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Soil nematodes status of crude oil polluted sites in Bodo community, Gokana Local Government Area, Rivers State, Nigeria

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Crude oil spill destroys biodiversity and adversely impacts the physicochemical characteristics of the terrestrial and aquatic environments. The population characteristics of soil nematodes can serve as indicators of alterations in the terrestrial environment. To determine the effects of crude oil spillage on the soil nematode fauna status; a study was carried out at an impacted area in Gokana Local Government Area of Rivers State, Nigeria. 60 soil samples were randomly collected vertically (30 from the unpolluted sites and 30 from the polluted sites) at designated depths of 0-5 cm, 6-10 cm and 11-15 cm with the aid of a calibrated soil corer. Soil samples were taken to the laboratory for nematode extraction using the Baermann's extraction technique and the sieving method. Physicochemical parameters of the soil samples were determined using standard laboratory techniques. A total of 340 nematodes (from 11 genera) were recovered in the soil samples comprising; 169 (49.7%) nematodes from polluted sites and 171 (50.3%) nematodes from the unpolluted sites. There was slight variability in nematode species diversity, richness and abundance in the polluted and unpolluted sites ($p > 0.05$). Crude oil pollution influenced soil nematodes community composition while anthropological interferences such as farming influenced the successional trend of which was reflected in the maturity index values obtained from the study.

Key words: Crude oil, soil nematodes, biodiversity, polluted sites and maturity index.

INTRODUCTION

The soil is a complex matrix that accommodates all flora and fauna in the terrestrial environment. Its ability to sustain biological productivity, maintain environmental quality and promote the health of plants and animals in a defined ecosystem is termed soil health (Wardle and Yeates 1993, Ferris, 2010; Bileva et al., 2013). However, the soil is constantly disturbed by anthropological operations such as mining, construction and agricultural

activities, to an extent that it fails to perform its ecological function that ensures the sustenance of life in the biome (Harris and Bezdicek, 1994; Abawi and Wilder, 2000; Bileva et al., 2013; Nzeako et al., 2019). The quality and integrity of the floral and faunal aspects of the soil ecosystem, indirectly reflects the status of the abiotic components of the soil ((Wardle and Yeates 1993, Nielsen and Winding, 2002, Wang et al., 2009a).

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To explain the relationship between the flora and fauna in the terrestrial biome, biologists use specific organisms endemic in specific terrestrial habitats to assess the overall integrity of such environments. This is achieved by carrying out comparative evaluations of the ambient ecological conditions of an impacted environment with established standards to determine variations in the biotic and abiotic components inherent in such environments. To this end, the determination of the sensitivity of some endemic organisms to minute alterations in the soil's physicochemical characteristics is one of the crux in environmental impact assessment studies. Several studies have explored the sensitivity of soil nematodes to minute alterations in the physicochemical characteristics of the soil biome to buttress their bioindicatorship (Bongers et al., 1997; Bongers, 1999; Ferris and Matute, 2003; Wang et al., 2009; Pen-Mouratov et al., 2010; Nzeako et al., 2011; Briar et al., 2012; Zhang et al., 2012). In view of this, Zhang et al. (2012) was able to distinguish forest types through canonical correspondence analyses of nematode genera to indicate the importance of nematodes in ecological studies.

Free living nematodes show positive responses in predictable ways to soil ecosystem disturbances (Freckman and Ettema, 1993; Moody and Aitken, 1997; Moura and Franzener, 2017). As the most important secondary consumer in the environment, nematodes are amongst the few meiofauna that readily respond to minute alterations in the physicochemical characteristics of the soil. Their sensitivity to disturbances in their environment is associated with their omnipresent nature, relative abundance, unique morphology, diversity, and wide range of trophic survival specialism (Bileva et al., 2013; Nzeako et al., 2015, 2019). Also, the ease in extracting them from the soil accentuates the use of their population parameters in the evaluation of soil health (Yeates and Bongers, 1999, Ferris and Matute, 2003) in a biome.

In the Niger Delta, where oil spills and improper disposal of hydrocarbon products are common occurrences, hydrocarbon leachates pollute groundwater and constitute a major public health concern in the region (Gogoi et al., 2003; Osuji et al., 2006; Abii and Nwosu, 2009). In addition to this, crude oil exploration and spills disturb the soil ecosystem impacting soil biodiversity adversely. Crude oil clogs the soil pores hampering physical processes such as capillarity, aeration and drainage as well as increased bulk density. Crude oil pollution actively influences soil nematodes community composition resulting in the negative correlation of trophic diversity and genus number with the duration of petroleum exploration in oil fields (Wang et al., 2009). Savin et al. (2015) in agreement, stated that crude oil pollution has long term negative effects on the faunal composition of the soil ecosystem. However, Dechang et al. (2011) observed that ecosystem instability common in crude oil impacted soils was due to pollution induced low

trophic diversity of resident basal organisms such as soil nematodes. According to Osuji et al. (2006), crude oil pollution increases the acidity of the top soil and shoots-up heavy metal profile of impacted sites, resulting in high electrical conductivity and moisture content. Naturally, the ambient physicochemical parameters of various ecological settings vary, however, oil spill exacerbates the acidity of impacted soil by reducing its cation exchange capacity (Abii and Nwosu, 2009).

Bongers (1990) used the maturity index of soil nematodes to evaluate the extent of disturbances in different ecological settings. Although, the maturity index of soil nematofauna is useful in evaluating the degree of colonization and succession in an ecological setting (Bongers et al., 1997). It should not be solely relied upon as the only effective way of evaluating changes in the biological aspects of the terrestrial environment (Bongers et al., 1997, Bongers, 1999). The task of determining the alterations in the successional process of an ecological setting must incorporate other ecological evaluations such as; enrichment Index (EI), structure index (SI) and channel index (CI), Briar et al. 2012.

An agro-productive soil is constantly disturbed through tillage, organic amendments, pest management strategies, nutrient enrichments, and construction activities. Disturbances in the soil ecosystem such as tillage and enrichment activities alter the structure of the soil ecosystem. The stated disturbances to the soil ecosystem reduce biodiversity and promote the population of short-lived *r*-strategist nematodes, such as the bacterial feeding Rhabditidae, Pangrolaimidae and Diplogastridae in relation to nematode in the other functional groups (Ferris and Matute, 2003; Nzeako et al., 2011, 2019).

In the Niger Delta, crude oil spillage is a frequent occurrence with devastating ecological impact on biodiversity, and the overall utility of the soil ecosystem (Popovici and Ciobanu, 2000; Zhang et al., 2012). It stimulates the adaptive survival strategies that lead to the development of tolerance and resistance in the endemic nematode meiofauna and other micro metazoans (Boucher, 1980; Wang et al., 2009b, Nzeako et al., 2011; Egwu, 2012). Numerous ecosystem services rendered by the soil are hampered due to crude oil spillage, of which trophic dynamics is one of the most affected. Nematodes are second in ubiquity to bacteria and occupy a central position in the soil food web, correlating with ecological processes such as mineral cycling, faunal and floral growth (Ferris et al., 1996). Therefore, there is a great need to relate the physical and chemical measurements of the soil ecosystem to the integrity of the biological components inherent, in order to understand the dynamics of the soil biome.

Considering the functional roles of the nematode fauna in the environment, they stand as good biological tools for assessing soil processes and plant conditions in the terrestrial ecosystem (Neher, 2001, Wang et al., 2009;

Pen-Mouratov et al., 2010; Zhang et al., 2012). Amidst their bioindicator quality, the use of nematodes as biological agents for environmental impact assessments is usually neglected in Nigeria (Nzeako et al., 2013, 2019). This work is designed to determine the successional progress of the Gokana crude oil spill sites in Rivers State of Nigeria ten years post impact.

MATERIALS AND METHODS

Study area

The study area is Bodo city located in Gokana Local Government Area, Rivers State, Nigeria. Gokana LGA has a population of 49,000, falls within latitude 40°39'N and longitude 7°16'E, bounded by two LGAs namely; Khana and Tia. The vegetation of the area is rainforest vegetation, annual mean rainfall range is 160-298 mm and the mean annual temperature range is between 24-32°C (UNEP Report, 2011). The community hosts the Shell Trans-Niger pipeline that accounts for 60% of Nigerias crude oil output, however, the natives' major occupation is subsistent farming. The sampling sites are designated as: 1) Trans-Niger pipeline, designated as Station A (polluted: indicating crude oil spilled); 2) Boogugoi; Station B (relatively undisturbed: indicating fallow condition and crude oil spilled) located at latitude 4°38'25" and longitude 7°43'20" served as the control for the crude oil; Kolgba village; 3) Station C (disturbed- indicates cultivated land and no crude oil spilled) located at latitude 4°37'25" and longitude 7°44'18"; Numuu Agbigel -River Bank, Bodo Creek; 4) Station D (polluted) located at latitude 4°37'24" and longitude 7°44'19" upland farm; Station E; (disturbed and unpolluted) in Numuu Agbigel, Kolgba village located at latitude 4°37'25" and longitude 7°44'12" (Figure 1).

Collection of soil samples

Soil samples were collected randomly from the designated sites with a calibrated soil corer (50 mm) in diameter. Collection of soil samples was vertical at 0-5 cm; 6-11 cm and 11-15 cm depths in both polluted and unpolluted areas. These were aggregated into three depth categories in each of the collection areas. A total of 60 soil samples were collected comprising 30 soil samples each from the polluted and unpolluted sites. These soil samples were composited separately according to the collection sites and depths respectively. These were put into well labeled black polythene bags with open mouths and transported to the laboratory for extraction of nematodes and physicochemical analyses. About 50g of the soil samples from each composite set was taken for physicochemical analyses. The modified Bearmanns' Extraction Technique (Thorne, 1961; Barker et al., 1985; Kimenju et al., 2007) and sieving method were adopted in the extraction of soil nematodes from the soil samples (Barker et al., 1970; Nzeako et al., 2014). Nematodes were identified to the genus level according to Goodey and Goodey (1963), Goodey et al. (1965) and Mai and Lyon (1975).

Evaluation of the nematode faunal characteristics in the study

The maturity and plant parasitic indices of the nematode fauna were determined according to Bongers and Bongers (1990) maturity index scale where soil nematodes were categorized to their colonizer-persister groups (c-p groups). Group c-p1 comprised nematodes with very short generation time, high colonization ability, tolerance to environmental stress, high metabolism, and increased

in population during nutrient enrichment, these constituted the colonizers or r-strategist. The c-p 2 group nematodes comprised nematodes that had short generation time, responded more slowly to environmental enrichment than c-p1 nematodes, reproduced more under stress conditions, occurred in all environments and very tolerant to pollutants and general disturbances (Herris and Bongers, 2009). The c-p 3 group nematodes are intermediates with longer generation time than the c-p 1 and 2, relatively sensitive to disturbances; e.g., nematodes in the family Chromadoridae. The c-p 4 group comprised nematodes with long generation time, permeable cuticle and are highly sensitive to stress and pollutants. Group c-p 5 nematodes are referred to as the k-strategists or persisters. They are nematodes with a generation time of one year, large sized (usually omnivores and predators, e.g. nematodes in the families; Enoplidae and Leptosomatidae), low colonization ability, low reproduction rates and have permeable cuticles. They are very sensitive to pollutants and general disturbances in the marine meiobenthos (Warwick, 1986). Shannon Wiener Diversity Index was used to assess the population characteristics of the nematodes meiofauna.

Laboratory soil analysis

100 g of the soil samples (composite) from designated sampling sites were transported to the Plant Science and Biotechnology Laboratory of the University of Port Harcourt, River State, Nigeria for the determination of their physical and chemical characteristics. The soil samples were air-dried and subjected to physicochemical analyses. The physicochemical tests conducted include; Total Organic Content (TOC), Total Hydrogen Content (THC), pH, temperature, moisture content (MC), conductivity, nitrate (NO_2^-) and determination of exchangeable ions of Magnesium (Mg), Potassium (K), Sodium (Na), Calcium (Ca), Iron (Fe), and Zinc (Zn), following the Association of Official Analytical Chemist Methods of Analysis (1975, 2016).

Determination of soil conductivity: A Jenway 4010 conductivity meter was used for the determination of the electrical conductivity at room temperature of 25°C. The platinum electrode was cleaned using Chromic acid and distilled water to keep the conducting surface free of contaminants. The samples were brought to room temperature and the meter and the electrode were set up to stabilize, then, the electrode was immersed in the test sample containing 1:1 volume suspension of soil and water. The set-up was allowed to stand for 15 min to stabilize before taking reading.

Determination of soil moisture content by air oven method: The moisture content of the soil sample(s) was determined using the Gallekamp hot air oven. About 2g of the soil sample was put into an evaporating dish and weighed. This was placed in the oven and heated to 105°C for about 6 hours. The heated soil sample(s) was put into a desiccator to cool to room temperature and was reweighed. The final weight was computed against the initial weight to determine the moisture content. Moisture content was computed as Percentage (%) of Moisture Content = $\frac{\text{Loss in weight (g)}}{\text{Sample weight (g)}} \times 100$.

Determination of soil sulphate using the turbidimetric method: About 5g of the soil sample was suspended in 200ml of distilled water and the debris filtered-off with 25µm sieve to obtain the soil extract. 10ml of the soil extract was added a pinch of Barium chloride crystals (BaCl_2) and stirred with a magnetic stirrer for 10min, after which, 2ml of concentrated H_2SO_4 was added and a white turbid precipitate was observed. Then, the white turbid precipitate was passed through a colorimeter at 420nm wavelength using distilled water as blank. The absorbance of the standard sulphate was read at 420nm at 30seconds interval for 4 min. The standard solution was prepared against variable concentrations and

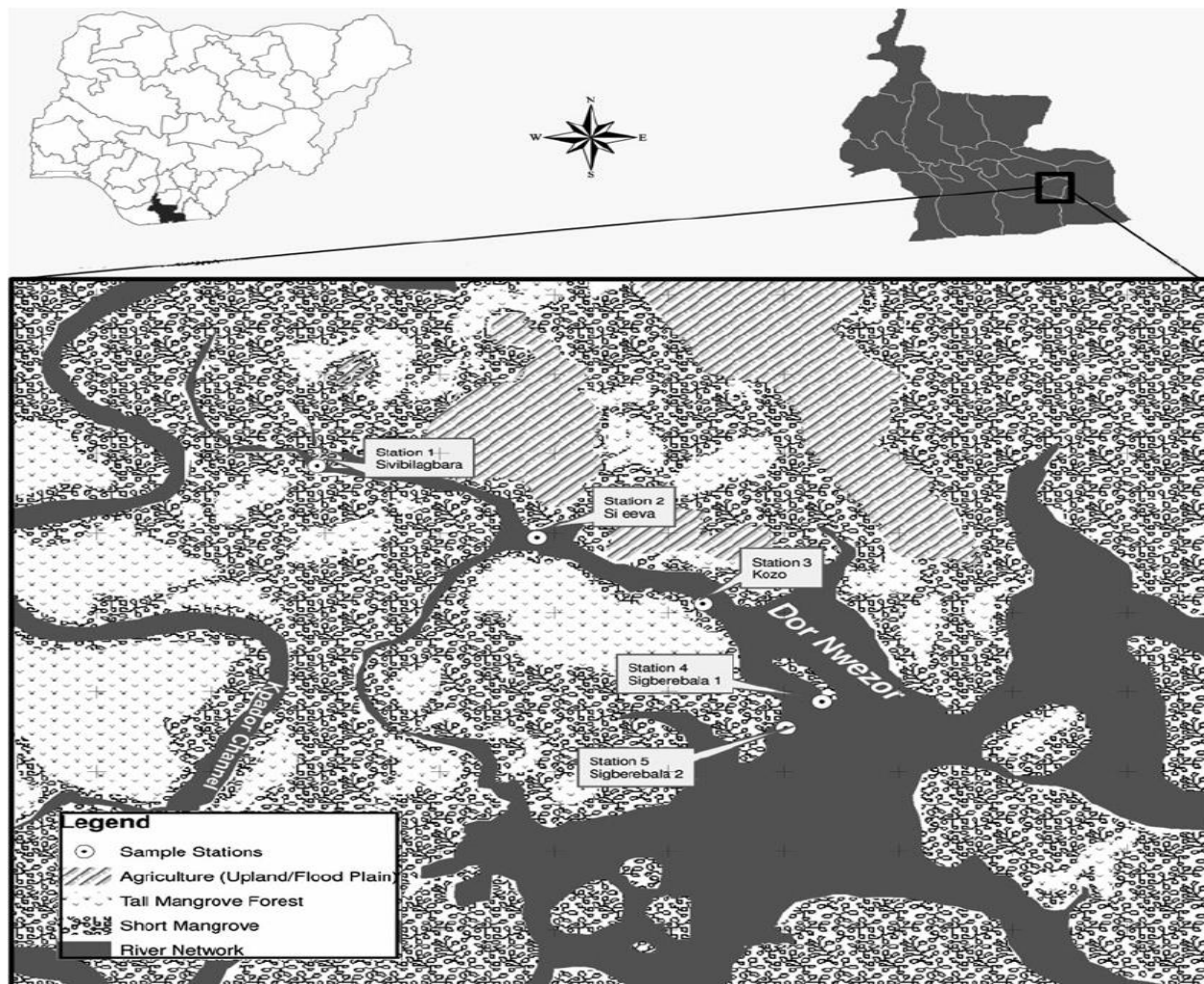


Figure 1. The map showing the physical features of the study area.

the sulphate concentration in the sample extrapolated from a standard curve.

Determination of phosphate using molybdenum blue method:

The molybdenum blue method was used for the 5g of each of the soil samples was weighed and 15ml of 2.5% acetic acid added. The solution was mixed thoroughly using a stirring rod and left to stand for 24h. To the extracts obtained through decantation, 1.5ml of molybdenum reagent was added. These were acidified with ascorbic acid and left to stand for 10 min for the molybdenum blue color to develop due to the reduction of the heteropolyphosphomolybdate compound. The absorbance of the characteristic molybdenum blue color of both the samples and standard were measured using the Atomic Absorption Spectrophotometer (Model: SpectrAA55B) at 880 nm.

Determination of soil nitrate: 1ml of the soil extract obtained from a 1: 10 ration soil to water mixture was transferred into a test tube and mixed with 0.5 ml of Bromine reagent (2.5% - 2.5 g Bromine in 100 ml of Glacial Acetic Acid (GAA)), and 2 ml of concentrated H_2SO_4 was added rapidly to the mixture. Then, the absorbance of each sample was measured at 470 nm using reagent blank as a reference solution (AOAC, 2016).

Determination of organic carbon: The organic carbon content of the soil samples was determined using the method proposed by the AOAC (1990). The weights of the crucibles were determined before being filled with soil samples and reweighed. The crucibles containing the soil samples were heated in the oven at a temperature of 630°C for three hours. These were cooled to room temperature and the weights of the residues (ash) determined. The percentage organic carbon content was estimated using the mathematical relationship; Total weight of crucible (TWC) - dry weight (DW) x 100/Sample weight. The organic matter was obtained by multiplying total organic carbon values by a conversion factor of 1.27.

The total hydrocarbon: The total hydrocarbon content of the soil was analyzed as described by Onianwa and Essien (1999) and Chukujindu et al. (2008). 100 g of the soil sample was refluxed with 100ml of methanol containing about 3.0 g of KOH for approximately 3h. The mixture was filtered and the filtrate was extracted with 2.5 ml of redistilled hexane. The combined extracts were evaporated to about 1.0 ml and then, subjected to clean-up in a silica column, eluted with n-hexane. The elute was subsequently evaporated to isolate the hydrocarbon oil that the weight was determined. Measurement of the THC was carried out using Atomic Absorption

Spectrophotometer (Model: SpectrAA55B). Total Organic Carbon (TOC) was determined with the ascorbic acid method according to Okalebo et al. (2002).

Determination of exchangeable cations: Exchangeable Calcium and Magnesium were determined with the Atomic Absorption Spectrophotometer (Model: SpectrAA55B) while Sodium and Potassium were determined on the JENWAY 6305 Spectrophotometer using Perchloric acid HClO_4 as extracting solution, because of its suitability for several elements, including; calcium, copper, iron, magnesium, manganese, potassium, sodium and zinc, and may be applicable to other elements (McLean, 1965). The soil sample was air-dried and crushed with a mechanical device and screened to pass a $20\mu\text{m}$ mesh sieve (Udo and Ogunwale, 1986). Weigh out 2.5g of soil in a 125ml Erlenmeyer flask and add 25ml of normal Ammonium acetate ($\text{NH}_4\text{O Ac}$) pH 7.0. Place the sample in a shaker for 15min. Filter the solution and analyse by flame atomic absorption spectrometer or by atomic absorption or emission. Using the routine procedure as stated in Cooksey and Bannett (1979) and David (1960). Standards were prepared by suitable dilution of stock standards with the extractant. Then, the concentration of calcium and magnesium by flame atomic absorption and sodium and potassium by atomic absorption (Plate 1). The standards for exchangeable cations considered in this study were prepared according to the procedures stated in the Analytical Methods for Atomic Absorption Spectroscopy (1996).

- (i) Dissolve 1000 g of iron wire in 50 ml of (1+1) HNO_3 to obtain 1000 mg/L by diluting this in 1 litre of deionized water.
- (ii) Cautiously dissolve 1000 g of magnesium ribbon in a minimum volume of (1+1) hydrochloric acid (HCl) and dilute this mixture to 1 litre with 1% volume by volume (v/v) of HCl to obtain 1000 mg/L.
- (iii) Dissolve 2.542 g of sodium chloride, NaCl, in deionized water and dilute to 1 litre with deionized water to obtain 1000 mg/L.
- (iv) Dissolve 1.598 g of lead nitrate, $\text{Pb}(\text{NO}_3)_2$, in 1% (v/v) HNO_3 and dilute to 1 litre with 1% (v/v) HNO_3 to obtain 1000 mg/L.
- (v) Dissolve 0.500 g of zinc metal in a minimum volume of (1+1) HCl and dilute to 1 litre with 1% (v/v) HCl to obtain 500 mg/L.

Data analysis

Data was analyzed using Analysis of Variance (ANOVA) while the Shannon Wiener Diversity Index was used to analyze nematodes community dynamics. Response of nematode to disturbances in the soil and parasitism in plant tissues was evaluated with Maturity Index and Plant Parasitic Indices.

RESULTS

Soil nematodes composition in the study

A total of three hundred and forty nematodes were recovered from the study comprising; 165 (48.5%) from polluted soil and 175 (51.5%) from unpolluted soil. Depth-related occurrence varied with the 0-5 cm depth recording a total of 96 (28.2%) nematodes out of which 50 (30.3%) and 46 (25.7%) were from the polluted and unpolluted sites respectively. At the 6-11cm core depth; a total of 124(36.5%) nematodes were recovered comprising; 53 (32.1%) and 71 (40.4%) from the polluted and unpolluted sites respectively. At the 11-15 cm core depth; a total of 120 (35.3%) nematodes were recovered with 62 (37.6%) and 58 (33.9%) occurring at the polluted

the unpolluted sites respectively (Table 1).

Variation in physicochemical composition of polluted and unpolluted soil

The total hydrocarbon concentration (THC) in the study was relatively higher in the polluted soil such that the mean differential between the soil samples from the polluted and unpolluted sites was 50 and 2.0 mg/kg respectively ($p < 0.05$). Percentage Organic Matter (% OM) declined as depth increased in both the polluted soil and unpolluted soil samples. In the study, (% OM) was higher in polluted soil samples, however, the (OM) mean differential between the polluted and unpolluted soil samples was 6.81% (Table 2, Figures 1 and 3). The mean differential of N_2 content between the polluted and unpolluted soil samples was 12.86%. There was slight depth related distribution in nitrogen (N) with the polluted soil harboring more nitrogen content (Table 2). In the unpolluted sites, there was depth related decrease in soil phosphorus (P) content, with the highest concentration at the 0-5 cm core depth (12.80%). pH was highest at the top soil in both the polluted (5.20) and unpolluted (5.12) sites. However, the mean pH of the unpolluted soil was higher than that of the polluted soil (Table 2 and Figure 1). Conductivity in polluted and unpolluted sites varied vertically with a mean differential of about $-29.65 \mu\text{s}/\text{cm}$. Conductivity increased as depth increased in the unpolluted sites, but, was the reverse in the polluted site. Percentage moisture content was higher in the top soil (6.90%) in both the polluted and unpolluted sites, however, moisture retention was more in the unpolluted sites (Figures 2 and 3). Higher temperature was recorded in the polluted soil with the mean temperature of 30.3 and 29.1°C in the polluted and unpolluted respectively. The polluted soil recorded the highest concentrations of the anion; SO_4^{2-} (mg/kg) and NO_3^- (mg/kg), however, the middle soil (5-10 cm depth) in both the polluted and unpolluted sites harbored the highest concentrations of anions in the study (Table 2).

Metal composition of soil samples in the study

Calcium ion (Ca^+) had the highest occurrence at the 6-10 cm depth in both the polluted (1283.9 mg/kg) and unpolluted sites (288.85 mg/kg), with a mean differential of $-740.04 \text{ mg}/\text{kg}$. Potassium (K^+) ion concentration was highest in the unpolluted soil (mean total; 183.57 mg/kg) with the 10-15 cm core depth exhibiting the highest concentration of 197.90 mg/kg and a mean differential of 44 mg/kg. Magnesium (Mg^+) concentration was higher in the polluted than the unpolluted site, with a mean differential of 35.83 mg/kg. However, the general concentration of Mg^+ was more at the 6-10 cm core depth in both sampling sites (Figures 4 and 5 and Table 3). Sodium ion (Na^+) occurred more in the polluted environment with a vertical pattern that increased as



Plate 1. a: Kelgdahl digester (Gallekemp digester); b: SpectrAA55B Atomic Absorption Spectrometer c: Jenway 6305 Spectrophotometer, d: +2 Gallekemp oven, and e: Modified Bearmanns soil extraction set-up for mobile soil nematodes.

Table 1. Composition of soil nematodes in the study.

Core depth (cm)	Polluted site (%)*		Total (%)	Unpolluted site (%)*		Total (%)	Overall total (%)
	A	C	(A+C)*	B	D	(B+D)*	(*+*)
0-5	30(60.0)	20(40.0)	50(30.3)	31(28.0)	15(21.9)	46(25.7)	96(28.2)
6-10	30(56.6)	23(43.4)	53(32.1)	43(40.2)	28(40.6)	71(40.4)	124(36.5)
11-15	32(51.6)	30(48.4)	62 (37.6)	34(31.8)	24(37.5)	58(33.9)	120(35.3)
Total (%)	92(55.6)	75(44.4)	165(48.5)	108(62.6)	64(37.4)	175(51.5)	340

*Total of polluted and unpolluted sites, ** Overall total of both sites; polluted and unpolluted.

Table 2. Depth related variations in physicochemical parameters in the study.

Soil parameter	Unpolluted site			Mean value	Polluted site			Mean value
	0-5 cm	6-10 cm	11-15 cm		0-5 cm	6-10 cm	11-15 cm	
THC (mg/kg)	1.50	1.50	3.00	2.00	1.50	73.50	75.0	50.0
Organic matter (%)	3.22	1.79	2.45	2.49	15.65	6.91	5.34	9.3
Nitrogen (%)	0.126	0.084	0.133	0.11	0.15	0.12	0.10	0.12
Phosphorus (%)	12.80	11.52	11.52	35.84	5.37	7.17	5.12	6.18
pH	5.20	4.17	4.47	4.6	5.12	3.90	4.58	4.53
Conductivity ($\mu\text{s}/\text{cm}$)	23.4	35.60	44.70	34.57	95.70	16.70	17.00	43.13
Moisture (%)	6.90	4.90	5.70	5.83	19.50	19.0	17.60	18.7
Temp ($^{\circ}\text{C}$)	29.9	29.9	29.1	21.8	30.4	30.3	30.5	30.3
SO_4^{2-} (mg/kg)	344.45	688.89	654.44	562.59	620.00	1308.89	688.89	1745.19
NO_3^- (mg/kg)	136.36	123.53	132.36	130.75	132.36	127.94	132.36	130.88

depth increased in both sampled sites. The mean total concentration of (Na^+) was 302.03 mg/kg and a mean differential of 198.32 mg/kg in the polluted and unpolluted

sites. Iron (Fe^{2+}) occurred more in the polluted environment without a consistent vertical variable pattern in the study. The mean total concentration of (Fe^{2+}) was

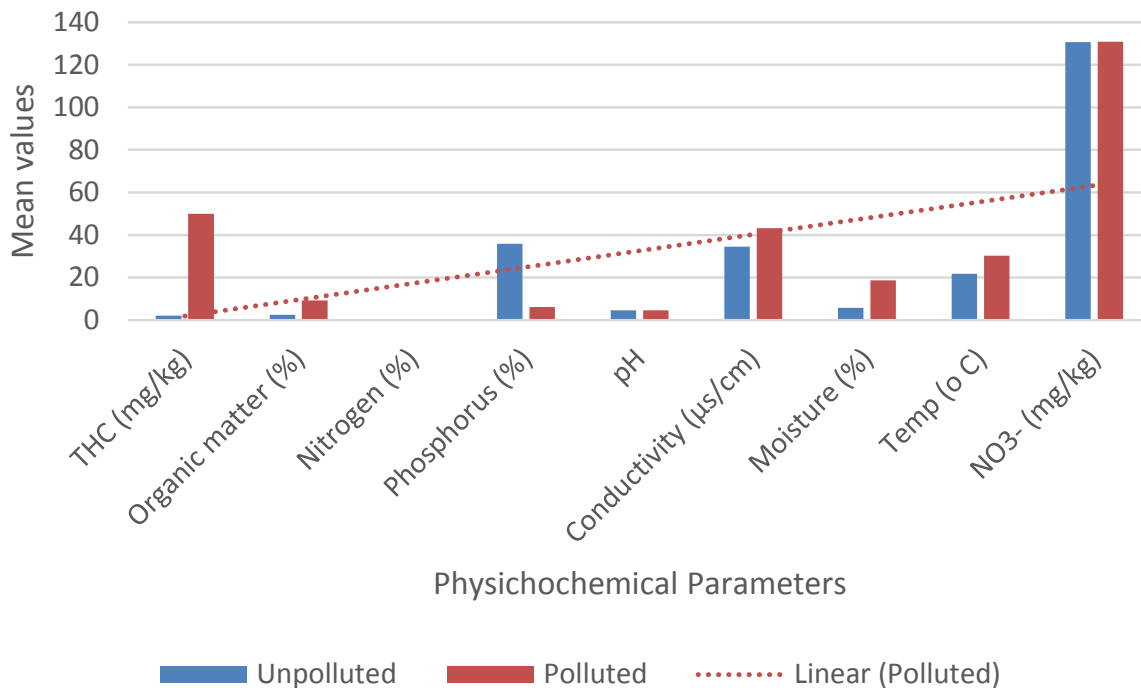


Figure 2. Physicochemical parameters in the study area.

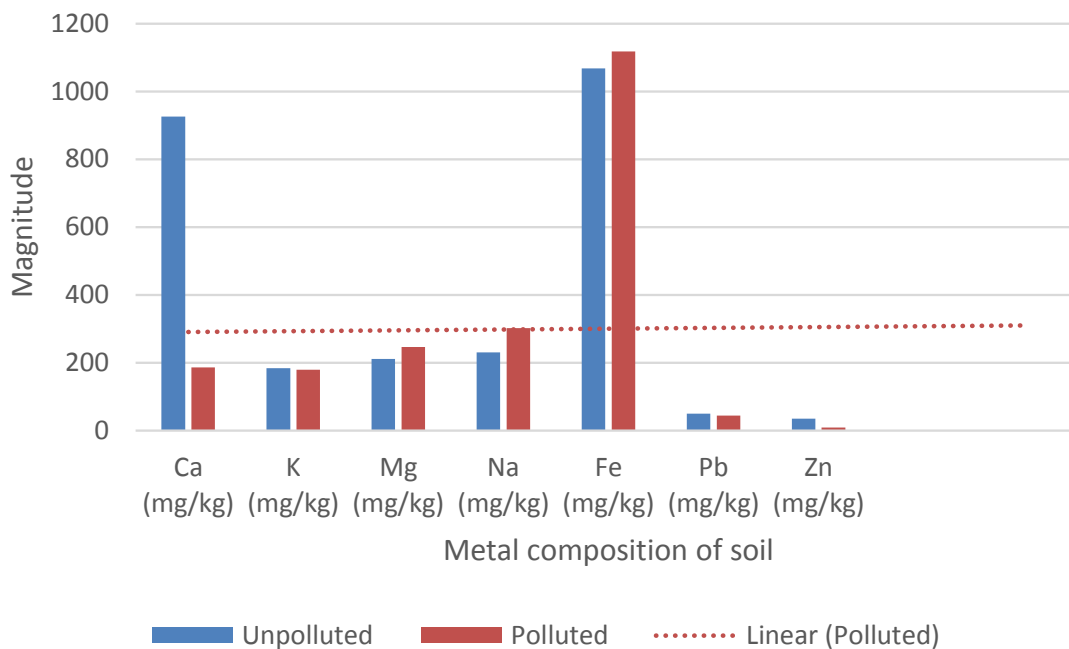


Figure 3. Showing variations of heavy metal composition in soil.

in the polluted and unpolluted are 302.03 mg/kg and a mean differential of 50.4 mg/kg respectively. Lead (Pb⁴⁺) occurred more in the unpolluted site with a mean total concentration of 49.63 mg/kg and a mean differential of

-561 mg/kg respectively. Zinc (Zn⁺²) also, occurred more (34.7 mg/kg) in the unpolluted soil with a mean differential concentration of 26.38 mg/kg (Tables 3 and 4, Figures 4 and 5).

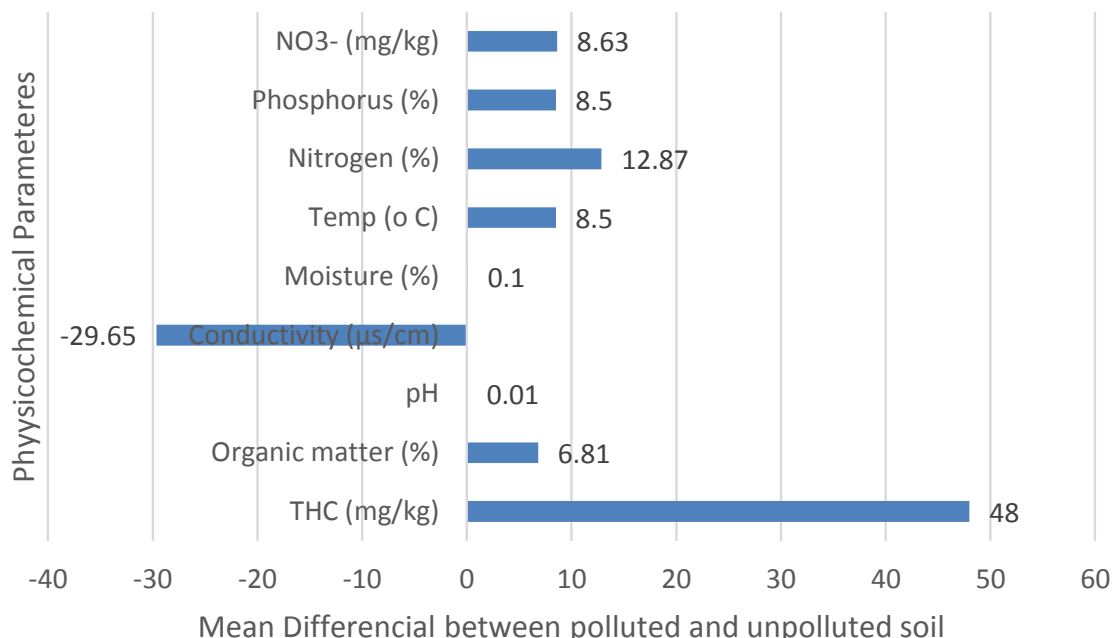


Figure 4. Mean differential between polluted and unpolluted soil in the study.
 *The negative values represent increase in the values of the physicochemical parameters of the unpolluted soil.

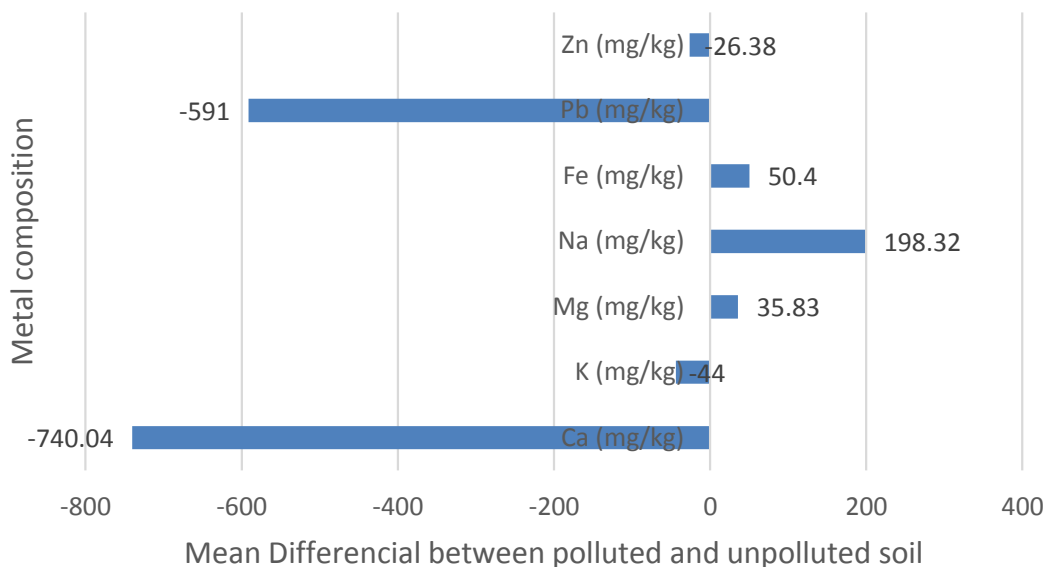


Figure 5. The mean differentials metal composition between polluted and unpolluted soil.
 *The negative values represent increase in the values of the physicochemical parameters of the unpolluted soil.

Vertical variation of soil nematodes species in the study area

Ten genera of soil nematodes; *Helicotylenchus* spp., *Aphelenchus* spp., *Longidorus* spp., *Xiphinema* spp., *Tylenchus* spp., *Hoplolaimus* spp., *Pratylenchus* spp.,

Tylenchorhynchus spp., *Dolichodorus* spp. and *Paratylenchus* spp., belonging to five families; Criconemoidae, Aphelenchoidae, Dolichodoridae, Dorylaimidae, and Tylenchidae were extracted from the soil samples in the study. Out of these, four genera occurred in unpolluted sites (Figure 6) with the exception

Table 3. Metal composition of the soil the soil in the study.

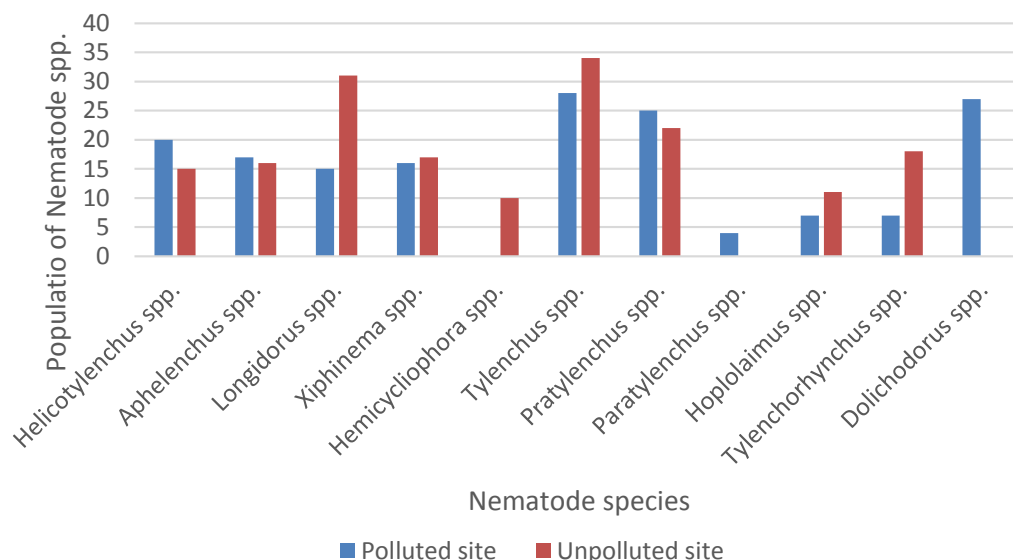
Soil parameter	Unpolluted Site			Mean value	Polluted site			Mean value
	0-5 cm	6-10 cm	11-15 cm		0-5 cm	6-10 cm	11-15 cm	
Ca (mg/kg ¹)	390.35	1283.90	1103.20	925.82	88.35	288.85	180.15	185.78
K (mg/kg ¹)	130.30	197.00	223.40	183.57	156.30	201.05	180.15	179.17
Mg (mg/kg ¹)	142.85	246.30	243.85	210.79	302.55	306.40	130.90	246.62
Na (mg/kg ¹)	152.90	266.70	272.25	230.62	285.60	287.10	333.40	302.03
Fe (mg/kg ¹)	1070.05	1084.85	1047.25	1067.38	1047.25	1283.05	1023.35	1117.78
Pb (mg/kg ¹)	47.80	53.30	47.80	49.63	36.80	47.80	46.55	43.72
Zn (mg/kg ¹)	18.75	26.90	16.00	34.78	14.40	0.75	10.05	8.4

*Global standard FEPA and WHO: pH range- (forest soil 3-5; Humid Climate soil arable soil 5-7 by McCauley et al. (2017)); % Organic Matter (OM) variable, Pb=100.0 mg/kg¹, Zn= 300-400mg/kg¹, Fe= 0- 300 (mg/kg¹), Mn= 2-200 (mg/kg¹), Cu= 70-80 (mg/kg¹), K=12-80 (mg/kg¹) Mg =30-150 (mg/kg¹), Na=2-200 (mg/kg¹).

Table 4. Vertical variation of soil nematodes in the study.

Soil depth in cm Nematode Genera	0-5 cm		6-10 cm		11-15 cm		Total (%)
	*P (%)	*U (%)	*P (%)	*U (%)	*P (%)	*U (%)	
<i>Helicotylenchus</i> spp.	60(30.0)	50(33.3)	60(30.0)	50(33.3)	80(40.0)	50(33.3)	350(10.3)
<i>Aphelenchus</i> spp.	50(29.4)	70(43.6)	20(11.8)	70(43.6)	100(58.8)	20(12.5)	330(9.7)
<i>Longidorus</i> spp.	60(40.0)	80(25.8)	20(13.3)	140(45.2)	70(46.7)	90(29.0)	460(13.5)
<i>Xiphinema</i> spp.	30(18.8)	80(47.1)	50(31.3)	60(35.3)	80(50.0)	30(17.6)	330(9.7)
<i>Hemicycliophora</i> spp.	0	0	0	50(50.0)	0	50(50.0)	100(2.9)
<i>Tylenchus</i> spp.	90(32.1)	70(20.6)	130(46.4)	130(38.2)	60(17.6)	140(41.2)	620(18.2)
<i>Pratylenchus</i> spp.	80(32.0)	20(9.1)	90(36.0)	110(50.0)	80(32.0)	90(50.0)	470(13.8)
<i>Paratylenchus</i> spp.	0	0	40(100)	0	0	0	40(1.2)
<i>Hoplolaimus</i> spp.	20(28.6)	30(42.9)	50(71.4)	30(42.9)	0	50(455)	180(5.3)
<i>Tylenchorhynchus</i> spp.	20(28.6)	40(21.1)	0	70(36.8)	50(71.4)	70(42.1)	250(7.6)
<i>Dolichodorus</i> spp.	100(37.0)	0	100(37.0)	0	70(25.9)	0	270(7.9)
Total (%)	510(30.7)	440(25.3)	560(33.7)	710(40.8)	590(35.5)	590(33.9)	3400

*P= polluted, U= unpolluted.

**Figure 6.** Overall population of soil nematodes in polluted and unpolluted soil.

of *Doclichdorus* spp.; 40 (1.2%) and *Paratylenchus* spp.; 250(7.6), while *Hemicycliophora* spp., was absent in polluted sites. Soil nematodes abundance was more in the unpolluted 165 (48.5%) than in the polluted 175(51.5) sites. *Tylenchus* spp., was the most abundant nematode genus 620 (18.2%) in the study with a prevalence of 28 (45.2%) and 34 (54.8%) nematodes in the polluted and unpolluted sites respectively. The variability in vertical distribution of soil nematodes showed species richness at the 0-5cm core depth at 510 (30.7%) and 440 (25.3%) nematodes for the polluted and unpolluted soils respectively. At the 6-10 cm core depth 560 (33.7%) and 710 (40.8%) soil nematodes were recovered from the polluted and unpolluted soil respectively (Tables 4 and Figure 6).

Trophic characteristics of soil nematodes in the study

Out of the 3400 nematodes extracted from both polluted and unpolluted soil, 27.94% were fungivores and 72.94% herbivores. The herbivores were strict obligates of the roots comprising 9 species of the 11 recovered from all sites. The soil from the polluted environment recorded the highest species diversity (10 species) of soil nematodes, but less abundance 165 (48.5%) while the polluted site recorded species diversity of 9. The non-obligates such as *Aphelenchus* spp., and *Tylenchus* spp., the fungivores occurred in both sites, however, their distribution in the study was arbitrary (Table 5 and Figure 5). The c-p values of the nematodes revealed a heterogeneous population with the dominance of the c-p 3 nematodes, followed by those of the c-p 2 and 5. The maturity indices of both the polluted and unpolluted sites were 2 while the plant parasitic indices was 3 and 3.7 for the polluted and unpolluted sites respectively (Table 4).

DISCUSSION

Total composition of soil nematodes in the study

There was variation in nematode abundance and species diversity in both the polluted and unpolluted sites that was statistically significant ($p > 0.05$). However, higher species diversity (10 species) and low abundance of nematodes was observed in the polluted environment (Table 2). Depth-related occurrence of nematodes varied between the polluted and unpolluted sites ($p < 0.05$) with the 6-10 cm core depth harboring the highest population and the 0-5cm core depth recording the least population in both sites (Figure 5). The high nematode abundance at the 6-10cm core depth may have been due to the attraction of plant parasitic nematodes to the rhizosphere where numerous rootlets release metabolites that attract them through chemotaxis (Imafidor and Nzeako, 2007; Nzeako and Imafidor, 2008; Elele et al., 2017). The specialist ectoparasites of rootlets; *Doclichdorus* Spp.,

and *Paratylenchus* Spp., occurred only in unpolluted sites because of their obligatory habit of herbivory and sensitivity to ambient physicochemistry of their habitats. However, the prevalence of *Pratylenchus* Spp., in the unpolluted site indicated the composition of the vegetation in the sites, since, the Pratylenchoidea are affiliated to the graminacea plants which were numerous in the sampled sites (Goodey and Goodey, 1963; Zhang et al., 2012). The absence of *Hemicycliophora* spp., in the polluted sites was attributed to lack of suitable plant hosts in the impacted environment (Table 5 and Figure 5). The *Tylenchus* spp., adapted to both the polluted and unpolluted environments due to its trophic flexibility. Obligate nematodes dominated all the sampling stations, thus, the high plant parasitic index recorded was occasioned by the continuous tilling of the soil due to the agricultural activities of the indigenes that constantly altered the soil structure. According to Zhang et al. (2012), forest types could be distinguished through a canonical correspondence analysis of nematode genera. Tilling or sub-soiling is an effective way of homogenizing the concentration of materials between the top and inner soil to stimulate or accelerate biological and chemical processes (Barker et al., 1985, Nzeako et al., 2014). In this study, the tilling of the soil aside, the crude oil impact was the greatest anthropological influence that regulated the successional process (Ferris and Matute, 2003; McSorley, 2003; Ibiene et al., 2013). Although, tilling as it relates to this study, was not targeted towards remediation, but, sufficed as one based on the felt-need of the people to sustain subsistence farming for their livelihood.

Trophic characteristics of soil nematodes in the study

The fungivores (27.94%) and herbivores (72.94%) were the dominant nematodes in the study, with the herbivores, being the strict obligates of plants roots, occurring majorly at the 6-10 cm depth. The Obligates are also, plant parasitic nematodes belonging to the c-p 3 and 5 groups that are highly attracted by root metabolites (Imafidor and Nzeako, 2007; Nzeako and Imafidor, 2008; Elele et al., 2017). The fungivores; *Aphelenchus* and *Tylenchus* species occurrence and distribution was arbitrary in both the polluted and unpolluted sites, because the occurrence of both nematode species are induced by environmental factors such as; the presence of fungi and organic enrichment for *Aphelenchus* and *Tylenchus* species respectively (Table 5 and Figure 5). The c-p values of nematodes in the study sites revealed a heterogeneous population with the dominance of the c-p 3 nematodes, followed by those of the c-p 2 (fungivores) and 5 nematodes in that order. The maturity indices (MI) of both the polluted and unpolluted sites was 2, indicating a disturbed habitat (Bongers, 1990; Hennis

Table 5. Showing population structure of soil inhabiting nematodes in all sampling sites.

Nematode genera	c-p value	Feeding type	FG	Site of occurrence	FGD	Abundance (%)
<i>Aphelenchus</i> Spp ^a	2	Fungivores	2	Both	Hyphal feeding, Fungal feeding	330(9.70)
<i>Tylenchus</i> Spp ^c	2	Fungivores/algivores	2	Both	Associates of plant roots, mosses, algae	620 (18.20)
<i>Longidorus</i> Spp ^b	5	Herbivores	1d	Both	Plant feeding	460(13.53)
<i>Tylenchorynchus</i> Spp ^c	3	Herbivores	1d	Both	Migratory ectoparasites of roots	250(7.65)
<i>Hemicycliophora</i> Spp ^d	3	Herbivores	1d	Unpolluted only	Ectoparasites of roots	100(1.80)
<i>Dolichodorus</i> Spp ^b	2	Herbivores	1d	Polluted only	Migratory ectoparasites of roots	270(7.94)
<i>Pratylenchus</i> Spp ^c	3	Herbivores	1b	Both	Migratory endoparasites of roots	470(13.82)
<i>Paratylenchus</i> Spp ^d	2	Herbivores	1d	Polluted only	Ectoparasites of roots	4(1.20)
<i>Hoplolaimus</i> Spp ^c	3	Herbivores	1c	Both	Ectoparasitic or semi-endoparasitic on roots	180(5.30)
<i>Xiphinema</i> Spp ^b	5	Herbivores	1d	Both	Plant feeding	330 (9.71)
<i>Helicotylenchus</i> Spp ^c	3	Herbivores	1c	Both	Ectoparasitic or semi-endoparasitic on roots	350 (10.3)
Total (%)						3400(100)

Family grouping: a = Aphelenchoididae, b = Dorylaimoidea, c = Tylenchoidea, d = Criconematoidea, FG: Feeding Group (Source: Bongers and Bongers, 1998; Yeates et al., 1993), FGD: Feeding Group Description (Source: Bongers and Bongers, 1998; Yeates et al., 1993).

and Bongers, 2009). Plant parasitic index (PPI) ratings of 3 and 3.7 was determined for the polluted and unpolluted sites respectively, which showed that the sites were continuously cultivated upon (Bongers, 1990; Bongers and Korthals 1995; Balsamo et al., 2012; Nzeako et al., 2019). This result varied with a previous study by Nzeako et al. (2011) where very few population of persisters and plant parasitic nematodes were discovered in a survey of the same study area. This study agrees with the report of Ferris and Bongers (2006), who stated that nematodes are clear indicators of organic enrichment thereby, are reliable bioindicators of organic influx in both the aquatic and terrestrial environments. The absence of *Rhadinis terrestris*; a short generation life nematode in c-p 1 group indicated poor organic enrichment in the study sites (Bongers, 1990; Bongers et al., 1995; Zheng et al., 2012; Moura and Franzener, 2017; Nzeako et al., 2019). Nematode community sensitivity in the presence of stress factors in the soil could play a major role in determining the health of the soil ecosystem (Moreno et al., 2011). Concentration of soil nematodes at the polluted sites revealed the ability of nematodes to tolerate harsh environments conditions. This might be due to the reduction in the concentration of oil contamination over the years. Bongers et al. (1997) and Urkmez et al. (2014) in their works showed that ecosystems with relatively high MI value of 2 usually show good ecosystem quality that tended towards stability through strategic successional processes.

Variation in physicochemical composition of polluted and unpolluted Soil

The physicochemical characteristics of the soil varied

significantly ($p < 0.05$) between the polluted and unpolluted sites. The total hydrocarbon content (THC) of the polluted sites decreased with increase in depth, due to poor solubility of the crude oil. Clogging of the surface soil by crude oil in the polluted sites hindered aeration, drainage, porosity and solubility of salts (Osuji et al., 2004; Oyem and Oyem, 2013; Rashid and Tanveer, 2016). The hydrology, topography and precipitation characteristics of the study area had a great impact on the distribution of the biotic and abiotic constituents of the habitat, irrespective of the pollution by crude oil (Th. Abdel-Moghny et al., 2012). This is due to the overlap in the concentrations of cations and anions ($p > 0.05$) in the sampled sites (Tables 2-3, Figures 2-6). The pH value was more acidic in the unpolluted sites which is in line with Zhang et al. (2012) and Otaiku (2019) who attributed soil acidity to high solubility and electrical conductivity, due to the availability of cations and anions (Table 5). Moisture content was more in polluted sites than the unpolluted which agrees with Klamerus-Iwan et al. (2015) who stated that capillary water capacity of the soil increases with increase in oil contamination. The moisture difference in this study was attributed to reduced drainage, porosity and aeration capacity of the soil occasioned by oil clogging especially at the polluted sites (Osuji et al., 2004; Kuyukina et al., 2005; Khamchian et al., 2007; Abosede, 2013; Oyem and Oyem, 2013; Rashid and Tanveer, 2016), High precipitation in the study area influenced its hydrological characteristics, causing aggressive leaching, speedy run offs and inflow of nutrients from higher contours to the natural drainage systems. This ecological idiosyncrasy, such as; elevation and hydrology in the study area resulted to the overlap in the values of the physicochemical parameters as earlier stated. This

assertion is in line with Zhang et al. (2012) who stated that soil nematode species richness is influenced by elevation amongst other physical attributes. It was clear that the two sites experienced relatively similar supply of water-soluble nutrients that are beneficial to soil nematodes (Murphy and Riley, 1962; Keith et al., 2009) which accounted for the near similarity observed in the distribution of the physicochemical parameters in the study (Shrivastava, 1996; Walls-Thumma, 2000). The polluted sites had more nitrogen concentration which suggested a slow conversion of nitrogen to nitrate compounds naturally (Boag and Yeates, 1998; Sohlenius, 1979; Khatoun et al., 2001).

Metal composition of polluted and unpolluted soil in the study

There was variability in the concentration of essential metals such as calcium (Ca), sodium (Na), iron (Fe), potassium (K) and magnesium (Mg), in the unpolluted and polluted sites, but Zinc (Zn) and Lead (Pb) occurred more in the unpolluted soils (Nwaichi et al., 2014). The relatively higher concentration of macro-elements that characterize enriched soils, confirmed the existence of enrichment interventions at specific intervals within the ten years of pollution which was primarily occasioned by farming activities (anthropological interference). The polluted sites may have suffered poor cation exchange capacity (Abii and Nwosu, 2009) due to moisture related issues. The concentration of Lead (Pb^{4+}) was relatively high and similar in both sites and this similarity, was attributed to flooding or run-off effects that distributed soluble fractions of crude oil from impacted sites to unpolluted sites. This phenomenon also, influenced the distribution of Zinc (Zn) which was more concentrated in the unpolluted sites confirming the role of ecological factors such flooding in the distribution of pollutants in the study area (Mohammed and Folorusho, 2015).

Conclusion

The study area has undergone series of natural and man-made remediation interventions that inadvertently influenced the successional pattern. The successional pattern influenced biodiversity which supported the occurrence of resilient basal floral and r-strategist nematodes in the MI 2 group that depict interference. However, the dominance of the obligate plant parasites may have been anthropologically induced by intensive farming by the indigenes and did not represent a stable environment as suggested by the presence of few nematodes in the MI 5 group. The overlap in physicochemical parameters of the actual spill sites and the none-impacted lands showed that ecological factors such as precipitation, flooding, porosity, leaching and topography greatly influenced the distribution of soluble

factors in the environment. There was no strict demarcation between the polluted and unpolluted areas in the study, suggesting that the environment is generally polluted even in the absence of oil spillage. However, the maturity index and c-p values of the resident soil nematode indicated that successional processes are ameliorating the effects of oil pollution in the area, but, is greatly hampered by the modifications in the endemic biological agents (flora and fauna) through farming processes. This study indicates that the environment has the capacity to rebuild itself but requires augmentation of inherent biological agents to facilitate the process.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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