THE USE OF FREE-LIVING ESTUARINE NEMATODES AS POLLUTION INDICATORS IN THE INCOMATI RIVER ESTUARY, MOZAMBIQUE.

by

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DECLARATION

I declare that the thesis hereby submitted to the University of South Africa for the degree of Doctor of Philosophy in Environmental Science has not been previously submitted by me for a degree at this or any other university and that it is my own work and that the sources used or quoted have been indicated and acknowledged by means of complete references.

Signature:  **Date:** 15 February 2020

Soko Mthobisi Innocent (48197319)

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ABSTRACT

The Incomati River Estuary is prone to pollutants from anthropogenic activities such as agricultural and industrial activities in the upper catchments. The main aim of the study was to use free-living nematodes as pollution indicators in the Incomati River Estuary. The main objectives were to determine the relationship between nematodes assemblage and environmental variables, and to identify environmental factors that play a role in nematodes community structuring. Lastly, it was to identify free-living nematode genera that can be used as pollution indicators in the Incomati River Estuary. Four sites were selected following the salinity gradient of the Incomati River Estuary. Site E1 with a salinity range of 0-3NST (Oligohaline Zone), E2 with a salinity 3-5NST (Euhaline Zone), E3 with a salinity 6-18NST (Mesohaline Zone), and E4 with 20-27 NST (Polyhaline Zone) were selected and monitored bi-monthly from June 2017 to April 2018. Two sediments samples were collected per site during neap tide using a handheld perplex corer which was 1m long with a 3.6 diameter and 10cm penetration height. Plastic bottles with a height of 13cm and a diameter of 7cm were used to store the sediment samples. One of the two sediment samples was used for free-living nematodes, and the other bottle was used for the analysis of environmental factors. All environmental factors were analysed at Labserve Laboratory, Mbombela Town, Mpumalanga Province. Sediment particle size and organic matter analyses were done following the procedure set by Parker (1983) and Buchanan (1971) respectively. Metal analysis was done following the procedure used by Gyedu-Ababio *et al*.1999. Nutrients were done using different methods. For nitrates (NO_3) analysis, a copper cadmium method by Bate and Heelas, 1975 was used, while a method designed by Strickland and Parson, 1972 was used to analyse orthophosphate. A mixed acid digestion procedure of Oles and Dean 1965 was followed for total phosphate. A method by Lorenzen and Jeffrey, 1980 was used for the analysis of chlorophyll-*a*. Heterotrophic bacteria analysis were done following a procedure by (Atlas, 1997). Nematodes were extracted using a method by Furstenberg *et al*.1978, with sucrose as a separating agent. Nematodes were counted following a procedure by Giere, 1993. Nematodes feeding types were investigated using Wieser, 1953 procedure. Different statistical packages including PRIMER version 6 were used to analyse the data. A Bray-Curtis Cluster analysis indicated a similarity between sites E1 (Oligohaline Zone) and E2 (Euhaline Zone), and between site E3 (Mesohaline Zone) and E4 (Polyhaline Zone)

which was attributed to similar sediment particle sizes distribution within the sites. There was no significant difference ($p>0.05$) of sediments particle size between the sites. The highest concentration of metals was found at site E2 which was situated in the Euhaline Zone, whilst the second highest concentration was found at site E1 which was situated in the Oligohaline Zone. A PERMANOVA analysis indicated that there was a significant difference ($p<0.05$) of Metal concentration between sites sampled. The PCA analyses indicated that there was a positive correlation between Metals and Sediment Particle Size such as Granules. It was observed that sediment particle size and organic matter influenced the distribution of metals in the Estuary. The highest concentration of chlorophyll-*a* and nitrates (NO_3) were found at site E3 which was situated in the Mesohaline Zone, and the second highest was found at site E4 which was situated in the Polyhaline Zone. There was a positive correlation between Heterotrophic bacteria and environmental factors such as zinc, fine sand, very fine sand and mud. This indicated that certain metals and sediment particles size played a role in structuring food source for meiofauna, especially nematodes. The number of free-living nematodes were found to decrease towards site E1. This indicated that salinity influenced the diversity and density of free-living nematodes in the estuary. Site E2 had the lowest diversity and richness followed by site E1. The lower diversity, richness and Maturity Index at site E2 and E1 indicated that these sites were under stress. A Bray-Curtis Cluster analysis indicated that there was a spatio-temporal variation of diversity and density of free-living nematodes in the estuary. All four nematodes feeding types were found in the Estuary and feeding type **1B** was the dominant feeding type at the sites, followed by feeding type 2A. The highest number of feeding type 1B (non-selective deposit feeders) was identified at site E2. The life strategy characterisation (colonizer-persisters) indicated that site E2 was dominated by colonizer and intermediate genera (c-p 2 and 3), which indicated that the site was under stress. The study found that genera such as *Terschellingia* and *Theristus* were pollution indicators because they were found in higher abundance at a site that was mostly polluted by metals, organic matter, and total phosphate. Further studies in other River Estuaries in South Africa and SADC should be undertaken to add to the findings of the current study.

Key words: free-living nematodes, nutrients, pollution, total organic matter, estuary and metals

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CHAPTER 1

1. GENERAL INTRODUCTION

Estuaries are situated between rivers and the sea. They receive pollutants which are derived from anthropogenic activities such as agricultural and industrial effluents (Lillebø *et al*. 2005; Paerl 2006). Increasing pressure due to natural and anthropogenic stressors on marine ecosystem have been observed worldwide (Dauvin, 2007). There are numerous anthropogenic stressors affecting estuarine environment by contributing to the change of habitat structure and dynamics of living communities (Webber, 1996; Hameedi, 1997). Estuaries are naturally disturbed systems with a high degree of variability in their physical and chemical features. According to Diaz and Rosenberg (2008), the main disturbances in the estuarine and marine environment are organic pollution of water and sediments. The Incomati River Estuary is also prone to these types of stressors due to the presence of impoundments and abstraction taking place in the upper catchment which reduces the flow regime, therefore, resulting in sediments fluxes. Moreover, anthropogenic activities such as mining, industrial, agricultural and afforestation exist in the upper catchment (Swaziland and South Africa) which also pose a threat to the estuary either through seepage, effluents, and run-offs from these activities. These further affects the estuarine ecosystem and other goods and services rendered by the estuary (Sengo, 2003).

To understand the environmental quality of estuaries, a Maturity Index (MI) was developed for years in terrestrial and freshwater habitat studies, and now it has been extended to marine and brackish water systems (Bongers *et al.*1991). The Index focuses on the ecological characteristics of nematodes taxa and the reproductive and life history strategies (Bongers, 1990). The taxonomic diversity of nematodes makes them to be a good tool in studies of environmental pollution (Platt *et al*. 1984). In a sea floor of many shallow subtidal areas, nematodes density comprises several millions per m^2 ,and represents a biomass of 0.2-0.5g and they are good response of impacts (Heip *et al.* 1992). Nematodes are present in all habitats that give life.

1.1. Global overview of meiofauna communities

Meiofauna are diverse group and they represent a wide range of invertebrate taxa. They are composed of organisms with a biomass size spectrum ranging from 0.01 to 50ug. Fleeger *et al*. (1988) classified meiofauna as meiobenthic organisms that are larger than 63um but smaller than 2mm, while another study reported meiofauna as organisms passing through a 0.5mm mesh sieve but are retained by sieve of 45µm (Kennedy and Jacoby, 1999). The agreement is that meiofauna pass through a 1mm mesh sieve and are retained on a 42 or 63µm sieve (Gibbons, 1991; Higgins and Thiel, 1988; Mare, 1942). Meiofauna life history and feeding characteristics makes them unique (Warwick and Gee, 1984), in such a way that their production in the estuarine environment in higher than that of macrofauna in shallow water to deep sea (Heip *et al.*1985; Coull, 1999; Gerlach, 1971; Schratzberger *et al*. 2002). Meiofauna are the main group of the benthic ecosystem and the most dominant taxa are nematodes and copepods (Urban-Malinga, 2013).

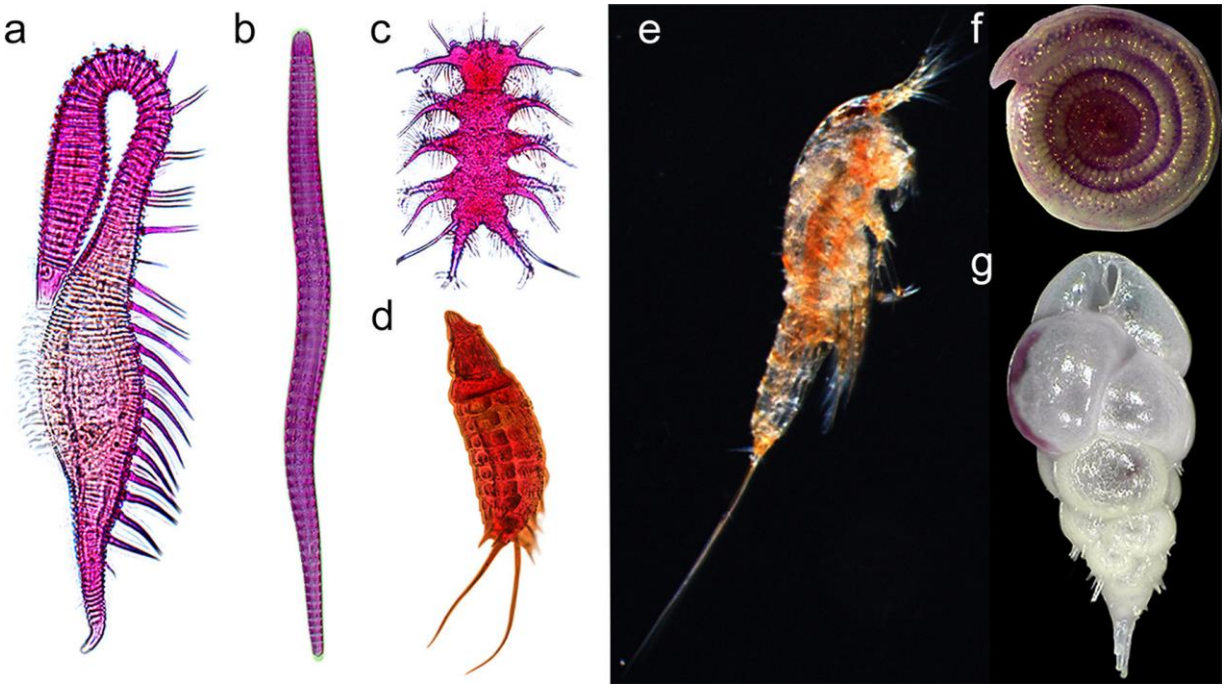


Figure 1: Microscopic view of meiofauna on selected meiofauna taxa: **a, b)** Nematoda, **c,)** Tardigrada, **d)** Kinorhyncha, **e)** Copepoda, **f, g)** Foraminifera (Zeppilli *et al.* 2013).

Nematoda is the dominant meiobenthos in sediment. In aquatic ecosystem nematodes live as free-living or parasitic nematodes, and they are the most representative meiofauna group in all aquatic ecosystems. Their morphology allows them to move between the particles in an aquatic environment (Heip *et al.*1985; Strayer, 1985; Traunspurger, 1996). The phylum Nematoda occurs in high densities and exceeds other communities (Coull, 1999), and it consists of two classes which are Secernenta and Adenophorea (Bongers, 1983). The class Adenophorea is sensitive to pollution, and it occurs in all habitats including freshwater and marine. On the other hand, the Secernenta occurs in terrestrial and respond rapidly to resource pollution. Other nematodes communities fit in the intermediate positions on the range of r- to k selected features, but still belongs to the Secernenta and Adenophorea.

Nematodes are everywhere and are known for their ecological roles in aquatic ecosystem (Platt & Warwick, 1983). Nematodes species of *Araeolaimida, Monhysterida* and *Chromadorida* are known to live in both freshwater and marine system (De Ley *et al.* 2005). The taxa of *Enoplida*, *Desmoscolecida*, *Chromadorida* and *Monhysterida* are mostly occurring in marine environment. There are about 4000 free-living nematodes species that have been found through different studies (Jensen, 1981; Sharma and Webster, 1983; Vanreusel *et al.*1992). According to Forster (1998) and Warwick (1981) estuarine nematodes have a taxonomic affinity to freshwater and they can tolerate changes in salinity. In temperate studies, meiobenthos were found to be good pollution indicators (Boyd *et al*. 2000), because they were able to assess the response of natural and disturbed gradients (Schratzberger, 2012). Free-living nematodes gives many advantages when used as biological indicators for pollution (Kennedy and Jacoby, 1999; Schratzberger *et al*. 2000). Apart from their dominance, nematodes play a significant role as transitional between the microbial and larger organisms (Danovaro *et al*. 2007) by influencing the diversity and structure of these communities (Heip *et al.1985*; Steyaert *et al.*1999). According to Mascart *et al*. 2013; Mascart *et al.* 2015; Lebreton *et al.* 2011; Vinagre *et al.* 2012 and Carpentier *et al.* 2014, meiofauna also play an important role in benthic food webs not only as consumers but also as producers. The state and structure of meiofauna assemblages can show the health of marine environment (Kennedy and Jacob, 1999).

Nematodes have indicated their importance in marine environment (Heip *et al*.1985; Yodnarasri *et al*. 2008; Adao *et al*. 2009; Hourtson *et al*. 2009). They have been

investigated in a variety of habitats including temperate estuarine and marine environment. They have been used in environmental studies and showed to be a good pollution indicator for induced disturbance of benthic ecosystems. However, only few studies have assessed the ecology of free-living nematodes in tropical habitats (Heip *et al.*1985; Coull and Chandler, 1992; Bongers and Ferris, 1999; Höss *et al*. 2011). In Tunisia and South Africa there have been few studies of nematodes (McLachlan, 1977; Furstenberg and Vincx, 1988) and only few of them used nematodes as pollution indicators (Gyedu-Ababio *et al*.1999; Mahmoudi *et al.* 2002; Gyedu-Ababio and Baird, 2006; Gyedu-Ababio, 2011, Hannachi *et al*. 2015; Jouili *et al*. 2017). In the Incomati Estuary, studies mostly focused on salt intrusion and environmental water pulse (Brockway *et al*. 2006; Sengo *et al*. 2005), but no meiofaunal studies have been undertaken.

1.2. Overview of Incomati River Estuary

The Incomati River Estuary is located on the east coast of Africa, in Southern Mozambique. It originates from South African Highveld and the Transvaal plateau at about 2000m altitude and it flows through South Africa, Swaziland and Mozambique to reach the Indian Ocean at Maputo Bay, Mozambique. The Estuary is about 40 to 50 km long and meanders within the coastal plain, separated from ocean by a narrow sand dune, a manifestation of the sluggish flow of the river (Hoguane *et al*. 2016). The side of estuary gradually converge upstream, resulting in a funnel type shape. The surface area of the mouth during high water is about 9000m^2 with a slope factor of about 0.1km^{-1}. The tides in the estuary are semi-diurnal, with a maximum range at the mouth of about 3 meters. The Incomati River Estuary is shallow and the deepest point at high water is no more than 10 meters. The estuary is classified according to Pritchard (1967), as a negative estuary because the annual evaporation exceeds the annual freshwater input. It is further classified as a lagoon-type based on their geomorphology and it is separated from the ocean by a sand dune and presents a spit at the mouth. According to Dyer (1997), the estuary may also be classified as a mixed to partially mixed based on their water circulation.

The weather in the Incomati basin changes greatly with the change in location of the basin. In the coastal plain and lowveld the climate is warm to humid, while in the

highveld it is a cooler dry climate. The Mozambican coastal plain is prone to tropical cyclones and rains in the basin is expected between October and March. The annual mean precipitation of about 740mm a^{-1} is lower than the mean annual potential evaporation of 1900mm a^{-1} in the basin resulting in an increasing deficit between rainfall and potential evaporation and higher demands for irrigation in the basin (Sengo *et al*. 2005). According to JIBS (2001), the estimated total runoff in the Incomati River basin is 3587Mm^3 a^{-1} of which 82%, 13% and 5% is generated in South Africa, Swaziland and Mozambique respectively. A higher percentage of all runoff is generated in the months of November and April. Disparity of discharge from year to year are noteworthy with floods and droughts occurring regularly, and the coefficient of disparity of annual discharge is 50-60% (Van der Zaag and Vas Carmo, 2003)

The Estuary has a mangrove forest covering approximately 5000ha on the mouth. The mangrove influences the wellbeing of the coastal zone and nearby marine habitat by protecting them from erosion caused by winds (Van der Zaag and Vas Carmo, 2003). The Incomati River Estuary provides water and ecological services to local people, and it is also a significant place for breeding colonies of aquatic birds (Sengo *et al*. 2005). In recent years, the reduction of the mangroves area, mangrove tree and biomass density has been reported to be attributed to the modification of river flow regime by dams (LeMarie *et al.* 2006). According to Van der Zaag and Carmo Vaz (2003), the upper reaches impoundments and abstractions have changed the flow regime with negative effects for the estuarine ecosystem. Hoguane (2002) also stated that impoundments constructed on the tributaries feeding to the Incomati River have an impact on the estuarine environment as it results to saltwater intruding inland. According to Hoguane (2002), salt intrusion in the Incomati Estuary take place between 40 km upstream to over 80 km. The impacts of salt intrusion in the estuarine environment are adverse in such a way that species less tolerant to salt content are highly affected and forced to migrate, resulting in changes in the estuarine species composition.

1.3. Study rationale

Meiofaunal taxa are the most significant metazoan in polluted sediments compared to macrofauna (Kennedy and Jacoby, 1999). They provide many advantages when used

as monitoring organism because of their high abundance and diversity, small size, short life cycle, rapid development, limited mobility, absence of pelagic life stages and the presence of both tolerant and sensitive species. According to Kennedy and Jacoby, (1999) and Frontalini *et al*. (2011), meiofauna gives more robust data sets which can show higher sensitivity, effects over small spatial scales, and a quicker response to disturbance. These characteristics makes meiofaunal organisms good biological indicators to test ecological hypotheses and theories (Nascimento *et al*. 2012 and Bonaglia *et al*. 2014). The use of nematodes and meiofauna in this study closes the gap in the current monitoring programs which is mainly based on macro-benthic invertebrates by showing different and complementary aspects of the factors structuring benthic ecosystem which are fundamental in ecological status assessment (Höss *et al.* 2011; Vanaverbeke *et al.* 2011). Moreover, this study is one of its kind to be conducted in the Incomati River Estuary as there was no studies undertaken in the Estuary using nematodes and meiofaunal communities as pollution indicators. The information that is gathered during the research will be used in the Incomati Catchment Water Quality Management Plan and it will assist in understanding the importance of free-living nematode as pollution indicators in water resources or estuaries in South Africa and Africa in general.

1.4. Aims and objectives of the study

The aim of the study was to use meiofauna groups, especially free-living nematodes as pollution indicator in an area strongly influenced by anthropogenic activities. The objectives of the study were to:

- Assess the relationship between nematodes assemblage and environmental variables.
- Investigate which environmental parameters influences nematode assemblages
- Identify free-living nematode genera which can be used as pollution indicators in the Incomati Estuary.
- Determine the spatial and temporal distribution of free-living nematodes feeding types along the salinity gradient.
- Investigate the influence of environmental factors to nematode feeding types.

- Evaluate the influence of environmental variables on nematodes population structure.
- Contribute to the water quality plan of the Incomati River Estuary.

1.5. Research questions

The following questions were to be answered at the end of the research:

- Can free-living nematodes be used as water pollution indicators in the Incomati River Estuary?
- Does nematodes composition changes due to change in environmental variables?
- Are there any spatial and temporal trends of free-living nematode in the estuary?
- Would trophic structure of free-living nematode communities indicate correlation with bacteria?
- Would specific nematode genera or trophic type have correlation to food abundance, which are not evident from an analysis at the higher taxon level only?

1.6. Thesis Outlines

Chapter 1 presents the general introduction of the study. It deals with the global overview of meiofauna and Incomati River Estuary; and other aspects such as study rationale, aims and objectives, and research questioners and hypotheses.

Chapter 2 provides a comprehensive literature review of free-living nematodes as a pollution indicator, the methodology used in nematode classification on feeding behaviour, the influence of different environmental factors on the distribution of nematodes in estuarine environment, the different sizes of corer used in nematodes sampling, and the location, site selection, the rainfall, land and water use in the study area.

Chapter 3 presents the comprehensive results and discussion of the environmental factors and the distribution of free-living nematodes. It provides the analysis of sediments, chlorophyll *a* and the relationship between free-living nematodes and environmental variables.

Chapter 4 presents the methods used for the analyses of meiofauna and nematodes. It also presents the results and discussion of the feeding types and life history strategy of free-living nematodes.

Chapter 5 provides a general conclusion and recommendations of the thesis.

Chapter 6 provides the comprehensive literatures used in preparation of the dissertation.

CHAPTER 2

LITERATURE REVIEW

2.1. INTRODUCTION

Estuarine areas are some of the most productive natural ecosystems because they provide important ecological functions and services such as habitat and shoreline protection, food for migratory and resident species, harbour and recreational utilities (Kennish, 2002; Dolbeth *et al*. 2003; Paerl, 2006). Because estuaries are situated between land and the sea, they receive large number of nutrients and pollutants derived from anthropogenic activities such as agricultural and industrial effluents (Lillebø *et al.* 2005; Paerl 2006). Although it is difficult to distinguish between the impact of anthropogenic from natural variable effects in field investigation (Tietjen, 1977; Platt *et al*. 1984; Travizi and Vidakovic, 1994), several studies have emphasized the sensitivity of the nematode community to various kinds of anthropogenic disturbances, which according to Mirto *et al*. (2002); Schratzberger (2012); Fraschetti *et al*. (2006); Moreno *et al*. (2008, 2009); Vezzulli *et al*. (2008), Gyedu-Ababio and Baird, (2006) seem to alter nematode communities structures, taxonomic diversity and functional group.

Nematodes are incapable of escaping from bottom pollution impact because, they are permanent members of benthos, and they offer a potential means of assessing sediment quality in the area where pollution level in water and sediment is not evenly balanced. According to Heip *et al.* (1985); Moens *et al.* (2005); Kennedy and Jacoby, (1999); Schratzberger *et al.* (2000); Austen and Widdicombe (2006) meiobenthic assemblage provide good information because of their ecological features which gives them several benefits over macrofauna communities as monitoring organisms. According to Kuipers *et al*. (1981); Coull, (1999) in an estuarine and marine environment, meiobenthos are the most abundant metazoans and has a density typical in the boarder of 10^6 Ind m^{-2}, and they are crucial in benthic fluxes of carbon and nutrients. The assessment of benthic assemblage's composition is a valuable tool for determining and describing environmental changes of estuarine and marine system (Heip *et al*.1992).

Most researches in marine nematodes and meiofauna assemblages focus on the intertidal, sublittoral or in deep sea sites and a considerable number of these studies

showed the importance of environmental impacts on meiofauna assemblages such as nematodes (Heip *et al*.1985; Giere, 1993). Different researchers use different sizes of hand-held cores for the sampling of meiofauna from sediments. According Buchanan, 1971, the most efficient sampling method for meiofauna from sediment is by using hand-held corer with an internal diameter of 3 to 4 centimetre (Heip *et al.*1985). The same centimetre range of hand-held corer was used in a study conducted (Anbuchezhian *et al*. 2010), but Nicholas and Stewart (1993) used a small size hand-held core with an internal diameter of 2.5 centimetre in a study conducted in South Coast of New Wales mud flat. In another study conducted in Swartkops Estuary, a different size of hand-held corer with an internal diameter of 6.5 centimetre and a 10 centimetre depth was used (Gyedu-Ababio *et al*.1999) and this was attributed to the fact that meiofauna are mostly found in the top ten centimetres of sediment (Smol *et al.* 1994). The instruments used for meiofauna sediment sampling depends on the purpose of that specific study (Heip *et al.*1985). These includes the extraction of nematodes from sediment in the laboratory as different authors used different extraction solution. Other authors (Gyedu-Ababio *et al*.1999; Lackey and May 1971) used sucrose solution; while others used Ludox-TM50 or Ludox-HS 40% (De Jonge and Bouwman, 1977; Furstenberg and Wet 1982; Anbuchezhian *et al.* 2010). For this study nematodes were sampled using a hand-held perspex corer with a 3.6 internal diameter and 10-centimetre depth. For extraction, a centrifugal floatation method with sucrose solution as the separating agent was used and a Rose Bengal was used for meiofauna staining.

2.2. NEMATODES AS POLLUTION INDICATOR IN ESTUARINE ECOSYSTEM

Biological indicators provide a comprehensive evaluation of ecological health than physico-chemical variables (Balsamo *et al*. 2012). Meiofauna have been thoroughly studied in Bohai Sea (Zhang *et al*. 2001; Guo *et al*. 2002 and Zhou *et al*. 2007), and in the Huaghai Sea in China (Zhang *et al*. 2001; Liu *et al*. 2005 and Liu *et al.* 2007). Other studies on meiofauna and free-living nematodes have been conducted in Changjiang Estuary areas (Zhang *et al*. 2004; Lin *et al*. 2004 and Hua *et al*. 2006). In Tunisia and South Africa there have been few studies of nematodes (McLachlan, 1977; Furstenberg and Vincx, 1988) and only few of them used nematodes as pollution indicators (Gyedu-Ababio *et al*.1999; Mahmoudi *et al.* 2002; Gyedu-Ababio and Baird, 2006; Gyedu-

Ababio, 2011; Hannachi *et al*. 2015; Jouili *et al*. 2017). All these studies have concluded that meiofauna especially nematodes can be used as pollution indicators in marine environment, and they can be used in the assessment of sediment quality in coastal marine ecosystem (Ryu *et al*. 2011; Lee *et al*. 2016 and Bae *et al*. 2017). Due to their high abundance and species richness in estuarine environment, meiofauna have advantage over macrofauna in monitoring the ecological health of an ecosystem. Studies focusing on meiofauna and macrofauna indicated that meiofauna are responsive in the initial influence of disturbance (Schratzberger *et al*. 2000; Whomersley et al. 2009). The high abundance and diversity of free-living nematodes found in sediments and the fact that they are always in aquatic ecosystem gives them an advantage to be used as biological indicators (Vranken and Heip, 1986), and in the assessment of ecological health within the Water Framework Directive (Danovaro *et al*. 2008; Moreno *et al*. 2011). Thus, environmental change is shown in their faunal analysis, and short-term studies can effectively show variations in their community assemblage (Liu, 2009). Simple indices of nematodes abundance by trophic group were previously suggested, a Maturity Index (MI) was also developed for terrestrial nematodes (Yeates, 1970, 1984; Bongers, 1990) and it was then extended to marine and brackish sediments (Bongers *et al*. 1991).

2.3. MATURITY INDEX

Maturity Index has been used in marine environment to assess the impact caused by metals, organic enrichment and eutrophication towards which nematodes responded positively (Tietjen, 1980; Bongers *et al*. 1991; Essink and Keidel, 1998; Fraschetti *et al*. 2006, Moreno *et al*. 2008, and Semprucci *et al*. 2013). In a study conducted in the New York Bight by Tietjen (1980), Maturity Index had a significant correlation with metal concentration such as chromium, copper, nickel, lead, and zinc. Hopper and Meyers (1967) when studying strong tidal action to sheltered habitats also found that Maturity Index was lower at sites which were subjected to sedimentation. Another study conducted in brackish condition in the Netherlands, Maturity Index was found to be low at sites which had silt sedimentation (Bongers and Van de Haar, 1990). According to Coull and Chandler (1992); Somerfield *et al*. (1994); Austen and Somerfield *et al*. (1997); Kennedy and Jacoby, (1999); Zeppilli *et al*. 2018; Carugati *et al*. 2018, meiofauna and nematodes have indicated to be useful biological indicators to assess

the environmental impacts of anthropogenic disturbance. The index has been acknowledged for its usefulness of the assessment of the ecological quality status of marine environment (Moreno *et al*. 2011; Ürkmez *et al*. 2014; Semprucci *et al*. 2015). Maturity Index takes into consideration the life history strategies of nematodes and it pays attention of the biological features and reproductive strategies (Bongers, 1990). The life history strategies of nematodes are r-strategies species (c-p=1) and K-strategies species (c-p=5) which has been distinguished from one another by the c-p values. The c-p value equal to 1 (c-p=1) represents extreme colonisers species which are tolerant to changes in environment. These species are represented by *Rhabditidae*, *Neodiplogasteridae* and *Monhysteridae*. The species represented by c-p value equal to 5 (c-p=5) are extreme persisters. These species are intolerant to environmental changes and they are mostly represented by *Enoplidae* and *Leptosomatidae* (Bongers, 1990). In polluted habitat, the Maturity Index value tends to decrease, or the K-strategist's species disappears and replaced by r-strategist species (Bongers, 1990). It is an important tool that gives researchers and opportunity to assess the ecological health of different habitats.

2.4. ESTUARINE ENVIRONMENTAL VARIABLE STRESSORS ON FREE-LIVING NEMATODES AND MEIOFAUNA.

As a naturally disturbed system, estuaries have high degree of variation in physical and chemical features. Depending on the variety of factors such as land runoff, range of tides, soil type, and wind pattern, an estuarine salinity may vary from 0.5 to about 40% and the main disturbances in estuarine and marine environment are organic pollution of water and sediments (Diaz and Rosenberg, 2008). Studies of meiobenthic dominance and composition have indicated the prime importance of salinity and sediment properties on the spatial distribution, abundance and species composition of free-living nematodes (Austen and Warwick, 1989; Vincx *et al.* 1990; Coull, 1999). Wieser (1959); Everard (1960); Warwick (1971); Warwick *et al. (*1990) and Soetaert *et al*. (1995) have concluded that salinity is a governing factor for nematodes distribution; and assessing the composition and regulating the species structure, abundance, and diversity in an estuary. Meiobenthic density and number of species deteriorate towards freshwater in an estuarine area (Austen and Warwick, 1989). In a study conducted in Exe Estuary in Britain, dorylaimids, rhabditids, and species of *Tripyla* were found in low

salinity and species such as *Anoplostoma viviparum*, *Hypodontolaimus geophilus*, *Sabatieria vulgaris* and *Theristus oxycerus* decreased in low salinity (Warwick, 1971). Gyedu-Ababio *et al*.1999 also found that nematodes density and diversity decrease with the decreasing salinity gradient in an estuary. Although studies have been done on intertidal nematodes communities, these studies were conducted within a limited salinity range (Austen and Warwick, 1989, Warwick and Gee, 1984). Studies on the spatial distribution of subtidal estuarine nematodes along the salinity gradient are also very scarce (Soetaert *et al.*1994) and there is limited information about the impact of salinity gradients on nematodes populations' quantitative parameters and studies only focuses on intertidal sediments. Within an area of uniform salinity and grain size of sediments are the dominant factor in determining the composition of nematodes communities as well as communities of other meiofauna (Tietjen, 1977; Ward, 1975).

Sediments variables concentration such as grain size, organic content, chlorophyll-*a* and phaeo-*a* are other important factors that contributes to the distribution of nematodes in estuarine environment (Levin *et al*. 1991). The relationship between estuarine nematode distribution and sediment grain size can be attributed to difference in buccal morphology and feeding preferences (Wieser and Kanwisher, 1961; Tietjen, 1969). In a study conducted in southern North Sea, nematodes diversity was found to be lowest at a station with low median grain size (Heip and Decramer, 1974), and in another study undertaken in Liverpool bay, the dominance diversity for nematodes was low in a muddiest habitat (Ward, 1973). A study conducted in the Swartkops River in South Africa (Gyedu-Ababio *et al*.1999), sediment particle size was found to influence nematodes density, and the number of nematodes were low at sites dominated by both finer, and coarse sands. de Beer *et al*. (2005) found that density and diversity of nematodes was higher in coarse sediments. Warwick and Buchanan (1971) also found that the diversity of nematodes was high at the sandiest station and low at the siltiest station in a study conducted in Northumberland coast (Britain). These findings were also supported by Vanaverbeke *et al.* (2011) and Fonseca *et al.* (2014) who also found that density and diversity of marine nematodes increase with increase sediment grain size, but different findings were observed by Maria *et al*. (2013) with a notion that nematodes response is species specific. There is supporting evidence that mud environment supports deposit feeders, and omnivores or predators increase in wet sand (Warwick, 1971; Perkins, 1974), and that the species found in mud environment

tend to be very small and with short setae, while species found in sand environment vary in size, depending on the amount of interstitial space, and have longer setae. According to Tietjen (1971); Marcotte and Coull (1974) and Ward (1975), in a marine environment the influence of grain size can also be an indirect result of the nature of available food supply. Organic carbon and chlorophyll-*a* concentration also influence nematodes density, and genus such as *Monhystera*, *Theristus* and *Calyptronema* were found to prefer silty environments which is rich in chlorophyll-*a* (Gyedu-Ababio *et al*.1999). Other studies conducted by Vincx *et al*. (1990); Vanreusel (1990); Vincx and Vanreusel (1989) found different genus such as *Terschellingia*, *Sabatiera*, *Metalinhomeous*, *Sphaerolaimus*, *Spirinia*, and *Dorylaimopsis* as silt bottom lovers. Gyedu-Ababio *et al*. (1999) and Losi *et al*. (2013), found genus such as *Axonolaimus* and *Sabatieria* at sites under pollution stress, and Bongers (1990) reported that these species are pollution resistant. Sediment analysis are useful in assessing the burden of anthropogenic component cover and above the lithogenic background, in some instances, trace the sources of pollution long after input has taken place (Frignani *et al*.1997; Buccolieri *et al*. 2006). Organic content and metals contribute in the structuring of nematode community in an estuarine environment.

Not all metals play a significant role in the structuring of nematode communities. Metals such as titanium, iron, chromium and tin were found to play an important role in the distribution of nematodes, while manganese, lead and zinc together with salinity were not significant as the other metals, and a negative correlation of manganese and iron was observed (Gyedu-Ababio *et al*. 1999). Mahmoudi *et al.* (2002) reported a negative correlation between metals such as copper, lead and zinc with the diversity of nematodes. Numerous studies and laboratory experiments on the effect of nematode communities were conducted and reported by Austen & McEvoy (1997); Austen & Somerfield (1997b); Boufahja *et al.* (2011); Derycke *et al*. (2007); Guo *et al*. (2001); Gyedu-Ababio *et al.* (1999); Hedfi *et al*. (2008); Hermi *et al*. (2009); Mahmoudi *et al*. (2007); Millward and Grant (1995); Somerfield *et al*. (1994). These studies found that nematodes are affected in many ways by different metals. According to Howell (1983), nematodes absorb metals via cuticular mucous secretions, and it differ from different species and genus. The influence of metals on nematodes depends on salinity, temperature and their trophic structure (Coull & Chandler, 1992). Several studies on the response of nematode assemblages to different types of contaminate have been

conducted, and species such as *Ptycholimellus ponticus, S. Pulchra, Molgolaimus demani* and *Axonalaimus paraspinosus* have been found to be tolerant to different metals (Somerfield *et al*. 1994; Austen & Somerfield, 1997b). Austen and Somerfield (1997b) further found that *Terschellingia* genus lived well in all microcosm's treatments including those containing the highest metal dose. Nematodes can accumulate metals such as cadmium, copper, lead and zinc from sediments (Fichet *et al*. 1999). According to Lampadariou *et al*. (1997); Gyedu-Ababio & Baird (2006), nematode genera such as *Theristus*, *Microlaimus*, *Paramonohysthera* and *Sabatiera* are common in habitants of sediments polluted with metals. In a study conducted in southern Caspian Sea, species such as *A. spinosus, H. minusculus, H. brachstoma*, and *T. flevensis* tolerated high concentration of metals such as chromium, vanadium, and cobalt, while the abundance of species such as species such as *A. elagans, Chromadorita sp.1, D. curticauda, D. karabugasensis, D. robustus, D. setosum, D. tenuispiculum, M. naidinae* and *S. cuneatus* decreased with an increase in metals (Bastami *et al.* 2017).

Studies on the impact of metals on nematode composition have been thoroughly conducted and the analysis of c-p composition were valuable tool to detect metal pollution (Nagy, 1999; Nagy *et al*. 2004; Georgieva *et al.* 2002; Yeates *et al*. 2003; Bakonyi *et al*. 2003). According to Somerfield *et al.* (1994); Heip *et al.* (1985), nematodes are good biological indicators of metals contamination in aquatic ecosystem because they are sensitive to changes than any other meiofaunal group.

2.5. DISTRIBUTION PATTERNS OF FREE-LIVING NEMATODES

The distribution and environmental factors affecting free living nematodes are the key information in understanding the ecology of their communities and the role in dynamics of the ecosystems. According to Snelgrove and Butman (1994), evidence of the availability of a specific factor such as grain size or organic content of sediment that attributed the distributional patterns of nematodes is not enough. Instead different studies Danovaro & Gambi (2002); Forster (1998); Moens & Vincx (2000); Ward (1975); Schratzberger *et al*. (2004); Shimanaga *et al.* (2015); and Gao and Liu, (2018), have concluded that nematodes respond to complex setting of environmental factors (e.g. food availability, salinity, depth, water temperature and grain size). Sediment characteristics such grain size analyses, grain shape, sorting and pores space

influence the diversity and abundance of nematodes in a soft bottom environment (Gray, 1974; Vincx *et al.*1990; Vanaverbeke *et al*. 2002). Median grain size of sediments is the primary influence on meiofaunal density and diversity (Coull, 1988; Heip *et al*.1985; Van Averbeke *et al*. 2000; Kim *et al*. 2020). In a case study conducted in the Swartkops River system, in South Africa, nematodes distribution was found to be attributed to food distribution patterns and other factors such as organic carbon and chlorophyll *a* (Gyedu-Ababio *et al*.1999).

The distributions of meiobenthic over a space and time is influenced by changing hydrodynamic environments caused by flow of current over transient bed frames, effects of physical disturbance and effects of pollution (Aller and Aller, 2004; Warwick *et al*.1990; Dittman, 2000; Armenteros *et al.* 2008). A significant spatial heterogeneity has been found on the distribution of nematodes on a small scale of a few meters and the dominant nematodes species found were *Sabatieria pulchra* and *Terschellingia longicaudata* which are tolerant to organic enrichments (Armenteros *et al*. 2009; Perez-Garcia *et al*. 2009). These spatial patterns were attributed to organic matter and metal content in sediments and water depth. Numerous studies on the seasonal distribution of nematodes have been documented from numerous lakes (Biro, 1973; Holopainen and Paasivirta, 1977; Raspopov *et al*. 1996; Traunspurger, 1996; Bergtold and Traunspurger, 2004; Wu *et al*. 2004; Michiels and Traunspurger, 2005).

Most meiofauna can be found in the upper four centimetres of sediment and the density disappear along the depth (Fleeger *et al*. 1995; Soltwedel, 997). The penetration of dissolved oxygen has been found to be the environmental factor that plays a role in the diversity and density of vertical distribution of nematodes (Coull, 1999). Another study done by Moodley *et al.* (2000), found that oxygen penetration has a limited direct effect on vertical distribution of nematodes hence, it conceded more importance the availability of trophic resource. However, other studies conducted by Ansari *et al*. (1980); Shirayama (1984); Alongi and Pichon (1988), Danovaro *et al.* (1995); Lambshead *et al*. (1995) and Vanreusel *at al*. (1995) indicated that the presence of food and oxygen in the sediments are the important factors that limit meiofaunal penetration into sediments. Evidence of vertical migration of nematodes following food sources were found in sublittoral setting (Graf, 1992). Several authors Ingels *et al*. 2011; Lizhe *et al*. 2012; Ngo *et al*. 2013; Zeppilli *et al*. 2013; Górska *et al*. 2014; Pusceddu *et al*. 2014, Nascimento *et al*. 2011; Braeckman *et al*. 2013 and Boldina *et al*. 2014,

Shimanaga *et al*. 2015 and Gao and Liu, 2018, concluded that abundance, diversity, distribution and functional properties of meiofauna can be affected by temperature, salinity, depth, hydrodynamic and sedimentary processes, sediment grain size, oxygenation level, food availability predation and competition.

2.6. CLASSIFICATION OF NEMATODES BY FEEDING BEHAVIOUR

Interpretation of nematodes feeding behaviour focuses on stoma and pharyngeal morphology. Nematode feeding groups and types are classified according to their buccal cavity structure (Wieser, 1953). According to Wieser (1953), nematodes have diversified structures related to food ingestion and adapted to a diversified spectrum of food items and factors. This was also confirmed in studies conducted by Alongi and Tietjen (1980) and Schiemer (1984), where nematode feeding types was approached through experiments and their results indicated that nematodes are highly selective with respect to size, shape and quality of food offered (Alongi and Tietjen, 1980; Schiemer, 1984). Marine nematodes are divided or classified in to four trophic groups (Wieser,1953).

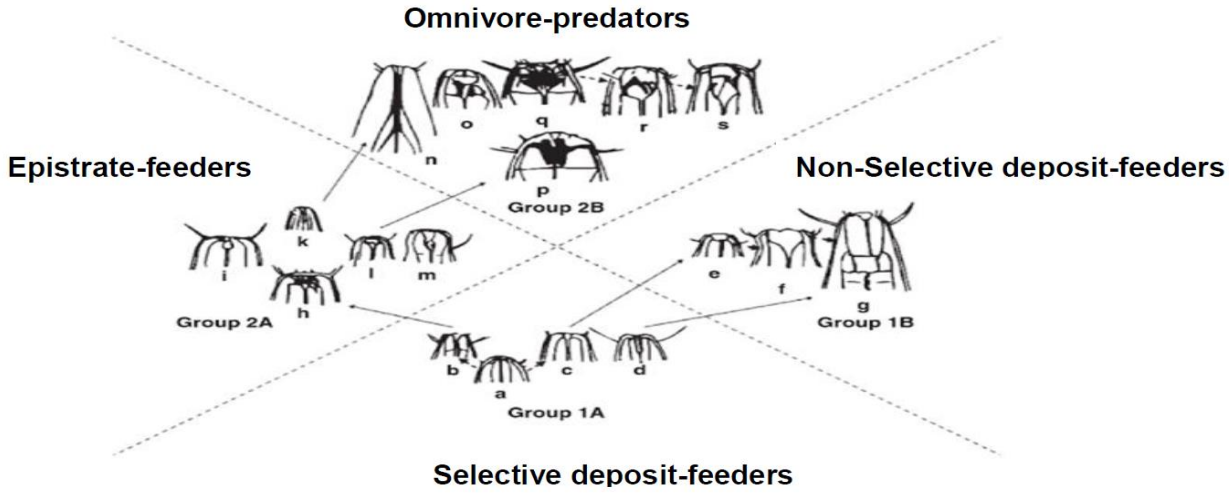


Figure 2.1: The schematic diagrams indicating the four main morphological feeding groups for free-living nematodes (Wieser 1953). **a:** *Oxystomantina*, **b:** *Anticoma*, **c:** *Terschellingia*, **d:** *Parachromagasteriella*, **e:** *Sabatieria*, **f:** *Paramonhystera*, **g:** *Bathylaimus*, **h:** *Paracanthonchus*, **i:** *Linhomoeus*, **k:** *Onchium*, **l:** *Chromadorea*, **m:**

Microlaimus, **n:** *Siphonolaimus*, **o:** *Halichoalaimus*, **p:** *Enoplus*, **q:** *Oxynchus*, **r:** *Oncholaimus*, **s:** *Eurystomatina*.

Many studies such as Wieser (1960); Wieser & Kanwisher (1961); Boucher (1973); Platt (1977); Romeyn & Bouwman (1983) and Bouwman *et al*. (1984) used these classifications and adjusted them in subsequent years. They even go so far as to include the cephalic setation as an additional character of importance in nematode feeding strategy. Jansen (1986) rejected the arbitrary subdivision of deposit feeders because experimental evidence is lacking, and the range of sizes of mouth openings and buccal cavities is as large in other feeding groups as in deposit feeders.

The feeding ecology of soil nematodes was further reclassified (Yeates *et al*. 1993). The study classified all nematodes based on their food source as inferred from ecological setting of nematode family or genus and eight feeding groups were identified. These groups were: plant feeders, fungal or hyphal feeders, bacterial feeders, substrate ingestion feeders, carnivores, unicellular eukaryotes feeders, parasites, omnivores. Since then refinement of the feeding classification continued (Moens and Vincx, 1997; Moens *et al*. 2004). This study emphasised that the current feeding classification of nematodes is reviewed because other studies came into light. Moens *et al.* (2004) emphasized that nematode feeding habit relies on the ecological settings of species, genus and family of nematodes. The study further indicated that food switching is a common phenomenon in other nematodes and has been found to switch food and predate on amoebae and other protozoa when soil pores become small for access to the bacteria (Elliot *et al*.1980). Thus, Moens *et al*. (2004) found terminology such as nonselective deposit feeder by Wieser (1953) and omnivores by Yeates *et al*. (1993) limiting. A further study conducted by Moens and Vincx (1997) emphasised that other nematodes species assemblage in Wieser (1953) had diverse feeding habits. According to Moens and Vincx (1997), assessing feeding guilds based on morphological characteristic provide information on a nematodes ability to handle food rather than on any feeding habit. Although several modification and alternative classification have been proposed, but the one by Wieser (1953) remains the most frequently applied (Moens *et al.* 2013). Free-living nematodes present an important several morphological characteristics thought to be related to important ecological functions mouth structure, tail shape and length-width ratio (Wieser, 1953; Thistle and Sherman, 1985; Thistle *et al.* 1995; Jensen, 1987; Vanaverbeke *et al.* 2004). According

to Vanhove *et al*. (2004), functional method in terms of feeding types provide alternative insight on the effects of the environmental parameters.

2.7. STUDY AREA AND SITE SELECTION

2.7.1. Incomati estuary

The Incomati River Basin originates at an altitude of 2000m and reaches the Indian Ocean in Maputo Bay in Mozambique. The Basin is located in the South-Eastern part of Africa and it covers a land area of about 46700km^2 in three different countries (Ref). In South Africa the Basin covers a land area of about 28681km^2, in Swaziland the land cover is 2560km^2, and in Mozambique it covers an area of about 15510km^2. The different rivers flowing to the Incomati River are Crocodile, Komati, Sabie, Massintolo, Uanetse and Mazimechopes. According to Sengo *et al*. (2005) constructed dams in these rivers have changed the flow in the lower reaches, affecting the ecological goods and services, therefore results in salt intruding in the inland (Hoguane, 2002). The Incomati River Estuary is about 40-50 km long and meanders within the coastal plain. It is separated from the ocean by a narrow sand dune, a manifestation of the sluggish flow of the river.

In the escarpment region of the Incomati Catchment, the main land uses are commercial forest plantations of exotics. In the Highveld region drylands crops such as maize and grazing are taking place, while in the Lowveld region only irrigated agriculture such as sugarcane, vegetables and citrus are taking place (Riddell *et al*, 2014). In the Mozambican coastal plain, sugarcane and subsistence farming dominate. A substantial part of the basin has been declared a conservation area, which includes the recently established Greater Limpopo Transfrontier Park (the Kruger National Park in South Africa and the Limpopo National Park in Mozambique are part of it) (TPTC, 2010).

During the study four sites were selected from the Incomati River Estuary. These sites were selected following the estuarine division (Teixeira *et al*. 2008).

Table 2.1: Sites selected to sample meiofauna in the Incomati Estuary from June 2017 to April 2018.

Site Names	**Salinity Ranges**	**Estuarine Zone**	**Co-ordinates**	
			Latitude	**Longitude**
E1	0-3NST	Oligohaline	-25.7198611	32.6982694
E2	3-6NST	Euhaline	-25.733775	32.680644
E3	10-18NST	Mesohaline	-25.7622361	32.729275
E4	20-27NST	Polyhaline	-25.8324361	32.73435

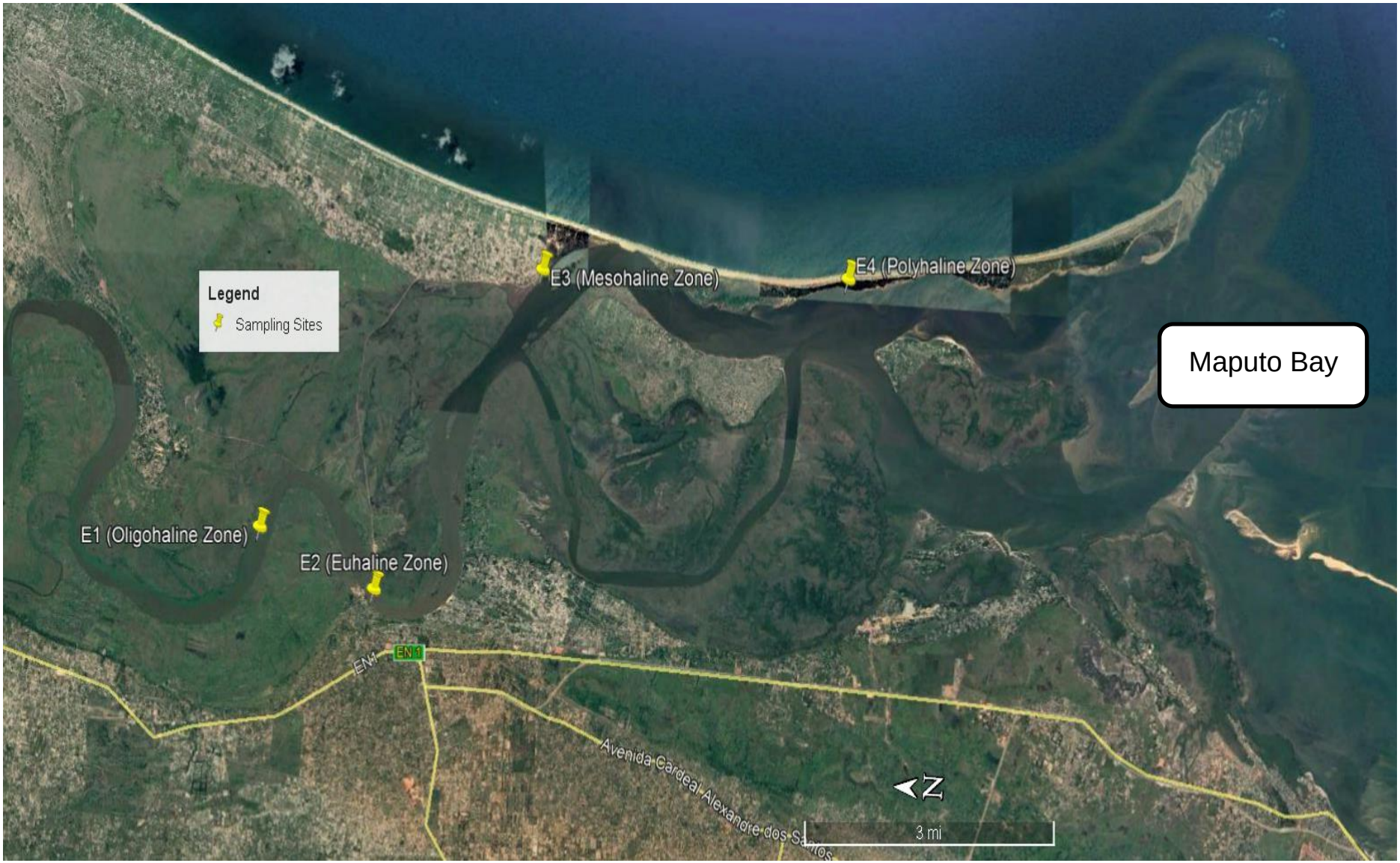


Figure 2.2: Google map indicating sampling sites in the Incomati River Estuary in Mozambique.

CHAPTER 3

ENVIRONMENTAL FACTORS INFLUENCING NEMATODE COMMUNITIES IN ESTUARIES.

3.1. INTRODUCTION

Marine environment is considered repository for waste disposal coming from various anthropogenic activities (Abdullah *et al*.1996). In shallow estuaries metals have a potential to be resuspended and become bioavailable (Wepener and Vermeulen, 2005). Significant increase in soil metal content are found in area of high industrial activity (Geng *et al*. 2005). Trace metals such as chromium, manganese, iron, nickel, copper, zinc, as, lead, cadmium, titanium, and vanadium are introduced in the marine environment through natural process and pollutants released by human activities (Garcia-Montelongo *et al*. 1994; Jordão *et al*. 2002). Most of the contaminants available in the water column are adsorbed on suspended particulate matter and get deposited in sediments by flocculation and sedimentation process. Sediments preserve contaminants in riverine ecosystem and have been used to identify source of trace metals in aquatic environment because of their high accumulation rates (Förstner *et al*. 1981).

Although sediment analyses do not represent the extent of toxicity, but they are useful to assess the burden of anthropogenic component cover and above the lithogenic background and in some instances, trace the sources of pollution long after input has taken place (Frignani *et al*. 1997; Fukue *et al*. 1999; Buccolieri *et al*. 2006). Studies conducted in the Swartkops River (Gyedu-Ababio *et al*. 1999) and Jinhae Bay (Kim *et al.* 2020) , sediment particles size distribution was found to be the most representative parameter together with other environmental factors such as chlorophyll-*a* concentration and organic carbon for the structuring of nematodes. During the study, trace elements such as manganese, titanium, zinc and iron were also found to affect the density, diversity and community structure of nematodes at specific sites. Another study conducted in the Lynher Estuary by Austen and Somerfield (1997), found that the meiobenthic communities' structure correlated with metal concentration in the sediment.

Studies of nematodes dominance in marine and estuarine environments illustrate the prime importance of salinity and sediment properties on spatial distribution, abundance and species composition of free-living nematodes (Austen and Warwick, 1989; Vincx *et al*. 1990; Coull, 1999; Shadrin, 2018; Mayer and Pilson, 2019). Salinity regime in estuaries is a key independent factor assessing the assemblage composition and regulating the species structure, abundance, and diversity (Soetaert *et al*. 1995). There are other sediments variables concentration such as grain size, organic content, chlorophyll-*a* and phaeo-*a* which are also important factors (Levin *et al*. 1991). The distribution and composition of meiofauna have been investigated in different habitats and it has been concluded that at a large scale, non-living parameters such as sediments composition, salinity, temperature fluctuation and tide action controls meiofaunal distribution along the estuarine salinity gradient, while at micro-scale, meiobenthic distribution patterns are based on food supply, predation competition and reproductive behaviour (Sandulli and Pickney, 1999; Steyaert *et al*. 2003). Special estuarine characteristics results in different meiofaunal composition. Other studies of sandy beach nematodes Sharma and Webster (1983); Gourbault and Warwick (1995); Nicholas and Hodda (1999); Gheskiere *et al*. (2004); Urban-Malinga *et al*. (2004); Calles *et al*. (2005); Hourston *et al*. (2009); Moreno *et al*. (2006) and Mundo-Ocampo *et al*. (2007) indicated that the dominance of nematode family is related to granulometric features.

Because of their numerous advantages as a biological indicator, nematodes can be useful in assessing human and natural impacts in sediment (Kennedy and Jacoby, 1999; Schratzberger *et al*. 2000; Schratzberger, 2012; Alves *et al*. 2013). Free-living nematodes have been used to evaluate soil pollution produced by metals, and they have been found to be dominant in finer sand less than 300µm other than copepods which have been found to be dominant in sediment coarser than 500µm (Vanaverbeke *et al*. 2000; Yeates *et al*. 2003; McLachlan and Brown 2006; Zhang *et al*. 2006).

3.2. MATERIALS AND METHODS

Samples for the analysis of environmental factors such as metals, particle sizes, organic matters, chlorophyll-*a*, and nutrients were collected bi-monthly from June 2017 to April 2018. Sediment samples were collected using a 3.6 cm diameter corer to a

depth of 10cm and stored in clean plastic bottles in a cold room until the analysis was carried out**.**

3.2.1. Metal Analyses

Sediments samples were dried for 48hrs at 80^{o}C in a petri-dishes. The dried samples were then crushed and about 2g of each sample was taken into a glass beaker with 20ml Aqua Regia (1:3 HNO_3: HCL) and allowed to react overnight. The mixture was heated to near dryness and allowed to cool, before 20ml of a 5M HNO_3 solution was added. The samples were left to react overnight and were then filtered using a Whatman no. 41 filter paper. The filtrates were transferred to a 100ml volumetric flask and made up the mark with 0.5M HNO_3. Metal determinations of the solutions were perfomed on Shimadzu sequential plasma spectrometer (ICPS-1000II) using the calibration curve method. Concentration of the following Metals: manganese, aluminium, vanadium, copper, chromium, iron, cadmium, lead and cobalt were determined using this method.

3.2.2. Sediment Particle Analyses

Sediment particle size analysis was done using the method described by Parker (1983). The samples were oven-dried at 60^{0}C for 48 hrs. A 30-g portion of the sediment from each site was washed with tap water and reweighed after drying. The dry samples were put on the topmost of a nest of sieves (with mesh size ranging from 0.002μm to 2mm) and sieved by a machine for 8 minutes. The fractions of each sieve were weighed. The median grain size, sorting values, mud composition and all the other sand fractions were assessed using a computer programme, SANDX (SANDSTA.BAJ).

3.2.3. Chlorophyll-*a*

A 10g of each sediment sample collected from the sites was weighed and placed in a 20ml screw cap vial. About 10ml of 90% acetone was then added in the vials containing sediments and swirled once gently (Lorenzen and Jeffrey, 1980). The vials were placed in a 5C incubator overnight. After incubation, the solution was filtered through a Whatman GF/C and placed in a screw cap test tube. The filtered solution was adjusted

to pH 9 using sodium hydroxide (NaOH) as a buffering solution. This was done to reduce the interference of pheophytin with spectrophotometric analysis of chlorophyll. A spectrophotometry with a 1nm spectral band width and optically matched 4cm cuvettes was used. 3ml of extracts from each sample was poured in to the 4cm cuvettes and the absorbance was measured at 664 and 750nm before acidifying. This was done very quickly to prevent light from breaking down the chlorophyll. The absorbance was blanked at 664nm using the 90% acetone solution with a second recorded at 750nm to correct for primary pigments absorbance. After taking the initial measurements, a 0.1ml of 1N HCl was added directly to the cuvettes to estimate the amount of phaeopigments Plante-Cuny (1974) and the acid was allowed to react for 90 seconds and the absorbance was recorded at 665nm and 750nm. The following equation was used to calculate Chlorophyll-*a*.

Chlorophyll-*a* (mg/m^3) =

$$\frac{26.7\,(E_{664b} - E_{665a}) \times V_{(extraction)}}{V_{(sample)} \times L}$$

Where:

b: Before acidification

a: After acidification

E_{664b}=**[(Abs**$_{664b(sample)}$**-Abs**$_{664b(blank)}$ – (**Abs**$_{750b(sample)}$**-Abs**$_{750b(blank)}$**]**

E_{665a}=**[(Abs**$_{665a(sample)}$**-Abs**$_{665a(blank)}$ – (**Abs**$_{750a(sample)}$**-Abs**$_{750a(blank)}$**]**

$V_{(extraction)}$: Volume of 90%acetone used in the extraction(ml)

$V_{(sample)}$: Volume of water filtered

L: Spectral path length

3.2.4. Total Organic Matter

A 10% hydrochloric acid was used to remove carbonated that could interfere with organic matter assessment in sediment samples (Buchanan, 1971). Total organic matter of sediment samples was calculated as the difference between the dry weight obtained when the samples were heated at 80°C for 24hrs and the residue left after combustion at 450°C for 2hrs (Parker, 1983).

3.2.5. Sediment Nutrients

3.2.5.1. Nitrates (NO_3)

A 25ml of 1M NaCl solution was added to 1g of undried sediments and shaken on Kotterman's mechanical shaker for 30 minutes (Bate and Heelas, 1975). The supernatant was then pass through Whatman's no.1 filter paper, and only 3ml of the filtrate was used for analysis in a test tube. A buffer solution (2ml) was added to the filtrate and shaken for 10 minutes. Standard solutions were then made in series: 0; 0.5; 1.0; 1.5; 2.0 and made up to a volume of 100ml with distilled water. A 1ml sulphanilimide was added to 1ml of the sample followed by 1ml solution of diamine-hydrochloride. The mixture stayed for 5 minutes and the nutrient concentrations were then read on a Pye Unicam Spectrophotometer SP 1800 at 540 nm.

3.2.5.2. Orthophosphate

A five gram of sediment was put in 50ml extracting solution of sodium hydrogen carbonate (0.5M $NaHCO_3$) adjusted to a pH of 8.5 with 1M NaOH and shaken on Kotterman's shaker for 30minutes (Strickland and Parson, 1972). The supernatant was filtered through Whitman's no. 40 paper. One ml ascorbic acid (2g/100ml) and 2.5ml molybdate ($Mo_7O_{24}.4H_2O$) reagent were added to 20ml of the filtrate in test tubes. The tubes were shaken and made up to 25ml with distilled water. A standard solution series of 0; 5; 10; 15; 20 were made up to 100ml with distilled water and read at 880nm with a spectrophotometer.

3.2.5.3. Total Phosphorus

Sediment was dried in an oven at 80˚C and sieved through a 4µm sieve (Olsen and Dean 1965). 1g of the sieve sieved sample was heated in a 50ml conical flask on a digestion rack in a 4ml mixed acid digester for 2hours. The mixture was cooled down, and then diluted to 50ml with distilled water. 10ml colour reagent (1.06g ascorbic acid, 1.2g ammonium molybdate ($(NH_4)_6Mo_7O_{24}.4H_2O$) and 27mg of potassium antimony oxide tartrate in 180ml distilled water) were added to 1ml of the solution/mixture. This is followed by the addition of 9.5ml concentrated H_2SO_4. The mixture was left to stand

for an hour for the colour to develop. Standard series; 0, 0.5, 1.0, 1.5, 2.0, 2.5 was made and observed at 690nm using a spectrophotometer. A phosphorus standard curve was used to assess the total phosphorus content in the sediment sample.

3.2.5.4. Heterotrophic Bacteria

In each sediment samples collected, a 1g of sediment was removed to make serial dilutions. About 99ml of sterilised filtered seawater was added, mixed and sonicated for 2min using a Cole-Parmer 8890 sonicator. Different dilutions were prepared with the same seawater, and then plated onto seawater agar and marine agar media (Atlas, 1997). The plates were incubated at 30°C for 4 days, and the colonies were counted and expressed as colony forming unit (cfu/mg).

3.3. DATA ANALYSIS

3.3.1. Environmental Variables

To find the pattern of multidimensional data of the environmental variables, a Principal Component Analyses (PCA) using PRIMER version 6 software was performed to reduce the number of dimensions with a minimal loss of information. Environmental variables including Sediments Particle Size were transformed in to a square root then normalised. The calculation of environmental variables similarity matrix was based on Euclidean distance. A one-way ANOSIM was used to indentify the significant difference between sites based on their environmental variables.

3.4. RESULTS AND DISCUSSION

3.4.1. Environmental Variables

3.4.1.1. Sediments Analysis

A variation of sediment particle sizes was found at the four sites sampled in the Incomati River Estuary (Figure 3.1). Site E1 was situated in the Oligohaline Zone (0-3 NST), and the main activities around this site were agricultural and human settlement. Site E1 was characterised mostly by fine sand with 46.32%, followed by granules (>2.0mm), and mud and fine sand (<212µm) with 21.31% and 19.6% respectively. The percentage of fine sand particle size was higher towards fresh water where deposition was higher. Site E2 was situated in the Euhaline Zone (3-5NST) and the main activities at this site were fishing, swimming and settlements. The site was mostly characterised by granules sand (>2.0mm) with 32.28%, followed by medium grain size, and mud and fine sand with 30.42% and 14.7%, respectively (Figure 3.1).

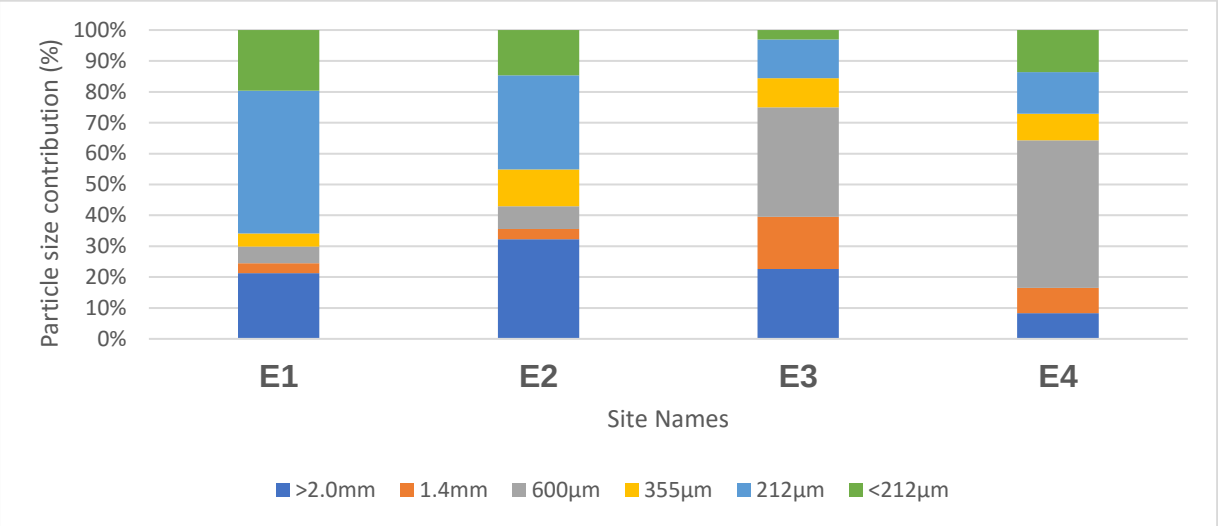


Figure 3.1: Results of sediments particle size analysis in the Incomati River Estuary.

Site E3 was situated in Mesohaline Zone (5-18NST), and the main activities at the site was fishing and informal settlement along the site. The most dominant particle size at this site was coarse to very coarse sand (600µm) with 35.47%, followed by granules (>2.0mm), very coarse sand (1.4mm) with 22.64% and 16.91% respectively. Fine and medium sand particles (212-355µm) contributed 12.56% of sediment particle sizes. Site E4 was situated in the Polyhaline Zone (20-30NST). The main activities at this site were

human settlement and boat fishing. The site was mostly characterised by coarse to very coarse sand particles with 47.79%. The higher percentage of coarse and very coarse particle size at both sites E3 and E4 was due to tidal action that washes the sand from small particles. Sediment grain sizes are important environmental factor especially that also help in the structuring of meiofauna.

To identify similarity and dissimilarity amongst the four sites sampled, a Bray-Curtis Cluster Analysis was used. The cluster analyses of the sites sampled (Figure 3.2), indicated similarity between sites E1 and E2 which were situated in the Oligohaline and Euhaline Zones.

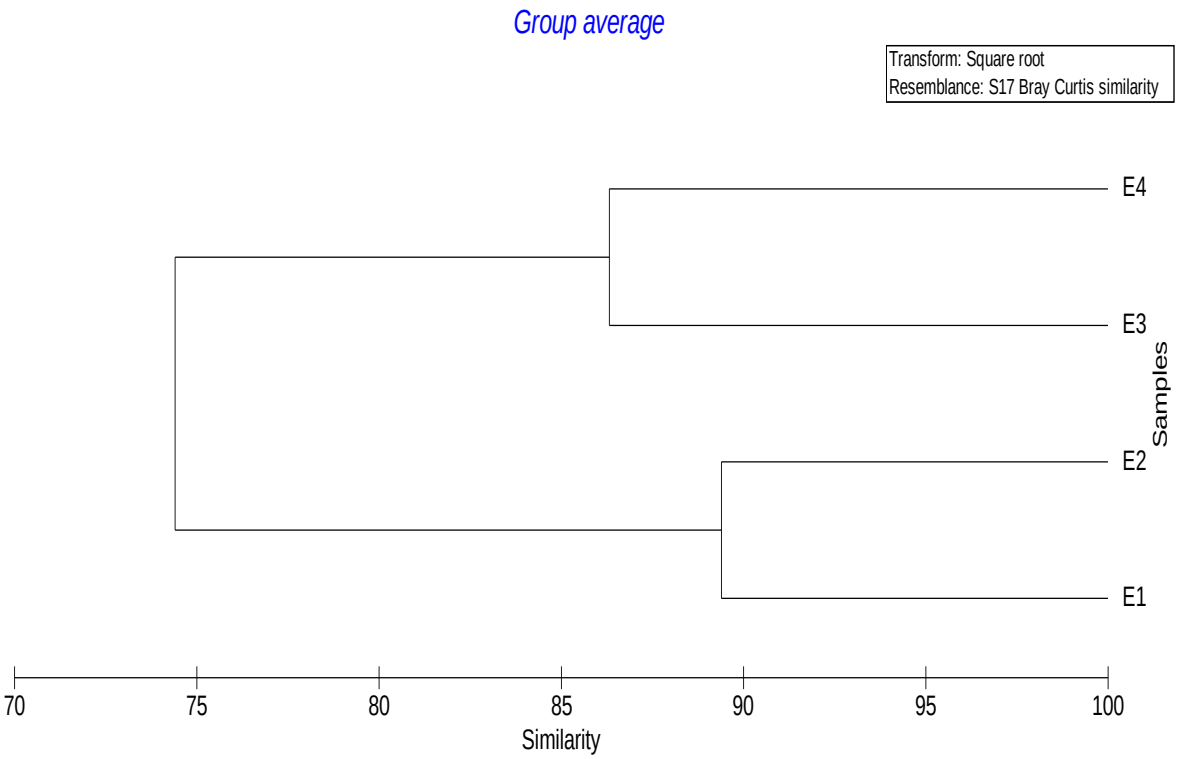


Figure 3.2: A Bray-Curtis Cluster Analysis indicating the Similarity and Dissimilarity between the sites.

Another similarity of sites E3 and E4 was also observed. Hence the sites that were situated in both Oligohaline and Euhaline Zones were separated from the sites situated in Mesohaline and Polyhaline Zones. From the sites sampled, there was no significant difference between them when using a two-way ANOVA ($p>0.05$). The result indicated that sediment composition in terms of particle size of the sites was not very

heterogeneous. Sediment particle size are well known to play a role in the distribution of Metals and meiofauna in estuarine environments.

3.4.1.2. Metal Analyses

The concentrations of ten metals (cadmium, cobalt, chromium, copper, iron, manganese, nickel, vanadium, zinc, and aluminium) were found to be above detectable limits (Annexure 1: Table 3.1) in the Estuary. A box and whisker plots were used to summarise the data for the metals. In all ten metal concentrations, median, mean and various percentiles were calculated (Figures 3.3 to 3.12). The median of the data is the horizontal bold line of the box, the top and bottom of the boxes are the 25^{th} and 75^{th} quantiles (percentiles) of the boxes. The ends of the box and whisker plots are the minimum and maximum values or the 10^{th} and 90^{th} percentiles.

Vanadium

Concentration of vanadium was found to vary between sites. The highest concentration was found at site E2 with a concentration of 5.1 ppm at 25^{th} percentile. Site E1 was the second highest with a concentration of 4.1ppm in the same percentile. Both sites E3 and E4 had low concentration at the 25^{th} percentile. At 75^{th} percentile, the concentration of vanadium was still higher with a concentration of 21.8ppm at site E2. Site E2 had the highest mean value of 12.3ppm and lowest mean concentration was observed at site E4 with a value of 1.43ppm (see Figure 3.3)

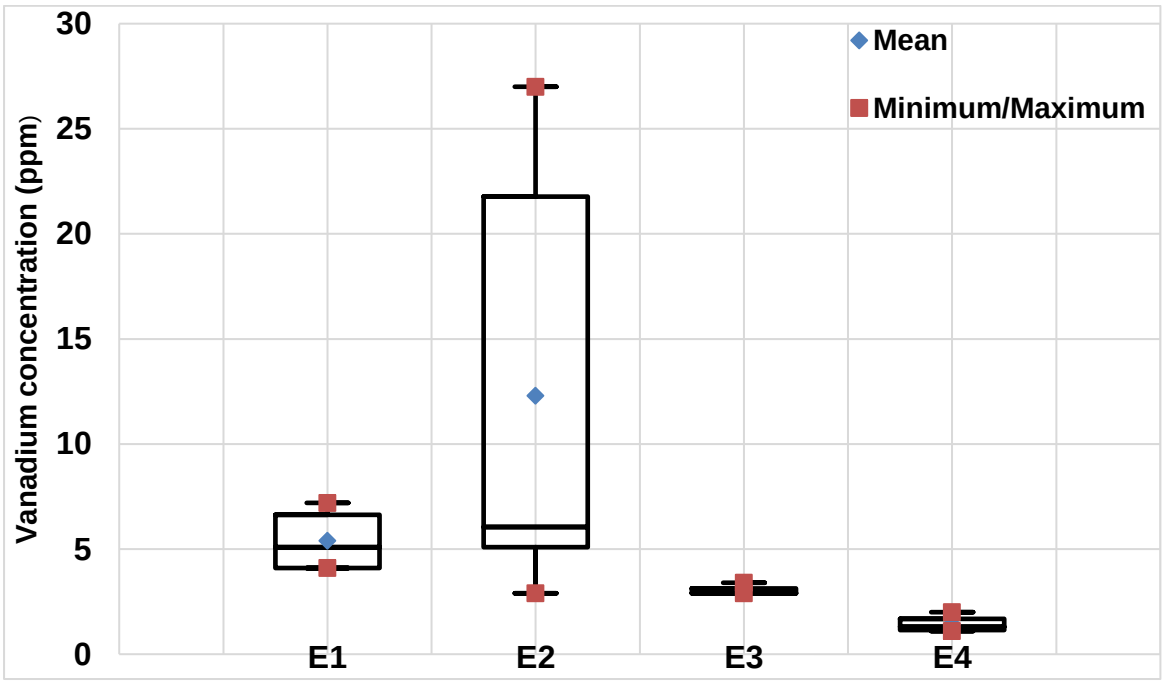


Figure 3.3: Box and whisker plot for vanadium concentration in the Incomati River Estuary.

The upper limit at site E2 was 27 ppm and the lower limit was 2.9ppm. The upper and the lower limit of concentration were found in August 2017, and October 2017 respectively.

Cobalt

Cobalt concentration varied between the sites indicating that the sites received different concentrations of cobalt in the estuary (Figure 3.4). At the 25th percentile site E1 had the highest cobalt concentration with a value of 2.7ppm, followed by sites E2 with a concentration of 1.6ppm, site E3 with concentration of 1.2ppm, and the least concentration was found a site E4, 0.5ppm.

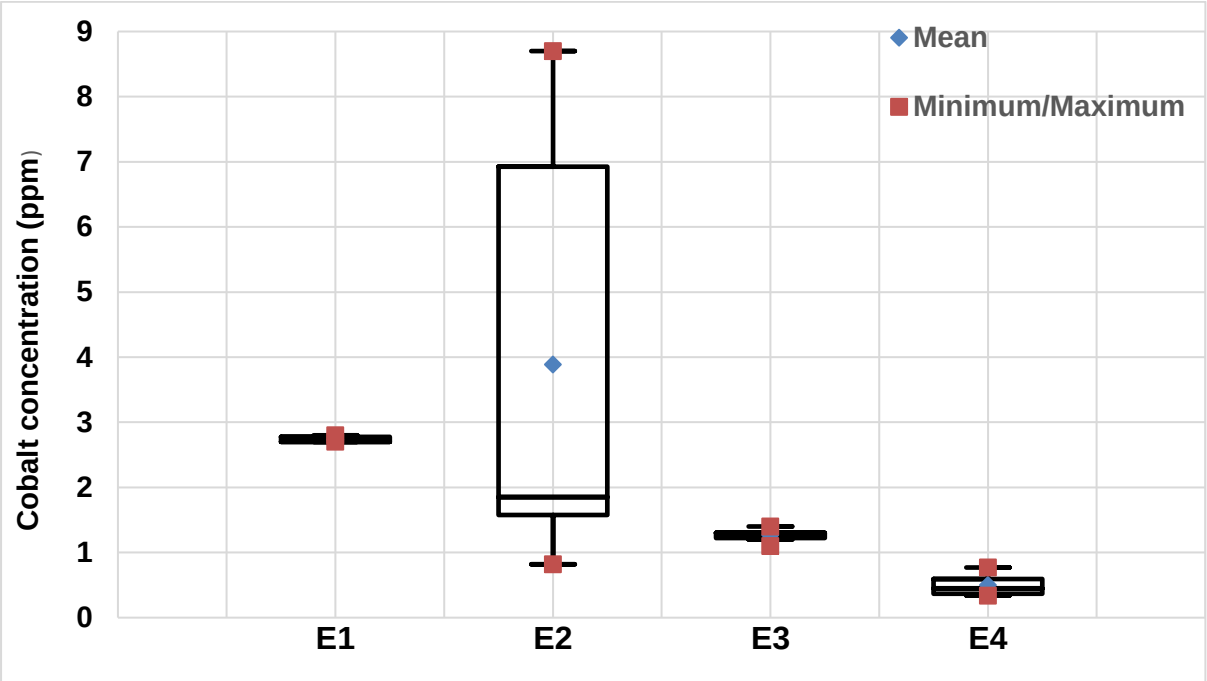


Figure 3.4: Box and whisker plot for cobalt concentration in the Incomati River Estuary.

At 75th percentile site E2 had the highest cobalt concentration with a value of 6.9ppm, with an upper limit of 8.7ppm. The mean concentration at site E2 was 3.9ppm which was also higher, followed by site E1 with a mean concentration of 2.8ppm.

Chromium

Figure 3.5 indicates that the highest mean concentration of chromium was found at site E3 with a mean concentration of 16.8ppm, followed by site E2 with a mean concentration of 14.9ppm, site E1 with a mean concentration of 8.2ppm, and site E4 with a mean concentration of 7.0ppm.

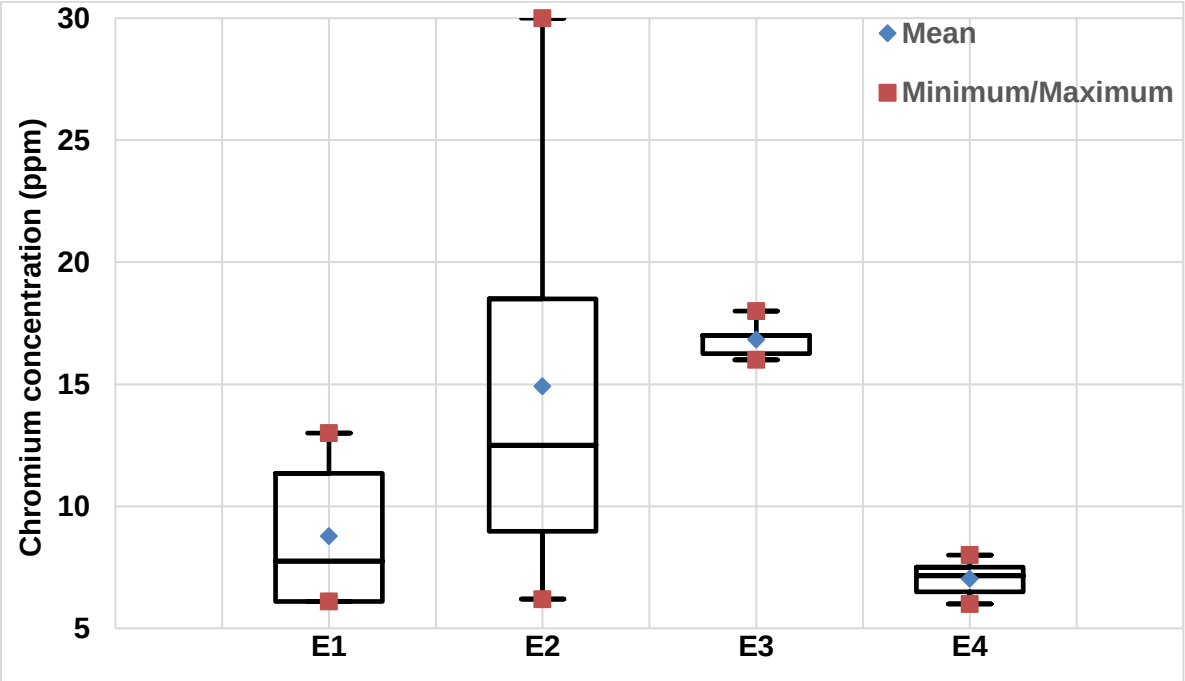


Figure 3.5: Box and whisker plot for chromium concentration in the Incomati River Estuary.

The data indicated that at 25th percentile site E3 had a concentration of 16.3ppm, followed by site E2 with a concentration of 8.9ppm, site E1 with a concentration of 6.5ppm. At 75th percentile site E2 had the highest concentration of chromium with a value of 18.5ppm. The data indicated that the distribution of chromium in the study was between sites E1, E2, and E3.

Zinc

The highest concentration of zinc was observed at site E1 with a concentration mean of 14.3ppm (Figure 3.6). Site E2 had a mean concentration of 12.6ppm. Site E3 and E4 had a mean concentration of 6.8ppm and 7.2ppm respectively. At 25th percentile, site E1 had the highest concentration of zinc with a value of 14ppm, followed by sites E2, E3, and E4 with concentrations of 5.95ppm, 4ppm, and 3.7ppm in the order (Figure 3.6).

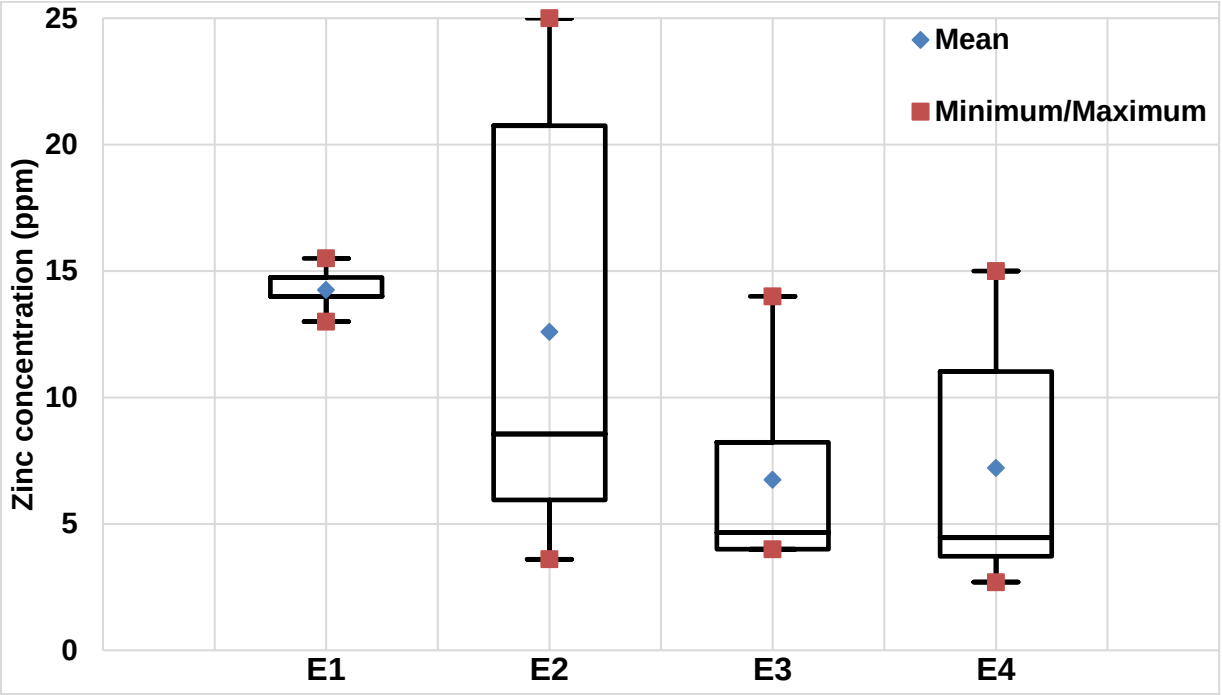


Figure 3.6: Box and whisker plot for zinc concentration in the Incomati River Estuary.

At 75th percentile, site E2 had a concentration of 20.8ppm which was the highest zinc concentration, followed by site E1 with a concentration of 14.8ppm, site E4 with a concentration of 11.05ppm, and site E3 with a concentration of 8.2ppm. There was not much difference between sites E3 and E4 as their median were similar.

Iron

Iron concentration varied between the sites sampled, and site E2 had the highest mean concentration of with a concentration of 9125.2ppm. Site E2 had the second highest concentration of iron with a mean of 3630ppm, followed by site E3 and E4 with mean concentration of 2188.5ppm and 1537ppm in that order.

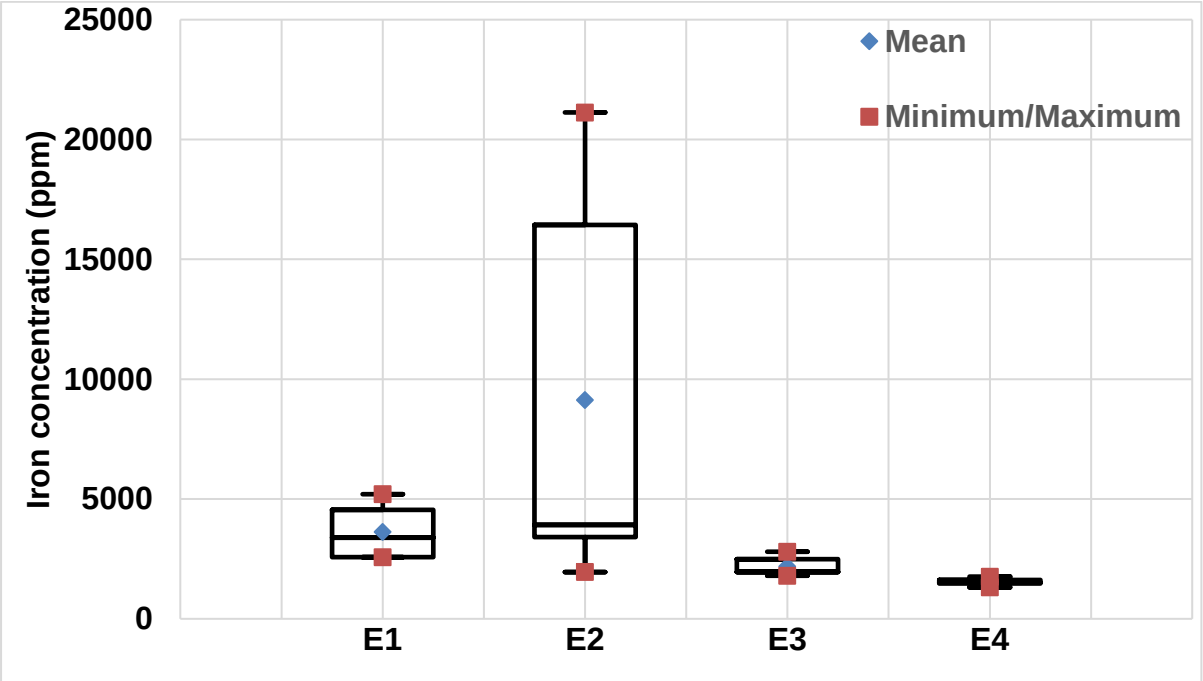


Figure 3.7: Box and whisker plot for iron concentration in the Incomati River Estuary.

A lower limit of Iron concentration at site E1 was 2574ppm, at site E2 was 1952ppm, at site E3 was 1799 ppm and at site E4 was 1315ppm. Sites E1 and E3 had upper limits which were outliers. The upper limit at site E2 was 21130ppm which was the highest upper limit concentration observed in August 2017. At 25th percentile Iron concentration at site E2 was 3406.8ppm, at site E3 was 1952ppm and at site E4 was 1513ppm. At 75th percentile site E2 had the highest concentration with a value of 16430.8ppm.

Copper

There was no significant difference between sites E3 and E4 because their median was similar. The mean concentration at the sites sampled was 4.7ppm at site E1. Site E2 had the highest mean concentration of copper with a value of 7.9ppm, and sites E3 and E4 had mean values of 4.0ppm and 4.1ppm, respectively.

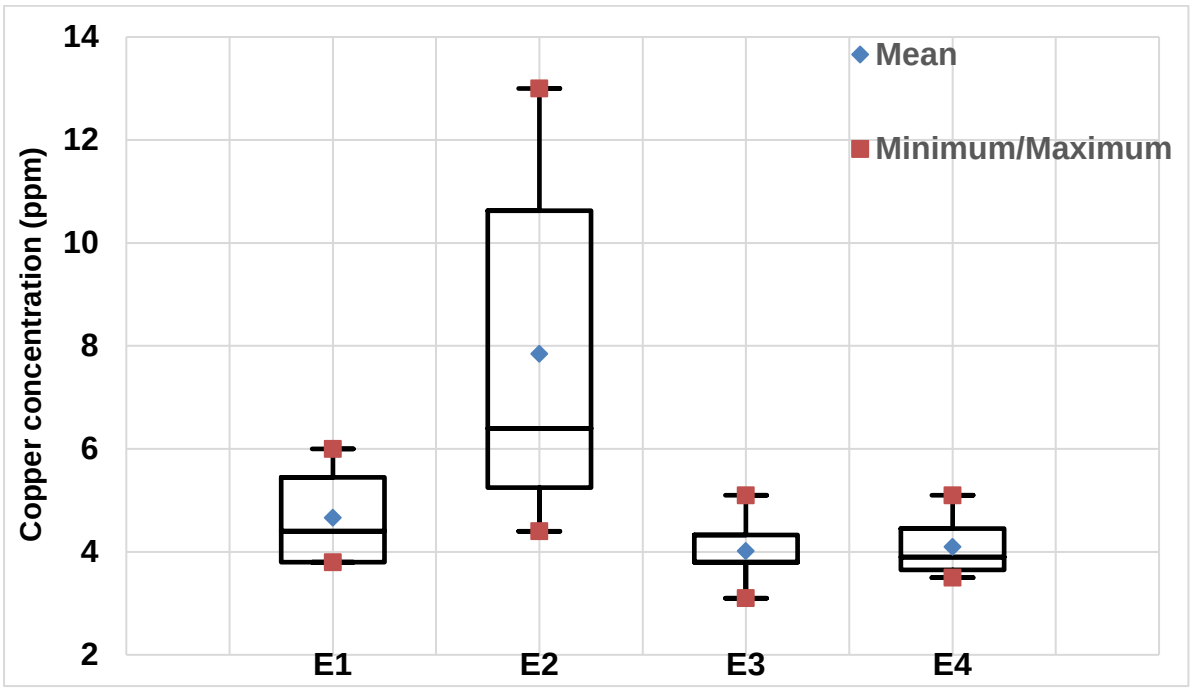


Figure 3.8: Box and whisker plot for copper concentration in the Incomati River Estuary.

At the 25th percentiles, both sites E1 and E3 had copper concentration of 3.8ppm, and sites E2 and E4 had concentrations of 5.3ppm and 3.7ppm in that order. At the 75th percentiles copper concentration at site E1 was 5.5ppm, at site E2 was 10.6ppm, at site E3 was 4.3ppm and at site E4 it was 4.5ppm. The lower and the upper limits of copper concentration at site E2 ranged from 4.4ppm to 13 ppm and this was the only site with a higher concentration of copper during the study, followed by site E1.

Aluminium

The lower and upper limit concentration of aluminium at site E2 were 2236ppm and 16945ppm respectively. The upper limit was observed in August 2017, and the lower limit was observed in October 2017. The mean concentration of aluminium at site E1 was 3844ppm, at site E2 was 7935ppm, at site E3 it was 1671ppm and at site E4 was 904ppm.

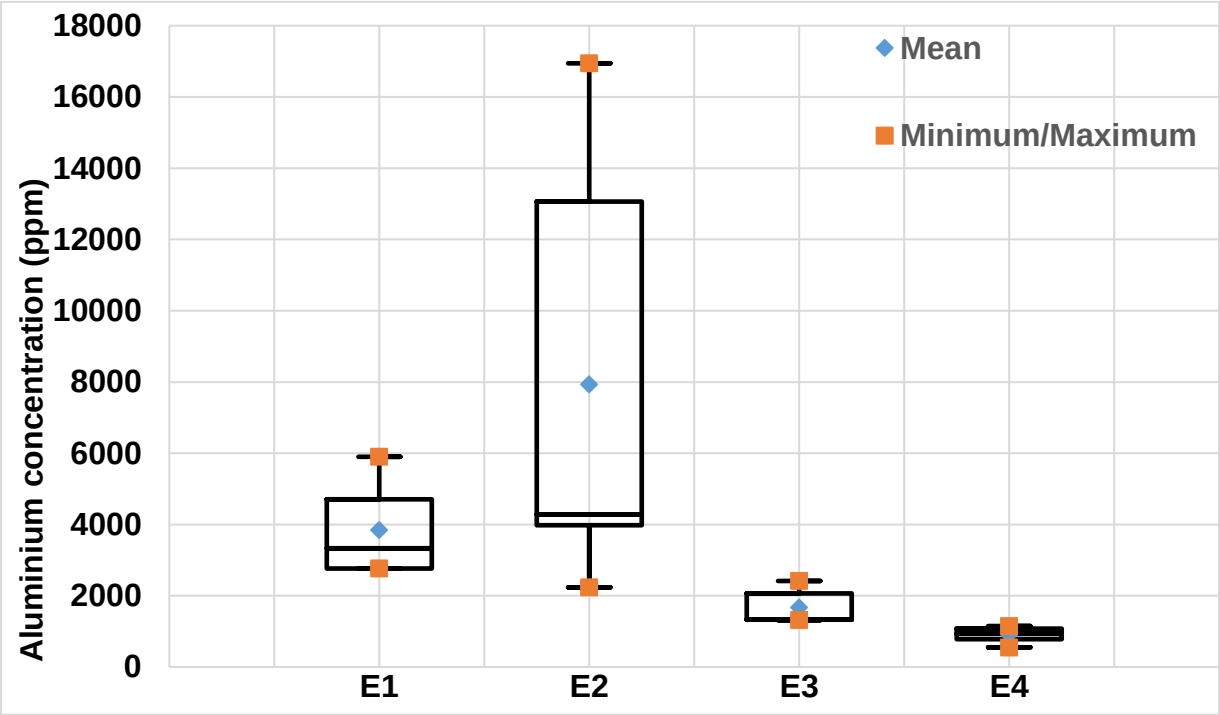


Figure 3.9: Box and whisker plot of aluminium concentration in the Incomati River Estuary.

At the 25th percentiles site E1 had a concentration of 2764ppm, site E2 had a concentration of 3978.3ppm, site E3 had a concentration of 1316ppm, and site E4 had a concentration of 779ppm. At the 75th percentile site E2 had the highest Iron concentration of 13062.5ppm which was higher than the other sites sampled.

Nickel

The highest mean concentration of nickel was observed at site E2 with a concentration of 11.9ppm and the lowest was observed at site E4 with concentration of 3.5ppm.

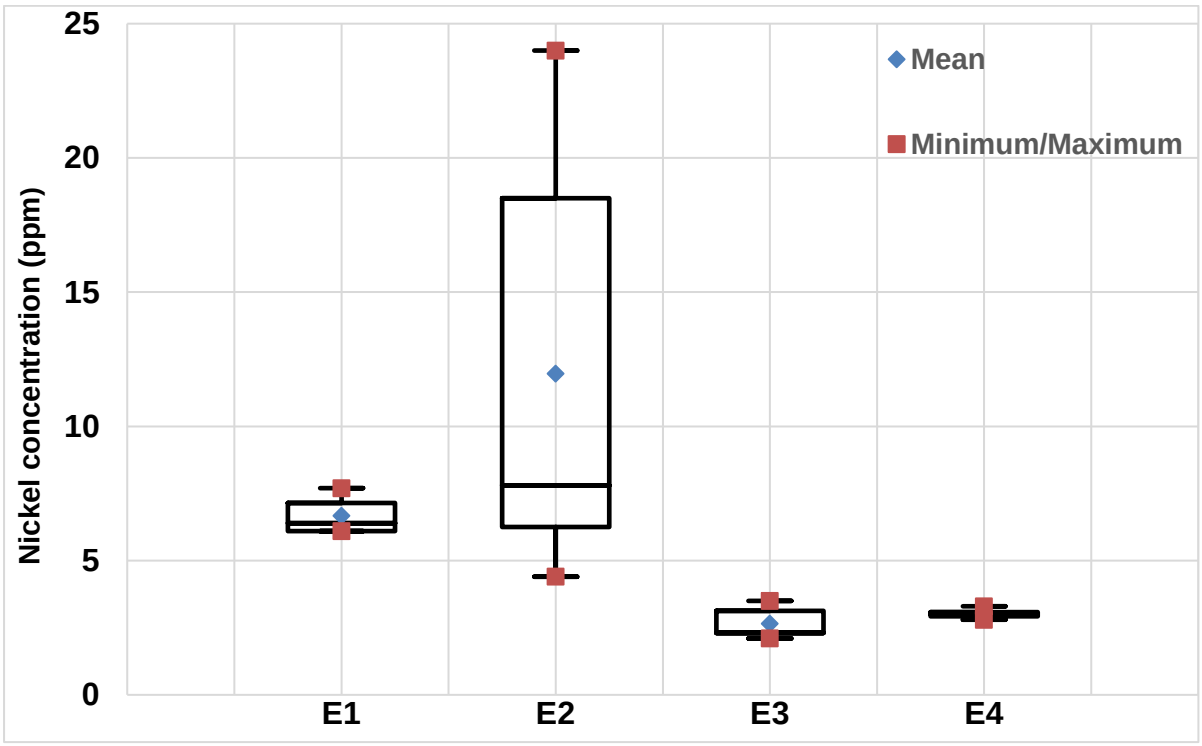


Figure 3.10: Box and whisker plot for nickel concentration in the Incomati River Estuary.

At 25th percentiles site E1 had a concentration of 6.1ppm, site E2 had a concentration of 6.3ppm, site E3 had a concentration of 2.1ppm, and site E4 had a concentration of 2.8ppm. At the 75th percentiles site E2 had the highest concentration with a concentration of 18.5ppm which was higher than the concentration at the other sites.

Manganese

The lower and upper limits concentration of manganese at sites E1 was 68ppm and 242ppm, while at site E2 the lower and upper limits were 55ppm and 391ppm respectively. The highest mean manganese was observed at site E2 with a concentration of 194ppm, followed by site E1 with a mean value of 123ppm.

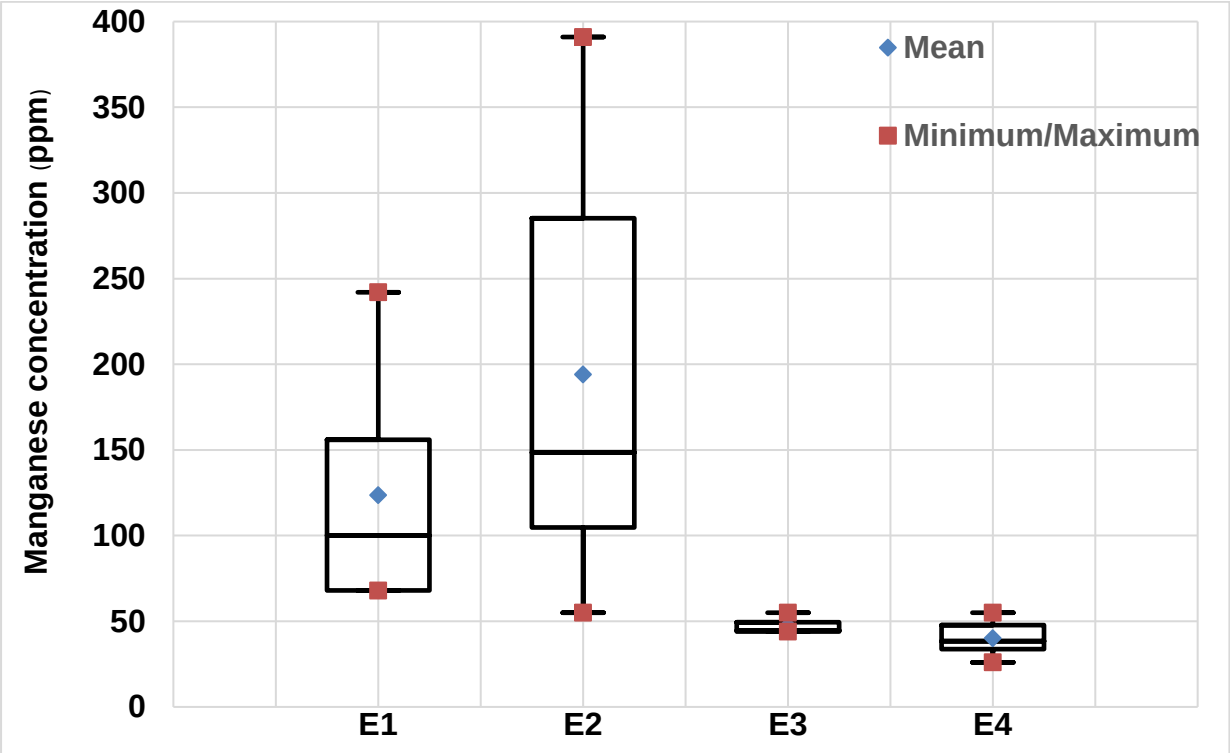


Figure 3.11: Box and Whisker plot for Manganese concentration in the Incomati River Estuary.

The concentration of manganese at 25th percentile was 68ppm at site E1, 104.8ppm at site E2, 44ppm at site E3, and 33.8ppm at site E4. Site E2 had the highest concentration of manganese at the 75th percentile with a concentration of 285.3ppm, followed by site E2 with a concentration of 156ppm. Site E2 had the highest concentration of manganese in the study period.

Cadmium

There was a variation of data between the sites sampled. The highest mean concentration of cadmium was observed at site E2 with a concentration of 0.17ppm and the lowest was observed at site E4, 0.09ppm.

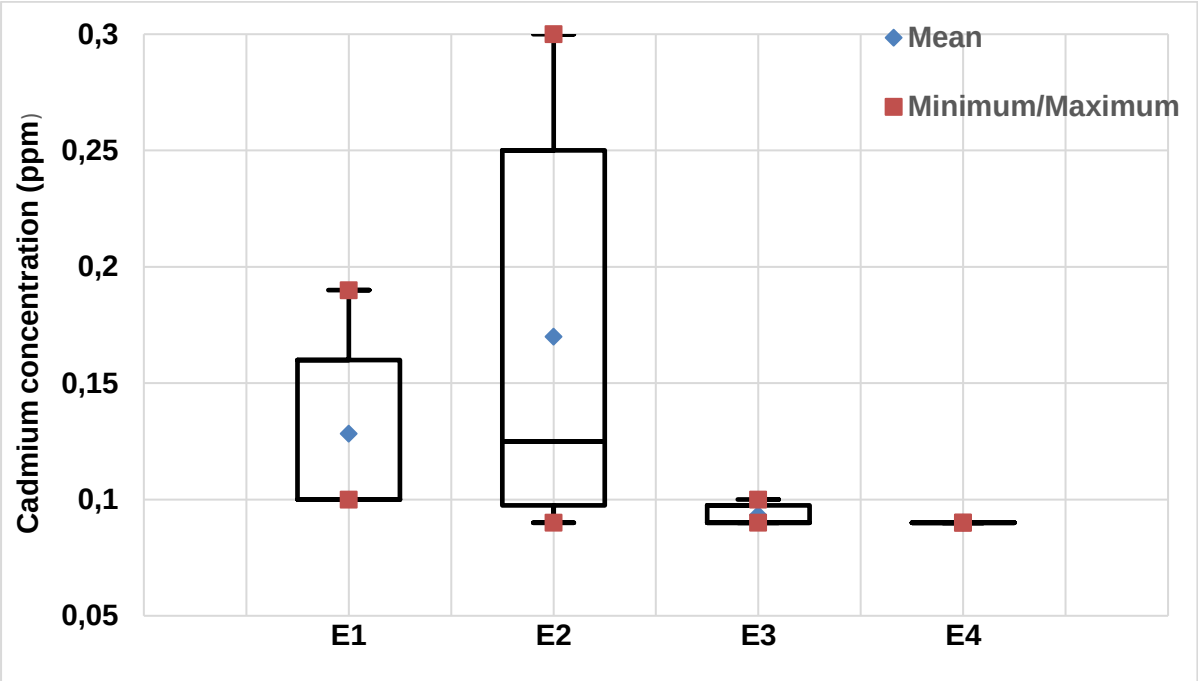


Figure 3.12: Box and whisker plot for cadmium concentration in the Incomati River Estuary.

At 25th percentiles site E1 had a concentration of 0.1ppm, site E2 had a concentration of 0.097ppm, site E3 had a concentration of 0.09ppm, and site E4 had a concentration of 0.09ppm. At the 75th percentiles site E2 had the highest concentration of 0.25ppm which was higher than the concentration at the other sites.

PERMANOVA results indicated that there was a significant difference (p<0.05) between sites sampled, but not between months sampling. These results showed that Metal concentration in sediments increase gradually over time. The higher concentration of metal in the study area especially at sites E1 and E2 was attributed to different anthropogenic activities from the upper catchments and local informal settlements. In South Africa, there are different anthropogenic activities such as mining, industries and agricultural activities which pose a threat in the upper catchment, as well as the estuary. In Mozambique there are smelter industries taking place in the Maputo Bay which were also believed to have influence in the study area.

3.4.1.3. Organic Matter

The highest percentages of organic matter in the study period were found at site E2. The percentage of organic matter at site E2 ranged from 1% to 4% with a mean value of 2%, and the months of June 2017 and October 2017 were found to have the highest percentages (Figure 3.13). The higher percentage of organic matter at this site was attributed to the presence of fine sand particles which have a higher surface area for organic adsorption (Parsons *et al*.1990; Patricio *et al*. 2009). The lowest percentage of organic matter was found at site E1 with a range of 1% to 2%. The mean percentage organic matter at site E1 was 1.2%.

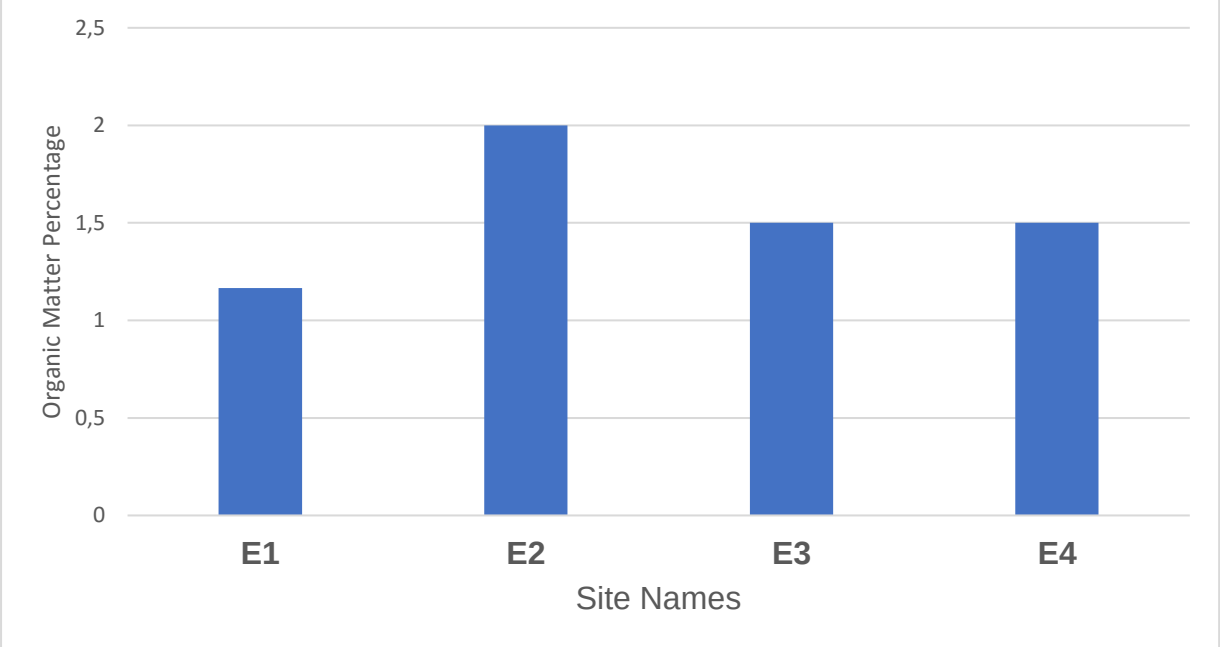


Figure 3.13: A graph representing the organic matter concentration in the Incomati River Estuary.

Sites E3 and E4 had a similar mean percentage of 1.5%. At site E3, the percentage of organic matter ranged from 1 to 3%. The months of December 2017 and February 2018 were found to have higher percentage of organic matter, while at site E4 the percentage of organic matter ranged from 1% to 2%. A one-way ANOVA indicated that there was no significant difference ($p>0.05$) of organic matter concentration between the sites sampled.

3.4.1.3. Total Phosphates

A variation in total phosphate concentrations was observed at the study area during the sampling months (Figure 3.14). Site E2 had the highest concentration of total phosphate. The concentration of total phosphate at site E2 ranged from 36ppm to 300ppm, with a mean of 134ppm. Site E1 had the second highest concentration of total phosphate ranging from 27ppm to 110ppm, with a mean of 50.8ppm (see Figure 3.14).

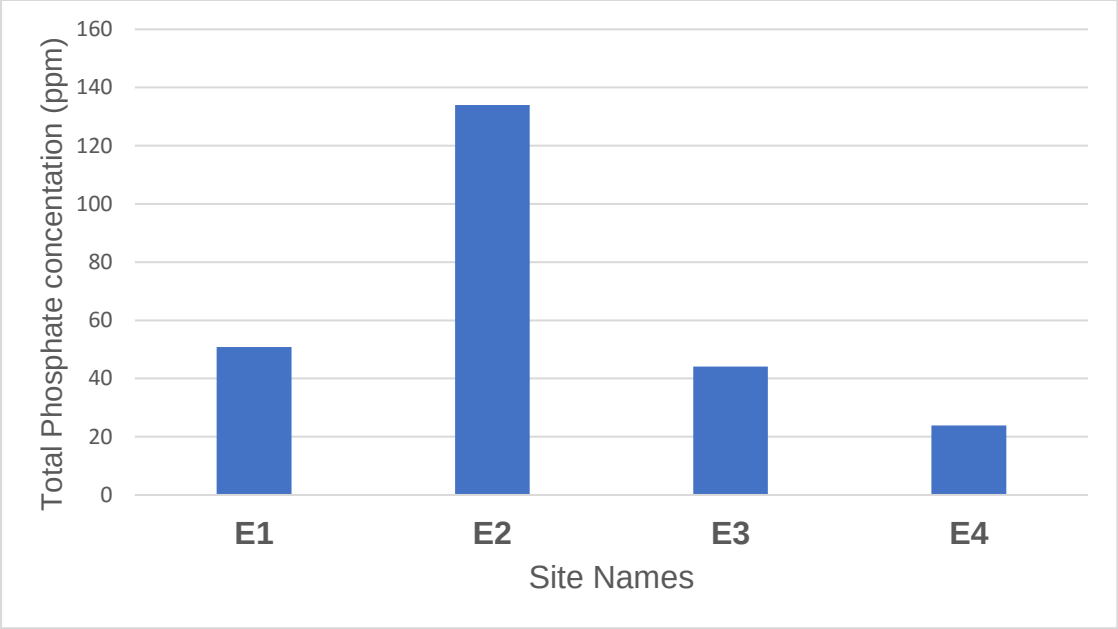


Figure 3.14: Total Phosphate concentration in the Incomati River Estuary.

The mean concentration of total phosphate at site E3 was 44.2ppm whilst site E4 recorded 27ppm. The highest concentration of total phosphate in the study was attributed to agricultural activities taking place upstream of the sites.

3.4.1.4. Nitrate (NO_3)

As indicated in Figure 3.15, the lowest concentration of nitrates in the study was observed at site E2 with a mean concentration of 0.01mg/l, and its concentration didn't change throughout the sampling months. This was the same case at site E1 which remained constant throughout the sampling period with a mean concentration of 0.05mg/l. Nitrates concentration started to increase from sites E3 to site E4 (Figure 3.15).

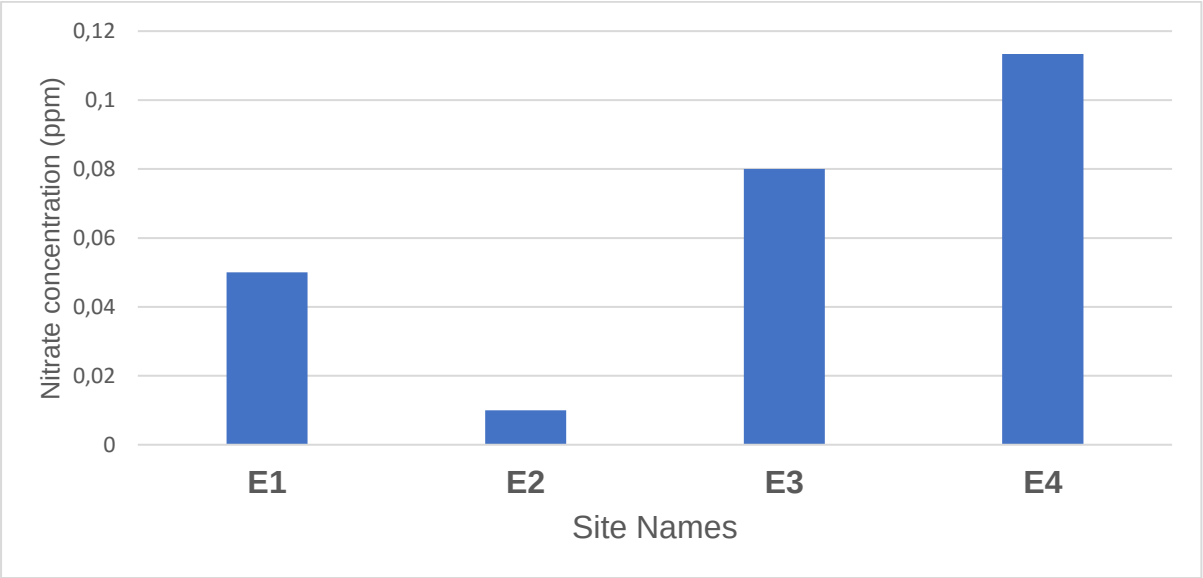


Figure 3.15: Nitrates concentration in the Incomati River Estuary.

At site E3, there was variation in nitrates concentration ranging from 0.07mg/l to 0.1mg/l with a mean concentration of 0.08mg/l. Similarly, at site E4, there was a variation in nitrate concentration. It ranged from 0.06mg/l to 0.14mg/l, with a mean concentration of 0.11mg/l. The high concentration of nitrates in high salinity zone sites E3 and E4 was believed to be from effluent from septic tanks used by lodges and informal settlements. Other source of nitrates in the study area were agricultural activities along the Incomati River Estuary.

3.4.1.5. Chlorophyll-*a*

Chlorophyll-*a* analysis from sediments was conducted in all four sites sampled bi-monthly Figure 3.16. The highest chlorophyll-*a* concentration in the estuary was found at site E3. Chlorophyll-*a* concentration at site E3 ranged from 0.7mg/cm^3 to 4.9mg/m^3, with mean concentration of 3.2mg/m^3.

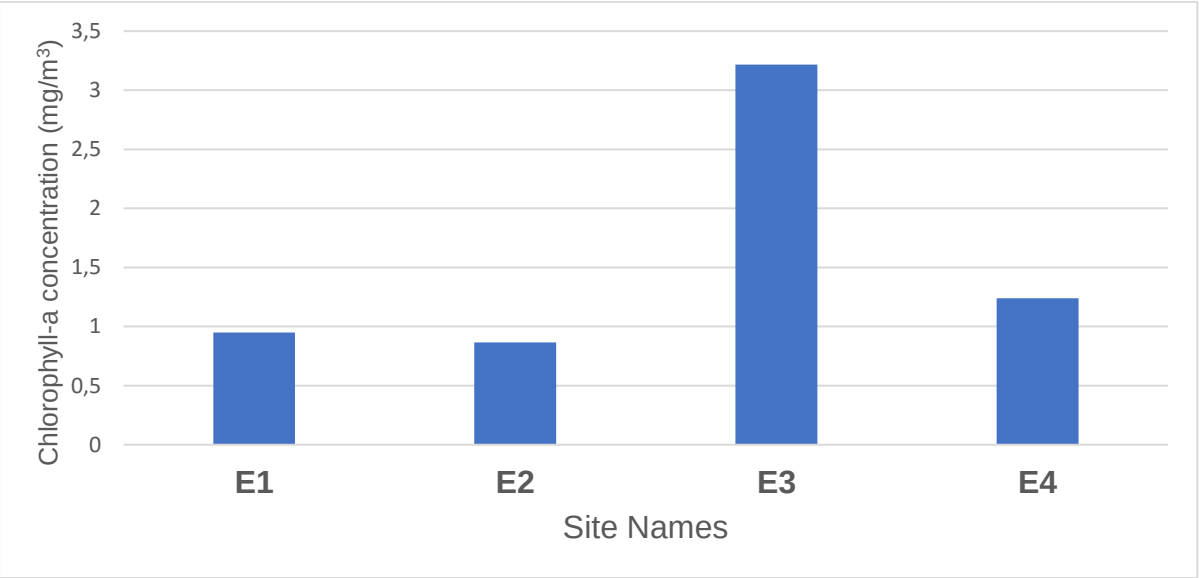


Figure 3.16: Chlorophyll-a concentration in the Incomati River Estuary.

Chlorophyll-*a* concentration at site E1 ranged from 0.6mg/m^3 to 1.5mg/m^3, and the mean concentration was 0.95mg/m^3. The mean concentration of chlorophyll-*a* at site E2 was 0.87mg/m^3 with a concentration range from 0.5mg/m^3 to 1.6 mg/m^3. At site E4 chlorophyll-*a* concentration ranged from 0.04mg/cm^3 to 2.2mg/m^{-3} with a mean concentration of 1.24mg/m^3. The higher concentration of chlorophyll-*a* at site E3 followed by site E4 indicated a potential fresh carbon source for bacteria and meiofauna.

3.4.1.6. Heterotrophic Bacteria

Heterotrophic bacteria from sediment were counted but not identified to genus level as that was not the focus of this study. The highest number of heterotrophic bacteria were found at site E1 with a count of 1x10^3cfu/mg to 1.2X10^7cfu/mg, and a mean value of 6.34X10^6cfu/mg (Figure 3.17). The second highest count of heterotrophic bacteria were found at site E2 with a mean value of 6.07X10^6cfu/mg.

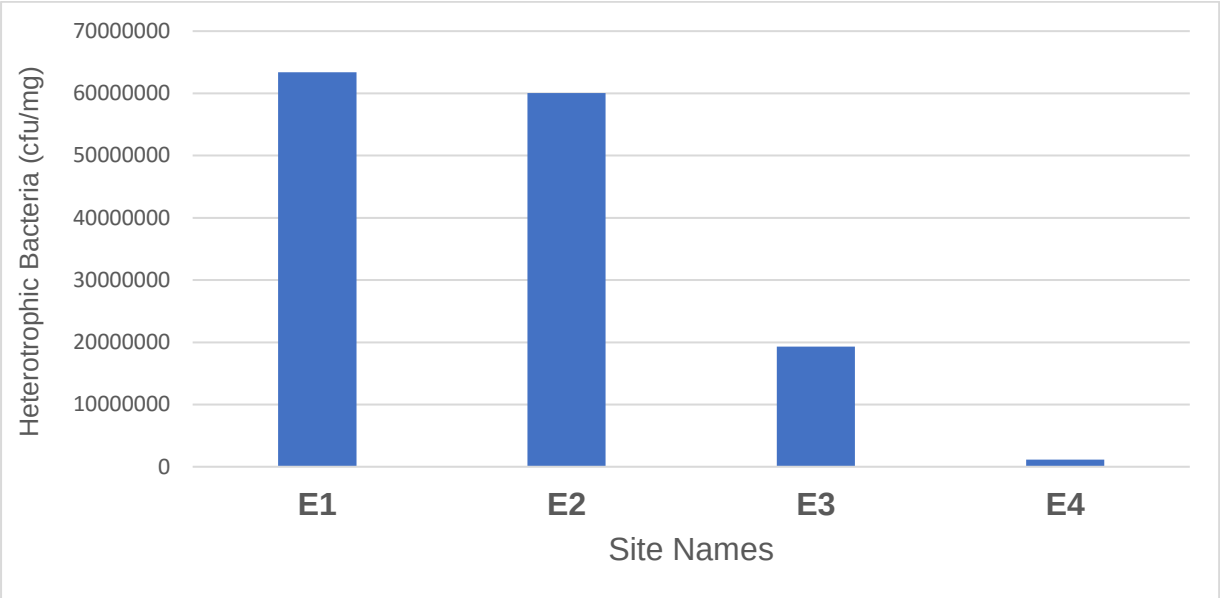


Figure 3.17: Monthly mean heterotrophic bacteria counts in the Incomati River Estuary.

The lowest counts of heterotrophic bacteria were found at site E4 with a range of $2.4X10^5$cfu/mg to $1X10^2$cfu/mg, and a mean value of $1.13X10^5$cfu/mg, followed by site E3 with heterotrophic bacteria mean value of $1.93X10^6$cfu/mg. This result indicated that heterotrophic bacteria in this study decreased towards the sea or towards higher salinity.

A PRIMER version 6, using Principal Component Analyses (PCA) was used to identify the spatial patterns of environmental factors. The PCA ordination constructed from the environmental factors showed that the first two components accounted for 71.9% of the variability in the data (Figure 3.18). The first and the second Axis explained 54.5% and 17.4% respectively. The PCA shows the eigenvector numbers graphically, with most environmental factors increasing towards site E2 (Euhaline Zone). In contrast, nitrates, gravel, fine medium sand, zinc, fine sand and chromium all had the large eigenvector on the PCA2 axis, thus their vectors increased on the PCA2. Fine sand, fine medium sand and zinc increased on the direction of the Oligohaline Zone at site E1. Nitrates and coarse very coarse sand increased towards the Polyhaline Zone (E4) and chlorophyll-*a* and very coarse sand increased towards the Mesohaline Zone (E3). The PCA analyses lastly indicated the separation of sampling sites or Zones within the Estuary and the ANOSIM formally confirms this with an overall ANOSIM R of 0.501, reflecting the pairwise R values of the sites.

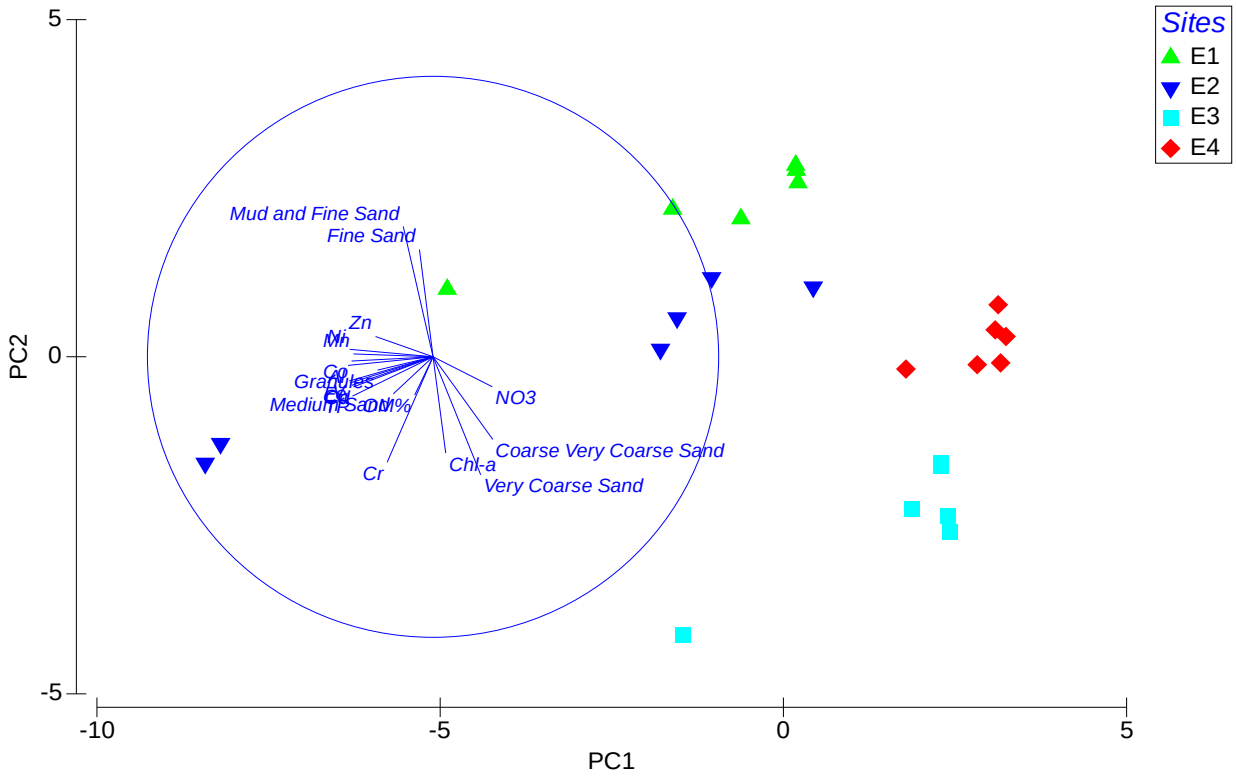


Figure 3.18: Principal Component Analyses indicating the spatial pattern of environmental variables in the Incomati River Estuary.

A strong correlation of sediment particles size such as granules with metals such as cobalt (r=0.87), copper (r=0.83), cadmium (r=0.88), iron (r=0.88), manganese (r=0.77), nickel (r=0.77), vanadium (r=0.92), aluminium (r=0.88); medium sand with metals such as cadmium (r=0.76), cobalt (r=0.52), copper (r=0.88), iron (r=0.86), manganese (r=0.76), nickel (r=0.70), vanadium (r=0.78) and aluminium (r=0.74) were observed. The positive correlation between metals and sediments particle size indicated that metals were distributed based on sediment particles sizes. A study conducted by Cox and Preda (2005) found that particle size, such as fine sediment contributes to the distribution of metal by giving enough specific areas for metal attachments. Another strong correlation between organic matter with metals such cadmium (r=0.56), copper (r=0.72), iron (r=0.69), vanadium (r=0.59) and aluminium (r=0.53) was also found. In another study conducted at Mhlathuze Estuary, Mzimela *et al.* 2014, also found high concentration of Metals at a site characterised by higher organic content.

3.5. CONCLUSION

The aim of this chapter as to use statistical analysis to examine the distribution of sediment particle size, metals, nutrients, organic matter, bacteria and chlorophyll-*a,* which were believed to play a role in structuring free-living nematodes in the study area.

Sediment particle size were found to vary from site to site. A Bray-Curtis cluster analyses showed that there was both similarities and dissimilarities between sites at different percentages of groupings. A two-way analysis of variance indicated that there was no significance difference between sites sampled on monthly basis.

A significant difference ($p<0.05$) of metals was found between sites, and the highest concentration was found at site E2. A strong correlation between metals, total organic matter with certain sediment particles size was established. This finding indicated that metals were distributed according to sediment particle sizes, and that total organic matter also played a role in the distribution.

Total phosphate concentration was found to be higher at site E2 while nitrate (NO_3) concentration was found to be higher at sites E3 and E4 which was believed to be due to agricultural activities, informal settlement and tidal wave in the estuary.

Chlorophyll-*a* concentration which was found to be higher at sites E3 and E4 indicated a potential fresh carbon source for meiofauna at these sites. The highest number of heterotrophic bacteria was observed at site E1 and decreased towards the sea.

A principal component analyses indicated that there was a strong correlation between nitrates and chlorophyll-*a*, and total phosphate and organic matter in the estuary. Different environmental factors play different roles in meiofauna, especially, nematodes structuring in the estuary.

CHAPTER 4

FEEDING TYPES AND LIFE HISTORY STRATEGY OF FREE-LIVING NEMATODES IN THE INCOMATI RIVER ESTUARY.

4.1. INTRODUCTION

Natural and anthropogenic stressors have been the results of increasing pressures to the marine ecosystem worldwide (Dauvin, 2007). Estuaries receives many pollutants from anthropogenic activities such as agricultural and industrial effluents (Lillebø *et al*. 2005; Paerl, 2006). It is difficult to distinguish between anthropogenic impacts and the natural variables in field investigation (Tietjen, 1977; Platt *et al*.1984; Travizi and Vidakovic, 1994). But several studies have highlighted nematodes sensitivity to various kind of anthropogenic disturbances which changes the nematodes assemblage structures, taxonomic diversity and functional group (Austen *et al*. 1994; Mirto *et al*. 2002; Schratzberger *et al*. 2002; Fraschetti *et al*. 2006; Moreno *et al*. 2009; Vezzulli *et al.* 2008; Gyedu-Ababio and Baird, 2006). Amongst the biological indicators, meiobenthos communities are important in assessing the response of natural and disturbance gradients (Schratzberger, 2012), because they provide significant morphological characteristics such as mouth structure, tail shape and length width ratios, which are related ecological function (Wieser, 1953; Thistle and Sherman, 1985; Thistle *et al.*1995; Jensen, 1987; Vanaverbeke *et al*. 2003; Vanaverbeke *et al.* 2004). Another important ecological characteristic of free-living nematodes is the life history strategy, as it gives information of the condition of the habitats (Bongers, 1990).

Interpretation of nematodes feeding behaviour is generally based on stoma and pharyngeal morphology (Wieser, 1953). Nematode feeding groups and types are classified according to their buccal cavity structure. The following are the four-trophic groups of marine nematodes used by marine and estuarine nematologists (Wieser, 1953). **Group 1A:** this group includes all the nematodes with small buccal cavities and have no teeth. The group is also seen to be selective deposit feeders as they just suck soft food into the intestine. **Group 1B:** this group represent nematodes with a cup-shaped cylindrical buccal cavity with no teeth. It is also a non-selective deposit feeding group, assisted by lips and anterior part of the buccal cavity. **Group 2A:** nematodes belonging to this group have buccal cavities armed with small teeth. They eat food by

scraping it off from the surface or cells are pierced and the cell fluid is suck out. **Group 2B:** this group has a large buccal cavity, armed with large teeth or stylet. Nematodes in this group are predacious, feeding by ingesting the prey whole or piercing the prey over the large teeth. These feeding groups designated by Wieser, 1953, are used to investigate the trophic composition of nematodes assemblage genera.

Free-living nematodes provide several advantages for their use as monitoring organisms (Kennedy and Jacoby, 1999; Schratzberger *et al*. 2000; Alves *et al*. 2013). In several studies Coull and Chandler (1992); Somerfield *et al*. (1994); Austen and Somerfield *et al*. (1997) and Kennedy and Jacoby (1999) meiofauna and nematodes were useful indicators in the evaluation of the environmental impacts of human disturbance. Before the Maturity Index came in to play simple indices of abundance, proportions, or ratios of nematodes by trophic group were used. The Maturity Index was developed for terrestrial nematodes (Yeates, 1984; Bongers, 1990). Then the Maturity Index was extended successfully to assess the marine and brackish sediments (Bongers *et al*.1991). The use of Maturity Index focuses on the ecological characteristics and reproductive strategies of nematodes and it is therefore important as it provides researchers with an opportunity to assess the ecological health of different habitats. The r-strategies species (c-p1) has been distinguished from the K-strategies species (c-p 5). Studies conducted by Bongers *et al*. (1991); Essink and Keidel, (1998); Fraschetti *at al*. (2006); Moreno *et al*. (2008); Semprucci *et al*. (2013) and Balsamo *et al.* 2012; used Maturity Index in marine ecosystem to assess the impacts caused by metals, organic enrichments and eutrophication, successfully nematodes showed good response.

4.2. MATERIALS AND METHODS

4.2.1 Meiofauna field collection

A 6% $MgCl_2$ was used to rinse the inner diameter of the corer as to allow meiofauna to relax during sediment sampling. Sediment samples were taken from the four sites selected in the salinity gradient of the Incomati River Estuary. In each site, a duplicate of sediment samples was taken using a 1m long PVC corer with an inner diameter of 3.6 cm, corresponding to a surface area of 10 cm^2.

4.2.2 Meiofauna Laboratory analysis

In the laboratory, sediment samples were transferred to centrifugal bottles and weighed. A sucrose solution of 589g prepared in a 1L bottle was added in the centrifugal bottles containing sediments to separate meiofauna from the sediments (Anderson, 1959; Heip *et al*. 1974; Esteves and Saliva, 1998). The sediment samples were centrifuged at 35000rmp for 5 minutes and the supernatant were decanted to another jar. After the supernatant was decanted, the bottles were weighed, and an amount of sucrose solution was added again. The samples were centrifuged for another 5 minutes. The final supernatant was decanted in to the same jar and sieve on a mesh aperture of 1mm followed by a sieve with a mesh aperture of 63um. Detritus and macrofauna were retained in the upper sieve were discarded, and meiofauna were retained by the lower sieve. Meiofauna were then collected in 5% formalin with Rose Bengal for staining and easier identification.

Nematodes were then counted under a stereo microscope at 40x magnification using a counting petri-dish or a sorting tray (Giere, 1993). They were then placed into solution of 5 parts glycerine, 5 parts ethanol and 90 parts distilled water. Finally, nematodes were mounted on a glass slides and identify to genus level using the pictorial keys of Warwick *et al*. (1998). The functional feeding groups designated by Wieser (1953) was used to investigate the trophic composition of nematodes assemblages' genera and the sex of all specimens was examined.

4.3. DATA ANALYSIS

4.3.1. Meiofauna Assemblages

A total meiofauna density and density of individual major meiofauna taxa were calculated for each sampling site. To test the notion that meiofauna communities change over a space and time, a two-way PERMANOVA analysis was performed with sites and month as fixed factors. Meiofauna groups were square root transformed to scale down their densities to increase the importance of less abundance group analysis. A Bray-Curtis similarity matrix was used to perform the PERMANOVA test and the residuals were permutated under a reduced model, with 999 permutations. A significance level of $p<0.05$ indicated that the null hypothesis was rejected. The Monte

Carlo permutation **p** was then used if the number of permutations was lesser than 150. A Non-Dimensional Scaling (NMDS), using Bray-Curtis as a similarity measure was also preformed (Clarke and Gorley, 2006).

4.3.2. Free-living Nematodes Assemblages

4.3.2.1. Nematode feeding types

To investigate the trophic composition of nematodes assemblage, the features of buccal cavity morphology was followed (Wieser 1953). According to this approach, four groups of feeders were defined: Selective deposit feeders **(1A)**, non-selective deposit feeders **(1B)**, epigrowth feeders **(2A)**, and predator or omnivores **(2B)**. The Index of Trophic Diversity was calculated as **ITD=** $\Sigma\theta^2$ where θ was the density contribution of each trophic diversity (Heip *et al.*1985). The ITD was used to compare the sites in terms of sediment contamination by metals.

4.3.2.2. Life history strategy of nematodes

To analyse nematodes life strategy, the Maturity Index was used (Bongers, 1990; Bongers et al.1991), and a value on a scale (**c-p** score) was assigned to nematodes genera. The scale of **c-p** score was according to the nematodes ability for colonizing or persisting in a certain habitat and the index was expressed as a **c-p** values ranging from 1 (extreme colonizer) to 5 (extreme persisters). The **c-p** score represented the life history features related with **r-** and **k-** selection (Bongers and Ferris, 1999). The Maturity Index formula

$$\mathbf{MI} = \sum_{i=1}^{n} \mathbf{v(i)}.\mathbf{f(i)}$$

was used to calculate the weighted average of the individual colonizer-persisters (c-p) values. The following symbols in the formula: **v(i)** represented the c-p value of the taxon, then **i** and **f(i)** was the frequency of that taxon.

4.3.2.3. Statistical analyses of nematodes

Univariate and multivariate statistical analyses were used to assess the structure of nematodes. Nematode abundance, composition, and biological indices such as Margalef's Richness and Shannon-Wiener Diversity Index were used as univariate measures of the community structure using PRIMER 6 software. The significant differences in univariate measures between sites were tested using two-way ANOVA. A multidimensional scaling ordination analyses (MDS) was applied to nematode genera density data transformed through square root, and a Bray-Curtis similarity index was used. The formal significance test for difference between sites and sampling months was performed using the two-way ANOSIM permutation test. The two-way ANONISM permutation test was further used to test the difference between monthly sampling, and sites for the relative abundance of the four feeding types. SIMPER analyses were done to find the dominant genera in the study area. K-dominance curve was plotted for the comparison of species composition at the sampling sites. A RELATE analyses was performed to identify whether the patterns based on environmental variables were significantly related to the pattern inherent in nematodes community both in species community and feeding types. A BIOENV procedure using a spearman's correlation was then used to determine the relationship of nematode species assemblage and functional traits with environmental variables (Clarke and Ainsworth, 1993). A DISTLM analyses based on the AIC model selection criterion was done following a Stepwise selection procedure (Burnham and Anderson, 2004). This analysis was done to identify the set of environmental variables that predicted the multivariate in nematode species assemblages. A distance-based redundancy analysis (dbRDA) was used to visualise that the results (Anderson *et al.* 2008) and a Euclidian distance was used as a resemblance measure in all DISTLM procedure. The above analyses were conducted using PRIMER 6.0 which is a multivariate statistical package developed by Plymouth Marine Laboratory (Clarke and Gorley, 2006).

4.4. RESULTS AND DISCUSSIONS

4.4.1. Meiofauna Assemblages

Meiofauna density and their composition followed a clear pattern along the estuarine salinity gradient in the Incomati River Estuary. During the study period nine meiofaunal taxa (Nematodes, Copepods, Turbellarians, Amphipods, Polychaeta, Kinorhynchia, Oligochaete, Gastrotricha, Ostracods) and other insects were found in the Incomati River Estuary. A total of 6655 meiofaunal specimens were collected from June 2017 to April 2018. A highest meiofauna density of 910 individual/10cm^2 was observed in December 2017 at site E4 which was situated in Polyhaline Zone. Site E3 which was situated in the Mesohaline Zone had the second highest meiofauna density of 631 individual/10cm^2 in December 2017. The lowest meiofauna density of 65 individual/10cm^2 was observed in June 2017 at site E1 which is situated in Oligohaline Zone (Table 1). A highest nematode mean density of 211 individuals/10 cm^2 was found at site E4, and the second highest nematodes mean density was found at site E3 with a mean density of 156 individuals/10cm^2. The lowest nematodes mean densities were found at sites E1 (Oligohaline Zone) and E2 (Euhaline Zone) with mean density of 70 and 74 individuals/10cm^2 respectively. The decrease in meiofauna density from the Polyhaline to Oligohaline Zones was attributed to salinity change along the estuary, and this indicates that meiofauna density in an estuarine environment increases with high salinity. A similar pattern has been reported in subtidal sediments of the Westerschelde Estuary Soetaert *et al*. (1994), and in intertidal sediments in the Chernaya River (Udalov *et al*. 2005).

Table 4.1: Meiofauna community identified along a salinity gradient in the Incomati River Estuary from June 2017 to April 2018

MEIOFAUNA	E1						E2						E3						E4					
	Salinity Range																							
	0-3NST						3-5NST						5-18NST						18-26NST					
	Jun-17	Aug-17	Oct-17	Dec-17	Feb-18	Apr-18	Jun-17	Aug-17	Oct-17	Dec-17	Feb-18	Apr-18	Jun-17	Aug-17	Oct-17	Dec-17	Feb-18	Apr-18	Jun-17	Aug-17	Oct-17	Dec-17	Feb-18	Apr-18
Nematodes	61	110	100	150	200	100	70	160	150	123	157	141	200	200	350	680	252	180	321	250	400	890	422	322
Copepods	7	2	2	1	0	0	0	11	12	6	0	8	8	10	7	32	21	1	7	3	16	13	10	0
Turbellarians	3	9	6	7	1	6	0	3	3	6	0	0	5	0	2	0	0	4	0	3	9	6	8	3
Amphipods	0	2	0	6	1	1	2	0	0	1	9	3	6	2	2	5	0	1	0	0	3	0	0	0
Halocarida	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Polychaetas	2	1	6	4	0	4	4	2	11	12	18	6	0	0	2	0	0	0	0	0	0	2	0	0
Kinorhynchia	0	0	0	2	2	0	3	2	1	0	1	2	2	0	0	0	2	1	7	0	2	2	4	3
Oligochaetes	2	4	0	3	5	0	1	7	8	0	0	0	0	0	0	2	0	0	0	0	0	0	0	0
Gastrotrica	0	0	5	0	3	0	0	3	7	1	0	0	4	5	0	0	3	0	4	2	0	3	0	0
Ostracods	2	0	6	13	0	0	8	9	0	9	2	4	3	11	21	8	8	9	1	2	5	19	6	4
Ciliophora	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Cladocera	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Insects	3	0	7	0	0	0	0	0	0	5	0	0	5	0	0	1	3	0	0	3	0	0	2	0

In all sites sampled, nematodes were the most dominant meiofauna taxa with a density of 6311 individual/10cm^2 and contributed about 92% of the total meiofaunal abundance. Copepods were found to be the second dominant meiofaunal group in the estuary. They had a density of 177 individuals/10cm^2 contributing 2.7% of the total meiofauna group. Ostracods, and Turbellarians contributed 2% and 0.9% of the total meiofauna group respectively. The dominance of free-living nematodes in estuarine environment were also reported in other studies conducted along the French beach (Renaud-Debyser and Salvat, 1963), and Yorkshire sandy coastlines (Gray and Rieger, 1971). In another study conducted in European estuaries (Portugal) similar results were obtained (Patricio *et al*. 2009; Alves *et al*. 2015). These studies found that nematodes were dominant along the estuarine gradient in Mira and Mondego and represented about 95% and 88% of the total meiofauna respectively.

To test the notion that meiofauna composition changes over a space and time, a Bray-Curtis similarity was used to complete a two-way PERMANOVA analysis (Table 4.2). The analyses showed a significant difference of meiofauna in monthly sampling, and at the sites sampled. A higher significant difference of a p value of 0.001 was obtained in monthly sampling, and a significant difference p value of 0.02 was obtained at the sites sampled in the estuary.

Table 4.2: Summary of PERMANOVA results indicating the significant difference between sites, and between Months.

Source	**df**	**SS**	**MS**	**Pseudo-F**	**P (perm)**	**Unique Perms**	**P (MC)**
Months	5	6117.4	1223.5	3.7336	0.001	999	0.0001
Sites	2	1485.1	742.54	2.2659	0.02	998	0.0359
Res	16	5243.1	327.7				
Total	23	12846					

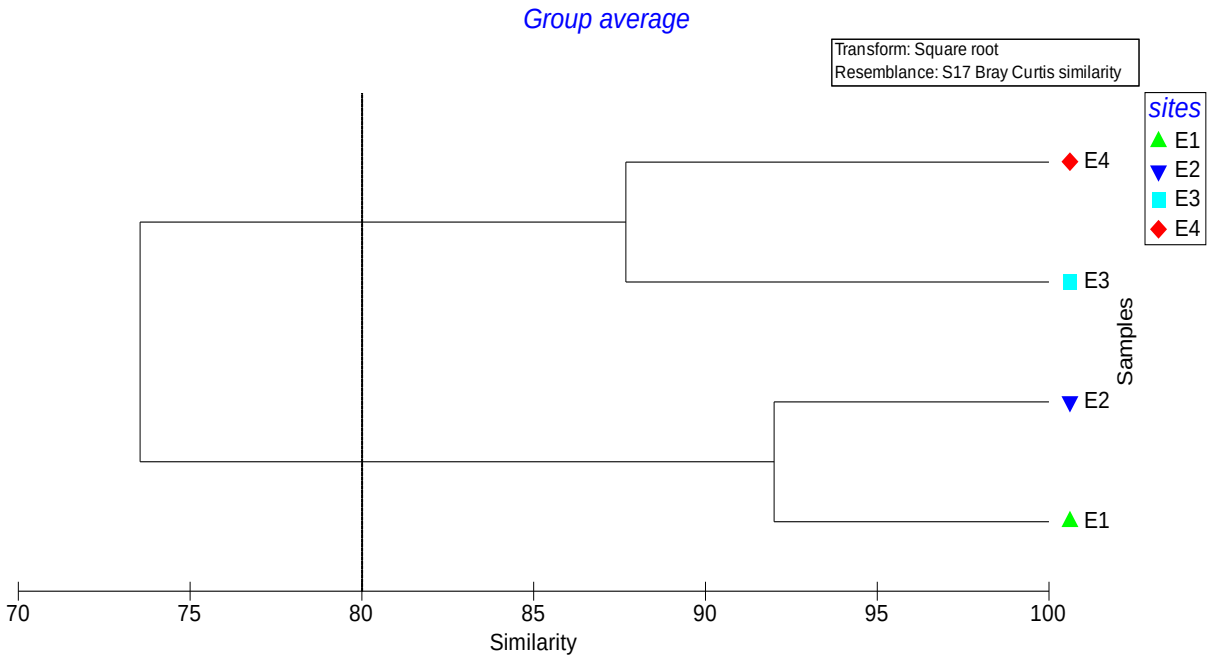
df= degree of freedom; **SS**= sum of square; **Pseudo-F**= F value by permutations.

The PERMANOVA analysis indicated that there was a spatio-temporal trend of meiofauna in the estuary. The trend was attributed to different environmental factors in the estuary.

PRIMER version 6 was used to perform a Bray-Curtis Cluster analysis and NMDS ordination to obtain an indication of the spatial trends of meiofauna in the Incomati River

Estuary (Figure 4.1. **A** and **B**). The results obtained from the MDS ordination and the Cluster Analysis revealed sites grouping in different months of sampling study period. The sites grouping in the study period was obtained at 80% and 90% (Figure 4.1. **A** and **B**). Two group formations were achieved at a similarity of 80%. Site E1 which was situated in the Oligohaline Zone and E2 which was situated in the Euhaline Zone were grouped together indicating that these sites had similar meiofauna communities. Sites E3 which was situated at the Mesohaline Zone and E4 which was situated in the Polyhaline Zone were also grouped together at similarity of 80%

A



B

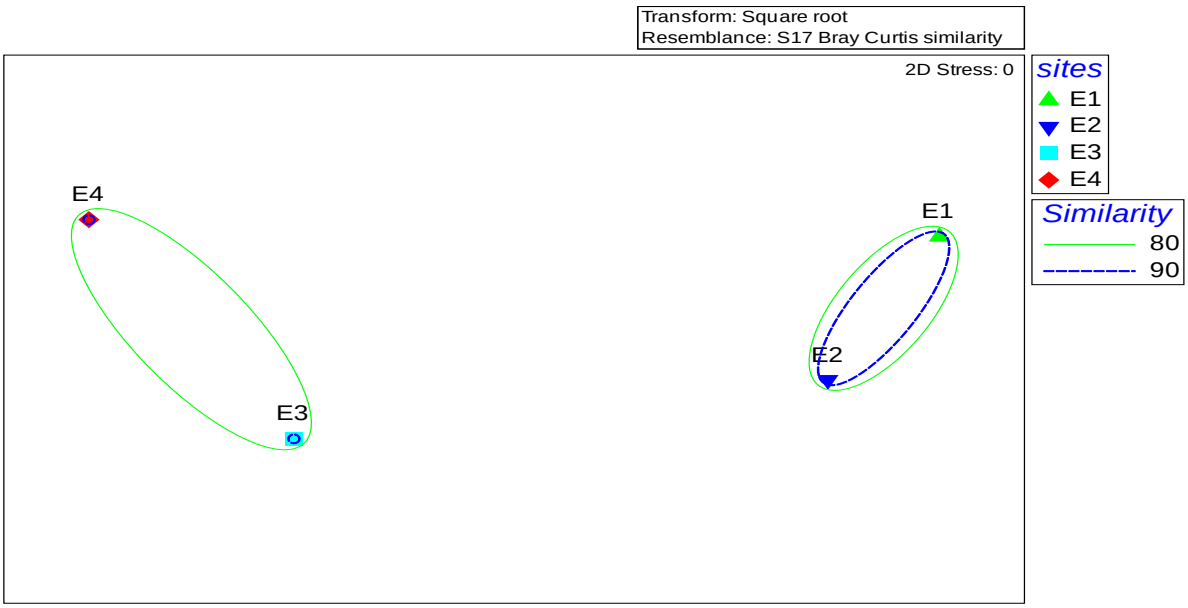


Figure 4.1: Bray-Curtis similarity matrix-based cluster analysis **(A)** and two-dimensional representation of the NMDS ordination **(B)** of meiofauna communities collected in the Incomati River Estuary. The NMDS ordination was completed with 25 iterations and showed a stress of zero.

At a similarity of 90% only sites E1 and E2 were grouped together, while sites E3 and E4 were separated Figure 4.1B. The separation of sites from another indicated that there was a dissimilarity of meiofauna communities between the sites sampled or it was attributed to change in meiofaunal taxa within the sites

4.5. FREE-LIVING NEMATODES ANALYSES

4.5.1. Nematodes Density

A total of 5989 nematodes individual/10 cm^2 were found in the Incomati River estuary. The highest nematode density of 2605 individual/10cm^2 was found at site E4 which is situated in the Polyhaline Zone of the estuary, while a lowest density of 721 individual/10cm^2 was found at site E1 situated in the Oligohaline Zone Figure 4.2. These findings indicated that nematodes density decrease with decrease in salinity.

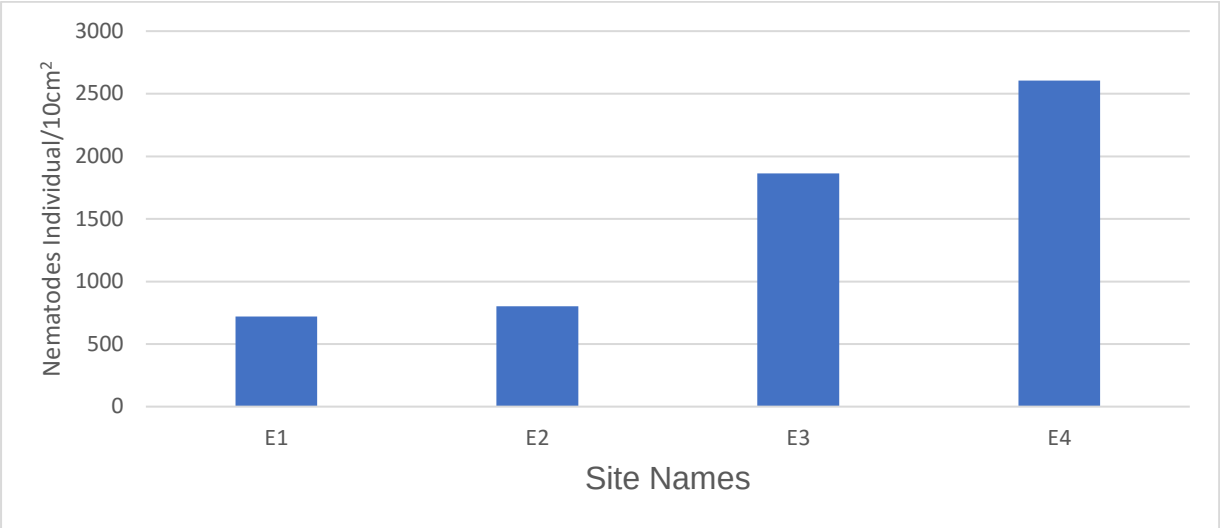


Figure 4.2: Mean nematodes density sampled in the Incomati River Estuary.

At all the sites sampled, the highest nematodes densities were observed in February 2018 at site E1, in August 2017 at site E2, and in December 2017 at sites E3 and E4. The lowest nematodes density at all the sites was observed in June 2017 except at site E4 which was observed in August 2017. The higher and the lower density of nematodes in these months of sampling indicated a change in the estuarine environment which was attributed to change in food availability in the system. Similarly, in a study conducted in the Swartkops River System, South Africa (Gyedu-Ababio *et al*. 1999), nematodes density was found to be higher in the Polyhaline Zone and lower in the Oligohaline Zone. Similar finding was made in Mondego estuary (Alves *et al*. 2013). In this study, the highest total nematodes density was observed at site E4 (Polyhaline Zone), and the lowest as site E1 (Oligohaline Zone). A significant difference ($p<0.05$) of nematodes density between the sites sampled during the study period was observed.

4.5.2. Nematode Diversity

A total of 2363 nematode taxa were identified using a compound microscope, and total of 39 nematode genera were found in the Incomati River Estuary (Annexure 1: Table 4.3). At site E1 (Oligohaline Zone), the number of nematodes genera diversity ranged from 4 to 13 genera, and the highest number of genera was found in December 2017, while the lowest number of genera was found in April 2018. At site E1 nematode genera such as *Haliplectus* dominated nematode communities with 41% of the total nematode genera sampled, followed by *Axonolaimus* with 13.2%. At site E2 (Euhaline Zone), the

number of nematodes genera ranged from 4 to 12, and the highest number of genera were observed in October 2017, and the lowest in February 2018, and April 2018. The dominant genera at site E2 were *Terschellingia* with 47.5%, followed by *Theristus* with 20.8% of the total nematode genera sampled at the site. Other nematode genera that were present at site E2 were *Axonolaimus*, *Sabatiera*, *Daptonema*, and *Parodontophora* which may indicate pollution and disturbance (Bongers *et al*. 1991; Lampadariou *et al.* 1997; Liu *et al*. 2016; Moreno *et al.* 2008). Therefore, dominance of nematode genera such as *Axonolaimus*, *Terschellingia* and *Theristus* at sites E1 and E2 indicated that these sites were polluted than the other sites. The pollution at these sites were attributed to agricultural, industrial activities from upstream catchments, and informal settlements along these sites.

At site E3 (Mesohaline Zone), nematode genera ranged from 11 to 18. The highest number of genera was observed October 2017, and the lowest number of genera was observed in April 2018. The dominant nematode genera at the site were *Sabatiera* with 8.5%, and Theristus with 8.2% of the total nematodes genera. Although *Sabatiera* and *Theristus* were dominating at site E3, their dominance was not significant. Their dominance was attributed to the high concentration of nutrients at this site. Nematode genera at site E4 (Polyhaline Zone) ranged from 13 to 21. The highest number of genera was observed in December 2017 and August 2017, and the lowest was observed in April 2018 and October 2017. The diversity and richness index used confirmed that site E4 with was situated in the Polyhaline Zone was had higher diversity than the other sites sampled.

4.5.3. Diversity and Margalef's Richness Index

Shannon-Wiener Diversity Index and Margalef's Richness Index were done using a PRIMER version 6. From Figure 4.3, both the Shannon-Wiener Diversity and Margalef's Richness Index showed that site E2 had the lowest diversity and richness of nematodes.

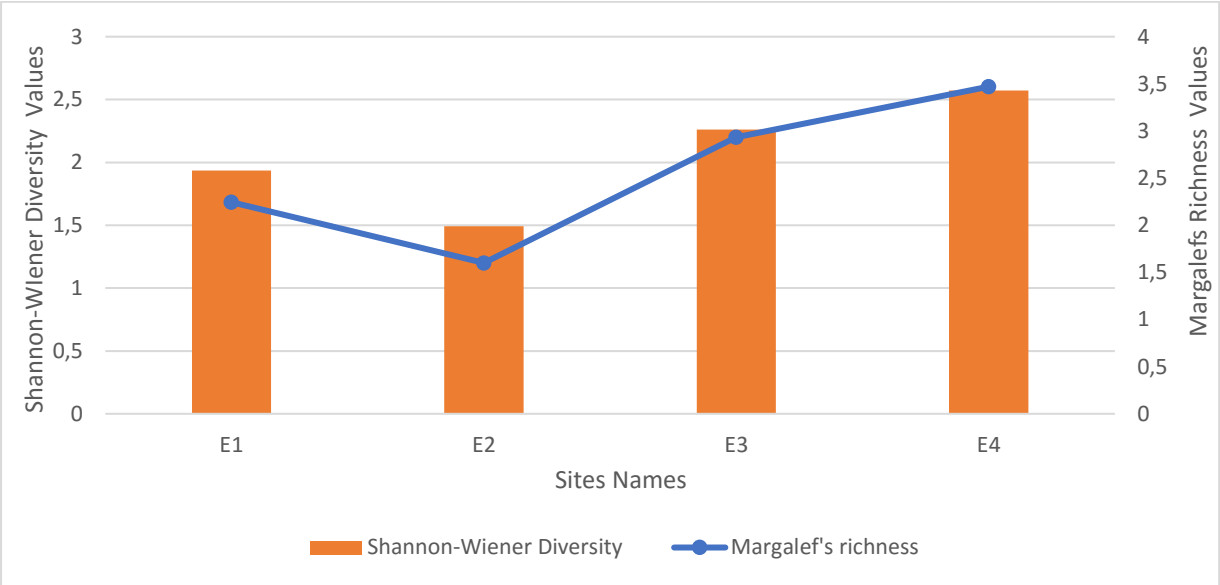


Figure 4.3: A diversity and richness index graph for free-living nematodes sampled.

The highest diversity and richness of free-living nematodes was observed at site E4 which was situated in the Polyhaline Zone, followed by sites E3 and E1 which were situated in Mesohaline and Oligohaline Zones respectively. The lowest diversity and richness at site E2 were attributed to higher concentration of metals, nutrients, and organic matter.

4.5.4. Maturity Index

The Maturity Index (MI) which is a potential indicator of nematode assemblage under stress were calculated for the four sites sampled (Figure 4.4). The MI values for sites E3 situated in Mesohaline Zone, and E4 situated in Polyhaline Zone were 2.67 and 2.66 respectively. The higher value of Maturity Index values at these sites indicated that nematode genera were not under stress.

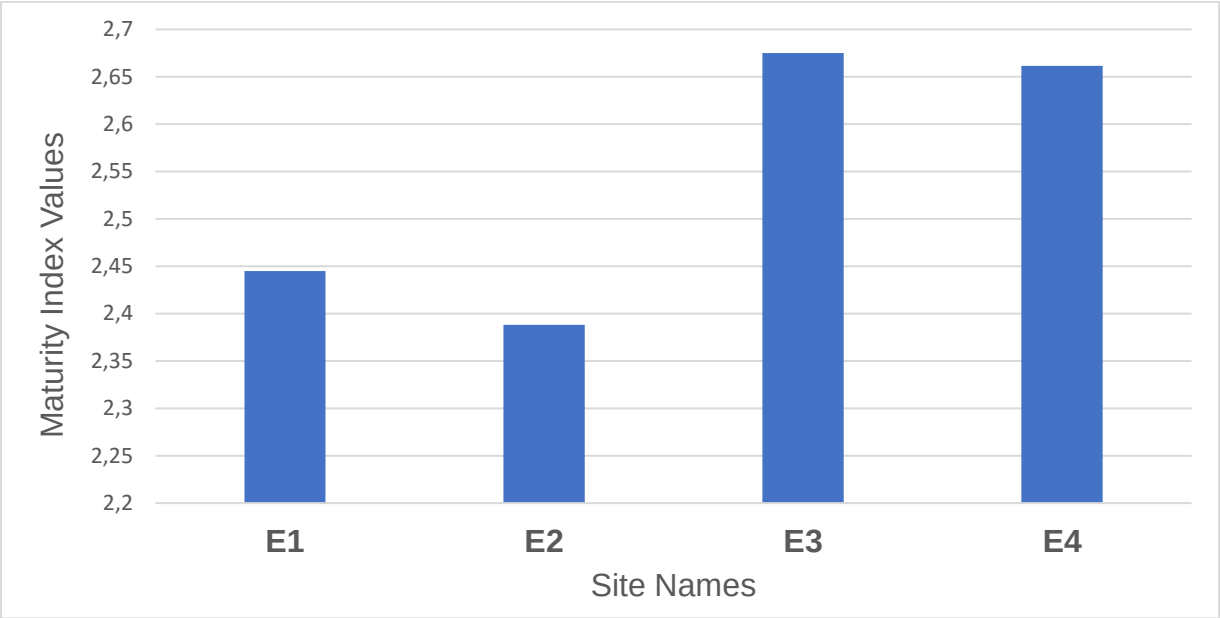


Figure 4.4: Maturity Index results indicating the polluted sites in Incomati River Estuary.

The values of Maturity Index at sites E1 which was situated at Oligohaline Zone and E2 which is situated at the Euhaline Zone were 2.44 and 2.38. The lower Maturity Index indicated that these sites were under stress, especially at site E2 which had higher concentration of metals and total phosphate throughout the sampling period. A correlation between Maturity Index and Shannon-Wiener Diversity was found using ANOVA.

4.5.5. Nematodes population

To indicate nematodes population in the study, free-living nematodes were divided into three groups (Table 4.4). The highest count for juveniles at sites E1 and E2 were observed in February 2018. Both these sites had juveniles count of 65% and 82% in the same months. The highest count of juveniles at site E3 was observed in April 2018 (78%). At site E4, the highest juvenile percentage count of 70% was observed in June 2017 and April 2018. The average means of juvenile counts at sites E1, E2, E3, and E4 was 57.4%, 68%, 69.2% and 64.7%, respectively.

Table 4.4: Mean nematodes population structure sampled from June 2017 to April 2018

Site Names	Female (F)	Male (M)	Juveniles (J)	Population Ratio (F:M:J)
E1	23.4	19.1	57.4	23.4:19.1:57.4
E2	18.7	9.8	68	18.7:9.8:68
E3	19.7	11.2	69.2	19.7:11.2:69.2
E4	21	14.3	64.7	21:14.3:64.7
Total Average	**20.7**	**13.6**	**64.8**	**20.7:13.6:64.8**

The highest male counts at sites E1, and E2 were observed in the months of June 2017 with a percentage count of 25.8%, and 18% respectively. At sites E3, and E4 the highest count of males was observed in the months of February 2018 (14%), and August 2017 (18%) respectively. The average means for male count at sites E1, E2, E3, and E4 were 19.1%, 9.8%, 11.2%, and 14% in that order. The highest female nematodes percentage count at sites E1, E2, and E3 was observed in the months of June 2017 (30.6%), October 2017 (30%), and August 2017 (26%). At site E4 the highest count was observed in the months of October 2017, December 2017, February 2018 with a percentage count of 24%. The mean average percentage of females count at site E1 was 23.4%, at site E2 was 18.7%, at site E3 was 19.7%, and at site E4 was 21%. From the mean average of the population juveniles were the dominant population contributing 64.8% of the population structure, followed by female population which contributed 20.7% of the total population structure. The dominance of juveniles in the study area may be partly due to mortality of adult nematodes. Bouwman *et al*. (1983) found that juveniles were dominate with a count of 75% of the nematode community which was similar to the finding of this study. Bouwman *et al.* (1983), also suggested that there was no exact period for nematodes reproduction, and that species reproduce throughout the year.

ANOVA correlation results indicated that male population correlated with environmental factors such as zinc, nitrates (NO_3), fine sand, very fine sand and mud, and bacteria. Female population was found to correlate with the same environmental factors as the male population except that there was no correlation between female population with very fine sand and mud. Although Juveniles were found to correlate with most of the environmental factors, a strongest correlation was found between juvenile population

with chromium, very coarse sand, medium sand, total phosphate, chlorophyll-*a*, and organic matter. A PERMANOVA analysis for spatial and temporal trends of the population structure indicated that there was no significant difference ($p>0.05$) between monthly sampling, but significant difference ($p<0.05$) existed between the sites sampled.

4.5.6. Nematode feeding types

All nematodes feeding types were observed in the Incomati River Estuary during the study period, and their trophic diversity percentage were calculated for their dominance between the sites. The Trophic Diversity Index percentage (Figure 4.5) indicated that throughout the study, nematodes feeding type 1B (non-selective deposit feeders) were dominant in all sites sampled. The highest percentage contribution of feeding type 1B at sites E1, E2, E3, and E4 was observed in the months of December 2017 (51%), February 2018 (98%), June 2017 (64%), and December 2017 (46%) respectively. The mean percentage contributions of feeding type 1B at sites E1, E2, E3 and E4 was 44.7%, 88.9%, 44.7%, and 37.8% respectively. The highest percentage contribution of feeding type 2B (omnivores-predators) at site E1, E2, E3, and E4 was observed in the months of June 2017 (45%), August 2017 (11%), February 2018 (39%), and April 2018 (41%). The mean percentage contributions of feeding type 2B at sites E1, E2, E3 and E4 were found to be 3.2%, 6%, 22.5%, and 24.3%, respectively (Figure 4.5).

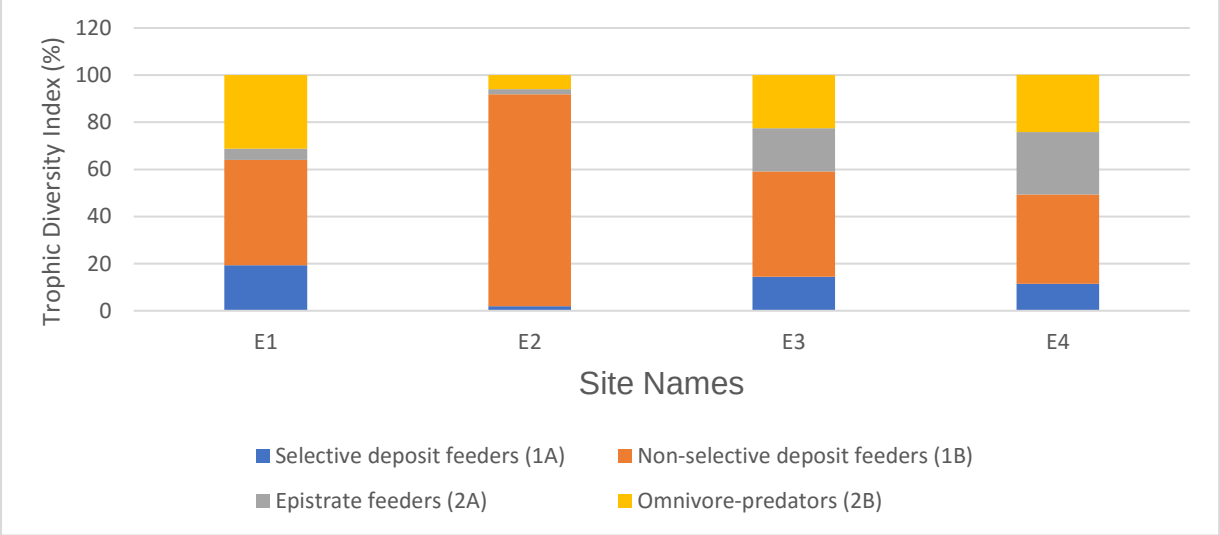


Figure 4.5: Trophic Diversity Index percentage of nematode feeding types in Incomati River Estuary.

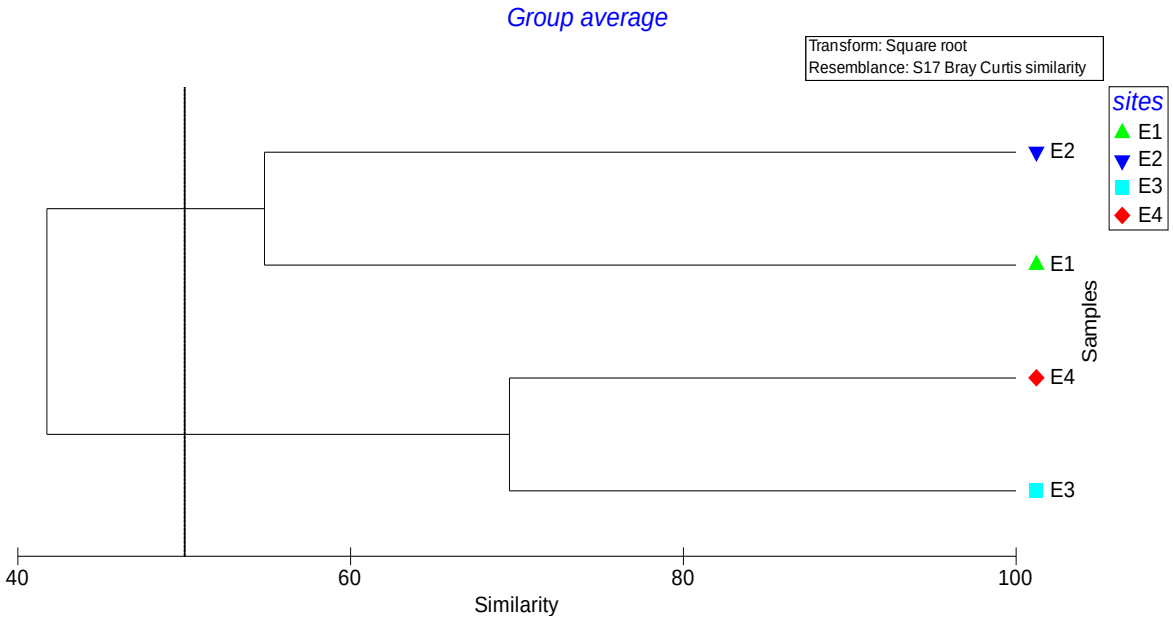
Feeding type 2A (epistrate feeders) were found to be dominant at sites E4 and E3 (Figure 4.5). The highest percentage contribution epistrate feeders were observed in the month of February 2018 at sites E4 and E3 a contribution percentage of 36% and 40% respectively. The mean percentage contribution of feeding type 2A at sites E1, E2, E3, and E4 were found to be 4.8%, 2.2%, 18.3% and 26.5% in that order. The highest contribution of selective deposit feeders was observed at sites E1, E3, and E4 in the months of February 2018 (40%) and August 2017 (20%, and 19%) respectively. The mean percentage contribution of feeding type 1A at sites E1, E2, E3, and E4 were found to be 19.3%, 2%, 14.5%, and 11.5% in that order. The mean percentage of the feeding types indicated that feeding type 1B was the dominant group, followed by feeding type 2B. A two-way ANONISM permutation test used to test the difference between sites, and between months for the relative abundance of the four feeding types indicated that significant difference existed between sites (rho=0.221; p=0.043), and between months sampled (rho=0.688; p=0.001). The grouping of nematodes according to feeding groups indicated that buccal cavity structure is an important criterion for understanding and explaining the food availability (Wieser, 1953; Chinnadurai and Fernando, 2007; Shabdin and Othman, 2008).

The life strategy characterization was calculated as it provides crucial additional information to that given by the feeding types regarding disturbance. At site E1 which was situated in the Oligohaline Zone, the dominance of colonizer and intermediate (c-p 2 and 3) genera was observed with 40.95%. Similarly, at site E2 which was situated in Euhaline Zone, the dominance of colonizer and intermediate (c-p 2 and 3) genera was observed with 50%. The presence of colonizer and intermediate genera indicated a high stress level with an increase in opportunistic nematodes at site E2. At site E3 which was situated in the Mesohaline Zone, the dominance of intermediate and (persisters) (c-p 3 and 4) nematodes were observed with 36.35%. The presence of intermediate and (persisters) indicated that site E3 was dominated by sensitive genera which may include several predators as well as bacterial feeders. Similar results were observed at site E4 which was situated in the Polyhaline Zone.

A Bray-Curtis Cluster Analysis and NMDS ordinations (Figure 4.6) were performed to investigate the spatial trend of free-living nematodes. Both Figure **A** and **B** indicated sites grouping at a similarity of 45%. Sites E1 and E2 were grouped together, and sites E3 and E4 were also grouped together. The grouping of sites at the estuary indicated

that the sites had similar free-living nematodes while the separation of grouping indicated that at different time of sampling free-living nematodes genera were not the same.

A



B

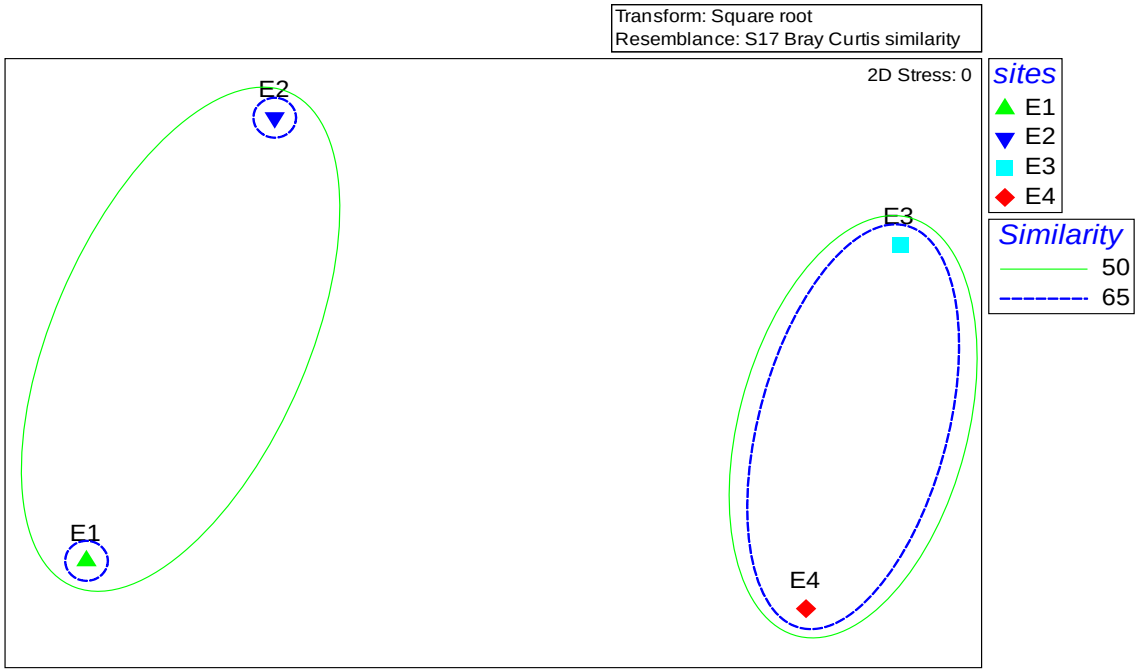


Figure 4.6: Bray-Curtis similarity matrix-based cluster analysis **(A)** and two-dimensional representation of the NMDS ordination **(B)** of free-living nematodes genera

collected in the Incomati River Estuary. The NMDS ordination was completed with 25 iterations and showed a stress of zero.

At 65% similarity site E3 and E4 were grouped together, while sites E1 and E2 were separated from the group and from each other Figure 4.6B. The grouping of sites indicated that these sites E3 and E4 were sharing similar genera. The separation of sites E1 and E2 from the other group indicated that these sites had different nematode genera from the other sites or other group. This was attributed to the fact that these sites were situated in a low salinity zone.

Nematodes genera contributed to the within-group similarity of sites were done using a SIMPER analysis (Table 4.5). SIMPER analysis indicated that they were four within group similarity of sites in the study. Nematode genera that have a cumulative contribution of 50% and above may indicate the disturbance with the groups. An average similarity of 63.89% was found for Group E1. Nematodes genera contributed to the similarity at this group were *Haliplectus*, *Axonolaimus*, *Anoplostoma*, *Adoncholaimus* and *Terschellingia* which had a cumulative contribution of 74.44%. Dissimilarity between group E1 and other groups existed.

Table 4.5: Summary of SIMPER results for free-living nematodes genera: average abundance (% cover) of nematodes genera in each site sampled, their contribution (%) within-group similarity, and cumulative total (%) of contributions (90% cut-off).

Groupings	Nematodes Genera	Average abundance	Average similarity	Contribution %	Cumulative %
Group E1	*Haliplectus*	6.12	20.73	32.45	32.45
	Axonolaimus	3.32	9.43	14.77	47.22
	Anoplostoma	2.83	8.19	12.82	60.04
	Adoncholaimus	2.40	5.43	8.49	68.53
	Terschellingia	1.65	3.77	5.91	74.44
	Viscocia	1.52	3.44	5.38	79.82
	Rhabditis	1.55	3.39	5.30	85.12
	Theristus	1.32	2.52	3.95	89.07
		1.56	2.46	3.85	92.93
Group E2	*Terschellingia*	6.85	26.58	40.75	40.75
	Theristus	4.44	15.55	23.85	64.60
	Axonolaimus	2.83	8.44	12.94	77.54
	Paramonohystera	2.34	6.33	9.71	87.26
	Daptonema	1.32	2.83	4.33	91.59

Table 4.5: continues

Groupings	Nematodes Genera	Average abundance	Average similarity	Contribution %	Cumulative %
Group E4	*Viscocia*	2.99	6.59	13.27	13.27
	Terschellingia	2.41	4.56	9.17	22.43
	Metachromadora	2.15	3.84	7.73	30.16
	Dichromadora	2.10	3.40	6.84	37.00
	Anoplostoma	1.82	3.23	6.49	43.49
	Aegialoalaimus	2.01	2.86	5.75	49.25
	Microlaimus	1.62	2.53	5.10	54.34
	Spirinia	1.35	2.31	4.65	58.99
	Sabatieria	1.91	2.31	4.65	63.64
	Axonolaimus	1.75	2.23	4.49	68.13
	Daptonema	1.75	2.12	4.26	72.38
	Paramonohystera	1.19	1.96	3.94	76.33
	Scaptrella	1.40	1.90	3.81	80.14
	Pomponema	1.92	1.88	3.79	83.93
	Monhystera	1.34	1.62	3.25	87.18
	Leptolaimus	1.12	1.36	2.73	89.92
	Paracyatholaimus	1.46	1.18	2.38	92.30

The average dissimilarity between group E1 and E2 was 63.25%, between Group E1 and E3 was 76.98%, between Group E1 and E4 was 70.26%. Group E2 had an average similarity of 65.22%, and the nematodes genera contributing within-group similarity were *Terschellingia*, *Theristus*, and *Axonolaimus* which had a cumulative contribution of 77.54%. The higher percentage contribution of *Terschellingia*, *Theristus*, and *Axonolaimus* in Group E2 indicated that this group was most polluted than its counterpart groups. The dissimilarity between Group E2 and E3 was 77.37%, between Group E2 and E4 was 70.47%. Group E3 had an average similarity of 42.20%. Nematodes genera contributing within-group similarity were *Paramonohystera*, *Neochomadora*, *Viscocia*, *Oxystomina*, *Sabatiera*, *Theristus*, *Daptonema*, and *Filoncholaimus*, which had a cumulative contribution of 73.31%.

A cumulative dominance percentage of free-living nematodes genera was plotted using a K-dominance curve (Figure 4.7). The K-dominance curve indicates that, at site E1 a single nematode genus (*Haliplectus*) dominated the nematodes community at cumulative dominance percentage of 40%.

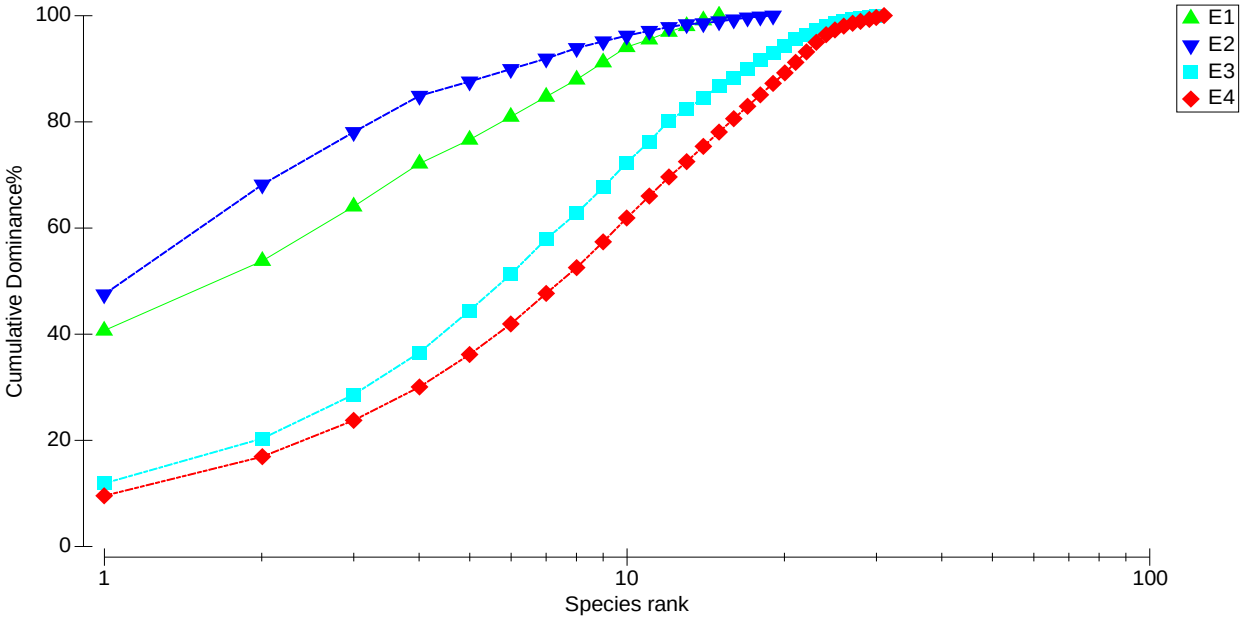


Figure 4.7: Ranked species K-dominance curves for the free-living nematode genera identified at the Incomati River Estuary.

Above 40% cumulative dominance of species was observed at site E2. These genera were *Terschellingia* and *Theristus*. Other nematodes genera identified at site E2 which were believed to be a pollution indicator were *Paramonohystera*, *Sabatiera*, *Synonchium*, *Viscocia*, *Daptonema*, and *Axonolaimus*. The dominance of individual genera at sites E1 and E2 indicated that nematode diversity at these sites were low. At sites E3, *Batylaiumus* dominated the nematode community, while at site E4, *Viscocia* dominated the nematode community. At both sites E3 and E4, the dominance of individual genera was below a cumulative dominance of 20%. The low cumulative dominance percentage at sites E3 and E4 was attributed to the fact that these sites were situated in a Mesohaline Zone (site E3) and Polyhaline Zone (site E4) which were known to have higher salinity than the Oligohaline Zone (site E1) and Euhaline Zone (site E2). The K-dominance curve showed that the higher the salinity the lower the dominance of individual genera, and the higher the diversity of individual genera. Moreover, it showed that sites E2 and E1 were more polluted than sites E3 and E4 because they had lower nematode diversity.

4.5.7. Environmental Factors and Nematodes Communities

A RELATE analyses done using PRIMER software indicated that patterns based on environmental variables were significantly related to the patterns inherent in nematodes community structure both taxonomically (r=0.353, *p*=0.01) and functionally (r=0.314,

p=0.03). The BIOENV analysis revealed the relationship of species abundance and biological traits with environmental parameters (Table 4.6). Feeding types have best correlated (rho=0.797, *p*=0.01) with coarse very coarse sand, medium sand and fine sand. Nitrates (NO_3), very coarse sand, coarse sand, and fine sand were best correlated (rho=0.693; *p*=0.01) species abundance. Wieser (1960); Tietjen (1977); Ward (1975); Ingels *et al*. 2011; Lizhe *et al*. 2012; Ngo *et al*. 2013; Zeppilli *et al*. 2013; Górska *et al*. 2014; Pusceddu *et al*. 2014, Nascimento *et al*. 2011, indicated that within an area of uniform salinity, grain size of sediments is a dominant factor in determining the composition of nematodes communities as well as communities of other meiofauna

Table 4.6: Summary of BIOENV analysis indicating the environmental factors influencing nematode structures.

Environmental Variables		**Correlation or Rho**
Species	Nitrate (NO_3), Very Coarse Sand, Coarse Sand and Fine Sand	0.693
Feeding types	Coarse Very Coarse Sand, Medium Sand and Fine Sand	0.797

According to Levin *et al*.1991; Ingels *et al*. 2011; Lizhe *et al*. 2012; Ngo *et al*. 2013; Zeppilli *et al*. 2013; Górska *et al*. 2014; Pusceddu *et al*. 2014, Nascimento *et al*. 2011, sediments particle size such as grain size, organic content, and chlorophyll-*a* are other important factors that contribute to the distribution of nematodes in estuarine environment. The relationship between estuarine nematode distribution and sediment grain size in the current study can be attributed to difference in buccal morphology and feeding preferences. Similar findings were obtained in a study conducted in the Swartkops River in South Africa Gyedu-Ababio *et al*. (1999) where sediment particle size was found to influence nematodes density, and the number of nematodes were low at sites dominated by both finer, and coarse sands. In another study conducted by de Beer *et al*. (2005) nematodes density and diversity were found to be in coarse sediments. Warwick and Buchanan (1971) also found that the diversity of nematodes was high at the sandiest station and low at the siltiest station in a study conducted in Northumberland coast (Britain). These findings were also supported by Vanaverbeke *et al.* (2011) and Fonseca *et al.* (2014) who also found that density and diversity of marine nematodes increase with increase sediment grain size.

Analyses using the distance-based linear model (DISTLM) indicated the environmental variables related to the variation in nematodes community structure (Table 4.7). These environmental variables were sediment particle size such as granules, medium sand, coarse very coarse sand, coarse sand, fine sand; metals such as cobalt, vanadium nickel, aluminium, manganese; and nutrients such as nitrates and total phosphate. All these environmental variables contributed significantly $p<0.05$ to the distribution of free-living nematodes when considered independently.

Table 4.7: Relationship between environmental variables and free-living nematodes based on AIC DISTLIM. Marginal test considers the importance of each variable in the absence of the other variables. Sequential tests consider the importance of variables in conjunction with the other variables starting with the variable with the variable explaining the greatest variance.

Variables	**SS**	**Pseudo-F**	**P values**	**Prop**	**Cumul.**	**res.df**
Marginal test						
Cobalt	5991	2.9802	0.006	0.1193		
Chromium	2130.3	0.97463	0.445	4.2422^{-2}		
Copper	3765.3	1.7833	0.088	7.498^{-2}		
Manganese	6333.4	3.1751	0.008	0.12612		
Cadmium	2703.7	1.2519	0.266	5.3841^{-2}		
Iron	4380.9	2.1027	0.04	8.724^{-2}		
Nickel	226.2	3.1138	0.007	0.12399		
Vanadium	5054.8	2.4624	0.018	0.10066		
Zinc	2991.2	1.3934	0.194	5.9565^{-2}		
Aluminium	6078.9	3.0299	0.004	0.12105		
Total Phosphate	4541.3	2.1874	0.045	9.0434^{-2}		
Nitrate	11925	6.851	0.001	0.23746		
Chlorophyll-a	3803.9	1.8031	0.067	7.575^{-2}		
Organic Matter	1353.1	0.6092	0.782	2.6945^{-2}		
Granules	8808.3	4.6798	0.001	0.17541		
Very Coarse Sand	13419	8.0227	0.001	0.26722		
Coarse Very Coarse Sand	14421	8.8628	0.001	0.28717		
Medium Sand	9313.9	5.0095	0.001	0.18547		
Fine Sand	11128	6.2632	0.001	0.2216		
Mud and Fine Sand	10863	6.0725	0.001	0.21632		
Sequential test						
Coarse Very Coarse Sand	179.38	14421	8.8628	0.001	0.28717	0.28717
Fine Sand	174.44	8990.1	7.0428	0.001	0.17902	0.46619
Mud and Fine Sand	171.83	4682	4.2325	0.001	9.3236^{-2}	0.55943
Medium Sand	171.83	2.597^{-11}	0	1	5.1716^{-16}	0.55943
Overall best solution						
AIC	**R^2**	**RSS**	**Number of Variables**			
171.83	0.55943	22124	4			

*indicate significant, SS: sum of squares, Prop: proportion of explanation, Cumul: cumulative proportion of explanation, res.df: residual degree of freedom.

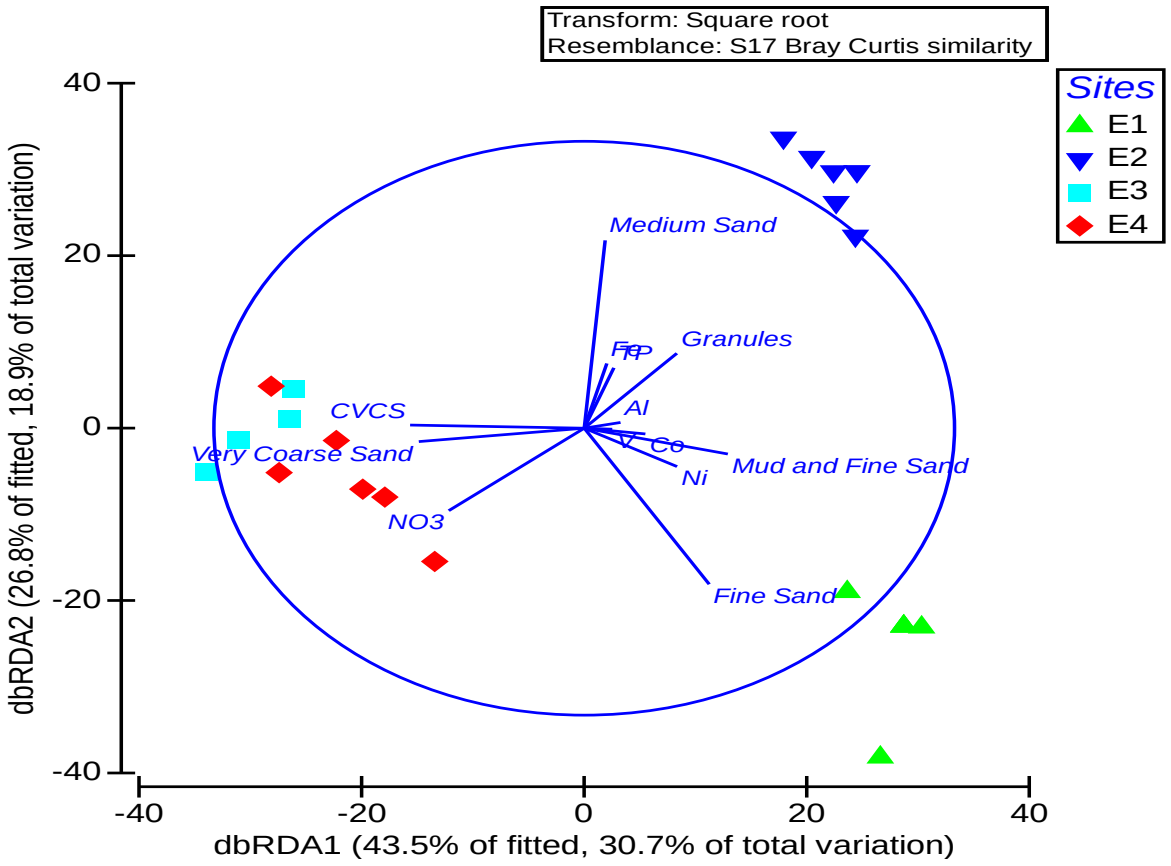


Figure 4.8: Distance-based redundancy (dbRDA) plot illustrating the DistLM model based on the free-living nematodes assemblages data and fitted environmental variables with their vectors (strength and direction of effect of the variable on the ordination plot)

The stepwise procedure selected coarse very coarse sand, fine sand, mud and fine sand and medium sand as variables that determined the composition of free-living structure in the Estuary (AIC=171.83; R^2 =0.55943, number of variables = 4). The dbRDA ordination figure 4.8 showed how sites were clearly separated by their location along the longitudinal gradient and the principal contributing environmental variables. The first two dbRDA axes explained 70.3% of the relationship between free-living nematodes assemblage and the environmental variables, and 49.6% of the total variability in the assemblage data. The first dbRDA axis was strongly related mud and fine sand while the second axis was related to medium sand.

4.6. CONCLUSION

The aim of this chapter was to identify environmental factors that contributes to the distribution of free-living nematodes and to identify nematodes which can be used as pollution indicators in monitoring.

Free-living nematodes density and diversity were found to decrease from site E1 to site E4 due to salinity change. A spatial and temporal trend in nematodes density and diversity were found to exist between the sites and sampling months. All the indices, Shannon-Wiener Diversity, Margalef's Richness and Maturity Index indicated lower diversity and richness at site E2 which had the highest concentration of environmental factors such as metals, total phosphate and total organic matter. A strong correlation between all the indices were found in this chapter and a K-dominance curve indicated that site E2 was dominated by genera such as *Terschellingia* and *Theristus*, which are known to be tolerant to polluted sites or environments (Gyedu-Ababio *et al.* 1999). These results indicated that environmental factors influence the diversity and richness of nematodes in estuarine environment.

A population structure of nematodes was found to be dominated by juveniles. A PERMANOVA analysis indicated that significant difference (p<0.05) existed between the sites sampled, but not between monthly samples. An ANOVA analysis indicated that juvenile, female and male populations were structured by different environmental factors as they had positive correlation with different environmental factors.

Nematode feeding types were found to be mostly dominated by non-selective deposit feeders such as *Terschellingia* and *Theristus* at site E2. The highest percentage of colonizer and intermediate (c-p 3 and 2) were also found at site E2 which indicated a high stress level with an increase in opportunistic nematodes. A spatial and temporal trend of nematode feeding types existed between the sites and monthly samples. A correlation was also found between nematode feeding type 2A and 1A with Heterotrophic Bacteria, while nematode feeding type 2B and 1B, were found to correlate with chlorophyll-*a*. This finding indicated that nematodes were also distributed based on their preference for their food source.

The BIOENV analyses indicated that free-living nematodes and their feeding types were structured by sediment particle size and nutrients (NO_3) in the Estuary. A DISTLM

analyses confirmed that sediment particle size played a huge role in nematodes community structure in the Estuary. This chapter succeeded in identifying environmental factors that contribute to the structuring of free-living nematode in the Incomati estuarine environments and the relationship between free-living nematodes and environmental factors were also established.

CHAPTER 5

5. GENERAL CONCLUSION AND RECOMMENDATIONS

The aims and objectives of the study were successfully achieved using the specified methods and analyses.

The study found that nematodes were the dominant group of meiofauna in the estuary, and their numbers decreased towards the Oligohaline Zone which was attributed to decrease in salinity. A spatio-temporal trend of free-living nematodes which was attributed to different environmental factors was found in the estuary. Certain individual environmental factors were found to have influenced free-living nematodes distribution in the Incomati estuary. A BIO-ENV analyses indicated a correlation between environmental factors such as Nitrates (NO_3), very coarse sand, coarse sand and fine sand in the estuary. These findings showed that environmental factors play a role in nematodes diversity and density. Thus, changes in environmental variables result in changes in nematodes composition.

All four nematode feeding types were identified in the study area. Non-selective deposit feeders were found to be dominant at all sites except at site E1. A two-way ANONISM permutation test of the four feeding types indicated that significant difference existed between sites (rho=0.221; p=0.043), and between months sampled (rho=0.688; p=0.001). These results indicated that there was a spatial and temporal distribution of feeding types in the estuary. A strong correlation between nematodes feeding types 2A and 1A with heterotrophic bacteria, and between nematode feeding type 2B and 1B with chlorophyll-*a* was observed in this study. This indicates that nematode feeding types in the estuary were structured based on different food sources.

Nematodes population structure was found to be dominated by juveniles, and that different environmental factors influenced the population structure. A PERMANOVA analysis showed that a spatial distribution of nematodes population structure exists in the estuary. Different environmental factors influenced the population structure.

All the three indices used in the study (Maturity Index, Shannon-Wiener Diversity Index and Margalef's Richness Index) had lower values on sites which were polluted. A higher

abundance of colonizer and intermediate genera (c-p 2 and 3) were found in polluted site of the Estuary. Nematodes genera such as *Terschellingia*, *Theristus* and *Halalaimus* were found to be dominant at the polluted sites or sites with the highest concentration of metals, organic matter and total phosphate. Other nematode genera which were found in lower abundance at polluted site were *Paramonohystera*, *Sabatiera*, *Synonchium, Viscocia, Daptonema*, and *Axonolaimus*.

A relationship between Maturity Index, Shannon-Wiener Diversity Index and Margalef's Richness Index, indicated that these tools can be used to assess pollution in estuarine environment because they gave similar findings in this study. These findings confirmed that nematodes can be used as good pollution indicators in the Incomati Estuary. Further studies confirming the findings of this study must be done in the Incomati River Estuary, and on the African Coast in order to understand free-living nematodes so as to close the gap in our monitoring strategies. Governments, researchers and the communities must work together in order to achieve this goal.

CHAPTER 6

6.1. REFERENCES

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6.2. APPENDICES

APPENDIX A

Table 3.1: Environment factors analyse in the Incomati River Estuary from June 2017 to April 2018

Environmental Factors	E1						E2						E3						E4					
	Jun-17	Aug-17	Oct-17	Dec-17	Feb-18	Apr-18	Jun-17	Aug-17	Oct-17	Dec-17	Feb-18	Apr-18	Jun-17	Aug-17	Oct-17	Dec-17	Feb-18	Apr-18	Jun-17	Aug-17	Oct-17	Dec-17	Feb-18	Apr-18
Cd ppm	0.27	0.12	0.1	0.1	0.1	0.1	0.3	0.29	0.13	0.12	0.09	0.09	0.09	0.09	0.09	0.09	0.17	0.1	0.09	0.09	0.09	0.09	0.09	0.09
Co ppm	6	2.7	2.8	2.7	2.7	2.7	8.6	8.7	1.9	1.8	0.82	1.5	1.3	1.3	1.3	0.95	3.6	1.2	0.34	0.77	0.62	0.38	0.52	0.36
Cr ppm	22	12	9.4	6.1	6.1	6.1	20	30	11	14	8.3	6.2	17	17	17	16	44	9.3	6	13	7.6	6.3	7.2	7.1
Cu ppm	9.5	5.6	5	3.8	3.8	3.8	12	13	6.5	6.3	4.4	4.9	3.8	3.8	3.8	3.1	7.2	4.5	3.8	5.1	3.6	3.5	4	4.6
Fe ppm	9549	4667	4191	2574	2574	2574	20595	21130	3938	3892	1951	3245	1952	1952	1952	1799	6336	2676	1648	1531	1761	1472	1495	1315
Mn ppm	242	164	132	68	68	68	320	391	116	101	55	181	44	44	44	45	101	51	26	172	50	33	41	36
Ni ppm	18	7.3	6.7	6.1	6.1	6.1	22	24	7.6	8	4.4	5.8	2.3	2.3	2.3	2.1	8.3	3.4	2.1	7.5	3	2.8	2.9	3.1
V ppm	16	6.8	6.1	4.1	4.1	4.1	27	27	6	6.1	2.9	4.8	2.9	2.9	2.9	2.9	10	4.1	1.1	1.1	1.8	1.3	2	1.3
Zn ppm	15	18	7.1	14	14	14	24	25	5.9	6.1	3.6	11	4	4	4	5.3	14	9.2	13	25	2.7	3.7	3.8	5.1
Al ppm	11648	4974	3898	2764	2764	2764	16945	15945	4415	4150	2236	3921	1328	1328	1328	1316	5976	2312	748	551	1101	1003	1150	872
TP	110	79	35	27	27	27	273	300	68	68	36	59	34	34	34	34	83	46	25	15	30	24	25	24
NO_3 (mg/l)	0.05	0.05	0.05	0.05	0.05	0.05	0.01	0.01	0.01	0.01	0.01	0.01	0.07	0.07	0.07	0.07	0.1	0.1	0.14	0.14	0.14	0.1	0.06	0.1
Chl-a (mg/m^3)	0.8	0.6	1.2	0.9	0.7	1.5	0.6	1.6	0.9	0.5	0.9	0.7	0.8	0.7	4	4.3	4.6	4.9	0.04	2.2	1.8	0.7	2	0.7
TOM%	1	1	3	1	1	1	4	4	1	1	1	1	1	1	1	3	2	1	2	1	1	2	2	1

APPENDIX B

Table 4.3: Feeding types, c-p values, salinity ranged, and Nematodes Genera identified in the Incomati River Estuary from June 2017 to April 2018.

NEMATODE GENUS	c-p values	Feeding types	E1						E2						E3						E4					
			Salinity range amongst the sites																							
			0-3NST						3-5NST						5-18NST						18-26NST					
			Jun-17	Aug-17	Oct-17	Dec-17	Feb-18	Apr-18	Jun-17	Aug-17	Oct-17	Dec-17	Feb-18	Apr-18	Jun-17	Aug-17	Oct-17	Dec-17	Feb-18	Apr-18	Jun-17	Aug-17	Oct-17	Dec-17	Feb-18	Apr-18
Adoncholaimus	3	2B	13	12	15	3	0	0	0	0	1	0	0	0	0	1	2	10	11	0	0	6	0	1	2	0
Aegialoalaimus	4	1A	3	0	0	4	2	0	0	0	2	0	0	0	0	0	3	0	10	0	0	11	12	6	0	8
Anoplostoma	2	1B	10	15	0	9	13	11	0	0	2	0	0	0	2	0	0	6	0	0	0	9	3	6	4	3
Axonolaimus	2	1B	3	15	16	12	3	26	0	10	9	10	10	20	0	0	0	0	0	0	0	11	0	10	4	4
Batylaiumus	2	1B	0	0	0	0	0	0	0	0	0	0	0	0	10	0	0	11	0	50	0	0	0	0	0	4
Camacolaimus	3	2A	0	0	0	0	0	8	3	2	0	1	0	0	0	0	0	0	0	2	0	0	0	0	0	0
Cephalainticoma	2	2A	0	0	0	0	0	0	0	0	0	0	0	0	2	0	0	0	0	0	0	0	0	2	0	0
Daptonema	3	1B	0	3	1	2	0	0	1	1	1	10	3	0	10	0	0	9	5	5	10	0	0	6	12	2
Dichromadora	2	2A	0	0	0	0	0	0	0	0	0	0	0	0	3	0	0	0	20	0	12	2	0	3	10	8
Dolicholaimus	2	2B	0	0	0	0	0	0	0	0	0	0	0	0	4	0	1	0	0	0	0	0	0	2	10	0
Enoplus	5	2B	0	0	0	0	0	0	0	0	0	2	0	0	6	0	0	0	0	0	0	3	0	0	0	0
Filoncholaimus	4	2B	0	0	0	0	0	0	0	0	0	4	0	0	6	20	5	0	9	0	2	0	0	0	0	0
Halalaimus	4	1A	0	0	0	0	0	0	0	0	0	0	0	0	5	0	0	2	3	0	0	3	0	0	2	0
Haliplectus	2	1A	23	29	35	34	54	55	0	3	0	5	0	0	0	0	0	1	0	0	0	0	0	0	0	0
Leptolaimus	2	1A	0	0	0	0	0	0	0	0	0	0	0	0	3	0	1	3	0	0	1	5	3	3	0	0
Metachromadora	3	2A	0	0	0	0	0	0	0	0	0	0	0	0	0	8	0	0	0	5	5	7	10	4	8	0
Metacyatholaimus	3	2A	0	0	0	0	0	0	0	0	2	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Microlaimus	2	2A	0	0	0	0	0	0	0	0	2	0	0	0	3	2	1	1	3	0	1	6	5	7	2	0
Monhystera	2	1B	0	0	0	0	0	0	0	0	6	0	0	0	0	2	2	0	0	0	5	2	0	7	3	0
Neochomadora	3	2A	0	0	0	0	0	0	0	0	0	0	0	0	0	3	7	6	9	4	0	0	0	13	0	0

Continued

NEMATODE GENUS	c-p values	Feeding types	E1						E2						E3						E4					
			Salinity Range																							
			0-3NST						3-5NST						5-18NST						18-26NST					
			Jun-17	Aug-17	Oct-17	Dec-17	Feb-18	Apr-18	Jun-17	Aug-17	Oct-17	Dec-17	Feb-18	Apr-18	Jun-17	Aug-17	Oct-17	Dec-17	Feb-18	Apr-18	Jun-17	Aug-17	Oct-17	Dec-17	Feb-18	Apr-18
Oncholaimellus	3	2B	0	0	0	0	0	0	0	0	0	0	0	0	0	1	2	1	0	0	0	2	0	0	0	0
Oxystomina	4	1A	0	0	6	0	0	0	0	0	0	0	0	0	0	20	10	9	0	8	3	0	0	0	0	0
Paracyatholaimus	2	2A	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	0	4	10	0	0	13	0
Paramonohystera	4	1B	0	4	3	4	13	0	14	4	9	6	8	0	1	19	12	5	2	3	2	1	0	3	4	1
Pomponema	3	2B	0	0	0	0	0	0	0	0	0	0	0	0	0	0	3	2	5	0	6	0	0	2	4	32
Pseudochromadora	3	2A	0	0	0	0	0	0	0	0	0	1	0	0	4	0	10	7	5	2	2	1	12	0	0	0
Rhabditis	1	1A	1	3	4	8	3	0	0	0	0	0	0	0	0	0	0	3	0	5	6	0	2	0	0	4
Sabatiera	2	1B	0	0	0	4	1	0	8	3	0	0	0	0	5	12	14	20	0	0	10	0	5	3	0	19
Scaptrella	2	2B	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	3	4	6	5	0	0
Spirinia	3	2A	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	3	1	0	2	3	5
Synonchium	3	2B	0	8	12	2	4	0	6	6	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Terschellingia	3	1B	2	5	2	8	4	0	56	56	50	30	52	41	0	4	9	0	0	0	10	6	10	10	8	0
Theristus	2	1B	6	3	3	4	0	0	12	10	13	31	25	34	36	3	5	4	0	1	0	5	10	0	0	1
Viscocia	3	2B	1	3	3	6	5	0	0	5	3	0	2	5	0	5	12	0	16	15	16	3	12	6	11	9
Xyala	3	1B	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	2	10	1	0	0