Hydrobiologia **397:** 155–169, 1999.

Nematodes as indicators of pollution: a case study from the Swartkops River system, South Africa

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Received 7 January 1999; in revised form 4 March 1999; accepted 21 March 1999

Key words: nematodes, estuary, pollution, indicator, density, diversity, sediment

Abstract

Nematodes from the sediments of the Swartkops estuary in Port Elizabeth, South Africa were investigated at 10 selected sites along a salinity gradient in the subtidal region at neap tide. The relation between nematode density, genera, community structure and environmental parameters including concentrations of seven heavy metals, Mn, Ti, Cr, Pb, Fe, Sn and Zn in the sediment were investigated. The nematode community structure was significantly influenced by the chlorophyll a concentration and sediment particle-size distribution. The number of genera had significant negative correlation with chlorophyll a and two heavy metals, Fe and Zn in the sediment. The habitat preferences of the genera were also assessed. A combination of the Shannon-Wiener Diversity Index (H') and the Maturity Index (MI) proved to be very useful in assessing polluted or stressed sites. The nematode communities at sites which are affected by pollution, were found to be under stress according to the density, diversity and other indices used in this study. At sites where relatively higher heavy metal concentrations occurred, variation in the nematode densities and diversity were observed. Nematode community structure at polluted sites differed significantly ($p < 0.05$) from those at less or no polluted sites. *Monhystera* spp. and *Theristus* spp. were found to be colonisers, and thus indicator genera for polluted sediments in this study.

Introduction

Estuaries are among the most productive ecosystems in the world; they are however, also vulnerable to man's activities. Due to industrialisation and agricultural activities in their catchments and floodplains, estuaries are subject to development pressures, such as harbour development, land reclamation, recreational projects and anthropogenic inputs (Grindley & Heydorn, 1979; Evans, 1981; Meire & Kuyken, 1984; Humme et al., 1986; Branch & Branch, 1981) . The develoment pressures have resulted in an escalation of interest in estuarine ecology and conservation over the past two decades (Martin, 1977).

The Swartkops estuary is an integral part of Port Elizabeth and surrounding areas. It is a valuable aesthetic, ecological and recreational asset, and due to its geographical position in a rapidly expanding urban area, subjected to influences and effects of various developments (Baird et al., 1987).

Benthic fauna have been found to vary greatly in sensitivity to various types of pollution (e.g. Cairns & Dickson, 1971). Benthic invertebrate species and communities have often been regarded as good indicators of organic pollution because of their constant presence, relatively long lives, sedentary habits and differing tolerances to stress. Whilst less is currently known about meiofaunal responses to pollutants, meiofauna have certain inherent advantages over macrofauna in the determination of the effects of biological pollutants at the community level. Unlike macrofauna there are a few meiofaunal species that rely on pelagic dispersal phases in their life history, thus all life stages must withstand the conditions of the area under study. Meiofauna also has a higher species richness than macrofauna (Heip et al., 1988; Moore & Bett, 1989).

Different species also exhibit different responses to different kinds of stress, and meiofauna may be more sensitive to sediment pollution than macrofauna.

The use of meiofauna in pollution monitoring studies started over a decade ago and at that time their potential was assumed to be quite high. Marine nematodes were thought to be sensitive biological indicators of pollution because they are very diverse taxonomically and occur everywhere, usually in great numbers and often exceeding other taxa by orders of magnitude (Croll & Mathews, 1977; Platt et al., 1984). Many authors do agree that nematodes are ecologically important and their exact role in ecosystems where they occur in abundance is still being investigated. Food availability has been cited many times as a factor for nematode distribution in both phytal and benthic habitats (Tietjen, 1967; Jensen, 1984; etc.)

According to Vanreusel (1991), the habitat preferences of most of the nematode species in the Dutch Delta area (Belgian Coast) are mostly related to sediment characteristics. For example, particle size of the sediment appears to be a particular factor influencing the distribution patterns. Where this is not so, factors other than typical sediment characteristics may be responsible for a shift in the distribution of species along environmental gradients. Apart from the sediment composition, salinity also influences species composition of nematode communities (Heip et al., 1985; Coull, 1988, Vanreusel, 1990; Soetart et al., 1995).

At present, there is little empirical evidence to suggest that ecologically similar species, belonging to the same genus or family, respond differentially to pollution effects. Also, there are strong indications that pollution effects are detectable at even higher taxonomic levels (Warwick, 1988). It has further been observed that, to be of value in a pollution assessment context it may not be necessary to work at the species level (Heip et al., 1988; Warwick, 1988; Danovaro et al., 1995).

Maturity Index (Tom Bongers, 1990) has been used as a tool in assessing polluted sites. Based on the colonising characteristics of the nematodes, they are grouped into colonisers or persisters with c-p values ranging from 1 through to 5. *Monhystera* which was originally asssigned a c-p value of 1 has now been given a c-p value of 2 (De Goede et al., 1993; Bongers et al., 1995). The reasons being that the genus is a general opportunist.

The main objective of this study was to assess the effect of pollution on the nematode community structure in the Swartkops estuary. Inorganic (heavy metal) and organic pollutants were investigated at 10 sites situated along the salinity gradient of the estuary. Since factors other than the above pollutants could affect the structure of nematode communities, the impact of salinity, particle size and seasonal changes were also investigated. The relationship between nematode density, diversity, community structure and environmental parameters including several measures of pollution, was established in the mildly polluted Swartkops estuary. This is in an attempt to ascertain the potential of nematodes as pollution indicators, in particular, of sediment contamination by e.g. heavy metals.

Materials and methods

The Swartkops estuary is situated about 15 km north of the Port Elizabeth Harbour at 33° 52′ S and 25° 38′ E (see Figure 1).

The estuary is 16 km long from the mouth to the head of the estuary (Dye, 1977). Ten sampling sites, L, K, J, CRM, I, H, KC, BR, D and C in order of decreasing salinity were selected to include a range of salinities as well as ‘polluted’ and unpolluted areas.

The study was conducted from May 1995 to March 1996. Sampling was done at neap tides in the subtidal region using a hand held perspex corer, 1m long and 6.5 cm diameter down to a depth of 10 cm. Most of the meiofauna are normally found in the top 10 cm of the sediment (Heip et al., 1982; Smol et al., 1994). Four sediment samples per site were taken approximately 10 centimetres apart. According to Sun & Fleeger (1991 cited by Giere, 1993), it is indispensable for reliable quantitative meiofauna studies to take parallel samples, since patchiness is a characteristic of meiofauna.

Two of the four samples at each site throughout the sampling period (May 1995–March 1996) were used for meiofaunal analysis. Two 10.0 cm^2 sediment cores proved to be adequate for quantitative analysis (Danovaro et al., 1995) of sublittoral meiofauna. The cores were treated with 6% $MgCl_2$ on the field to facilitate relaxation of the meiofauna. Back in the laboratory the meiofauna in the sediment samples were fixed with 5% formalin with Rose Bengal solution added for staining of the nematodes.

The nematodes were extracted using the centrifugal flotation method with sucrose solution as the separating agent (Lackey & May, 1971). This method

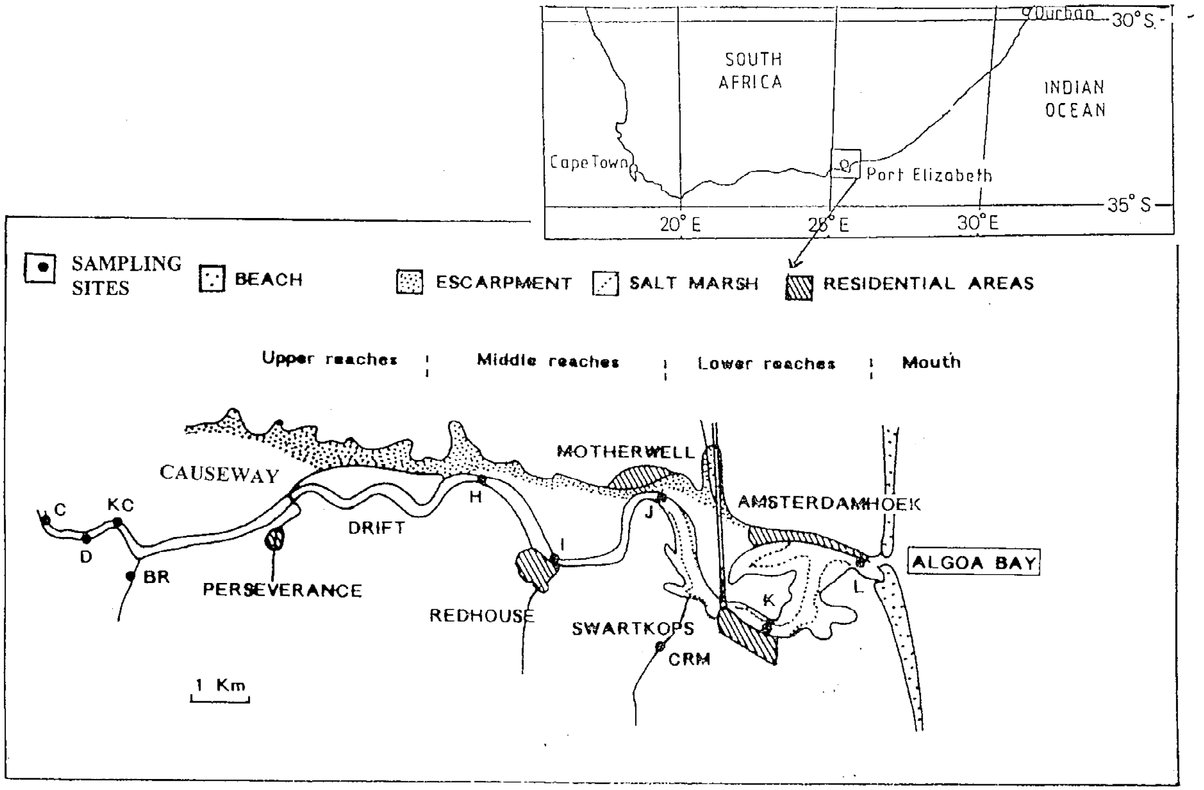

Figure 1. Map of the Swartkops estuary showing sampling sites.

has an extraction efficiency of about 90% (Furstenberg et al., 1978). The nematodes were collected in 5% formalin, and counted under stereo microscope at 40× magnification using a counting petri dish or a sorting tray (Giere, 1993). Counts were converted to numbers per m^2. The first hundred specimens in each replicate were removed at random and mounted onto wax ring slides for identification using the pictorial key of Platt & Warwick (1988).

Additional core samples were taken at each site for sediment particle size, organic carbon, chlorophyll *a* and heavy metals analyses. The sediment particle analysis was done using the method described by Ingram (1983). The median grain size, sorting values, mud composition and all the other sand fractions were determined using a computer programme, SANDX (Sandsta.Baj) by Olivetti, 1984. The organic carbon content was determined using the ash method described by Parker (1983) and the chlorophyll *a* content determined by the method of Rodriguez (1993). The actual concentration of the chlorophyll *a* was calculated by the formula

$$CC = Abs - (Abs/1.7)29.6,$$

where CC = chlorophyll *a* concentration; and Abs = absorbency reading.

For the determination of heavy metal concentrations samples were dried at 80 °C and digested in concentrated HNO3. Metal determinations of the solutions were performed on a Shimadzu sequential plasma spectrometer (ICPS-1000II) using the calibration curve method. Salinity was measured in the field on every sampling trip using refractometer.

Various statistical methods including Analysis of variance (ANOVA), Kruskal-Wallis and Post-hoc (LSD) tests; Spearmans-Rank Correlation and Regression Analysis were used to establish temporal and spatial differences and relationships between the environmental factors and nematode attributes.

The Maturity Index (MI) (Bongers, 1990) and Shannon-Wiener's Diversity Index were also used establish sites under stress. The Maturity Index (MI) is calculated as the weighted average of the individual

Table 1. Sediment particle size distribution at the ten study sites (mean values)

Fraction	L	K	J	CRM	I	H	KC	BR	D	C
Median	2.28	2.32	2.72	2.48	2.62	2.24	1.26	0.76	1.04	2.65
Mean	2.3	1.96	2.29	2.45	2.68	2.23	1.25	0.86	0.98	2.52
Sort	0.31	0.86	1.14	0.52	0.57	0.9	1.21	0.95	0.99	0.81
Skewn	0.06	−0.28	−0.38	−0.06	0.11	3.E-9	−0.01	0.1	−0.06	−0.16
Kurt	0.96	1.2	0.79	1.52	0.64	0.47	0.46	0.63	0.46	0.86
>VCS	4.12	23	10.8	6.32	0.78	0	27.9	29.66	63.58	18.1
VCS	0.24	6.08	3.04	0.54	0.12	0.14	10.54	15.72	5.7	0.98
CS	0.38	2.1	2.06	0.58	0.84	5.84	15.14	22.68	9.2	2.2
MS	11.6	7.98	6.54	2.8	6.58	27.18	15.44	20.70	14.22	6.9
FS	79.5	46.06	15.94	15.7	54.2	32.78	15.88	6.82	6.38	16.68
VFS	1.68	5.28	17.52	2.56	22.3	17.5	4.82	2.28	0.54	16.26
Mud	2.5	9.6	44.48	71.56	15.2	16.54	10.38	2.04	0.42	38.92

>VCS – More than very coarse sand; VCS – Very coarse sand; CS – Coarse sand; MS – Median sand; FS – Fine sand; VFS – Very fine sand; Mud = Silt and Clay.

coloniser-persister (c-p) values:

$$\mathrm{MI} = \Sigma \nu(\iota) f(\iota),$$

where ν is the c-p value of the taxon (genus) (ι) and $f(\iota)$ is the frequency of the taxon in the sample. The Maturity Index (MI), is proposed as a semiquantitative value which indicates the condition of an ecosystem based on the composition of the ‘nematode community’. Shannon-Weiner’s Diversity Index (H') was calculated for each site for every month.

$$H' = -\Sigma \rho(\iota).\log \rho(\iota),$$

where ρ is the ratio of the number of a particular species to that of the total number of species (nematodes) (i) at a sampling site.

Multivariate Analysis were applied on the data in various ways. They include (a) PRIMER (developed at the Plymouth Marine Laboratory, Plymouth, U.K.), used to group the study sites based on the nematode attributes and environmental factors. A similarity matrix was constructed using the Bray-Curtis measure of similarity on 4th root transformation. (b) CCA (Canonical Correspondence Analysis). The relationship between the community structure and environmental parameters was analysed using CCA option from the programme package CANOCO (Ter Braak, 1988). This package was used on the data to asses which of the environmental factors were important in the structuring of the nematode community.

Results

Sediment

Apart from site C which had the low median grain size of 6.9, the upper reaches had higher median grain sizes (see Table 1).

There was no significant difference between the sampling months in as far as specific size fractions are concerned. The sorting values followed a similar trend; higher sorting values at the upper reaches as compared to the middle and lower reaches. Figure 2 shows the variations in the sediment composition at the sites during the period of the study.

Sites K, J and C were the only sites which showed marked variations in the sediment grain size composition. Site K is used extensively for angling purposes and the search for baits in the sediment might have caused the wide variations in the sediment composition. Site J is a sewage output point and the discharge of various organic matter and silt could cause the changes in the mud component over time. Site C located at the head of the estuary is mainly fresh water and the deposition and washing away of sediment by currents of runoff water is a contributing factor to the wide variations in the sediment composition.

Results of the sediment analysis performed on the primer programme indicated that the 10 sites could be grouped into four clusters. The fresh water sites D & BR had low silt fractions and larger sand particles compared to the other sites. Only one site, L at the mouth of the estuary had very well sorted sediments. The remaining seven sites J, KC, C, CRM, I,

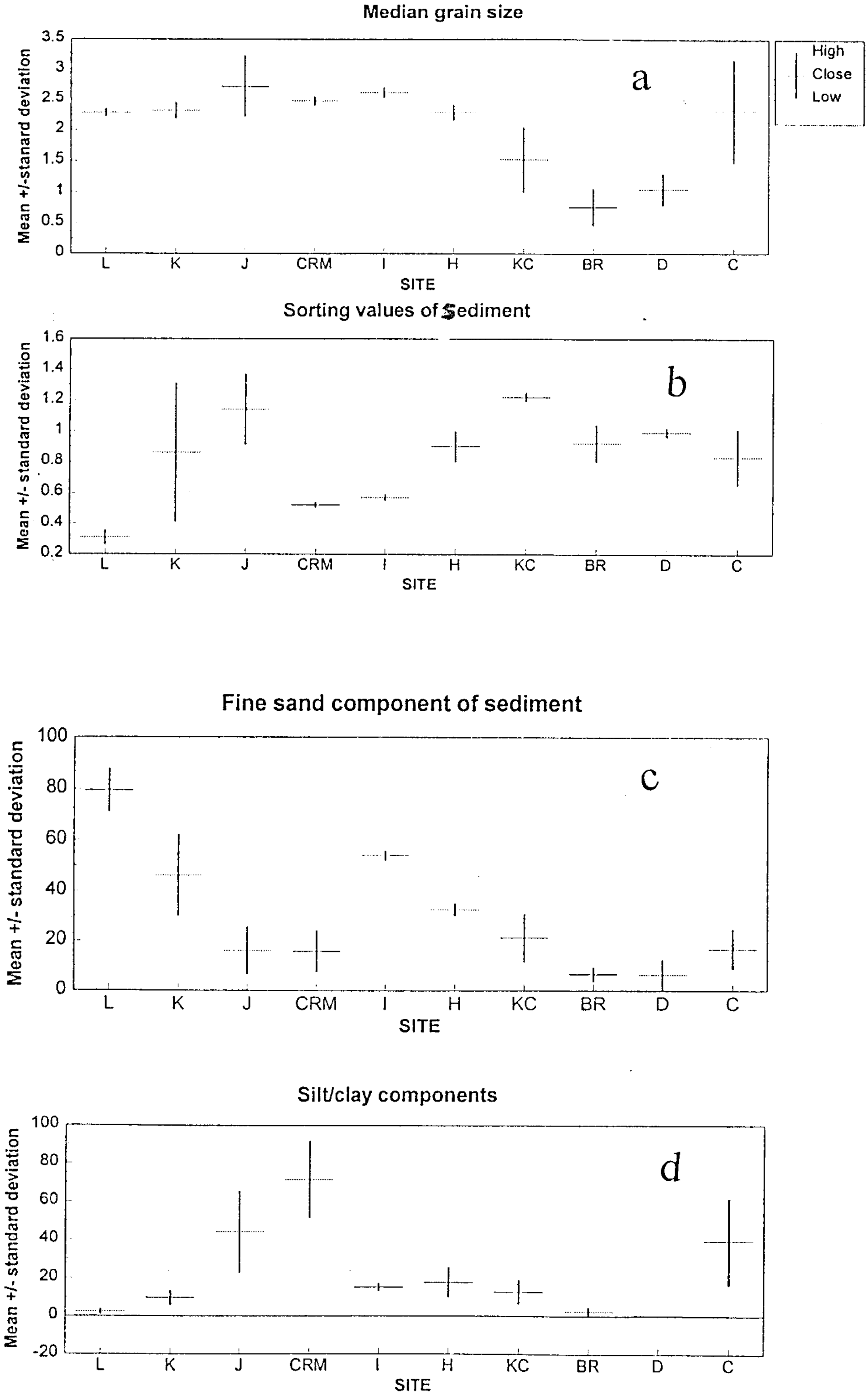

Figure 2. (a–d): Variation in sediment particle-size distribution at the study sites.

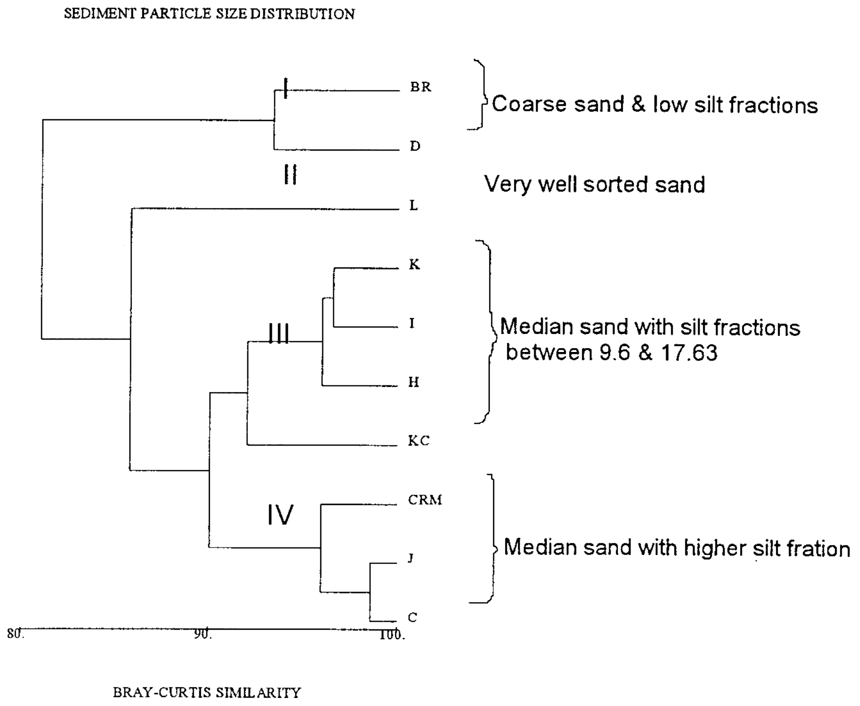

Figure 3. Bray-Curtis Similarity Index (4th root) of the sites based on sediment particle size composition.

H and K all had median grain sediments with varying proportions of silt. In terms of sediment particle size distribution, no significant differences ($p < 0.05$, Two-Way ANOVA) existed between the sites. The sediment composition in terms of particle size of the sites therefore was not very heterogeneous. Figure 3 illustrates the clusters indicating the dominant sediment fraction at various sites.

Salinity

The sampling sites are arranged in order of decreasing salinity as shown in Figure 1. Based on the mean salinity values, they can be grouped as follows: L, K, J & CRM are poly-haline (>18¡); I & H as alpha-mesohaline (10–18¡); KC as beta-mesohaline (5–10¡) and BR, D and C as beta-oligohaline (0.5–3¡). Table 2 shows the salinity ranges for all the sites during the sampling months. Apart from the salinity gradient between the sampling sites, significant temporal variation in salinity was observed during the study period.

Site C, however, never experienced any changes in its salinity values. It is purely a fresh water site at the head of estuary. One site which did not always follow the salinity gradient was CRM. It recorded abnormally high salinity values in July 1995 and March 1996 (see Table 1).

Table 2. Monthly salinity values (¡) at the sampling sites from May 1995 to March 1996

	Salinity values					
Site	MAY	JUNE	SEPT	NOV	JAN	MAR
L	35	38	37	35	35	38
K	30	35	37	33	34	36
J	–	31	37	14	30	33
CRM	23	44	–	12	21	42
I	10	23	33	8	26	29
H	6	23	27	8	26	28
KC	–	5	5	6	3	3
BR	0	0	4	1	0	4
D	0	0	3	0	0	2
C	0	0	0	0	0	0

Organic carbon & chlorophyll

Table 3 shows the organic carbon content of the

Table 3. Organic content (%) of the sediment at sampling sites from May 1995 to March 996

Site	JUL ’95	SEPT ’95	NOV ’95	JAN ’95	MAR ’95	MEAN
L	2.33	1.08	2.20	0.84	0.50	1.39
K	4.1	1.69	2.69	1.97	3.55	2.80
J	5.66	3.89	3.70	3.70	4.69	4.33
CRM	4.12	4.99	4.63	6.53	4.32	4.92
I	1.00	1.32	1.27	1.43	1.09	1.22
H	0.87	–	1.13	2.75	1.35	1.53
KC	–	1.47	2.72	2.06	–	2.08
BR	–	0.23	0.38	0.86	0.47	0.49
D	0.43	0.48	0.63	0.34	0.62	0.50
C	1.75	2.49	2.22	2.44	0.73	1.93
MEAN	2.03	1.76	2.16	2.29	1.73	2.12

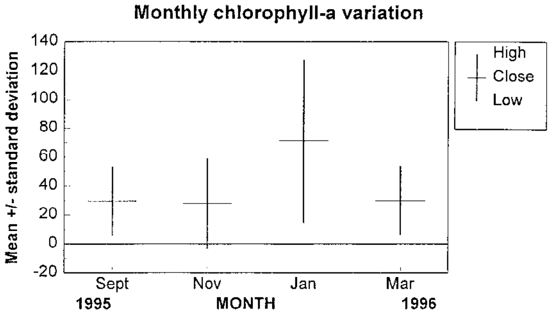

Figure 4. Variation in chlorophyll *a* concentrations (4 months analysed).

sediment at the ten sites during the sampling months.

Organic carbon concentration at sites K, CRM and J were the highest (see Table 3). Significant difference ($p < 0.05$, Two-Way ANOVA) existed between the sites in the concentration of organic carbon. There was however, no significant difference between the months in the organic content of the sediment. Sites L, I and H at the lower and middle reaches had lower values. Sites BR, D and C in the upper reaches also had relatively, lower values of the organic content. The remaining sites (estuarine) had comparatively higher organic content in the sediment.

Unlike the organic content of the sediment, chlorophyll *a* showed significant differences ($p < 0.05$, ANOVA) between the sites as well as the sampling months. Chlorophyll *a* showed fluctuations seasonally with summer months especially January 1996 having significantly higher concentrations. Figure 4 shows the seasonal variations in the chlorophyll *a* concentrations.

Table 4. Concentrations (μg/g) of 7 heavy metals at the study sites

Metals	Sampling sites									
	L	K	J	CRM	I	H	KC	BR	D	C
Mn	32.9	44.0	139.2	177.2	97.6	89.5	233	140	94.1	129.2
Ti	29.2	54.7	149.7	172.6	85.6	128.5	59.7	47.3	33.7	22.1
Fe	2942	5084	22385	31232	6659	5713	16052	5669	4812	4700
Cr	7.3	21.5	50.9	81.6	16.6	13.3	26.5	18.3	13.7	11.6
Pb	7.4	22.5	41.4	53.8	30.6	18.3	87.9	30.5	46.3	8.2
Sn	336.8	827	4389	6435	1180	860	2958.6	975	958.8	724.8
Zn	6.0	20.8	54.2	68.8	23	10.6	116.1	17.6	27.4	32.3

January 1996 had the highest variation in chlorophyll *a* concentrations. The post Hoc tests performed also showed that January 1996 was significantly different from the other months. Sites CRM, J, KC and BR relatively had higher chlorophyll *a* concentrations. See Table 9 in Appendix. Sites CRM, KC, BR, D and C also had wide variations in the chlorophyll *a* concentration. The remaining sites in the lower reaches, L, K, J, I and H had very little variations in their chlorophyll *a* concentrations.

Heavy metals

The concentrations of seven metals, Mn, Ti, Fe, Cr, Pb, Sn and Zn were obtained at all 10 sites and the values obtained from this analysis were not very different from those obtained by Watling & Watling (1983) from the same estuary. The concentrations of the named metals at the sites are given in Table 3. Lowest concentrations were obtained at site L whereas highest concentrations were recorded at sites CRM, J and KC (see Figure 1 and Table 4).

Sites K, I and H had moderate concentrations of the metals. These sites are in the middle reaches of the estuary.

Seasonality

Apart from chlorophyll *a* and salinity, all the other environmental parameters had no significant differences ($p > 0.05$, Two-Way ANOVA) between the sampling months. Mean values of the environmental parameters were subsequently used for further comparison. The chlorophyll *a* concentrations of the sediment, which showed a significant ($p < 0.05$) difference between the sites were mainly influenced by the seasonal effect with January 1996 (summer) being significantly different from the other months (see Figure 4).

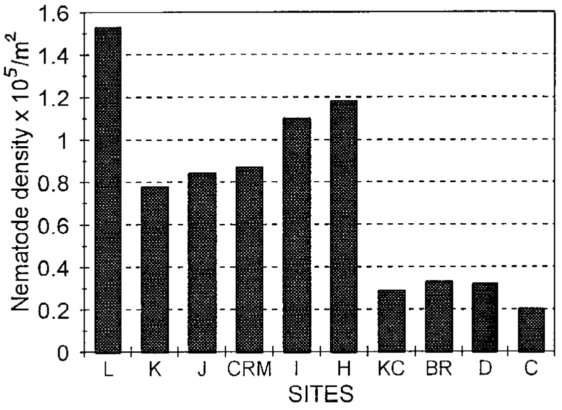

Figure 5. Mean nematode densities at the sites from May 1995 to March 1996.

Table 5. Maximum correlation of environmental factors with mean nematode density

Environmental factor	*n*	*r*
Chlorophyll *a*	39	−0.47
Sorting	45	−0.38
Fine sand	45	0.40
Median grain size	45	0.23
Mn	29	−0.50
Zn	29	−0.61
Ti	29	0.48

Nematode density

The nematode densities were not very high as compared to results from some parts of the world. This is, however, not abnormal as similar or even smaller numbers have been recorded in some estuaries (Ferris & Ferris, 1979). Nematode density (m^{-2}) are given in Figure 5.

Significant differences ($p < 0.05$, ANOVA) existed between the sites in their nematode densities. The upper reaches KC, BR, D and C had lower nematode numbers than the rest of the estuary studied. The lowest mean number was found at site C (204068 ind/m^{-2}) whilst the highest number was found at site L (1532666 ind/m^{-2}). The two sites, L and D experienced salinity extremes with mean values of 0¡ at site C and 35¡ at site L, respectively. Generally, the density of nematodes decreased along the salinity gradient. Thus, the lower reaches had higer nematode densities as compared to the upper reaches. Although higher numbers were recorded at some sites during certain months there was no significant difference ($p > 0.05$; ANOVA) between the months in terms of nematode numbers. A clear trend in seasonal abundance was not observed, although the mean numbers were high in September 1995 and November 1995 as compared to the other months. Variation in nematode densities was very high at sites J, H, BR and C. The mean spatial distribution of nematodes is presented in Figure 5, which illustrates that nematodes are not randomly distributed along the Swartkops estuary probably due to the environmental variability at particular sites. Spearman's Rank correlation indicated that the mean chlorophyll *a* and sorting values of the sediment had a significant ($p < 0.05$, $n = 10$) negative effect on mean nematode density. The organic carbon content had a significant negative correlation with the nematode density in September 1995 and January 1996. The chlorophyll *a* concentration, which was significantly high in January 1996, had a negative correlation with the nematode density but statistically not significant. A significant ($p < 0.05$) negative correlation however existed between the nematode density and chlorophyll *a* in September 1995. The metals, Zn and Mn also had significant negative correlation with the mean nematode density (see Table 5). The influence of the chlorophyll *a* concentration of January 1996 on the nematode density was felt in March 1996. Multiple regression showed a negative value for beta, (beta $= -0.54$, $p < 0.0034$).

Sediment fractions, median sand, fine sand and the metal Ti had positive correlations with nematode density. At individual sampling sites, chlorophyll *a* and median sand had the highest significant correlations ($p < 0.05$) with the nematode density as compared to the other parameters. Chlorophyll *a* influenced the density at J, CRM, I, KC, C and BR whilst median sand affected nematode density at I, H, KC and C.

Nematode diversity and genera composition

Fifty (50) nematode genera belonging to 19 families were identified in this study, all from only three orders namely, Enoplida, Chromadorida and Monhysterida (see Appendix, Tables 8 and 9). *Monhystera*, *Theristus*, *Viscosia*, *Adoncholaimus* and *Metalinhomoeus* were numerically the dominant genera. Their presence was recorded in almost all the sampling months in relatively larger numbers. *Monhystera* and *Viscosia* were widely distributed along the length of the estuary although their numbers varied from one sampling site to another (see Appendix, Tables 8 and 9). The

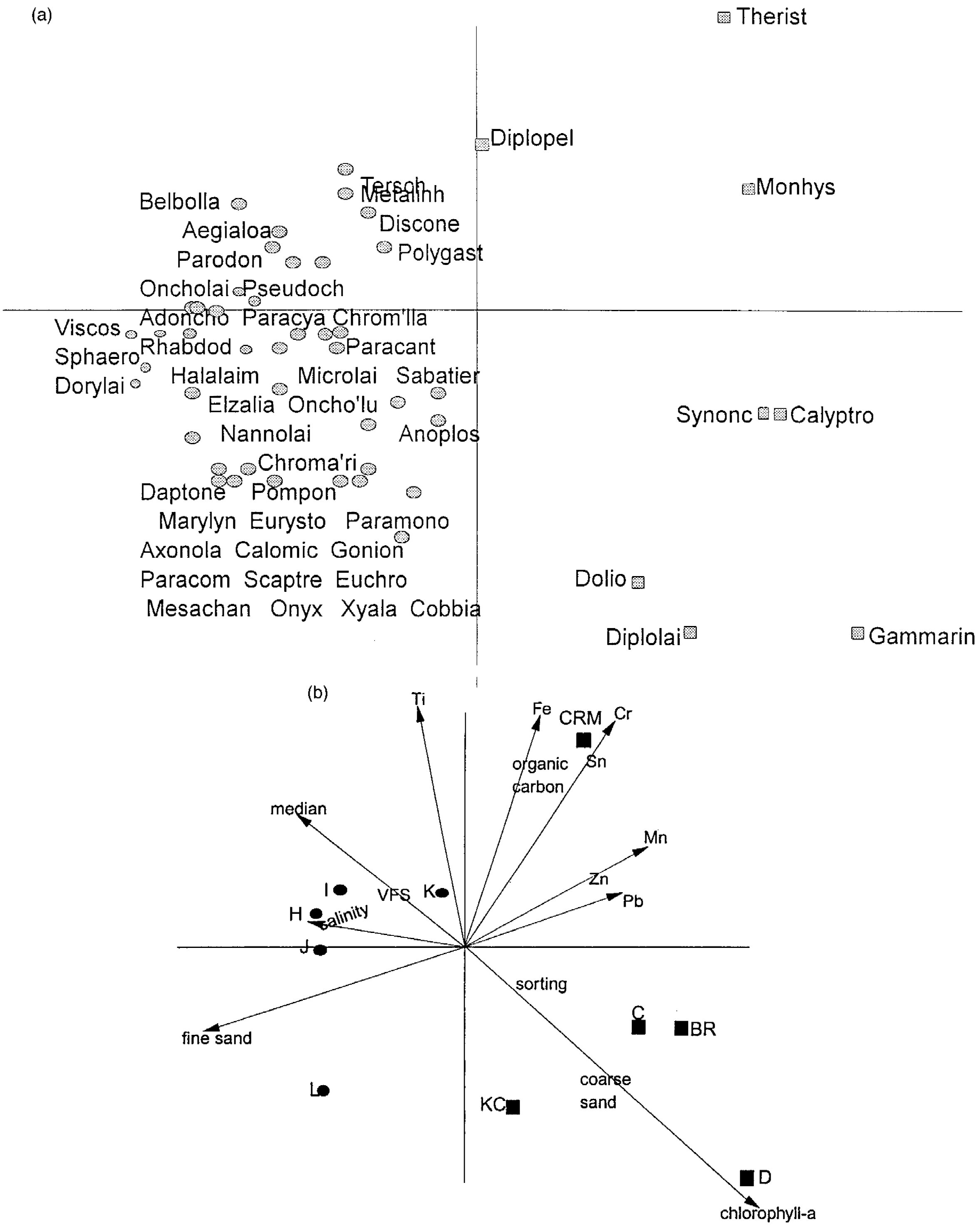

Figure 6. (a) A Canodraw diagram for the CCA results of the nematode community structure. (b) A canodraw diagram of the sites and environmental parameters from July 1995 to March 1996.

highest number of genera was recorded in November 1995. Genera such as *Onyx*, *Calomicrolaimus* and *Belbolla* were not only localised but their occurrence were also seasonal, indicating that there was seasonal influences on the nematode diversity and the composition of the genera. However, no significant difference was observed between the months in terms of number of nematode genera.

Generally, the diversity was highest in the lower reaches of the estuary (sites L, J, I and H) when compared with the upper reaches (sites KC, BR, D and C). Diversity ranged from 3 at BR, D and C to a maximum of 18 at L, the mouth of the estuary. There was a significant difference, ($p < 0.05$, ANOVA) between the sites in terms of diversity. Sites J, K, KC and C had wide variations in nematode diversity over the study period. Apart from the effects of salinity and that of seasonal changes, other factors were found to be influential in the structuring of the nematode community at the study sites. These include chlorophyll a which had a significant negative correlation with the nematode diversity ($r = -0.79$, $p < 0.05$). The significant increase in the chlorophyll a concentration in January 1996 affected the nematode diversity in January 1996 and in March 1996. Multiple regression analyses showed a more significant value for March 1996. The beta values are (-0.62, $p < 0.0007$ for January 1996 and -0.74, $p < 0.0005$ for March 1996). The particle size also had influence on the community structuring of nematodes in the Swartkops estuary during the period of the study.

Two of the seven metals, Mn and Ti, had maximum correlation with the genera distribution (BIOENV) at the sites. The CCA diagram in Figure 6 indicates that chlorophyll a was the environmnetal parameter with the greatest influence on the nematode diversity. The Canonical Correspondence Analysis (CCA) based on the absolute numbers of each genera at the sites are shown in Figure 6. The environmental parameters were superimposed on the genera distribution, after permutations of X-axis presented in Figure 6.

The relative position of the arrows reflects the relationship of the axes with the environmental parameters. Axes 1 and 2 are the most important here and therefore chlorophyll a sediment particle size, especially, fine sand fraction, median and coarse sand fractions played a very important role in the community structure. Four of the seven heavy metals analysed (Ti, Fe, Cr and Sn) and organic carbon content of the sediment also played a very important role in the structuring of the nematode community in this study. Although Mn, Pb and Zn together with salinity also played part in the structuring of the nematode community, they were not as significant as the factors mentioned earlier (see Figure 6). *Monhystera*, *Theristus* and *Calyptronema* preferred silty environments rich in chlorophyll a. The first two were mostly restricted to site CRM whilst *Calyptronema* was restricted to site BR. The sediment particle-size distribution and the chlorophyll a concentration of the sediment were the prominent parameters in the structuring of the nematode community.

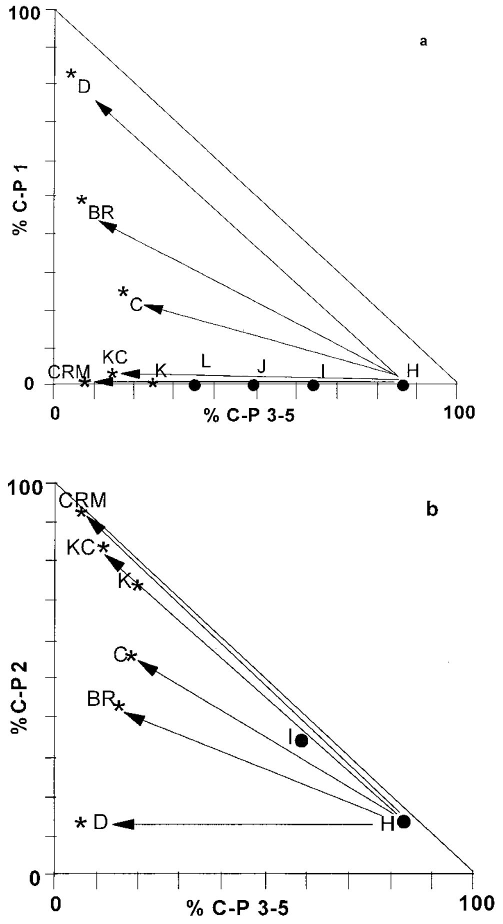

Figure 7. (a & b): c-p triangles illustrating changes in nematode fauna composition or nematode community structure at the sites.

Diversity index/maturity index

Apart from site CRM, the sites in the lower and middle reaches of the estuary, L, K, J, I and H had higher values of the diversity indices (see Table 6). Reduced values of Shannon-Wiener's Diversity Index

Table 6. Mean values of Shannon-Wiener Diversity Index (H') and Maturity Index (MI) at the various sites from May 1995 to March 1996

Sites	L	K	J	CRM	I	H	KC	BR	D	C
MI	2.39	2.08	2.62	1.70	2.60	2.85	1.93	1.36	1.20	1.47
H'	0.79	0.68	0.63	0.18	0.81	0.48	0.44	0.26	0.20	0.20

at CRM, KC, BR, D and C suggested that nematode assemblages at these sites were under stress. The Maturity Index which is a potential indicator of nematode communities under stress were calculated for each site for every sampling month. Mean values computed are shown in Table 6 together with mean diversity index.

The two indices were lowest at sites, CRM, KC, BR, D and C, which can be considered as indications of stress at these sites. These sites had higher concentrations of the heavy metals as compared to L, K, I and H which had lower concentrations of the heavy metals and consequently higher values of the two indices (Tables 4 and 6). Site J surprisingly, had higher values for the indices although it had very high concentrations of the heavy metals. It must also be stated that as far as the organic content of the sediment is concerned, sites CRM, KC and K ranked high among the other sites. This really explains the inclusion of site K among others like CRM, KC, BR, D and C on the c-p (%) triangle (see Figure 7).

Figure 7 (a and b) illustrates the c-p (%) triangle for the sites. Using sites I and H as reference sites (with little or less organic polltion), it can be seen that there was a shift from sites I and H to the more polluted sites , CRM, KC, K, BR, D and C.

Discussion

The sediment particle-size distribution is seen as probably the most representative parameter together with a few environmental parameters, e.g. the chlorophyll *a* concentration and organic carbon, for the structuring of the nematode communities in this study. The sites (D and C) with higher percentage of very coarse sands, had the lowest numbers of nematodes. In fact, the sediment particle size had the highest significant correlation with the nematode density. Site C, however, had finer sand but recorded very low nematode numbers, and factors other that the sediment particle size may have influenced the nematode density here. Sites K, J and C were the only sites which showed marked variations in the sediment grain size composition. Site K is used extensively for angling purposes and the search for baits in the sediment might have caused the wide variations in the sediment composition. Site J is a sewage output point and the discharge of various organic matter and silt could cause the changes in the mud component over time. Site C located at the head of the estuary is mainly fresh water and the deposition and washing away of sediment by currents of runoff water is a contributing factor to the wide variations in the sediment composition.

The patchiness in nematode distribution observed in this study could be due to food distribution patterns as suggested by Orren et al. (1979), Alongi & Tietjen (1980), Eleftheriou et al. (1982) & Hicks & Coull (1983). Organic enrichment has been seen to influence the density of nematodes (Orren et al., 1979; Eleftheriou et al., 1982, Gee & Warwick, 1985, Smol et al., 1994). We found organic carbon and chlorophyll *a* concentration as influential factors on nematode density. It has already been documented that densities of nematodes increase with increasing salinity (e.g. Heip et al., 1985; Coull, 1988; Soetart et al., 1995). However, whereas this could very well explain the presence and absence of species and hence diversity, it is unclear why genera like *Gammarinema* and *Synonchium* that have adapted to brackish and very low salinities can not attain large densities at sites KC, D, BR and C. Chemical contamination of the sediment might be the probable cause.

Seasonal influence on the diversity of nematodes was not significant. Two metals Mn and Fe, and chlorophyll *a* had significant negative correlations with nematode diversity. *Theristus* and *Monhystera* were two genera that were restricted to sites with high organic load and/or high chlorophyll *a* concentration in the sediment. Site CRM, which was dominated by only these two genera, had higher organic carbon and chlorophyll *a*. Some authors have found the following genera; *Terschellingia, Sabatieria* (Nicholas, 1975; Vincx et al., 1990; Vanreusel, 1990) *Metalinhomeous; Sphaerolaimus; Spirinia, Dorylaimopsis* (Vincx, 1989; Vincx et al., 1990) Daptonema (Vanreusel, 1989) as silt bottom lovers. Bongers (1990) reported that *Axonolaimus* and *Sabatieria* are pollution resistant. We found *Axonolaimus* and *Sabatieria* at a sewage outfall (site J) and other sites under stress. As indicated earlier in the results, some of the genera were in the intersection in terms of habitat preference, which supports the concept that besides sed-

Table 7. Nematode densities (m^{-2}) at the sampling sites from May 1995 to March 1996

SITES	MAY 1995	JULY 1995	SEPT 1995	NOV 1995	JAN 1996	MAR 1996	MEAN
L	1,742,127	1,421,655	1,995,516	1,173,825	984,728	1,878,143	1,532,666
K	982,204,	330,224	201,817	1,733,700	568,728	889,781	784,409
J	292,524	285,712	1,511,658	252,494	392,037	1,335,568	844,999
CRM	563,751	1,166,734	1,263,948	544,478	379,181	1,283,394	866,914
I	462,452	1,288,843	1,353,978	1,472,724	1,073,947	977,876	1,104,970
H	1,582,261	674,023	308,454	2,353,332	479,815	1,656,480	1,175,728
KC	546,796	403,339	187,867	N/A	177,906	113,843	285,950
BR	40,676	81,334	122,002	54,229	997,530	677,900	328,945
D	159,853	321,992	245,001	147,009	337,712	700,999	318,761
C	82,973	132,646	745,822	103,616	84,323	75,032	204,068

iment types, chemical and biological characteristics might be responsible for the structuring of nematode communities. It is evident from Figure 6 that chlorophyll *a* together with particle size distribution were the determining factors in the community structure of nematodes studied. We found out that significant changes in diversity and community structure are associated with organic enrichment and chlorophyll *a* concentration in the sediment. At particular sites e.g. at CRM, the diversity was reduced but the density was high illustrating the so-called ‘paradox of enrichment’ proposed by Hockin (1983).

The suitability of diversity indices as measurement of pollution effects on nematode communities is being appreciated by econematologists all over the world. The Shannon-Wiener Diversity Index (H’) and the Maturity Index (MI) calculated for all the sites confirmed that CRM, KC, BR, D and C are under stress. The c-p (%) triangles used also confirms that site K in addition to the affore-mentioned sites are under stress. At the generic level, the ‘indicator taxa’ in the Swartkops river system have been identified. Site K had *Monhystera*, *Metalinhomoeus* and *Paramonohystera* as the main genera throughout the study period. Site K is among the sites with high organic load. The variation in the nematode density and diversity at this site buttresses the point that this particular site is under stress as well. *Monhystera* constituted a great proportion of the genera at all the other polluted sites. *Theristus* was the only other genus at site CRM.

Monhystera and *Theristus* are considered indicators of stressed conditions especially, organic pollution in this study. They could be ‘colonisers’.

Organic carbon and or chlorophyll *a* concentration that become pollutants at a certain level in the sediment might increase or decrease the density of nematodes. Nematode numbers appear to increase up to a certain level of organic carbon and chlorophyll *a* concentration in the sediment, less than 3% and 60 micrograms (m^{-2}) respectively but at higher concentrations of more than those indicated, the numbers decrease.

We conclude that pollution of any kind does not necessarily cause the density of nematodes to increase. McLusky (1981) had made a similar observation after observing that nematode density was small in an anoxic area they studied. Organic carbon, chlorophyll *a* and chemical elements e.g. Mn, Ti, Zn and Fe were found to affect the density, diversity and community structure of the nematodes at specific sites. We found higher taxonomic levels convenient in indicating stress conditions. A combination of Maturity Index (MI), Shannon-Wiener Diversity Index (H’) and the c-p (%) triangle are good tools in pollution monitoring, especially, organic pollution involving nematodes. Although the c-p values used are based on the recent proposed changes by Bongers et al. (1995), the stressed sites were not obscured. Organic carbon, chlorophyll *a* concentration and metals, e.g. Ti, Fe, Cr and Sn, were very influential in the structuring of the nematode community in the Swartkops estuary. Our conclusions are based on the fact that sites CRM and KC that were the most polluted sites were confirmed by the nematode attributes used in the study. We suggest that, considering the results of this study, nematodes have a potential of being used in pollution monitoring in marine and estuarine environments.

Table 8. Absolute numbers of nematode genera used in figures and analysis

		SITE									
GENERA	c-p value	L	K	J	CRM	I	H	KC	BR	D	C
ADONCHOLAIMUS	3	54	6	24	0	177	156	0	0	0	0
AEGIALOALAIMUS	4	0	0	0	0	30	0	0	0	0	0
ANOPLOSTOMA	2	0	15	39	0	12	3	48	0	3	6
AXONOLAIMUS	2	111	0	3	0	6	0	0	0	0	0
BELBOLLA	4	0	0	0	0	24	6	0	0	0	0
CALOMICROLAIMUS	2	24	0	3	0	0	0	0	0	0	0
CALYPTRONEMA	4	0	0	0	0	0	0	0	6	6	126
CHROMADORELLA	3	75	21	222	0	216	72	9	3	0	0
CHROMADORINA	3	30	0	45	0	0	0	33	0	0	0
COBBIA	3	75	0	0	0	0	0	15	0	9	6
DAPTONEMA	2	189	57	0	0	18	30	48	0	0	0
DIPLOLAIMELLA	1	0	0	0	0	0	0	21	0	6	0
DIPLOPELTOIDES	3	0	51	0	42	15	36	0	0	0	0
DISCONEMA	2	0	6	0	0	0	0	0	0	0	0
DOLIOLAIMUS	2	3	0	0	0	0	0	390	3	0	126
DORYLAIMOPSIS	2	66	6	408	0	285	39	0	0	0	0
ELZALIA	2	24	0	0	0	27	0	0	0	0	0
ETHMOLAIMUS	3	0	0	102	0	132	180	3	0	0	0
EUCHROMADORA	3	30	0	0	0	0	0	0	0	0	0
EURYSTOMINA	4	36	0	0	0	6	0	0	0	0	0
GAMMARINEMA	1	0	0	0	0	6	3	0	330	912	249
GONIONCHUS	2	180	0	12	0	0	9	3	0	0	0
HALALAIMUS	4	0	0	33	0	6	15	12	0	0	0
MARYLYNNIA	3	9	0	3	0	0	0	0	0	0	0
MESACHANTHION	3	9	0	0	0	0	0	0	0	0	0
METALINHOMOEUS	2	6	579	3	0	345	15	3	0	0	0
MICROLAIMUS	2	138	33	42	21	42	9	0	0	0	0
MONHYSTERA	2	3	246	18	840	42	12	69	279	144	357
NANNOLAIMOIDES	3	60	21	0	0	21	0	0	0	0	0
ONCHOLAIMELLUS	3	3	0	0	0	3	0	0	0	0	0
ONCHOLAIMUS	3	0	0	0	0	0	3	0	0	0	0
ONYX	3	30	0	0	0	0	0	0	0	0	0
PARACANTONCHUS	2	9	15	0	0	18	0	6	0	0	0
PARACOMESOMA	3	75	0	3	0	0	6	3	0	0	0
PARODONTOPHORA	2	0	3	9	0	150	30	0	0	0	0
PARACYATHOLAIMUS	2	24	0	0	3	21	90	0	0	0	0
PARAMONHYSTERA	2	972	150	24	0	21	0	135	0	0	0
POLYGASTROPHORA	4	0	33	3	0	0	0	0	0	0	3
POMPONEMA	3	147	3	18	0	54	9	33	3	3	21
PSEUDOCHROMADORA	3	0	39	81	0	69	192	3	0	0	0
RHABDODEMANIA	4	0		3	0	0	0	0	0	0	0
SABATIERA	2	90	51	285	0	42	99	33	3	3	30
SCAPTRELLA	2	165	12	0	0	0	0	3	0	0	
SPHAEROLAIMUS	3	12	21	222	0	24	0	0	0	0	0
SPIRINIA	3	3	45	0	0	0	0	0	0	0	0
SYNONCHIUM	3	0	0	12	0	0	0	3	39	6	12
THERISTUS	2	9	9	6	729	48	12	3	0	0	0
TERSCHELLINGIA	3	0	78	24	75	420	0	3	0	0	6
VISCOSIA	3	336	54	24	3	570	1299	9	3	24	0
XYALA	3	36	0	0	0	0	0	0	0	0	0

Table 9. Monthly chlorophyll a concentrations (μg/m^2) at the sites

Month	L	K	J	CRM	I	H	KC	BR	D	C
September 1995	5.08	11.44	20.11	87.32	22.04	21.59	56.40	24.36	38.66	8.72
November 1995	10.97	10.97	15.85	103.6	15.85	14.50	9.14	14.63	9.75	73.14
January 1996	23.16	9.75	15.85	163.3	29.25	20.11	90.80	20.11	104.8	96.30
March 1996	23.94	3.05	21.34	48.84	7.93	4.26	39.63	4.26	65.12	73.14

Table 10. % c-p values at the sites

c-p	L	K	J	CRM	I	H	KC	BR	D	C
1	0	0	0	0	0	0	3	49	82	27
2	66	76	51	93	38	15	83	43	14	55
1 & 2	66	76	51	93	38	15	86	92	96	55
3–5	34	24	49	7	62	85	14	8	4	18

Acknowledgements

We are grateful to Magda Vincx and the department of Marine Biology (Morphology & Systematics), University of Ghent, Belgium for the provision of facilities during part of this study. We also thank staff and the Zoology Department, University of Port Elizabeth for their assistance in various ways during the course of this study. We thank the University of Port Elizabeth and the FRD for financial assistance towards this study.

References

Alongi, D. M. & J. H. Tietjen, 1997. Population growth and trophic interactions among free-living nematodes. In Tenore, K. R. & B. C. Coull (eds), Marine Benthic Dynamics. Columbia: Univ. of S. Calorina Press. 151–166.

Austen, M. C. & P. J. Somerfield, 1997. A community level sediment bioassay applied to an estuarine heavy metal gradient. Mar. Environ. Res. 43: 315–328.

Baird, D., P. E. D. Winter & G. Wendt, 1987. The flux of particulate material through a well-mixed estuary. Continental Shelf Res. 7: 1399–1403.

Bongers, T., R. G. N. De Goede, G. W. Korthals & G. W. Yeates, 1995. Proposed changes of c-p classification of nematodes. Russian Journal of Nematology. 3 (1): 61–62.

Bongers, T., 1990. The maturity index: An ecological measure of an environmental disturbance based on nematode species composition. Oecologia 83: 14–19.

Bongers, T. & J. Van de Haaar, 1990. On the potential of basing an ecological typology of aquatic sediments on the nematode fauna: An example from river Rhine. Hydrobiol. Bull. 24: 37–45.

Branch, G. & M. Branch, 1981. The living shores of Southern Africa. Cape Town: Struik Publ.

Cairns, J. J. & K. L. Dickson, 1971. A simple method for the biological assessment of the effects of waste discharges on aquatic bottom dwelling organisms. Journal WPCF 43: 755–772.

Canoco-Fortran Program for Canonical Community Ordination by (partial) detrended (Canonical) Corresponcence Analysis and Redundancy Analysis. 2.1. Ter Braak, C. J. F. 1987.

Coull, B. C., 1988. Ecology of marine meiofauna. In Higgins, R. P. & H. Thiel (eds), Introduction to the Study of Meiofauna. Washinton: Smithsonian Institute Press: 18–38.

Croll, N. A. & G. B. Mathews, 1977. Biology of Nematodes. Glassgow: Blackie & Sons Ltd.

Danovaro, R., M. Fabiano & M. Vincx, 1995. Meiofauna response to the Agip Abruzzo oil spill in the subtidal sediments of the Ligurian Sea. Mar. Poll. Bull. 30: 133–145.

Dye, A. H., 1977. An ecophysiological study of the benthic meiofauna of the Swartkops estuary. Zoo. Dept., Univ. of Port Elizabeth. General ecology of the nematodes.

Eleftheriou, A., D. C. Moore, D. J. Basford & M. R. Robertson, 1982. Underwater experiments on the effects of sewage sludge on a marine ecosystem. Neth. J. Sea Res. 16: 465–475.

Ferris, V. R. & J. M. Ferris, 1979. Threadworms (Nematoda). In Hart, C. W. Jnr. & S. L. H. Fuller (eds), Pollution Ecology of Estuarine Invertebrates. Academic Press: 1–33.

Furstenberg, J. P., A. H. Dye & A. G. De Wet, 1978. Quantitative extraction of meiofauna: A comprison of two methods. Zool. Afr. 13: 175–186.

Gee, M. J. & R. M. Warwick, 1985. Effects of organic enrichment on abundance and community structure in sublittoral soft sediments I. Mar. Biol. Ecol. 91: 247–262.

Giere, O., 1993. The microscopic fauna in aquatic sediments: In Meiobenthology. Springer-Verlag.

Goede, R. G. M., T. Bonger & C. H. Ettema, 1993. Graphical presentation and interpretation of nematode community structure: c-p triangles. Mededelingen Faculteit Landbouwkundige en toegepaste biologische weienschappen, Universiteit Gent 58/2b: 743–750.

Heip, C., M. Vincx & G. Vranken, 1985. The ecology of marine nematodes. Oceanog. mar. Biol. Ann. Rev. 23: 399–489.

Heip, C., M. Vincx, N. Smol & G. Vranken, 1982. The systematics and ecology of free-living marine nematodes. Commonwealth Institute of Parasitology: Helminthological Abstracts SeriesB-PlantNematology 51: 1–31.

Heip, C., R. M. Warwick, M. R. Carr, P. M. J. Herman, R. Huys, N. Smol & K. Van Holsbeke, 1988. Analysis of community attributes of the benthic meiofauna of Frierfjord/Langesundfjord. Mar. Ecol. Prog. Ser. 171–180.

Herman, P. M. J. & C. Heip, 1988. On the use of meiofauna in ecological monitoring: who needs taxonomy? Mar. Poll. Bull. 12: 665–668.

Hicks, G. R. F. & B. C. Coull, 1983. The ecology of marine meiobenthic harpacticoid copepods. Oceanog. mar. Biol. Ann. Rev. 21: 67–75.

Hill, M. O., 1979. Twinspan – A Fortran program for arranging multivariate data in an ordered two-way table by classification of the individuals and the attributes. In Anonymous, Ecology and Systematics. New York: Cornell Univ. 1–48.

Hockin, D. C., 1983. The effects of organic enrichment upon a community of meiobenthic harpacticoid copepods. Mar. Envir. Res. 10: 45–58.

Jensen, P., 1984. Ecology of benthic and epiphytic nematodes in brackish water. Hydrobiol. 108: 201–217.

McLusky, D. S., 1981. The Estuarine Ecosystem. Glasgow, London: Blackie & Sons Ltd.

Moore, C. G. & B. J. Bett, 1989. The use of meiofauna in marine pollution impact assessment. Zoological Journal of the Linean Society 96: 263–280.

Nicholas, W. L., 1975. The biology of free living marine nematodes. Oxford: Claredon Press.

Orren, M. J., G. A. Eagle, P. G. Greenwood & H. F.-K. O. Henning, 1979. Preliminary pollution surveys around the South-Western Cape Coast. Part 3: Hout Bay. S. Afr. J. Sci. 75: 459–461.

Parker, S., 1983. Determination of siol organic content. In Carver, R. E. (ed.), Procedures in Sediment Petrology. New York: John Willey & Sons: 389–401.

Platt, H. M., K. M. Shaw & P. J. D. Lambshead, 1984. Nematode species abundance patterns and their use in the detection of environmental perturbations. Hydrobiologia 118: 59–66.

Platt, H. M. & R. M. Warwick, 1988. Freeliving marine nematodes, Part II: British Chromadorids. Cambridge Univ. Press.

Rodriguez, F. D. G., 1993. The determination and distribution of chlorophyll *a* in selected South Cape estuaries.

Smol, N., K. A. Willems, J. C. R. Govaere & A. J. J. Sandee, 1994. Composition, distribution, biomass of meiobenthos in the Oosterschelde estuary (SW Netherlands). Hydrobiologia 282/283: 197–217.

Soetaert, K., M. Vincx, J. Wittoek & M. Tulkens, 1995. Meiobenthic distribution and nematode community structure in five European estuaries. Hydrobiologia 311: 185–206.

Tietjen, J. H., 1967. Observations on the ecology of the marine nematode, *Monhystera filicaudata* Allgen 1929. Trans. a. microsc. Soc. 86: 304–306.

Vanreusel, A., 1990. Ecology of free-living marine nematodes in the Voordelta (Southern Bight of the North Sea).I. Species composition and the structure of the nematode communities. Cah. Biol. mar. 31: 439–462.

Vanreusel, A., 1991. Ecology of free-living marine nematodes of the Voordelta (Southern Bight of the North Sea).II. Habitat preferences of the dominant species. Nematologica. 37: 343–359.

Vincx, M., 1989. Free-living marine nematodes from the southern Bight of the North Sea. Mar. Ecol. Prog. Ser. 46: 207–211.

Vincx, M., P. Meire & C. Heip, 1990. The distribution of nematode communities in Southern Bight of the North Sea. Cah. Biol. mar. 31: 107–129.

Watling, R. J. & H. R. Watling, 1982. Metal surveys in South African estuaries. I. Swartkops river. WaterSA. 8: 26–35.

Warwick, R. M., 1988. The level of taxanomic discrimination required to detect pollution effects on marine benthic communities. Mar. Poll. Bull. 19: 259–268.