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Free-Living Nematodes as Pollution Indicator in Incomati River Estuary, Mozambique

Mthobisi Innocent Soko*, Thomas Kwadwo Gyedu-Ababio

Inkomati-Usuthu Catchment Management Agency, Mbombela, South Africa
Email: *sokom@iucma.co.za; thomasga@iucma.co.za

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Abstract

Four sites following the salinity gradient of the Incomati River Estuary E1 (0-3NST), E2 (3-5NST), E3 (6-18NST) and E4 (19-27NST) were selected for the study. The aim of the study was to use free-living marine nematodes as pollution indicators in an area strongly affected by anthropogenic activities. Multivariate statistical analyses were used to determine the relationship between different environmental factors and with free-living marine nematodes. Metals such Cadmium, Colbat, Chromium, Copper, Iron, Manganese, Nickel, Vadium, Zinc and Aluminium influenced the diversity and density of free-living nematodes. Shannon-Wiener Diversity, Maturity Index and colonize-persisters percentage (c% - p%) were found to be good tools for use as pollution indicators in the study. Nematode genera such as Terschellingia, Theristus and Halalaimus were found to be dominant at a site strongly impacted by both metals concentration and organic matters. The three genera are believed to be good indicators of pollution in the Incomati River Estuary. It is recommended that further studies are done along the Mozambican Coast to identify nematodes that can be used as pollution indicators.

Keywords

Estuary, Nematodes, Chlorophyll-*a*, Metals, Organic Matter, Pollution

1. Introduction

The Incomati River Estuary is prone to anthropogenic activities such as agricultural and industrial effluents from the upper catchments of the Incomati Basin. The presence of impoundments and abstraction taking place in the upper catchment reduces the flow regime, therefore, resulting in sediments fluxes. These activities affect the estuarine environment by changing the habitat structure and dynamics of living communities [1] [2]. These further affect the estua-

rine ecosystem and other goods and services rendered by the estuary [3]. The main disturbances in an estuarine and marine environment are organic pollution and sediment [4].

To understand the environmental quality of estuaries, free-living nematodes provide advantages as biological indicators because of their morphological structures such as mouth structure, tail shape and length-width ratio which relate to ecological functions [5] [6] [7] [8]. Their diversity in aquatic environment and their response to pollution make them a good tool in studies of environmental pollution [9] [10]. Nematodes were used in studies conducted in temperate estuarine and marine environment, and showed to be good pollution indicators for induced disturbance. Thus, they have indicated their importance in marine environment [11] [12] [13] [14]. In studies conducted in tropical estuaries in Tunisia and South Africa nematodes were also found to be sensitive to pollution disturbance [15] [16] [17].

The distribution and environmental factors affecting free-living nematodes are the main information in understanding the ecology of their communities and the role in dynamics of the ecosystems. There is no enough evidence of the availability of a specific factor such as grain size or organic content of sediment that contributed to the distribution patterns of nematodes [18]. Instead, nematodes respond to complex setting of environmental factors such as food availability, salinity and grain size [19] [20] [21] [22]. Sediment characteristics such grain size analyses, grain shape, sorting and pores space influence the diversity and abundance of nematodes in a soft bottom environment [23] [24]. Median grain size of sediments has been found to be the primary influence on meiofaunal density and diversity [11] [25]. 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* [15]. The aim of this study was to use free-living marine nematodes as pollution indicators in an area strongly affected by anthropogenic activities.

2. Materials and Methods

2.1. Study Area

The Incomati River Estuary is about 40 - 50 km long and meanders within the coastal plain. It is located on the east coast of Africa, Southern Mozambique (**Figure 1**) and the main anthropogenic activities along the estuary are drylands crops such as maize, grazing, sugarcane, vegetables and citrus [26]. Four sites were selected from the Incomati River Estuary following the salinity gradient of the estuary and based on their prone to pollution (**Table 1**).

2.2. Free-Living Nematodes

The study was conducted from June 2017 to April 2018. Sampling was done during low tide in the subtidal region using a hand held perspex corer which was 1 m long and 3.6 cm diameter down to a depth of 10 cm. Most nematodes are

Table 1. Sites selected for meiofauna in the Incomati Estuary from June 2017 to April 2018.

Site Names	Salinity Ranges	Estuarine Zone	Co-ordinates	
			Latitude	Longitude
E1	0 - 3 NST	Oligohaline	−25.7198611	32.6982694
E2	3 - 5 NST	Euhaline	−25.733775	32.680644
E3	5 - 18 NST	Mesohaline	−25.7622361	32.729275
E4	18 - 27 NST	Polyhaline	−25.8324361	32.73435

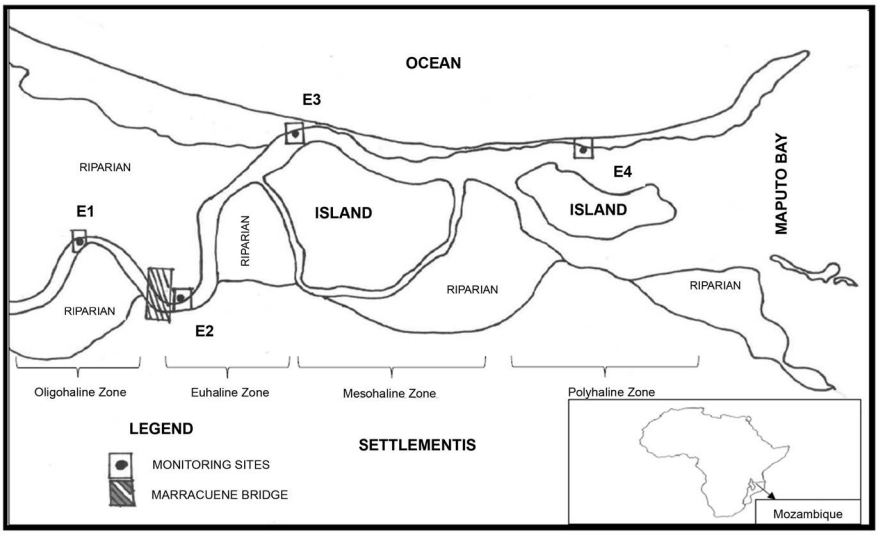

Figure 1. Map showing the monitoring sites in the study area.

mostly found between 4 cm to 10 cm of the sediment [27] [28] [29] [30]. One of the two samples taken at each site bi-monthly from June 2017-April 2018 was used for free-living nematodes analysis. Before sediment samples were taken, the sediments in the container was treated with 6% $MgCl_2$ on the field to facilitate relaxation of the meiofauna. In the laboratory the meiofauna in the sediment samples were fixed with 5% formalin with Bengal Rose solution added for staining of the nematodes. Meiofauna were separated from sediment using a centrifugal method with sucrose solution [31] [32] [33]. Nematodes were then collected in 5% formalin and counted under a stereo microscope at 40× magnification using counting petri-dish [34]. The first hundred counts of specimens in each replicate were randomly selected and mounted in wax ring slides for identification using pictorial keys [35]. Nematodes feeding types were investigated using their morphological structure [36].

2.3. Environmental Factors

The other corer sample was used for the analysis of Metals, Particle Size, Organic Matter and Chlorophyll-*a*. Sediment particle size and Organic Matter analysis were done following the procedure set by [37] [38]. Metal analysis was done fol-

lowing the procedure set by [15]. A method/procedure set by [39] was used for the analysis of Chlorophyll-*a*.

3. Data Analysis

A PRIMER 6.0 which is a multivariate statistical package developed by Plymouth Marine Laboratory [40] was used for the analysis of free-living nematodes data. A Shannon-Wiener Diversity Index was to determine the diversity of nematodes, while a Non-Multidimensional Scaling and Brait-Curtis Cluster Analysis were used to determine the similarities between the sites sampled based on their nematodes diversity, density and sediment particle sizes. The significant difference between sites was tested using a two-way ANOVA or PERMANOVA. A K-dominance curve was plotted for the comparison of genera composition at the sites. An RDA plot was done to determine the relationship between different environmental variables with nematode feeding types. A BIOENV procedure using a spearman's correlation was used to determine the relationship between environmental variables and the structure of nematodes community [41]. A Maturity Index was used to analyse the life strategy on free-living nematodes [42] [43] and a value on a scale (c-p score) was assigned to nematodes genera. The Maturity Index formular

$$\mathrm{MI} = \sum_{i=1}^{n} v(i) \cdot f(i)$$

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

4. Results and Discussions

4.1. Sediments

A variation of sediment particle sizes was found in the four sites sampled in the Incomati River Estuary (**Figure 2**). Site E1 was characterised mostly by fine sand with 46.32% and this was attributed to deposition taking place at the site.

Site E3 and E4 were mostly characterised by coarse and very coarse particle sizes which were attributed to tidal action that washes the sand from small particles. Sediment grain sizes are important environmental factor especially that help in the structuring of meiofauna.

4.2. Organic Matters

The highest percentage of Organic Matter was found at site E2 with a mean value of 2% (**Figure 3**). The highest percentage of Organic Matter was attributed to fine sand particles because they have higher surface area for organic adsorption. Similarly, in a study conducted in Southern European estuaries organic matters were higher at sites characterised by fine particle size [44].

The lowest percentage of Organic Matters was found a site E1with a mean value of 1.2%. At both sites E3 and E4, the mean percentage of Organic Matters

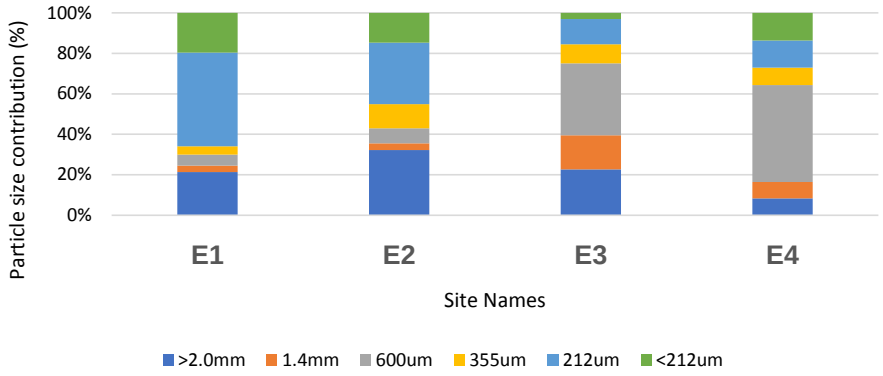

Figure 2. Sediments particle size analysis in the Incomati River Estuary.

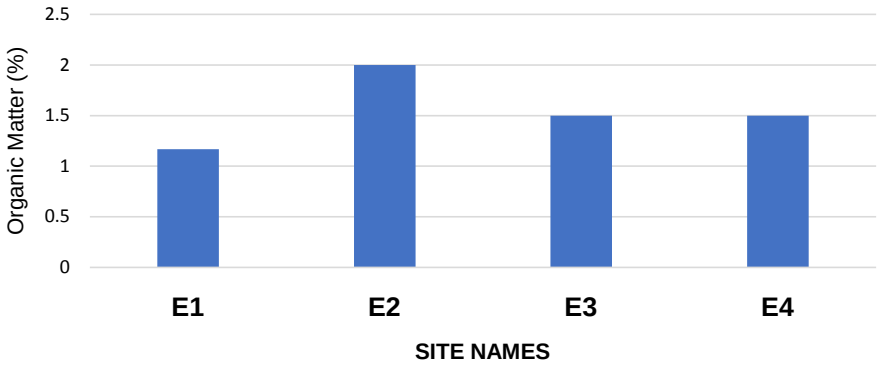

Figure 3. A graph representing the Organic Matter concentration in the Incomati River Estuary.

was 1.5%. A two-way ANOVA indicated that there was no significant different ($p > 0.05$) of Organic Matter concentration between the sites sampled.

4.3. Chlorophyll-*a*

The highest concentration of Chlorophyll-*a* was found at site E3 with a mean concentration value of 3.2 mg/m^3 (Figure 4).

The second highest concentration of Chlorophyll-*a* was found at site E4 with a mean concentration value of 1.24 mg/m^3. The lowest concentration of Chlorophyll-*a* was found at site E2 and E1 with a mean concentration of 0.87 mg/m^3 and 0.95 mg/m^3 respectively.

4.4. Metals

Ten metal concentrations (Cadmium, Colbat, Chromium, Copper, Iron, Manganese, Nickel, Vadium, Zinc, and Aluminium) were found (Table 2) in the estuary. The highest concetration of metals were observed at site E2, and the second highest concetration was observed at site E1. Site E2 is situated in the Euhaline Zone while site E1 is situated in the Oligohaline Zone. The lowest concetration was observed at site E4 and E3, which were situated in the Polyhaline and Mesohaline Zones respectively.

PERMANOVA analysis indicated that there was a significant different ($p < 0.05$)

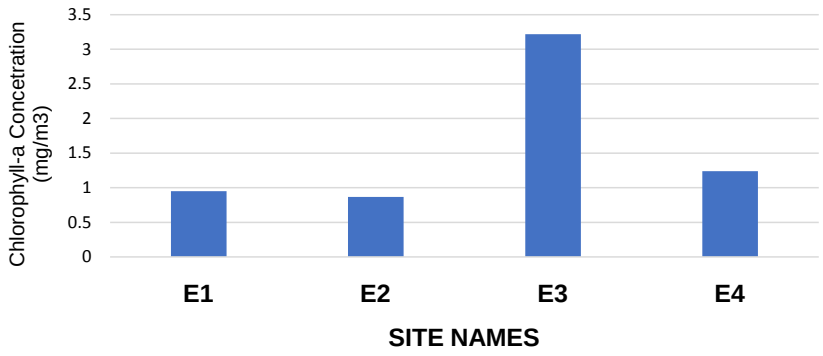

Figure 4. Chlorophyll-*a* concentration in the Incomati River Estuary.

Table 2. Mean concetration of metals from sediments sampled from June 2017 to April 2018.

Metals (ppm)	E1	E2	E3	E4
Cd	0.13	0.17	0.11	0.09
Co	3.27	3.89	1.61	0.49
Cr	10.28	14.92	20.05	7.87
Cu	5.25	7.85	4.37	4.10
Fe	4354.83	9125.12	2777.83	1537
Mn	123.67	194	54.83	59.67
Ni	8.38	11.97	3.45	3.57
V	6.87	12.30	4.28	1.43
Zn	13.68	12.6	6.75	8.88
Al	4802	7935.33	2264.67	904.17

between sites sampled, but not between months. These results indicated that the concentration of metals changes spatial, but not temporal. The higher concentration of heavy 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.

4.5. Nematodes Density

A total of 5989 nematodes individuals/10 cm^2 were sampled in the Incomati River estuary. The highest nematode density of 2605 individuals/10 cm^2 was found at site E4 which is situated in the Polyhaline Zone of the estuary, while a lowest density of 721 individual/10 cm^2 was found at site E1 situated in the Oligohaline Zone (**Figure 5**). These findings indicated that nematodes density decrease with decrease in salinity.

Similarly, in a study conducted in the Swartkops River System, South Africa [15], nematodes density was found to be higher in the Polyhaline Zone and lower in the Oligohaline Zone. Different findings were observed in another study conducted in Mondego estuary [45]. A two-way ANOVA indicated a significant different ($p < 0.05$) of nematodes density between the sites.

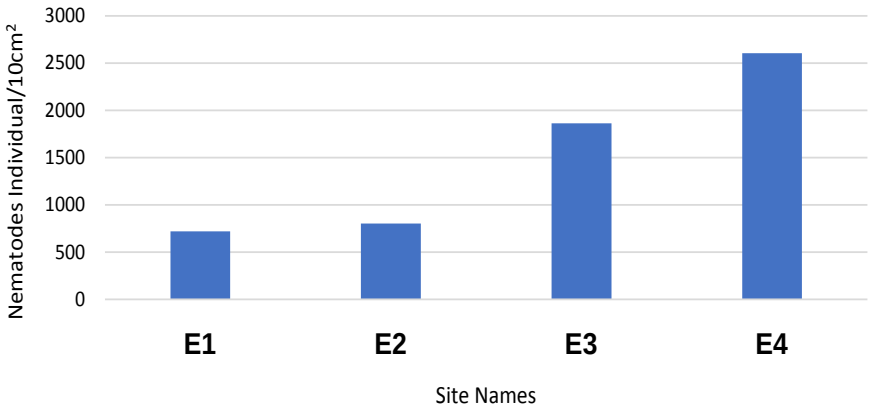

Figure 5. Mean nematodes density sampled in the Incomati River Estuary.

4.6. Nematode Diversity

A total of 35 nematode genera were found in the Incomati River Estuary (**Appendix A: Table A1**). The diversity of nematode in the Incomati River Estuary were found to differ from site to site. The lowest diversity of nematodes was found at site E1 with nematodes diversity range of 4 - 13 genera. Nematodes genera such as Haliplectus and Axonolaimus were found to be dominant at site E1 with nematodes diversity values of 41% and 13.2% respectively. The number of diversity genera at E2 was found to range from 4 to 12 genera, and the dominant nematodes genera were found to be Terschellingia and Theristus with 47.5%, and 20.8% respectively. Other nematode genera that were present at site E2 were Axonolaimus, Sabatiera, Daptonema, and Parodontophora which may indicate pollution and disturbance [43] [46] [47] [48]. Therefore, dominance of nematode genera such as Axonolaimus, Terschellingia, and Theristus at sites E1 and E2 indicated that these sites were more polluted than the other sites. Theristus has been found to be a good pollution indicator of organic matter [17]. Pollution at these sites was attributed to agricultural, industrial activities from upstream catchments, and informal settlements along these sites. Nematode density at site E3 ranged 11 to 18 and the dominant genera was Sabatiera with 8.5%, and Theristus with 8.2% of the total nematodes genera. Site E4 had the highest diversity of nematodes with a range of 13 to 21. The highest diversity of nematodes at site E4 and the lowest diversity of nematodes at site E1 indicated that nematodes diversity decreases from the Polyhaline zone to the Oligohaline Zone.

4.7. Maturity Index and Shannon-Diversity Index

The Maturity Index (MI) which is a potential indicator of nematode assemblage under stress and the Shannon-Diversity Index of the four sites sampled were calculated (**Figure 6**). 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 at these sites indicated that nematode genera were not under stress.

At sites E2 and E1 the Maturity Index were found to be lower with Maturity

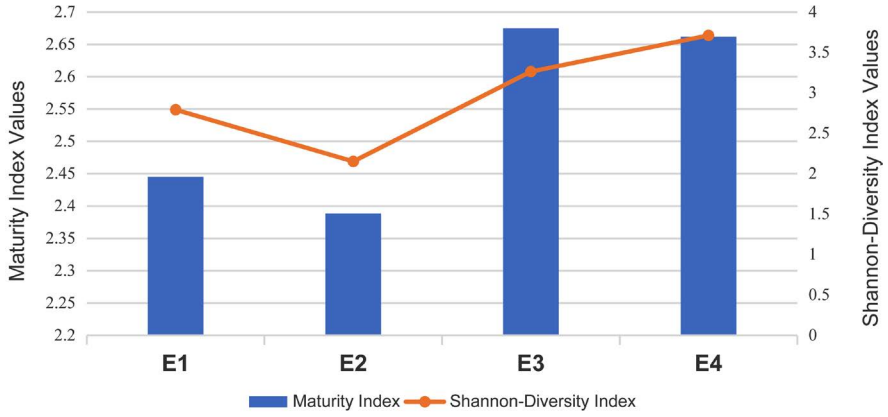

Figure 6. Maturity Index results indicating the polluted sites with Shannon-Diversity Index superimposed.

values of 2.38 and 2.44. The lower Maturity Index indicated that these sites were under stress, especially at site E2 which had higher concentration of heavy metals and total phosphate throughout the sampling period. Similarly, the Shannon-Diversity Index indicated the same finding as the Maturity Index.

A Bray-Curtis Cluster Analysis and NMDS ordinations (**Figure 7(A)** and **Figure 7(B)**) indicated a group formation of sites at similarities 50% and 65%. Group 1 was formed by sites E1 and E2 and group 2 was formed by sites E3 and E4 at similarity 50%. The similarities within the groups were attributed to similar meiofauna taking place and experiencing similar environmental factors within the sites.

Group 3 was formed by sites E3 and E4 at similarity 65%. The similarity at 65% indicated that the was no much change of meiofauna diversity and density at these sites, while the dissimilarities of sites E1 and E2 was attributed to the factors that these sites received different environmental factors, and meiofauna diversity changed at different period of sampling.

The K-dominance curve (**Figure 8**) indicated that at cumulative dominance of 40% Haliplectus dominated the nematodes communities at site E1. At a cumulative dominance of above 40% the K-dominance indicated that Terschellingia and Theristus were the dominant genera at site E2. The dominance of these nematodes at sites E2 and E1 indicated that they were tolerant to higher concentration of metals and organic matters at these sites, hence the dominance of single genera in polluted sites.

At both sites E3 and E4, the cumulative dominance was below 20% indicating that these sites were more diverse than sites E1 and 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.

An RDA triplot indicated that the lower diversity and density of nematodes at site E2 was attributed to high concentration of metals such as Cadmium, Colbat, Chromium, Copper, Iron, Manganese, Nickel, Vadium, Aluminium and Organic Matters with had strong correlation with nematode feeding type 1B (**Figure 9**).

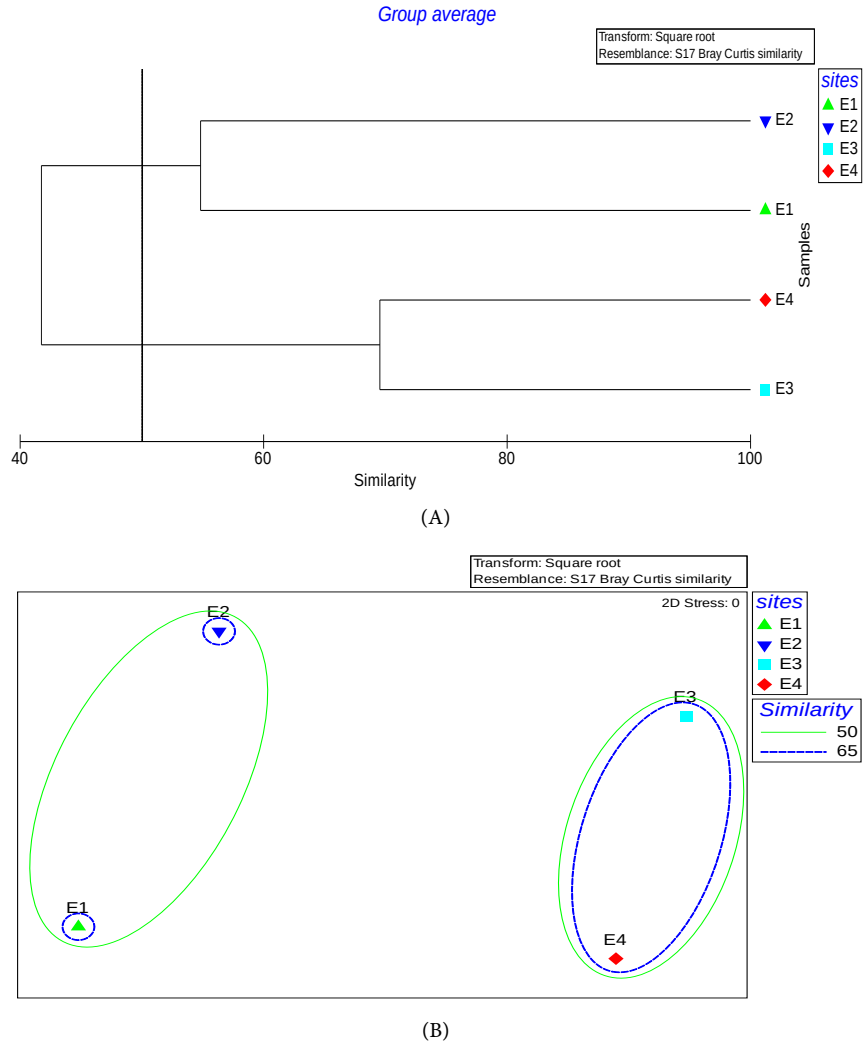

Figure 7. Bray-Curtis similarity matrix-based cluster analysis (A) and two-dimensional representation of the NMDS ordination (B) of free-living nematodes genera collected in the Incomati River Estuary. The NMDS ordination was completed with 25 iterations and showed a stress of zero.

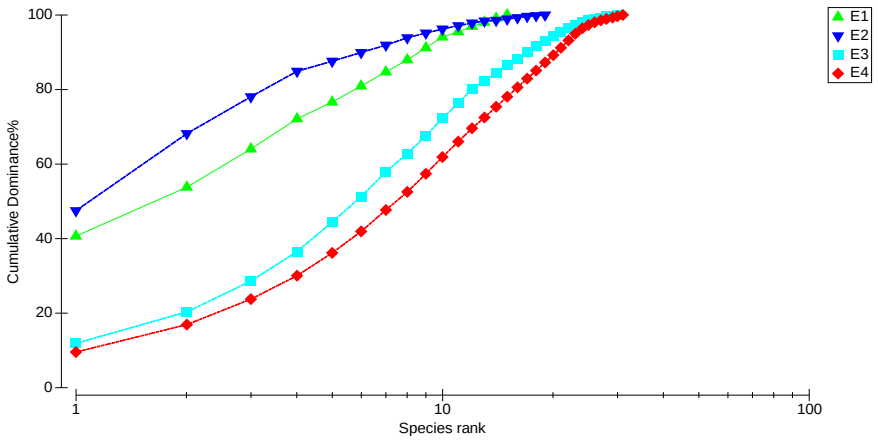

Figure 8. Ranked species K-dominance curves for the free-living nematode genera identified at the Incomati River Estuary.

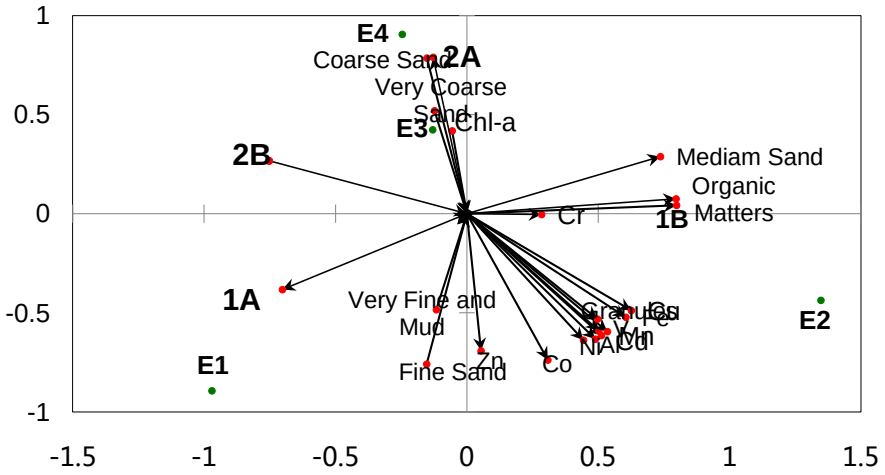

Figure 9. RDA tri-plot illustrating the similarities between the feeding types and the environmental factors found in the Estuary. The Tri-plot describes 99.86% of the variation with 66.93% described on the first axis and 32.93% on the second axis.

The higher diversity and density of nematodes at sites E3 and E4 were attributed to sediment particle size such as Coarse Sand, Very Coarse Sand and Chlorophyll-*a* because they had a strong correlation with nematode feeding types 2A and 2B.

Another strong correlation was observed between very fine sand, fine sand and Zinc with nematodes feeding types 1A.

4.8. Environmental Factors and Nematodes Communities

The BIOENV analysis indicated that although other environmental factors correlated with nematodes diversity, Nitrates (NO_3), Very Coarse sand, Coarse Sand, and Fine Sand were the most significant (Rho = 0.693; $p < 0.05$) environmental variables that structure nematodes community in the estuary especially when all environmental variables were combined (**Table 3**). [22] [49] [50] indicated that within an area of uniform salinity, grain size of sediments is a dominant factor in determining the composition of nematodes communities.

According to [51] sediments particle size such as grain size, organic content, and Chl-*a* are other important factors that contribute to the distribution and buildup of nematodes in estuarine environment. Similar findings were obtained in a study conducted in the Swartkops River in South Africa [15] where sediment particle size was found to influence nematodes density, and the number of nematodes was low at sites dominated by both finer, and coarse sands. In another study conducted by [52] nematodes density and diversity were found to be structured by coarse sediments. [53] 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 [54] [55] who also found that density and diversity of marine nematodes increase with increase sediment grain size.

Table 3. Summary of BIOENV analysis indicating the environmental factors influencing nematode structures.

	Environmental Variables	Correlation or Rho
Combined	Nitrate (NO_3), Very Coarse Sand, Coarse Sand and Fine Sand	0.693

5. Conclusion

Nematode diversity and density decrease with a decrease in salinity gradients in the study. Sites E2 and E1 were found to be the polluted sites with higher concetration of metals and organic matters. Nematodes genera such as Terschellingia, Theristus and Halalaimus were also found to be dominant at these sites E2 and E1. The positive correlation between nematodes genera such as Terschellingia, Theristus and Halalaimus with metals such as Cadmium, Colbat, Chromium, Copper, Iron, Manganese, Nickel, Vadium, Zinc, and Aluminium indicated that these nematode genera can be pollution indicators in the estuarine environments. A combination of Maturity Index, Shannon-Diversity Index and c-p values was good tool in identifying polluted sites in the study. It is recommended that further studies are done along the Mozambican Coast to identify nematodes that can be used as pollution indicators.

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Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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Appendix A

Table A1. 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 - 3 NST						3 - 5 NST						5 - 18 NST						18 - 26 NST					
			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
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

Continued

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
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