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Geochemical Characteristics of Soil in Relation to Occurrence of Entomopathogenic Nematodes (EPNs) in Osogbo, Osun State, Nigeria

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Abstract

The occurrence and abundance of entomopathogenic nematodes (EPNs) vary across locations, and this has been attributed to many factors including soil physico-chemical properties. This study examined the influence of selected soil chemicals on the occurrence of EPNs under different cultivated lands in Osun state, Southwestern Nigeria. EPNs were isolated from 23.33% of the soil samples in selected sites using decantation and centrifugation method and nematodes observed under standard microscopy were identified based on morphological analysis of infective juveniles (IJs). This study has revealed the presence of nematodes of the families *Steinernema* and *Heterorhabditis* in the sampled soils. Soil chemical properties, including sodium (Na), carbon (C), phosphorus (P), magnesium (Mg), potassium (K), calcium (Ca), zinc (Zn), and iron (Fe) of the soil samples containing EPNs were determined following standard laboratory methods. Soil moisture contents of the sampled soils were also determined. Association of soil chemical characteristic and soil moisture content with the occurrence of EPNs was determined. There were statistically significant differences between mean soil chemical properties such as Na, C, P, Mg, K, Ca, Zn and Fe and soil chemical levels of soil samples with EPNs and soil samples without EPNs at $P \leq 0.05$. However, soil moisture was found to significantly influence EPNs' counts in positive soil samples. EPNs were found in soils of varying soil chemical levels, with individual species preferring a certain degree of chemicals (potassium (K) 267.83 mg, sodium (Na) 190.01 mg and iron (Fe), 155.80 mg). Soil fertility management that includes the use of fertilizers that contain the specific soil chemicals is required for occurrence of the EPNs.

Keywords: Entomopathogenic nematodes, soil chemicals, Moisture content, influence.

Introduction

The entomopathogenic nematode species (EPNs) are effective biological control agents against major insect pests (Ehlers and Shapiro-Ilan 2005). These nematodes possess many desirable attributes including broad host range, safety to non-target organisms and the environment. They inhabit most terrestrial habitats, but their occurrence has been evaluated mainly in relation to soil type and habitat (Homininck and Briscoe 1990, Mráček and Becvar 2000). The correlations between soil physico-chemical properties and nematodes suggest that the soil physical and chemical characteristics play an important role in the abundance, distribution and structure of nematode communities (Kandji et al. 2001).

Entomopathogenic nematodes (EPNs) of families *Steinernematidae* and

Heterorhabditidae are used to control insect pests primarily in the soil. These nematodes use symbiotic bacteria in the genera *Xenorhabdus* and *Photorhabdus* to kill and develop inside their insect hosts (Burnell and Stock 2000). The symbiotic bacteria, *Photorhabdus* reside in the nematode intestine (for *Heterorhabditis*) and are released into the insect haemocoel when the nematode enters the target insect host (Dowds and Peters 2002). The bacteria multiply, producing septicemia, and kill the insect host within 24 to 48 hrs, allowing the colonizing nematodes to feed on both the bacteria and the digested insect tissues (Dunphy and Webster 1988) for provision of essential nutrients for nematode development (Campbell and Gaugler 1997). Once developed, infective juveniles (IJs) emerge from the cadaver and search for another host. The nematodes complete generally two to three generations within the host’s cadaver and emerge as infective juveniles which forage for new hosts. The soil serves as the EPNs’ most suitable habitat. A small sample of soil may contain several thousand round worms (Hominick et al. 1997). The use of entomopathogenic nematodes (EPNs) as biocontrol measures for insect pests provide several advantages including their ability to disperse in the soil long enough until hosts can be located (Ehlers and Shapiro-Ilan 2005). The ability of *H. bacteriophora* to uniformly disperse and persist in the soil was found to relate to its success which is dependent on the soil moisture and other physico-chemical properties. Several studies have demonstrated that IJs of different EPNs differ in their ecological and behavioral traits with regard to their persistence and survival in the soil (Kaya 1990; Koppenhöfer and Fuzy 2007). Motility and persistence of entomopathogenic nematodes (EPNs) may be influenced by internal parameters (e.g. behavioural, physiological, and genetic characteristics) and external parameters of abiotic (e.g. temperature, soil moisture, soil texture, relative humidity and ultra violet (UV) radiation (Shapiro et al. 1993, Dzięgielewska and Skwiercz 2018) and natural biota. The influence of soil environment and characteristics on host searching behaviour as reviewed by Kaya and Gaugler (1993) showed that soil physical characteristics can influence the movement of IJs toward insect hosts in the soil. Physical or chemical characteristics of soil could play an important role in regulating EPN populations.

Soil moisture is the most important factor that affects nematode activities, because nematodes need a thin film of water for effective propulsion (Wallace 1959). In the soil, IJs move through the water film that coats the soil particles surrounding the interstitial spaces. If this film becomes too thin (in dry soil) or the interstitial spaces are completely filled with water (in water saturated soil), nematode movement can be restricted. Soil moisture content is closely linked with soil texture, another important factor, because soil particle size composition and organic matter content in soil strongly influence the availability of water in a given soil. Nematode motility generally decreases as soil pores become smaller. Small soil pores, particularly in combination with high soil moisture, will also limit oxygen levels and the activities and survival of aerobically respiring EPNs (Kung et al. 1991, Koppenhöfer and Fuzy 2006).

These soil nematodes have been found worldwide, except in Antarctica. EPNs possess many desirable attributes such as broad host range, safety to non-target organisms and the environment, ease of mass-production and application, ability to search for pests, potential to recycle in the environment and compatibility with many agricultural chemicals (Nyasani et al. 2008). EPNs are attractive alternatives to chemical insecticides and are ideally suited for integrated pest management (IPM) and organic systems particularly for soil inhabiting pests. Even though EPNs are common, their efficacy has been reported to depend on soil characteristics, agricultural management practices as well as the competition with native EPNs. Previous studies have found that agricultural management affects both soil quality variables and EPN distribution. The uses of pesticides (e.g. insecticides, herbicides and fungicides), fresh manure, and chemical fertilizers have also been shown to have detrimental effects on the survival and efficacy of EPNs. Environmental variables, soil characteristics and anthropogenic activities are also known to affect their diversity and occurrence. The effects of agricultural management practices and soil pollutants have been extensively studied on other nematode species (Nyasani et al. 2008, Jaworska et al. 1997). However, works on the impacts of soil physical and geological characteristics on the occurrence of EPNs are still very scanty and none exist for the study area. The aim of this study was to determine the effects of soil chemical characteristics on the occurrence of EPNs in Osun State, Southwestern Nigeria that serves as a model for determining the factors that influence the presence and abundance of EPNs in the environment.

Materials and Methods

Study area

The study was carried out in Osun State University's farmlands (7°62'13.4″N and 4°60'1 3.8″E) in Osogbo Local Government Area (LGA) of Osun State, Southwestern Nigeria. Soil samples were collected from three farmlands namely: Zoological garden farmland (7°76'19.4″N and 4°60'3.18″E); URP/University Mosque farmland (7°76'19.4″N and 4°60'3.18″E); and Auditorium farmland (7°76'19.4″N and 4°60'3.18″E) within Osun State University.

Collection of soil samples

Soil samples were collected from three (3) cultivated vegetable farmlands within Osun State University, Osogbo campus in July, 2019. Ten (10) samples of soil were randomly collected at 20 cm depth from an area of 30 metres x 30 metres of land at every 10 metres in a 'Zigzag' pattern. The samples were placed in a bucket and mix thoroughly with hands before placed in a labelled plastic bag. This procedure was repeated in all three farmlands that were visited for soil samples collection where a total of 30 soil samples were collected. The collected soil samples were stored in a cooler to maintain standard temperature range.

Laboratory experiments on collected soil samples

A total of 30 samples from five study locations were processed to isolate EPNs using decanting and centrifugation method as described in Bedding and Akhurst (1975). In addition, microscopic examinations were performed to confirm the presence of entomopathogenic nematodes. A soil sample was poured in a bowl and all debris hand-picked off. Water was added to the pure soil to produce a soil-water mixture. The mixture was poured through a 600 µm sieve and the residue discarded. The filtrate was filtered again through a 250 µm sieve, the filtrate poured off and the residue transferred into a 15 ml centrifuge tube (17 mm x 120 mm) and centrifuged for 3 minutes at 1700 rpm. After centrifugation, the supernatant was poured off while the residue was mixed with sugar solution (prepared using 545 g of sugar to 1 litre of water). The mixture was then centrifuged for 3 minutes at 1700 rpm. After that, the supernatant was passed through a cloth sieve and 10 ml of water was sprinkled on it to allow the debris to spread more evenly over the filter as well as enable nematodes to be separated from particles that settle faster (Akyazi et al. 2015) The residue on the cloth sieve was transferred into a petri-dish containing 2 ml of water and viewed under light microscope for EPNs as described by Nguyen (2007). Morphological features such as tail shape of all the stages, presence and absence of mucron in adults, head shape, presence or absence of horn like structures on head, shape and colour of spicule and gubernaculum in males were used in the identification of the nematodes using Identification key guide for agriculturally important plant-parasitic nematodes: A manual for nematology (International Maize and Wheat Improvement Centre (CIMMYT) and the samples containing entomopathogenic nematodes were noted.

Counting of nematodes

The EPNs extracted from 0.5 kg of sampled soil were counted to determine the population per soil sample and per sampled area. The counting was done following Cobb's sieving and decantation method and the total population enumerated. The nematodes were poured into a dish and counted under a binocular microscope**.**

Statistical Analysis

All data from the soil samples that were either positive or negative for EPNs were subjected to ANOVA, and significant differences in soil sodium (Na), carbon (C), phosphorus (P), magnesium (Mg), potassium (K), calcium (Ca), zinc (Zn), iron (Fe), soil moisture content and EPNs Counts were determined by using the general linear model (GLM) procedure using SAS 9.4 (21). The least significant data (LSD) was set at $P \leq 0.05$. The difference was considered statistically significant when $P < 0.05$.

Results

Soil screening and determination of entomopathogenic nematodes in different sampled location

Of the thirty soil samples collected and screened for EPNs, only seven of them namely AF001, AF002, AF003, AF004, AF006, AF007 and AF010 had entomopathogenic nematodes (Table 1). All positive samples were obtained from Auditorium farmland. Number of EPNs per positive soil samples in relation to soil moisture contents showed AF006, AF007 and AF010 having the highest moisture contents of 26.60, 26.20 and 26.95% with EPNs counts of 43, 21 and 38, respectively. These were followed by AF003, AF001 and AF004 with respective moisture contents of 18.00, 8.60 and 7.19 and EPNs counts of 10, 19 and 11 while AF002 had the least moisture content of 2.20 and EPN count of 4 (Table 2).

The soil chemical properties, including sodium (Na), carbon (C), phosphorus (P), magnesium (Mg), potassium (K), calcium (Ca), zinc (Zn), and iron (Fe) of the soil samples containing EPNs are presented in Figure 1. Potassium (K) 267.83 mg/kg of soil; sodium (Na), 190.01 mg/kg of soil and iron, 183 mg/kg of soil were the highest amounts of these chemical properties. The mean amounts of potassium, sodium and iron in the soil samples containing EPNs were 206.67, 139.67 and 164.27 mg/kg of soil, respectively. The amounts of C, P, Mg, Ca and Zn in the soil samples containing EPNs ranged as follows: 3.47–4.64, 14.90–34.89, 12.13–17.86, 26.11–27.44 and 21.88–18.60 mg/kg of soil with mean values of 3.99, 21.18, 14.54, 26.62 and 22.68 mg/kg of soil, respectively. As shown in Figure 2, the soil samples without EPNs had the following maximum amounts of these chemicals (mg/kg soil): Na 165.45, K 238.64, Fe 197.88, C 5.70, Mg 19.54, P 46.24, Ca 29.48 and Zn 31.60. The average amounts of Na, K, Fe, C, Mg, P, Ca and Zn in the soil samples without EPNs were 140.67, 222.29, 182.52, 5.06, 17.73, 32.37, 28.39 and 27.81 mg/kg of soil, respectively. The soil chemical properties, including sodium, carbon, phosphorus, magnesium, potassium, calcium, zinc, and iron of the soil samples containing EPNs and the soil samples without EPNs varied in composition with the soil samples containing EPNs having lower mean amounts of Na (139.67), C (3.99), P (21.18), Mg (14.54), K (206.67), Ca (26.62), Z (22.68), and Fe (164.27) than the soil samples without EPNs with mean amounts (in mg/kg of soil) of Na (140.67), C (5.06), P (32.37), Mg (17.73), K (222.29), Ca (28.39), Z (27.81), and Fe (182.52).

Table 3 shows comparison of values of the soil chemical properties in the three farmlands sampled. Soil chemical properties showed that in auditorium farmland (AF) and URP/MF farmland were significantly higher than the ZNG farmland. Soil chemical properties, including sodium, carbon, phosphorus, magnesium, potassium, calcium, zinc, and iron of the soil samples in URP/MF and ZNG farmlands were significantly higher than soil samples at AF (Table 3). Soil phosphorus content was significantly higher at AF than at URP/MF and ZNG. Soil total potassium (TK) at the AF and URP was significantly higher than the ZNG and MF (Figure 2). Overall, all soil chemical properties were lowest in the AF soil compared to other farmlands sampled.

Results of the mean soil chemical compositions showed that soil chemical levels within each soil samples with different EPNs count were significantly different at $P \leq 0.05$. There were statistically significant differences between mean soil chemical compositions and soil chemical levels of soil samples with EPNs and soil samples without EPNs as determined by one-way ANOVA (Table 4). There were EPN counts at 139.67 mg/kg of mean Na level in the soil with EPNs compared with 140.67 mg/kg of mean Na level in the soil without EPNs. The trend is the same in other soil chemicals tested including K, Fe, C, Mg, Ca and Zn. The mean P (38.89 mg/kg) level in the soil with EPNs was higher than the mean P (32.37 mg/kg) level of the soil without EPNs.

Table 1: Presence of entomopathogenic nematodes in the sampled soils

Site	Samples and EPN status									
	001	002	003	004	005	006	007	008	009	010
Zoological Garden Farmland (ZNG)	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve
URP/University Mosque Farmland (URP/MF)	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve
Auditorium Farmland (AF)	+ve	+ve	+ve	+ve	-ve	+ve	+ve	-ve	-ve	+ve

Table 2: EPNs' counts per positive soil samples in relation to soil moisture contents

Soil code for positive samples	% Moisture $= (W_1 – W_2)/W_2 \times 100$	Nematode counts (Adults/kg of soil)
AF001	8.60	19
AF002	2.20	4
AF003	18.0	10
AF004	7.19	11
AF006	26.60	43
AF007	26.20	21
AF010	26.95	38

W_1 = weight of moist soil sample; W_2 = weight of oven dried soil

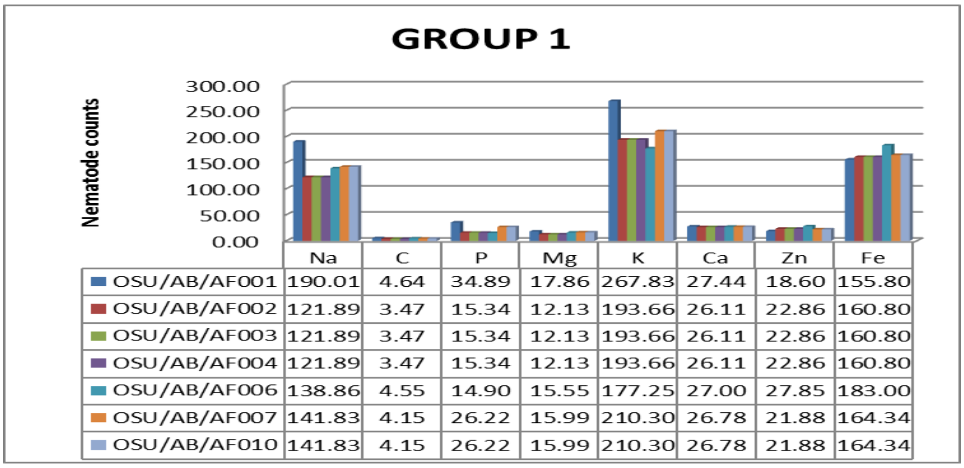

Figure 1: Chemical parameters of soil samples containing nematodes.

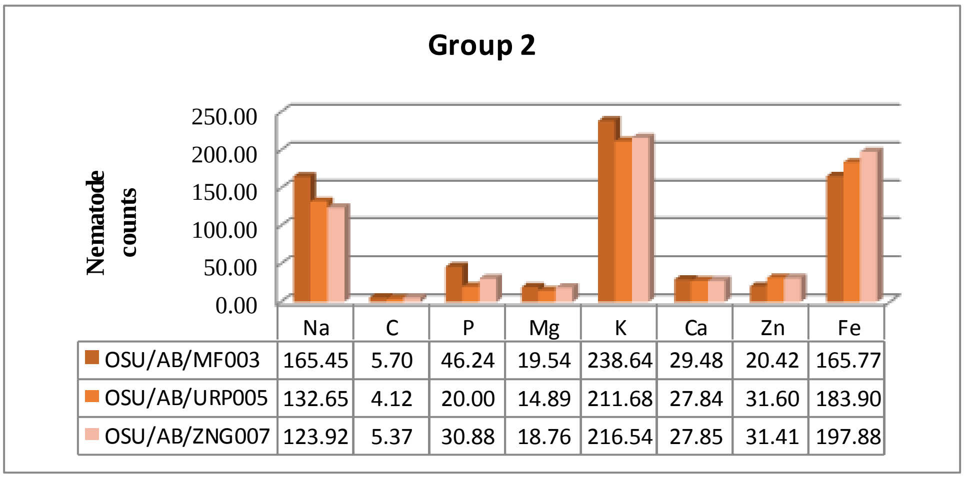

Figure 2: Chemical parameters of soil samples without occurrence of nematodes.

Table 3: Comparison of soil chemical properties at different farmlands and EPNs counts across farmlands

Element	Location code	Soil chemical (mg/kg of soil) / counts of EPNs	Location code	Soil chemical (mg)/kg of soil/ counts of EPNs
Na	AF001	190.01 (19)	MF003	165.45 (0)
	AF004	121.89 (11)	URP005	132.65 (0)
	AF006	138.86 (43)	ZNG007	123.92 (0)
	AF007	141.83 (38)		
C	AF001	4.64	MF003	5.70
	AF004	3.47	URP005	4.12
	AF006	4.55	ZNG007	5.37
	AF007	4.15		

Table 3 (Ctd)

Element	Location code	Soil chemical (mg/kg of soil) / counts of EPNs	Location code	Soil chemical (mg)/kg of soil/ counts of EPNs
P	AF001	34.89	MF003	46.24
	AF004	15.34	URP005	20.00
	AF006	14.90	ZNG007	30.88
	AF007	26.22		
Mg	AF001	17.86	MF003	19.54
	AF004	12.13	URP005	14.89
	AF006	15.55	ZNG007	18.76
	AF007	15.99		
K	AF001	267.83	MF003	238.64
	AF004	193.66	URP005	211.68
	AF006	177.25	ZNG007	216.54
	AF007	210.30		
Ca	AF001	27.44	MF003	29.48
	AF004	26.11	URP005	27.84
	AF006	27.00	ZNG007	27.85
	AF007	26.78		
Zn	AF001	18.60	MF003	20.42
	AF004	22.86	URP005	31.60
	AF006	27.85	ZNG007	31.41
	AF007	21.88		
Fe	AF001	155.80	MF003	165.77
	AF004	160.80	URP005	183.90
	AF006	183.00	ZNG007	197.88
	AF007	164.34		

Table 4: Analysis of soil chemical levels in soil samples that were either positive or negative for EPNs

Chemical	Mean soil chemical (mg/kg of soil) with EPN /*P* value	Mean soil chemical (mg/kg of soil) without EPN / *P* value
Na	139.67 (0.518)	140.67 (0.573)
C	4.64 (0.067)	5.06 (0.071)
P	38.89 (0.082)	32.37 (0.612)
Mg	17.86 (0.058)	17.73 (0.052)
K	206.67 (0.713)	222.29 (0.785)
Ca	27.44 (0.713)	28.39 (0.732)
Zn	18.60 (0.055)	27.81 (0.741)
Fe	164.27 (0.715)	182.52 (0.723)
Moisture contents	16.53	2.20
EPNs count	20.86	0

Discussion

The soil is an ecosystem with very complex relationships between EPNs and abiotic factors. It contains various components that are likely to influence the natural occurrence and population of EPNs. Our study showed that not all the farmlands harboured EPNs which may be attributed to significant differences in soil physico-chemical parameters. The presence of EPNs in Auditorium Farmland with low moisture (averaging 2 - 26%) and their absence in soil samples from Zoological Garden Farmland with average moisture content (of up to 36%) and URP/Mosque Farmland with more than 36% moisture showed that soil moisture is a critical abiotic factor affecting the distribution of EPNs. This is in line with the findings of Kaya and Gaugler (1993) who attributed behaviour, efficacy, survival and occurrence of nematodes to require water films of sufficient thickness and continuity in order to allow movement. In this study, EPNs were not found in soils with extremely low or high moisture contents. This agrees with the work of Wallace (1959) who attributed wet or saturated soils to be limiting in oxygen, and that nematode movement can be restricted due to lack of surface tension forces.

Results from the soil chemical compositions showed that soil chemical levels within each soil samples with different EPNs count are significantly different at $P \leq 0.05$. There were statistically significant differences between soil chemical compositions and soil chemical levels within each soil samples with different EPNs count as determined by one-way ANOVA (Table 4). Soil chemical properties such as Na, C, P, Mg, K, Ca, Zn and Fe within the soil samples with different EPNs counts were significantly different at $P \leq 0.05$. However, soil moisture was found to significantly influence EPNs' counts in positive soil samples. Samples from the Auditorium Farmland in the present study had high levels of sodium (Na), potassium (K) and iron (Fe) and moderate moisture contents than other farmlands (Figure 2). The amounts of these soil chemicals in the soil samples AF001, AF002, AF003, AF004, AF005, AF006, AF007, AF008, AF009 and AF010 were high and related to the occurrence of entomopathogenic nematodes. EPN population was higher in soil samples with potassium (K), 267.83 mg (43) followed by sodium (Na) with level of 190.01 mg (19) and iron with level of 155.80 mg (38) (Table 2 and Figure 1).

There were EPNs at 139.67 mg/kg of mean Na level in the soil with EPNs compared with 140.67 mg/kg of mean Na level in the soil without EPNs. The trend is the same in other soil chemicals tested including K, Fe, C, Mg, Ca and Zn. This means lower soil chemical properties favour the distribution of EPNs. The mean P (38.89) level in the soil with EPNs was higher than the mean P (32.37) level of the soil without EPNs, indicating that EPNs require higher levels of phosphorus.

The values of the soil chemicals also varied according to the number of nematode counts. The occurrence of EPNs in the soil with these degrees of soil chemicals may be attributed to the affinity of EPNs for potassium (K), sodium (Na) and iron (Fe). This is in line with the findings of Thurston et al. (1994) who reported high sodium content to influence survival and infectivity of EPNs and may contribute to provision of a good shelter for entomopathogenic nematodes.

In accordance with our predictions, most of the soil chemical properties showed significant differences between the three farmlands. Specifically, the K, Fe, C, Mg, Ca and Zn at the three farmlands vary and the differences between them were significant (Tables 3 and 4). There were no EPNs in soil samples collected from Zoological Garden Farmland and URP/ University Mosque Farmland with high soil chemicals of potassium (K), 238.64 mg/kg of soil followed by sodium (Na) with level of 165.40 mg and iron with level of 165.77 mg/kg of soil. The absence of EPNs in soil samples from Zoological Garden (ZGF) and URP/ Mosque farmlands despite high soil chemicals may be due to low moisture contents in these soil samples, a factor that is required for soil chemical movements in the ground.

The presence of high sodium (Na) in the Auditorium Farmland soil may be attributed to concentrated runoff of fertilizers from adjourning farmlands. Soil samples from

Zoological Garden (ZGF), URP and Mosque farmlands had no EPN but showed high levels of some soil chemicals such as C, P, Mg, K, Ca and Zn. The effects of these chemicals on the distribution of EPNs in ZGF, URP and MF require further studies.

Conclusion and recommendations

The findings of this study have indicated that many factors interact to determine the occurrence of EPNs in the environment. Soil properties such as soil chemicals, soil nutrients and land use systems may help to predict the natural occurrences of EPNs in various landscapes. Soil fertility and management practices should therefore be taken into account for occurrence of EPNs in the soil. The study has demonstrated that soil type and its physicochemical properties influence the presence of EPNs in the environment.

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