

# Oil Pollution Effects on Nematodes in Mangrove Sediment: A Microcosm Study

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## Research Article

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# Abstract

The effects of oil pollution on meiobenthic nematode assemblages in a mangrove sediment were investigated. Microcosms comprised 350 ml plastic jars that were filled with 200 g mangrove sediment and subjected to oiling, with or without addition of fertiliser. In the oiled treatments, 15 ml of Bunker fuel oil 180 and 5 ml/L fertiliser (N: P: K: 3: 2: 5) were added to the soil. After four weeks, nematodes were extracted and identified. In the unfertilised oiled treatment, nematode abundance and species richness were significantly reduced by 87% and 53%, respectively, compared to the control. In the fertilised oiled treatment, nematode abundance and species richness increased by 56% and 30% respectively. The eight taxa present in the control but absent in the oiled treatments (*Monhystera*, *Prodesmodora*, *Plectus*, *Rhabditis*, *Koerneria*, *Rotylenchus*, *Tobrilus*, and *Fictor*) were characterised as oil-intolerant. The seven taxa present in the oiled treatments (*Monhystera*, *Ethmolaimus*, *Panagrolaimus*, *Camacolaimus*, *Hemicycliophora typica*, and *H. ripa* and a species of the family Xyalidae) were characterised as oil-tolerant and resilient. In all treatments, the dominant species was *Ethmolaimus*. Taxa such as *Rhabditis*, *Koerneria* and *Rotylenchus* survived oiling, due to the addition of fertiliser. Fertilizer amendment favoured survival of *Rhabditis*, *Koerneria* and *Rotylenchus* and increased reproduction in *Camacolaimus*.

## Introduction

Mangroves, which occur between marine and terrestrial environments in tropical and subtropical areas, provide many ecosystem services. These include wave attenuation, stabilizing coastlines, carbon sequestration and nurseries for a wide variety of fauna (Friess et al. 2016; Barbier 2016). Mangroves are highly vulnerable to oil pollution because they occur close to industrial and urban areas (Lewis et al. 2011). Oil pollution of mangroves occurs by spills from ships, leakage from refineries, offshore production and improper disposal of used motor and lubricating oil. Polycyclic aromatic hydrocarbons (PAHs), which are the toxic components of oil, are highly persistent organic pollutants. Oil polluted mangrove habitats exhibit low biodiversity, genetic abnormalities and reduced ecosystem function (Veldkornet et al. 2020).

The effects of oil in marine environments are determined by monitoring changes in the community structure of macro- and meiofauna (Wei et al. 2012). Soil benthic meiofauna are an important link in the food web as they are an important food source for juvenile fish that use mangrove habitats as nurseries. Meiofauna, such as nematodes, are involved in sediment bioturbation and recycling of organic matter (Lindgren et al. 2012; Pinto et al. 2013). Benthic meiofauna pass through a 500–1000 µm mesh and are retained on a 44–63 µm mesh (Gyedu-Arabis and Baird 2006; Giere 2009). Nematodes are a widespread and taxonomically diverse group of organisms that are highly sensitive to anthropogenic disturbances such as oil spills, radiation leakage and suspended solids. They respond rapidly to oil spills, radiation leakage, large amounts of total suspended solids and are therefore important bioindicators (Beyrem et al. 2010; Wei et al. 2012). Moreover, nematodes are small, abundant, ubiquitous, reproduce continuously, have rapid turnover and high density and are sediment bound throughout their life history. In addition, they are easy to culture and suitable for microcosm studies. (Suderman and Thistle 2003; Gyedu-Arabis

and Baird 2006). Oil pollution effects on meiofauna, however, are dependent on the type and quantity of oil, the bioavailability of PAHs, the type of sediment and on the nematode species (Mahmoudi et al. 2005).

In the natural environment, degradation of spilled oil by soil microorganisms is dependent on levels of biologically available nitrogen and phosphorus. The addition of fertilisers can increase the rate of oil degradation by three to five-fold (Swannell et al. 1996). The effects of petroleum hydrocarbons on benthic fauna have been extensively studied following real oil spills, in field experiments and in mesocosms (Carman et al., 2000; Mahmoudi et al. 2005).

Nematodes are important biological indicators of anthropogenic disturbance and provide critical information on the ecological status of estuaries and aquatic ecosystems. Although several studies have examined the effects of oil pollution on macrofauna, little is known about meiofauna. Lack of knowledge on the benthic meiofauna of mangroves is common worldwide. There is a paucity of data on free-living nematodes in estuarine environments, including mangroves, specifically on the African continent. Despite the importance and diversity of microhabitats in mangrove, nematode studies generally focus on soft sediments and decaying leaves.

In this study, we determined the effects of oil pollution on meiobenthic nematode assemblages in a mangrove soil. Here, we present the results of a microcosm study designed to investigate the effects of oil, with or without fertiliser amendments, on the composition, abundance, richness, and diversity of meiobenthic nematode assemblages in a mangrove sediment. We tested the null hypotheses that there would be no differences in nematode assemblages between control and oil contaminated mangrove sediment in laboratory microcosms.

## Materials And Methods

### Description of study site.

Mangrove sediment was collected from the Isipingo Estuary (29° 59' S, 30° 56' E), Durban, South Africa. The estuary covers an area of approximately 3.8 ha and has mixed *Avicennia marina* (Forsk.) Vierh., *Rhizophora mucronata* Lam. and *Bruguiera gymnorhiza* (L.) Lam. mangroves. In this site, the three species co-occur under similar environmental conditions of salinity, tidal cycle, and substrate matrix. These species are taxonomically different and possess different salinity adaptations. *Bruguiera gymnorhiza* and *R. mucronata* are salt excluders, whereas *A. marina* possesses leaf salt glands and is a salt secretor. The species also have different tolerance levels to oil contamination (Naidoo et al. 2010; Ke et al. 2011). The mean annual temperature at this site is 20°C, mean annual rainfall 1027mm and maximal tidal range is 2.17m. Soil characteristics of the mangrove sediment are indicated in Table 1.

### Experimental design

Mangrove sediment containing natural nematode assemblages were collected at a depth of 15 cm with standard meiofauna cores with an inner diameter of 3.6 cm. The cores were placed in a thermostatic chamber and transported to an air-conditioned glasshouse. Microcosms consisted of 350 ml plastic jars, sterilized with ethanol and washed with double distilled water. Each microcosm was considered as an independent experimental unit. Natural mangrove sediment (250 g) was placed into each microcosm. In the experiment, there were three treatments, each with four replications: control (C): microcosms contained mangrove sediment; oiled (O): microcosms containing mangrove sediment were contaminated with 15 ml of Bunker fuel oil 180; Oiled +fertilizer (O+F): Five ml of a commercial liquid fertiliser (Biogrow Chemicals, 2007) were added to microcosms containing mangrove sediment which was contaminated with 15 ml of Bunker fuel oil 180. In the oil contaminated treatments, oil was added onto the soil surface.

The properties of the Bunker fuel oil used in this study were presented previously (Naidoo et al. 2010). The oil completely infiltrated the soil volume within one hour. In the fertilised treatments, an organic liquid fertiliser (Biotrissol, Germany, Table1) was added. The fertiliser (5 ml) was diluted with double distilled water (1 L) and the mixture sprayed onto the sediment surface weekly. All treatments were kept moist by spraying seawater (10 PSU) daily onto the soil surface. Microcosms were unsealed throughout the duration of the experiment to allow for ventilation. Each microcosm was run as an open system in an air-conditioned glasshouse at 25 °C (day), 18 °C (night) and 60% humidity for four weeks. The resulting samples were then sent to the nematode extraction laboratory at the ARC-Plant Health and Protection (ARC-PHP) and stored in a cold room at 10°C until processing.

### **Extraction of nematodes**

Nematodes were extracted from 250g soil by the sieving centrifugal flotation method (Swart and Marais 2017). The soil was washed through a 2mm sieve into a 5-liter bucket. The suspension was stirred, allowed to settle for 30 seconds and then poured through a 45 µm sieve. This procedure was repeated another two times for 20 and 10 seconds respectively. The residue was then transferred from the sieve to two centrifuge tubes. After the addition of kaolin, the suspension was centrifuged for 7 minutes at 3500 rpm. The supernatant was discarded. After the addition of a sugar solution (450 g sugar / L water) to the tubes, the contents were mixed and centrifuged for 3 minutes at 3500 rpm. The suspensions were passed through a 45 µm sieve and collected in a beaker. Nematodes were transferred to a De Grisse counting dish and, using a stereomicroscope (60x magnification), identified to genus level at the Nematology Unit, ARC-Plant Health and Protection (ARC-PHP). The genera were assigned to trophic or feeding levels by using the classification of Yeates et al. (1993). The number of nematodes in each genus was counted with a Laboratory DC counter.

### **Preparation of nematode slides**

After collection in a small volume of water in a Syracuse dish, the nematodes were fixed in hot FPG (100 ml of 40 % formalin, 10 ml propionic acid, 890 ml distilled water). The fixative was drawn into a pipette and decanted into a test tube. The test tube was placed in boiling water until the temperature

reached about 60 °C. The hot fixative was then poured onto the live nematodes within the Syracuse dish. The dish containing the nematodes, was placed in a small Petri dish, closed and placed in a desiccator. The desiccator was placed in an incubator for three days at 38-40 °C. Solution 1 (200 ml of 95 % alcohol, 10 ml glycerol, and 790 ml distilled water) was added after half the fixative was drawn off. The dishes were left open and placed in a desiccator for 12 hours. Solution 2 (950 ml of 95% alcohol and 50 ml glycerol) was added to the nematodes after half of Solution 1 was drawn off with a pipette. The dish with nematodes was partly covered to allow for slow evaporation and returned to the incubator until all the alcohol evaporated. The dish was then placed in a desiccator for dehydration at room temperature for 24 hours.

The fixed specimens were mounted on Cobb slides (Swart and Marais 2017). Firstly, a wax ring was made in the centre of a square coverslip. Then, a drop of glycerol was placed in the centre of the wax ring. Four to five fixed nematodes were placed in the glycerol drop and gently pressed down onto the surface of the square coverslip. A coverslip (15 µm thick, 19 mm round) was heated over a flame and placed onto the glycerol drop. The coverslips were placed on an electro-thermal slide drying bench to dissolve the wax and seal the coverslips. The sealed coverslips were placed in the aluminium slide. Then, carton squares were placed on each side of the coverslip and the aluminum bent to form a finished Cobb slide (Swart and Marais 2017).

## **Nematode identification**

Identification of nematodes to family, genus and species was undertaken with a compound oil immersion microscope (Zeiss Axioskop 40 equipped with a drawing tube) using various taxonomical publications and keys (Goodey 1963; Heyns 1971; Van den Berg 1987; Andrassy 2005) Nematodes were identified to the lowest taxonomic level possible, with most specimens identified to genus and some to putative species. Identification to genus level has been shown to be adequate for detection of ecological patterns. Juvenile specimens of plant parasitic nematodes that could not be identified to species level were recorded to genus level only. Specimens that proved difficult to identify were processed to permanent slides and deposited in the National Collection of Nematodes (NCN), ARC-PHP, Pretoria. These specimens were later identified by nematode taxonomists.

## **Data analyses**

Means and standard errors were calculated for all measurements. Resulting data were tested for normality using the Kolmogorov-Smirnov test and subjected to two-way ANOVA and Tukey's multiple comparisons test ( $P \leq 0.05$ ) using GraphPad Prism Version 6.05 (GraphPad Software, Inc., USA). Other data were subjected to one-way ANOVA and Tukey-Kramer multiple comparisons test ( $P \leq 0.05$ ) using MINITAB version 16 (Minitab Statistical Software, MINITAB Inc., USA).

# **Results**

Nematodes in South African mangrove sediments have not been investigated previously, so most taxa were identified to family and genus. Taxa were ranked based on their abundance in each treatment (Table 3) from 1 (highest dominance) to 5 (lowest dominance). Of the 15 taxa present in the control, *Ethmolaimus* sp. exhibited the highest dominance (Fig.4).

### Effects of oil on nematodes

Nematode abundance (Fig.1) and species richness (Fig. 2) were highest in the C and significantly lower in the O treatment by 87% and 53%, respectively. Species that were present in the O treatment were *Monhystera* sp., *Ethmolaimus* sp., *Panagrolaimus* sp., *Camacolaimus* sp., *Hemicycliophora typica*, and *H. ripa* and a species of the family Xyalidae (Fig. 2). In the O treatment, *Monhystera* sp. (13%), *Ethmolaimus* sp. (40%) and *Camacolaimus* sp. (40%) exhibited the highest abundance. In the O treatment, *Hemicycliophora typica*, *Hemicycliophora ripa*, *Panagrolaimus* sp., and species from the family Xyalidae had an abundance of 2 to 5% (Fig.1). The seven species present in the O treatment were characterised as oil-resistant and resilient. In the O treatment, *Ethmolaimus* and *Monhystera* decreased in abundance by 33% and 58%, respectively, compared to the control (Fig. 1). In the O treatment, abundance of *Camacolaimus* sp. increased by 79%, compared to the C treatment. These were characterised as opportunistic species (Fig. 1). There were eight genera present in the C but absent in the O treatment (Fig. 1). These genera (*Rhabditis*, *Monhystera*, *Prodesmodora*, *Plectus*, *Tobrilus*, *Koerneria*, *Fictor*, and *Rotylenchus*) were characterised as oil-intolerant.

### Effects of oil on adult and juvenile nematodes

In the O treatment, adults of five species were identified (Fig.2). These five species included *Ethmolaimus* sp. (50%), *Camacolaimus* sp. (36%), *Monhystera* sp. (7%), *Hemicycliophora ripa* (4%), and family Xyalidae (4%). Juvenile abundance of *Monhystera* sp. was highest in the C and significantly lower in the O treatment by 50% (Fig. 4). In the O treatment, *Camacolaimus* sp. exhibited the highest juvenile abundance (46%), while juveniles were absent in the control. In the O treatment, the juvenile abundance of *Ethmolaimus* sp. was 63% lower than that in the control (Fig. 4).

### Effects of oil with added fertiliser on nematodes

Nematode abundance (Fig. 1) and species richness (Fig. 2) were highest in the C and significantly lower in the O+F treatment by 69% and 33% respectively. Oil and fertiliser in combination increased nematode abundance and species richness by 56% and 30%, respectively, compared to the O treatment. In the O+F treatment, *Ethmolaimus* sp. (51%) and *Camacolaimus* sp. (27%), exhibited the highest abundance (Fig.1). In the O+F treatment, *Monhystera* sp., *Hemicycliophora typica*, *Hemicycliophora ripa*, *Rhabditis* sp., *Panagrolaimus* sp., *Desmodora* sp., *Rotylenchus* sp. and *Koerneria* sp. had low abundance (1 to 6%). Abundance of *Ethmolaimus* sp. and *Camacolaimus* sp. increased in the O+F treatment by 37% and 83% respectively, compared to the C treatment (Fig.1). The genera *Rhabditis*, *Koerneria* and *Rotylenchus* were present in the O+F treatment, but absent in the O and C treatments. These three genera were characterised as oil-sensitive (Fig.1). *Desmodora* was absent in the C and O treatments but present in the

O+F treatment. The following five genera, *Monhystera*, *Prodesmodora*, *Plectus*, *Tobrilus* and *Fictor* (Fig. 2) were present in the C but absent in both the O and O+F treatments and characterised as oil-intolerant.

### **Effects of oil with added fertiliser on juveniles and adults**

The highest juvenile abundance in the C treatment was exhibited by *Monhystera*, while in the O+F treatment, juvenile abundance of this genera was 60% lower (Fig. 4). *Camacolaimus* sp. was absent in the C but exhibited the highest juvenile abundance in the O+F treatment (25%). In the O+F treatment, adults of six species were present (Fig.1). These were *Ethmolaimus* sp. (57%), *Camacolaimus* sp. (32%), *Monhystera* sp. (4%), *Koerneria* sp. (4%), *Desmodora* sp. (2%) and *Hemicycliophora ripa* (2%). In the O+F treatment, adult abundance of *Ethmolaimus* sp. and *Camacolaimus* sp. increased by 44% and 75% respectively, compared to the C treatment (Fig.3). In the O+F treatment, juveniles of the genera *Rhabditis* (8%) and *Rotylenchus* (8%) were present, but absent in the O treatment (Fig. 4). There were no significant differences in juvenile abundance of *Ethmolaimus* between the treatments. Juveniles of species from the family Xyalidae and genera *Desmodora* and *Koerneria* were absent in all treatments (Fig. 4).

## **Discussion**

Nematodes have been used widely to evaluate soil conditions in agriculture and as long-term indicators of pollution and climate change. The nematode assemblage-level approach to the detection and monitoring of oil pollution effects has been validated in experimental microcosms (Beyrem et al. 2010) and in field studies (Lindgren et al. 2012). In our study, Bunker fuel oil decreased abundance, richness and number of species of natural populations of nematodes from a mangrove ecosystem. Several other studies also reported that oil decreases nematode abundance and species richness, alters community composition and reduces biodiversity (Lv et al. 2011; Beyrem et al. 2010). Little information is available on nematodes in the upper-littoral zone where mangroves occur. Upper-littoral zones are rarely sampled, so that composition of species that occur is largely unknown. Furthermore, estuarine nematodes are dispersion-limited, so that their occurrence is primarily influenced by local and regional environmental conditions (Brustolin et al. 2018).

Oil contamination may increase, decrease or eliminate meiofaunal communities (Mahmoudi et al. 2005; Beyrem et al. 2007; Kang et al. 2016). Most studies reported a reduction in the population of meiofauna immediately following an oil spill. In other studies, benthic communities responded positively and showed no oil-induced mortality (Kang et al. 2014). Factors that could contribute to differential effects of oil on nematode communities include type of oil and dosage, morphological and physiological differences of the nematode taxa and soil conditions (Mahmoudi et al. 2005; Thompson et al. 2007). Oil induced changes alter nematode community composition and reduce biodiversity (Beyrem et al. 2010). In addition, some nematode communities that occur in oil contaminated environments tend to become more oil tolerant over time (Carman et al. 2000).

### **Oiling and nematode community structure**

Nematode assemblages in marine sediments are generally resistant to environmental changes. In our study, oiling decreased nematode abundance and species richness significantly. Nematodes are particularly sensitive to PAHs because of their intimate association with soil particles (Lindgren et al. 2012). Nematodes possess permeable cuticles and lack a protective calcium or silicate casing making them vulnerable to oil penetration (Stringer et al. 2012). Other meiofauna, such as copepods, are more resistant as they possess protective casings. PAHs, which are non-polar and lipophilic, diffuse passively across exposed nematode membranes (Gauthier et al. 2014). Within the cells, PAHs increase expansion and fluidity of nematode membranes thereby disrupting membrane integrity, cellular function and causing mortality. PAH disruption of cell membranes leads to breakdown of ion-selective barriers, leading to loss of water and salt regulation (Stringer et al. 2012). PAH-induced damage to membranes also contributes to greater sensitivity of nematodes to soil salinity. Furthermore, nitrogenous wastes, which are usually excreted by diffusion through the outer membrane, tend to accumulate in damaged cells, contributing to waste build up and toxicity. For example, benzene increased membrane fluidity in the bacterium, *Rhodococcus* sp. (Gutierrez et al. 1999). In the mussel, *Mytilus edulis*, benzene disrupted ion transport and decreased calcium concentrations (Borseth et al. 1995). Furthermore, PAHs which are cytotoxic, cause intracellular damage by preventing the synthesis of proteins that are important for cellular repair and survival (Liuzzi et al. 2012).

In our study, four species of nematodes decreased with oiling (*Hemicycliophora ripa*, *Panagrolaimus* sp., *Camacolaimus* sp. and a species of the Xyalidae). In several other studies, nematode abundance and species richness also decreased when sediment was contaminated with diesel (Lindgren et al. 2012), crude oil (Lv et al. 2011) and lubricating oil (Beyrem et al. 2010). In the sediment, PAHs decrease dissolved oxygen concentrations leading to oxygen stress in nematodes (Carman et al. 2000; Neira et al. 2001). In low oxygen environments, nematode activities such as sediment bioturbation and recycling of organic matter are decreased (Neira et al. 2001; Sundbäck et al. 2010). Other studies showed that addition of the PAH, pyrene, to sediment decreased grazing activity of nematodes (Sundbäck et al. 2010). Reduced grazing activity decreases sediment bioturbation leading to increased soil anoxia (Lindgren et al. 2012). Soil anoxia, induced by oiling, probably contributed to the decrease in abundance and species richness in our study. Oil contamination of the sediment therefore altered nematode community structure, reduced competition and favoured resilient species.

Oil has been shown to enhance the retention of toxins such as heavy metals (Millward et al. 2004). Combination of heavy metals and PAHs therefore further reduce nematode assemblages (Beyrem et al. 2007). In addition, PAHs promote the growth of microalgae which produce mucous exo-polymers. The latter adsorb heavy metals that are present in the sediment, and when consumed, result in greater toxicity to nematodes (Carman et al. 2000). Nematode species vary in their sensitivity to PAHs at sub-lethal concentrations (Carman et al. 2000; Millward et al. 2004). Species that are sensitive decrease with oil contamination (Sundbäck et al. 2010; Lindgren et al. 2012). The elimination of sensitive species by oil was reported in several studies (Mahmoudi et al. 2005; Beyrem et al. 2010; Lv et al. 2011). PAHs are neurotoxic and adversely affect the nervous system of nematodes (Meyer and Williams 2014). PAHs

interfere with locomotion, feeding behaviour, brood size, growth, life span and ultimately lead to mortality (Meyer and Williams 2014).

In this study, *Ethmolaimus* sp. and *Camacolaimus* sp. were dominant and resilient to oiling. *Camacolaimus* sp. increased in the O treatment by 78% compared to the C treatment and were characterised as opportunistic (Millward et al. 2004; Mahmoudi et al. 2005). Oiling increased robust and opportunistic species, enabling them to become dominant. Oiling reduced nematode competition and favoured resistant species. In this study, oiling changed the abundance of dominant species and altered nematode community structure.

In the O treatment, *Camacolaimus* sp. exhibited the highest juvenile abundance, indicating that this species is highly tolerant and resilient to oil contamination (Mahmoudi et al. 2005). In contrast, juvenile abundance of *Monhystera* sp. was highest in the C treatment and decreased significantly in the O treatment suggesting that reduction in reproductive capacity affects community structure by changing species dominance (Beyrem et al. 2010). In terrestrial and marine environments, hydrocarbon degrading microorganisms that are capable of metabolizing oil compounds are ubiquitous (ITOPF 2014). In this study, it was assumed that these microorganisms increased in the sediment of oil treated microcosms. Degradation of PAHs occurs at different rates and is dependent on the structure of the PAH and the species of microorganisms.

### **Oiling with fertiliser amendment**

The addition of fertilisers to oil contaminated sediment is effective in bioremediation. Fertilisers provide sources of energy for PAH degrading microorganisms. Nutrient addition accelerates the degradation process in mangrove sediments (Xu et al. 2005). Generally, the addition of fertiliser to oiled sediments increases nematode abundance and species richness significantly (Eisentraeger et al. 2002). In our study, three genera (*Rhabditis*, *Koerneria* and *Rotylenchus*), which were absent in the O, but present in the O+F and C treatments, were characterised as oil-sensitive. Survival of these species was attributed to the addition of fertiliser. The addition of fertiliser also increased nematode abundance of *Ethmolaimus* sp. and *Camacolaimus* sp. Furthermore, the addition of fertiliser increased reproduction in *Camacolaimus* sp. In our study, addition of fertilizer increased the abundance of dominant and opportunistic species. Fertiliser additions also increased oil decomposition rate of microorganisms by stimulating bio-degrading bacterial activity, which in turn elevates N and P concentrations in the interstitial water (Xu et al. 2005).

Juveniles of the genera, *Rhabditis* and *Rotylenchus* were absent in the O but present in the O+F treatment, suggesting that reproduction was stimulated by fertiliser addition. In the O-F treatment, *Camacolaimus* sp. exhibited the highest juvenile abundance which suggests opportunistic behaviour. In the O+F treatment, there were no differences in juvenile abundance of five species. Similar results were reported for juvenile abundance of nematodes in fertilised, oiled treatments in field mesocosms (Schratzberger et al. 2003). Lubricating oil decreased the abundance of dominant nematodes in oiled treatments resulting in decreased competition (Beyrem et al. 2010). In the O+F treatment, the decrease in

juvenile abundance of *Ethmolaimus* sp. contributed to the increase in oil-sensitive species through reduced competition.

The food sources of nematodes, which include bacteria and microalgae, are negatively affected by oil contamination. The addition of fertiliser to oiled sediment increases resilient microalgae and bacteria, which provide a food source for oil-resistant nematodes (Sundbäck et al. 2010). In this study, most nematode taxa were bacterivores, but plant parasites and one predator (food consists of algae, diatoms, and small animal such as ciliates and rotifers) were also encountered. Other studies demonstrated that bacterivorous nematodes proliferated in soils contaminated with PAHs probably due to increased microbial activity and availability of food (Chen et al. 2009). Long-term studies showed that burrowing and bioturbating amphipods increased the availability of microalgae which in turn increased densities of nematodes, copepods and juvenile polychaetes (Fleeger et al. 2021). *Desmodora* sp. was present in the O+F but absent in the O and C treatments, suggesting that this species is tolerant to oiling if fertiliser is present. Although microcosms of natural nematode assemblages do not mimic natural conditions, the observed responses provide insight into the complex effects of oil contamination on natural meiofaunal communities. Moreover, toxicity of PAHs depends on their partitioning between the sediment, pore water, overlying water and sediment carbon.

## Conclusions

This study is the first to provide information on the identity of nematodes in a mangrove sediment in Africa. Most taxa were identified to family and genus because of the paucity of information on nematodes in mangrove ecosystems. In our study, Bunker fuel oil decreased abundance, richness and number of species of natural populations of nematodes. Of the nematode taxa identified in this study, seven were oil-resistant (*Monhystera*, *Ethmolaimus*, *Panagrolaimus*, *Camacolaimus*, *Hemicycliophora typica*, and *H. ripa* and a species of the family Xyalidae). The eight oil-intolerant taxa included *Rhabditis*, *Monhystera*, *Prodesmodora*, *Plectus*, *Tobrilus*, *Koerneria*, *Fictor*, and *Rotylenchus*. Oiling eliminated species that were oil-intolerant and favoured those that were resistant and resilient, thereby altering free-living nematode community structure. The addition of fertiliser to oiled sediments increased nematode abundance and species richness significantly. In our study, three genera (*Rhabditis*, *Koerneria* and *Rotylenchus*), were absent in the O, but present in the O + F and C treatments and characterised as oil-sensitive. Altered species composition and abundance of nematodes, induced by oil, could have repercussions on other meiofaunal communities and ultimately ecosystems.

The recovery of communities after an oil spill is generally slow. Differences in the response of nematodes may be attributed to the tolerance of some nematodes to pollutants. Most studies, however, suggest that nematodes are more resilient than other meiofaunal taxa. Although the abundance and diversity of nematodes decrease immediately after oil contamination, recovery is rapid in comparison to other meiofauna. Additional studies are required to determine the relative sensitivity of other components of the meiobenthos. Our study suggests that the nematode assemblage approach is a convenient and sensitive measure of estuarine pollution

# Declarations

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**Availability of data and materials** The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

**Author contribution** GN and KN contributed equally to the experimental design, data collection, interpretation and discussion of the results and writing of the manuscript. AS assisted in the identification of the nematodes and editing the manuscript.

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**Competing interest** The authors declare no competing interests

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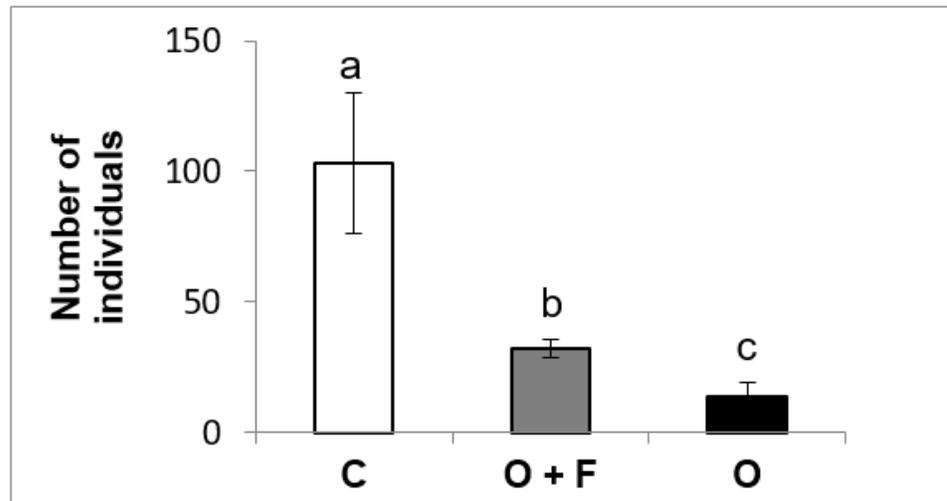
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## Tables

Due to technical limitations, table 1-3 is only available as a download in the Supplemental Files section.

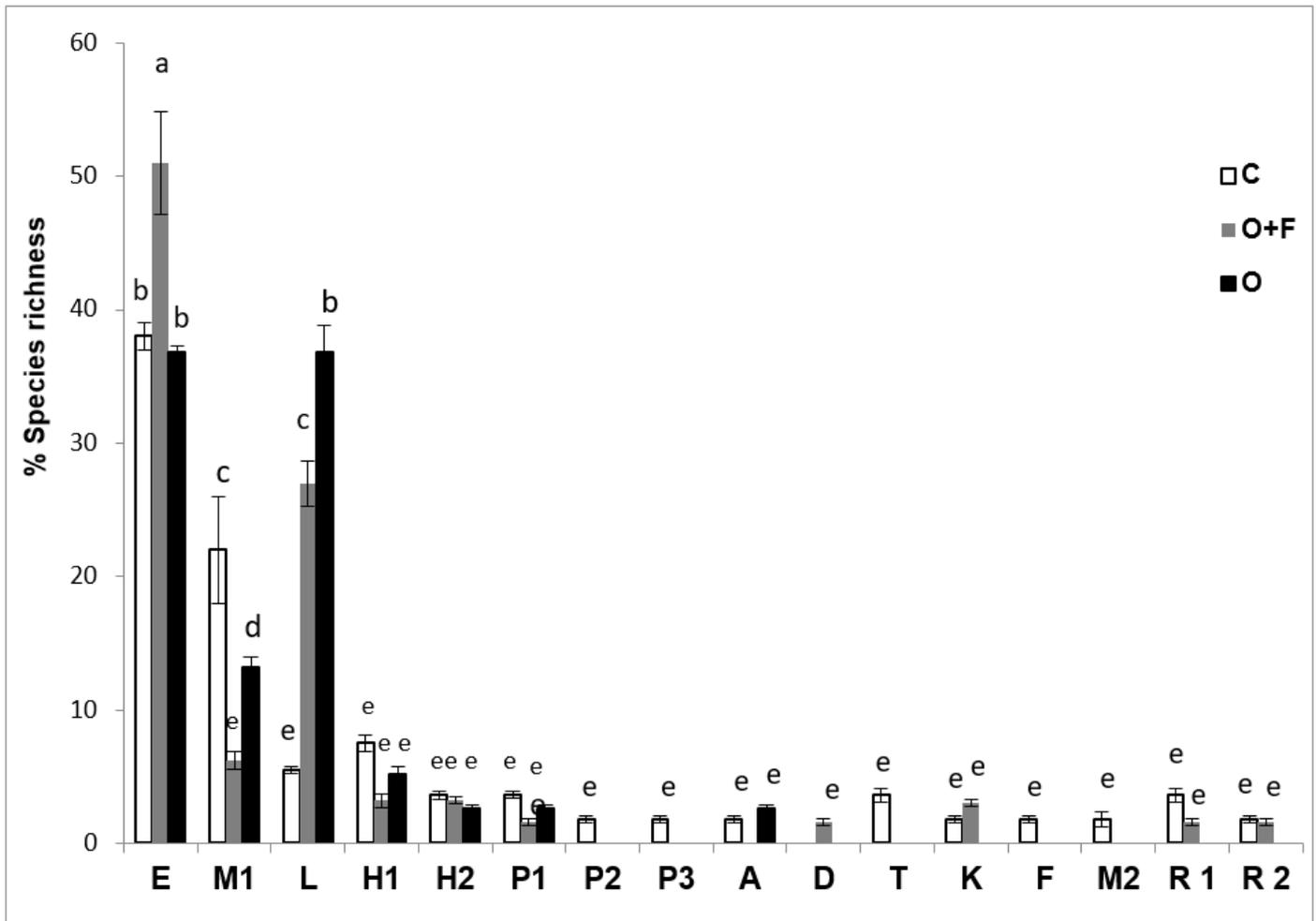
## Figures



**Fig 1.**

**Figure 1**

Effects of oil and added fertiliser on nematode abundance in mangrove sediment. Measurements were taken after four weeks of treatment, C = control, O = oiled, O+F = fertilised+oiled (n = 4). Means  $\pm$  standard error are given. Bars with different letters are significantly different at  $P \leq 0.05$  using Tu key-Kramer multiple comparisons test.



**Fig 2**

**Figure 2**

Effects of oil and added fertiliser on species richness in mangrove sediment. Measurements were taken after four weeks of treatment, C = control, O = oiled, O+F = fertilised+oiled (n = 4). Species names on the X-axis are listed in Table 1 (E1 to R2). No bars indicate absence of species in treatment. Means  $\pm$  standard error are given. Bars with different letters are significantly different at  $P \leq 0.05$  using two-way ANOVA and Tukey's multiple comparisons

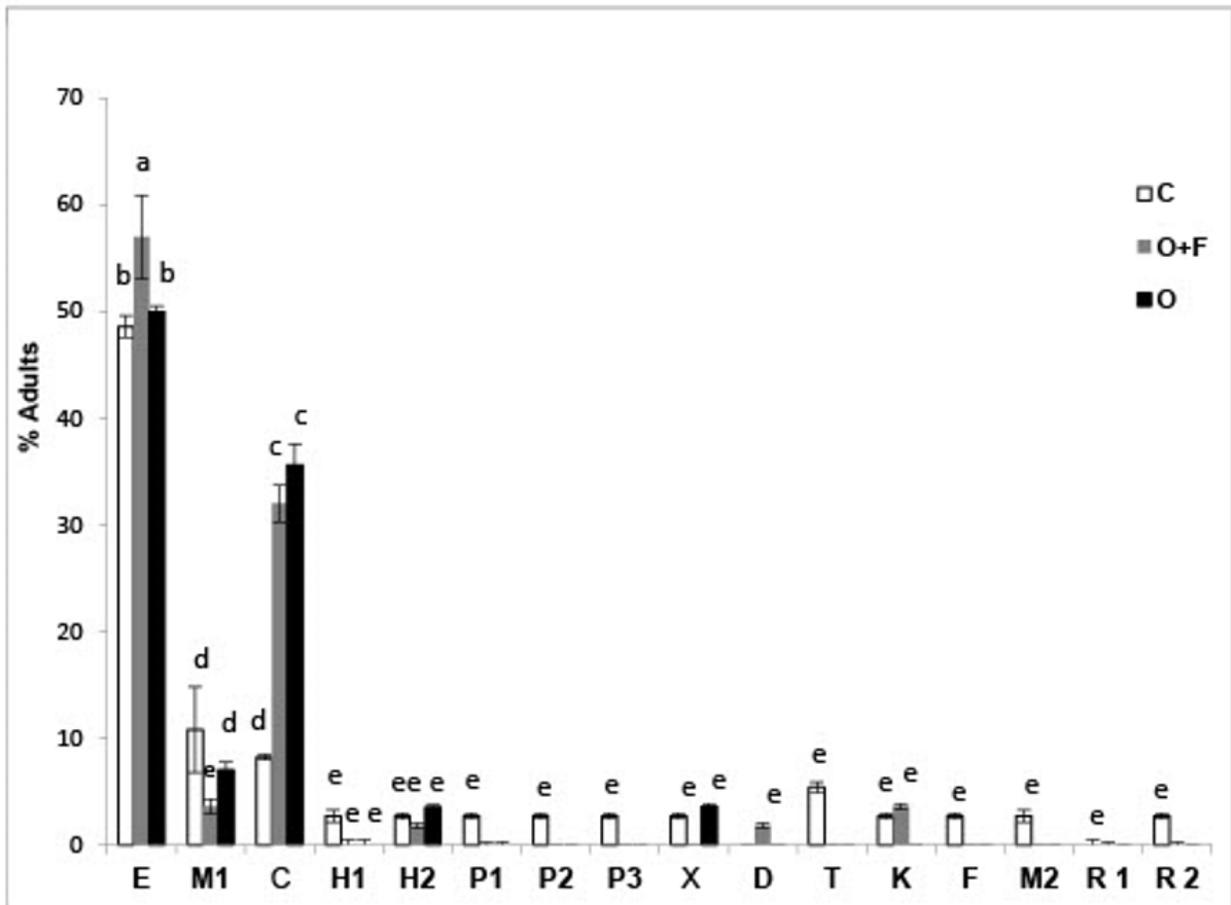


Fig 3

Figure 3

Effects of oil and added fertiliser on adult nematode abundance in mangrove sediment. Measurements were taken after four weeks of treatments, C = control, O = oiled, O+F = fertilised+oiled (n = 4). Species names on the X-axis are listed in Table 1 (M1 to R2). No bars indicate absence of species in treatment. Other details as for Fig. 1.

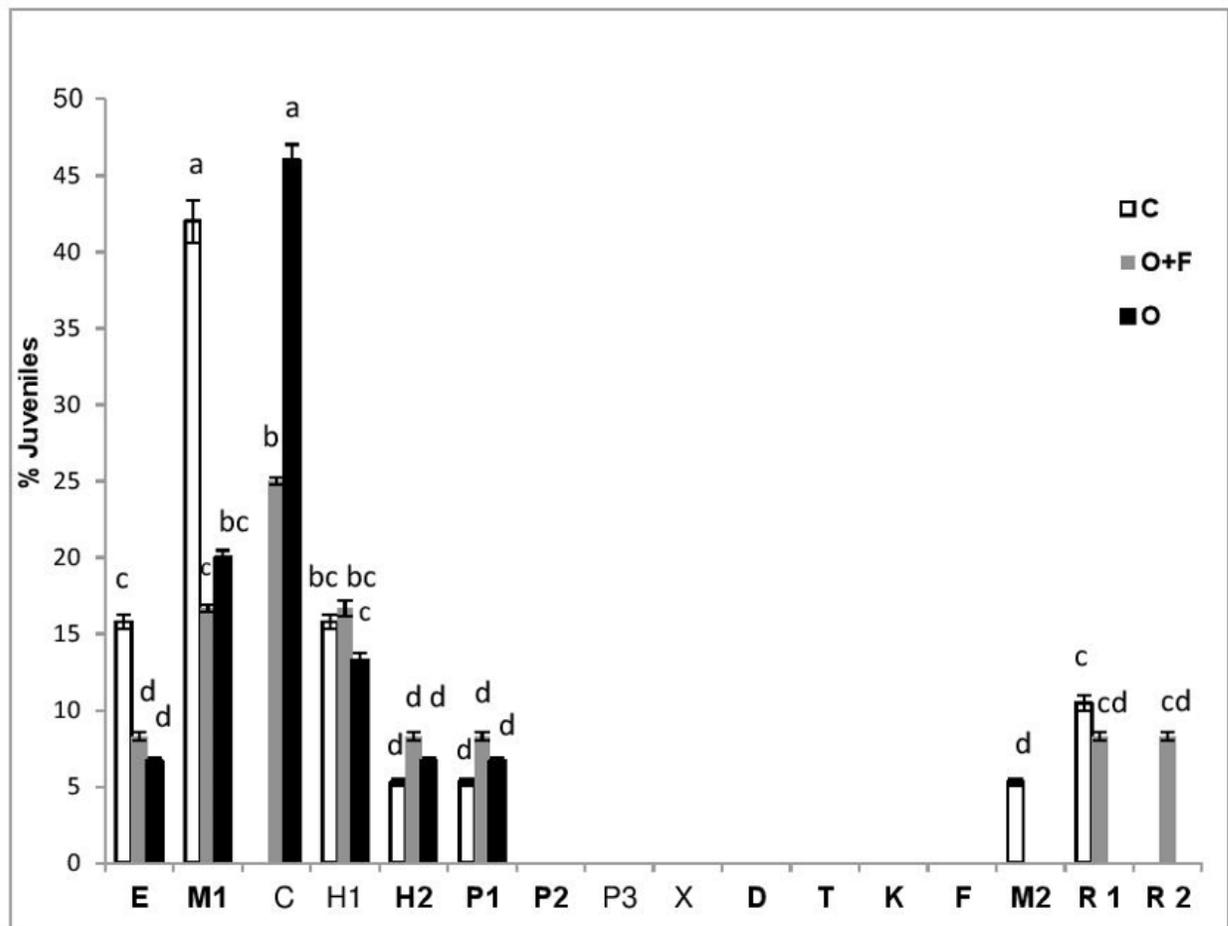


Fig. 4

## Figure 4

Effects of oil and added fertiliser on juvenile nematode abundance in mangrove sediment. Measurements were taken after four weeks of treatment, C = control, O = oiled, O+F = fertilised+oiled (n = 4). Species names on the X-axis are listed in Table 1 (M1 to R2). No bars indicate absence of species in treatment. Other details as for Fig. 1.

## Supplementary Files

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