational effects include additional mechanisms (cumulative damage, acclimatization, adaptation) that shape the nematode community and divert the direction of its development from that expected. Therefore, it is essential to consider the multigenerational effects to highlight the possible impacts of contamination over multiple generations and to include them in development predictions.

Standardization of indicating tools

Although the understanding of soil ecosystem functioning has improved significantly in the last decades, due to the high heterogeneity and dynamics of the soil ecosystem and the absence of baseline input data, comparison and evaluation is often a challenge. One step towards improving the robustness of the experimental data obtained and the applicability of nematodes as a tool for bioindication of soil stress is the harmonization of different methods and approaches used in ecological studies of ecosystem pollution. Obstacles to harmonization of biological methods often lie in the requirement for “fresh” samples of nematode communities for analyses and the lack of reference control material as a data source (Faber *et al.*, 2013). Therefore, standard meth-

odological approaches, as can be found in other scientific fields, are usually not applicable in the field of soil biology. Instead, the way to compare the relative efficiency and reliability of different biological methods is in their application under the same experimental conditions, i.e. concurrently on the same set of plots or samples. The harmonization step is necessary as part of the effort to achieve standardized methods at national and international level and to make progress in the use of native soil indicators in actual monitoring.

Morphological and molecular taxa identification

Another issue hampering the engagement of nematodes in soil monitoring is the extremely time consuming taxonomic determination using traditional morphological methods (Donn *et al.*, 2012). However, the recent increase in the development and use of molecular approaches, which are capable of processing large numbers of samples with high sensitivity in a relatively short time, should reduce the difficulties of identification based on morphology alone (Stone *et al.*, 2016). The low resolutions of morphological methods often led to misconceptions in taxa distribution, through numerous cryptic species that are relatively site specific (Taylor *et al.*, 2006). These subtle differences ultimately resulted in the incorrect assessment of the nematode community structure as well as an inability to recognize the uniqueness of each community and its proper reactions towards environmental conditions or stress (Stone *et al.*, 2016)

However, it should be noted that molecular methods are still under development and may not currently be able to fully incorporate nematode identification and generate accurate representations of nematode community diversity in a single step. Therefore, it is necessary to employ more than one technique to obtain valid results (Lott *et al.*, 2014). Stone *et al.* (2016) suggested that methods such as T-RFLP, which allow for quick but rather coarse analysis of large sample sets, could act as a sieve for selecting those to be analyzed in more detail at a later stage using additional methods. The main advantages of this approach lie in using rapid and cost-effective screening platforms without the considerable data processing and storage space requirements of most next-generation sequencing technologies, while yielding comparable resolution of community structure (Pilloni *et al.*, 2012). Rapid analysis opens up the possibilities of studying nematode assemblages and their spatial and temporal dynamics in sample-intensive studies not only in polluted soils, but also in natural or disturbed soils and in different agricultural environments. Although the entire nematode community is used in soil monitoring, for specific purposes, these methods are able to restrict the analysis to groups of interest or to be expanded and applied to the entire food web or eukaryotic faunal community of the soil (Donn *et al.*, 2012).

Conclusion

PAHs are an important class of environmental pollutants generat-

ed by both natural and anthropogenic processes. The importance of soil as a primary sink of PAHs means that they interact with soil and its constituents, leading either to their stabilization and persistence in the soil profile or to their loss, depending on the particular physical, chemical and biological conditions of the soil. Therefore, understanding the interactions between PAHs and soil should be one of the most important challenges in studying the impacts of organic pollutants in the soil environment. As this review shows, soil scientists are not always able to identify the negative impacts of PAHs on nematode communities, although many studies have observed negative effects on nematodes, including retarded development, reduced reproduction or activation of detoxification pathways in nematodes physiology. The ability of different nematode taxa to cope with the presence of contaminants in the soil usually results in altered species composition, which could significantly influence interactions within the nematode communities and interactions among other important soil taxa. The direct and indirect implications of soil contamination are that pollutants may be one of the driving forces of changes in the soil ecosystem, which could ultimately result in the alteration of entire communities and subsequently the entire ecosystem. Therefore, systematic and cost-effective monitoring of affected areas would greatly improve the possibilities of preventing negative developments in the soil ecosystem. This goes hand in hand with new techniques that open up new possibilities in analyzing large quantities of samples with relatively good resolution and will make it possible to detect threats more quickly and precisely.

Conflict of Interest

Authors state no conflict of interest.

Acknowledgments

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