

# Impact of sewage disposal on a nematode community of a tropical sandy beach

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## Abstract

*Free-living marine nematodes from a intertidal sandy beach from Goa near the Panjim city, central west coast of India was investigated along a gradient of sewage pollution. High nematode diversity (11 species) and abundance was observed near the sewage discharge point, which decreased gradually away from the discharge site. The salinity and dissolved oxygen of the estuarine water increased from the discharge point whereas reverse trend was observed for the sedimentary organic carbon. The total nematode densities indicated three-fold increase (from 523 to 1769 ind.10cm<sup>-2</sup>) in 25 years with a contrasting gradient of nematode abundance, spatially from the source point of sewage discharge. *Daptonema* sp is known to be a good indicator of stressed and polluted habitats and was observed to be the most dominant species at the study site. Being exposed to the domestic sewage, the area also has high counts of pathogenic bacteria (eg: *E coli* and other coliform types). *Daptonema* spp are known to consume bacteria and presence of high bacterial biomass due to nutrient enrichment from the discharged sewage enhanced their abundance. Thus, the increasing nematode densities specifically like *Daptonema* sp at organically polluted sites can be of immense aid to reduce pathogenicity and can potentially be applied in pollution management and act as agents of natural bio-remediation.*

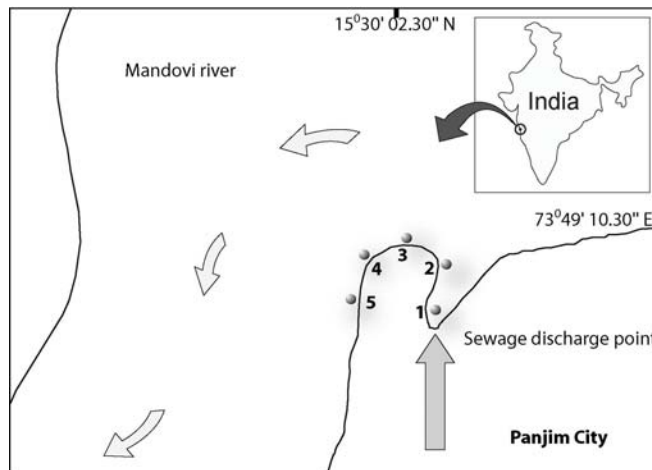
**Key words:** Free-living nematodes; *Daptonema* sp.; sandy beach; bacterial feeding; pathogen; sewage pollution; bio-remediation; west coast; India.

## INTRODUCTION

Free-living nematodes are ubiquitous and persistent as a taxon in all environmental conditions that can support metazoan life. Their small size facilitates very precise spot samples giving better picture of the environment. Nematodes have conservative life cycles (i.e. no highly mobile pelagic life stages) so local contamination effects should not be hidden by immigration. Ironically, the conceptual arguments against the use of these organisms for biomonitoring rest on this very property of diversity (Lambshhead, 1986). This, coupled with chaotic taxonomy, has made the taxon difficult for the non-specialist. However, better pictorial keys and descriptions (Lorenzen, 1981; Platt and Warwick, 1983; Platt *et al.*, 1984; <http://nemys.ugent.be/>) have started to rectify this situation bringing accurate nematode identification and application within the scope of extension workers. Now the nematode community attributes are turning out to be suitable for monitoring sediment quality, with generic composition being the most accurate indicator for assessing differences (Heininger *et al.*, 2007). Studies have shown that sewage can change structural and functional attributes of biodiversity, but effects can vary depending on the response variables considered and the type of analysis (Pearson and Rosenberg 1978, Chapman *et al.*, 1995, Otway *et al.*, 1996, Smith *et al.*, 1999).

Panjim is a valuable aesthetic city, ecological and recreational asset, and due to its geographical position in a rapidly expanding urban area, subjected to the influence of various developmental activities related to tourism industry. This has led to increase in domestic sewage discharge from the city into the pristine estuarine region. Even though, the development of strategies to mitigate the environmental effects caused by sewage disposal has initiated since long but were not very effective till recent. The sewage treatment was initialized in 1976, which had great difficulties in smooth running hence mostly sulfidic conditions remained throughout the sewage channel due to periodic stagnancy (Kamat, 2009). A new technologically improvised system was installed in 2005 with a sewage treatment capacity of 12.5 million litres per day (Annon, 2007). However, it normally handles 7-8 million liters, in a region where intense monsoon activity can cause floods (Annon, 2007). The present study was aimed to observe the free-living meiobenthic nematode community along a decreasing gradient from the sewage discharge outlet and comparing it with a previous study for the changing trends. Hence, the relationship between nematode community structure, environmental parameters and domestic sewage disposal gradient

was established in an organically polluted estuarine sandy beach of Panjim city on the west coast of India.



**Figure 1: Map of the study area with sampling location**

## MATERIALS AND METHODS

Mandovi estuary, which opens into the Arabian Sea near Panjim city on the west coast of India with an average annual river discharge of 6004 Mm<sup>3</sup> (Suprit and Shankar, 2008), used to receive about 1.3 Mm<sup>3</sup> liters of urban runoff annually in early 1980's (Ansari *et al.*, 1984). During the present study, a section of estuarine beach receiving domestic sewage through "St. Inez Nullah" was sampled during April 2007 (Figure 1). Nematode samples were collected from 5 stations along a decreasing gradient of sewage pollution. Station PM1 was at the discharge point, other four stations i.e., PM2-PM5 each at 100m interval towards the seaward side (Figure 1). Surface sediment was sampled with an acrylic core (4.5cm diameter) down to 5 cm depth in triplicates. Separate cores were taken for sediment organic carbon and grain size analysis. Organic carbon was analysed using wet Oxidation method (Wakeel and Riley, 1957) employing chromic acid as oxidizing agent. For grain size analysis approximately one gram of sediment after passing through 63µm was put in distilled water for disintegration. Later, complete disintegration of these samples was achieved by keeping them in ultrasound sonic bath for 10 min. These samples were analysed with a Malvern laser particle size analyzer (Master-Sizer 2000).

Overlying water was sampled for dissolved oxygen and was analysed using Wrinkler's method (Strickland and Parsons, 1972) The pore water temperature and salinity was measure in-situ using hand held mercury thermometer ( $\pm 0.5^{\circ}\text{C}$  accuracy) and Atago® make refractometer, respectively.

## Sample processing

Nematode samples were immediately preserved in 5% buffered formalin rose Bengal solution. In laboratory, the samples were sieved with 500 and 45 µm sieve.

**Table 1: Occurrence of nematode species at the sampling location with respective feeding types\*.**

	Feeding types*	PM1 PM2 PM3 PM4 PM5				
		PM1	PM2	PM3	PM4	PM5
<i>Actinonema</i> sp	2A	-	+	+	+	-
<i>Axonolaimus</i> sp	1B	+	+	-	-	-
<i>Bolbolaimus</i> sp	2A	-	+	+	-	-
<i>Comesoma</i> sp	2A	+	+	+	+	-
<i>Daptonema</i> sp1	1B	+	+	-	+	-
<i>Daptonema</i> sp2	1B	+	+	+	-	-
<i>Desmodora</i> sp	1B	+	-	+	+	-
<i>Dorylaimopsis</i> sp	2B	-	+	-	+	-
<i>Gammanema</i> sp	2B	-	-	+	-	-
<i>Oncholaimus</i> sp	2B	-	-	-	-	+
<i>Oxyonchus</i> sp	2B	-	-	-	-	+
<i>Paracyatholaimus</i> sp	1B	+	-	+	-	-
<i>Polysigma</i> sp	2A	-	+	-	-	-
<i>Praeacanthonchus</i> sp	1B	+	-	-	-	-
<i>Terschellingia</i> sp1	1A	+	+	-	-	-
<i>T. longicaudata</i>	1A	+	+	-	-	-
<i>Theristus</i> sp1	1B	+	-	+	+	+
<i>Trefusia</i> sp	1A	-	-	-	+	-
<i>Tripyloides</i> sp	1A	-	-	-	+	-
<i>Viscosia</i> sp	2B	-	-	+	-	-
Unidentified	1B	+	-	+	-	-
Abund. (ind.10cm <sup>-2</sup> )		1769	874	150	59	43

\*- Wieser (1953), 1A: Selective deposit feeders; 1B: Non-selective deposit feeders; 2A: Epigrowth feeders and 2B: Predatory/omnivores

The fauna retained on 45µm was considered for meiofaunal and nematode analysis. Nematodes were sorted and temporary glycerol mounted slides were prepared for identification. Identification was done using pictorial key of Platt and Warwick (1983), Warwick *et al.* (1998).

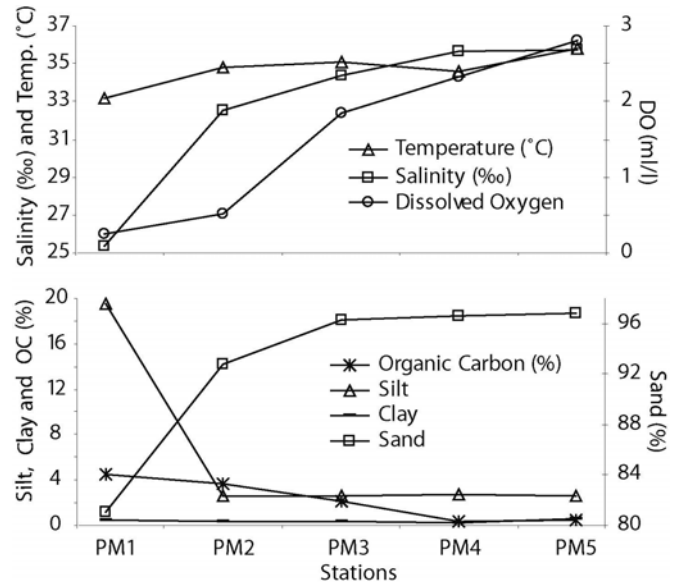
## Statistical analysis

Nematode species data for individuals (ind. 10 cm<sup>-2</sup>) were used to calculate the diversity as the number of species per sample (S), the Shannon-Wiener diversity index ( $H'$ ) and Simpson's diversity index. Species richness ( $d'$ ) was estimated from Margalef's formula as  $d' = (S-1)/\ln N$ , Evenness was calculated using Pielou's ( $J$ ). Diversity patterns were visualized by  $k$ -dominance curves. The nematode community structure was analyzed by cluster analysis using the untransformed species abundance Bray-Curtis similarity measure. The species contributing to dissimilarities between zones were investigated using a similarity-percentages procedure (SIMPER).

All the diversity functions for nematode species were calculated using PRIMER 6 (Clarke and Warwick 1994) statistical package. The maturity index (MI) was calculated as the weighted mean of the individual taxon scores (Bongers, 1990; Bongers *et al.*, 1991):

$$MI = \sum v(i) \times f(i)$$

Where,  $v$  is the colonisers-persisters ( $c-p$ ) value of taxon  $i$  (as given in Appendix A by Bongers *et al.*, 1991) and  $f$  is the frequency of that taxon in a sample. Nematode species were enumerated into functional groups according to Wieser (1953).



**Figure 2: Physico-chemical and sedimentary parameters at the sampling locations.**

## RESULTS AND DISCUSSION

The domestic sewage pollution is known to have considerable impact on the sediment dwelling fauna (Pearson and Rosenberg, 1978, Ansari *et al.*,

1984; 1986; Chapman *et al.*, 1995, Smith *et al.*, 1999, Otway *et al.*, 1996). Nematode community demonstrates a marked response to different pollutants (Heip *et al.*, 1985; Essink and Romeyn, 1999; Sommerfield, 2003) and in the present study, nematode community at the sewage discharge point seems to have benefited from the resultant enrichment of nutrients.

### Physico-chemical parameters

The station PM1 had the lowest temperature (32.2°C) and salinity (25.32 PSU) whereas the highest temperature (35.8°C) and salinity (35.71 PSU) was recorded at PM5 (Figure 2). The dissolved oxygen showed the gradient with lowest values (0.25 ml/l) recorded at PM1 and highest (2.80 ml/l) at PM5 (Figure 2). The sewage discharge at the study site was almost certainly responsible for the observed gradient in temperature, organic carbon and dissolved oxygen from the discharge point (Figure 2). Organic enrichment has been reported to influence nematode community structure (Orren *et al.*, 1979; Eleftheriou *et al.*, 1982, Gee and Warwick, 1985, Smol *et al.*, 1994). Moreover, the nematode numbers appear to increase up to a certain level of organic carbon concentration (<3%) in the sediment, (Gyedu-Ababio *et al.*, 1999) but in the present study, the nematode community seems to be flourishing even at higher (4.5%) content of organic carbon. Although this point needs further evaluation, it certainly suggests ability of certain nematode species to tolerate high organic load.

**Table 2: Diversity indices based of nematode species number.**

Stations--	PM1	PM2	PM3	PM4	PM5
Species number (S)	11	10	10	8	3
Total abundance (N)	1769	874	150	59	43
Species Richness ( $d'$ )	1.34	1.33	1.80	1.72	0.53
Species Evenness ( $J'$ )	0.37	0.38	0.50	0.70	0.65
Shannon-Weaver's ( $H'$ )	0.88	0.88	1.14	1.45	0.72
Simpson's (1-Lambda)	0.38	0.39	0.48	0.73	0.42
Maturity Index (MI)	2.03	2.03	2.18	2.81	2.37

Apart from the sediment composition and organic carbon, salinity also influenced the species composition of nematode communities (Heip *et al.*, 1985; Coull, 1988, Vanreusel, 1990; Soetart *et al.*, 1995). Salinity was low at discharge point and showed

a gradual increase, possibly due to mixing of fresh water with the sewage. The major effect of sewage is that it reduces the oxygen content and stimulates the formation of hydrogen sulfide. Such conditions can be disastrous to biota (Bozzini, 1975). This is clear at station nearest to the discharge point, where the oxygen level was low and the occurrence of a black sulfide layer below 5 to 6 cm sediment was visible (Ansari *et al.*, 1984). However, this layer was not found in the top 5cm at station 3 onwards which are away from the sewage outlet, perhaps due to better flushing by the riverine flow and the wave action. The gradient comparison for the physico-chemical parameters of the present study with the previous study (Ansari *et al.*, 1984) showed very similar trends depicting no significant change in parameters, except temperature.

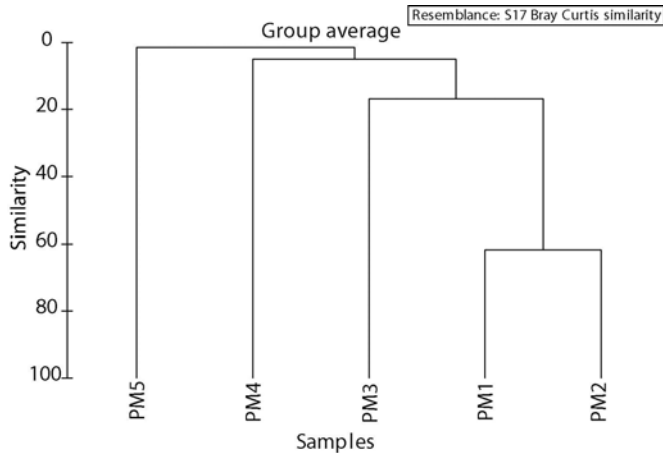
**Table 3: SIMPER analysis for the difference (%) between the stations based on species dominance.**

Between stations	Average dissimilarity	1 <sup>st</sup> two species contribution	% Dissimilarity
PM1 and PM2	69.13	<i>Daptonema</i> sp1	72.75
		<i>Daptonema</i> sp2	9.47
PM1 and PM3	92.04	<i>Daptonema</i> sp1	39.18
		<i>Daptonema</i> sp2	31.93
PM2 and PM3	79.30	<i>Daptonema</i> sp1	83.37
		<i>Dorylaimopsis</i> sp	5.67
PM1 and PM4	97.87	<i>Daptonema</i> sp1	47.19
		<i>Oxyonchus</i> sp	16.03
PM2 and PM4	95.50	<i>Daptonema</i> sp1	73.96
		<i>Daptonema</i> sp2	10.44
PM3 and PM4	92.34	<i>Daptonema</i> sp1	55.44
		<i>Trefusia</i> sp	11.40

### Nematode community

The occurrence of nematode species with their respective feeding types at all the sampling stations has been stated in Table 1. Highest (1769 nos.10cm<sup>-2</sup>) abundance was recorded at PM1 and lowest (43 nos.10cm<sup>-2</sup>) at PM5 (Table 1). The diversity indices revealed highest of 11 species at PM1 while, only 3 species were recorded at PM5. Shannon-

Wiener's ( $H'$ ) information function showed highest (1.72) values at PM5 and the lowest (0.88) at PM1 and PM2. But the Richness ( $d'$ ) was highest (1.72) at PM3 and lowest (0.37) at PM1. The species evenness ( $J'$ ) was highest (0.70) at PM4 and lowest (0.37) at PM1. The Simpson's Index and the Maturity Index (MI) also showed a gradient (Table 2).

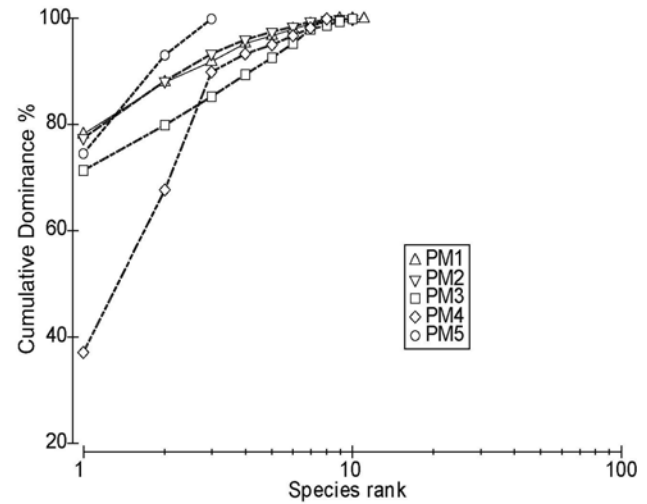


**Figure 3: Cluster analysis of the sampling locations based on nematode species abundance.**

The SIMPER analysis showed an average dissimilarity of more than 90% for most of the spatial comparisons of stations (PM1 and PM3, PM1 and PM4, PM2 and PM4 and PM3 and PM4). The species that contributed highest for the maximum difference between the stations was *Daptonema* sp1. The highest dissimilarity contributed by *Daptonema* sp1 was between PM2 and PM3 (Table 3). The other species that contributed after *Daptonema* sp1 at all the stations were *Daptonema* sp2, *Dorylaimopsis* sp, *Oxyonchus* sp, *Trefusia* sp (Table 3). The nematode community composition was very similar for stations near the discharge point and gradually differed away from the swage disposal point (Figure 3). This was due to the successful colonization of enrichment opportunist species. *Daptonema* sp1, *Daptonema* sp2, *Terschellingia* sp, *T. longicaudata* and *Axonolaimus* sp are actually known to thrive in degraded and stressful conditions (Bonger, 1990; Gyedu-Ababio *et al.*, 1999; Palacin *et al.*, 1992; Sommerfield *et al.*, 2003; Liu *et al.*, 2008; Essink and Romeyn, 1994).

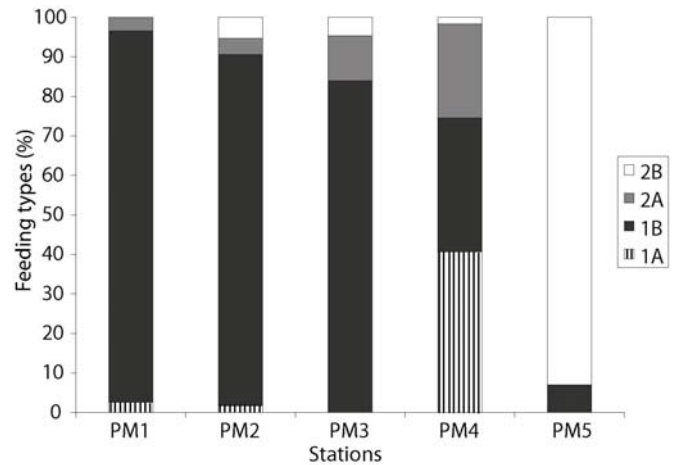
Overall, gradient of all the parameters structure the incline of nematode community spatially, and the species specialized in adapting in perturbed habitats, seems to have gained advantage due to the sewage discharge. The high abundance at the discharge point and very low density away from the discharge point revealed the increase of enrichment opportunistic species near the discharge point, which

are also resistant to pollution as well as lowered oxygen conditions.



**Figure 4: Cumulative dominance curve for nematode species at the sampling locations.**

A combination of Maturity Index (MI) and Shannon-Wiener diversity Index ( $H'$ ) are known to be good tools in pollution monitoring, especially, organic pollution involving nematodes (Gyedu-Ababio *et al.*, 1999) and in the present study MI showed low values at all the stations and  $H'$  decreased with decreasing pollution, except for the last station, which recorded only three species with very low abundance.



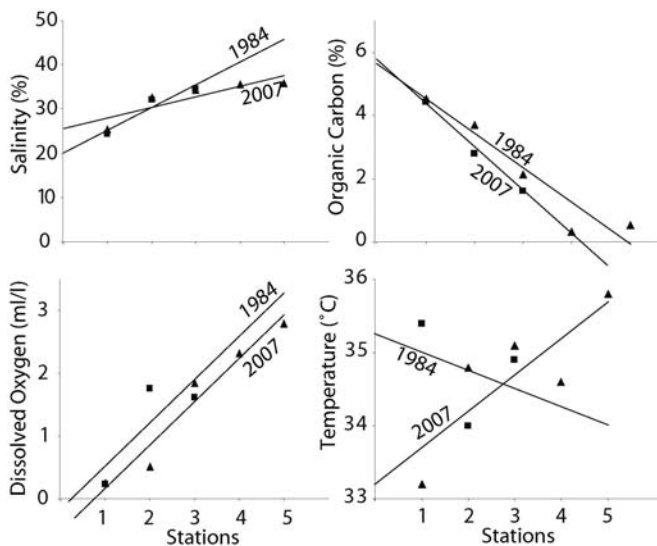
**Figure 5: Percent feeding type of nematode species based on Wieser's (1953) classification (1A- Selective deposit feeders, 1B-Non-selective deposit feeders, 2A- Epistrate feeders and 2B- Predatory/omnivores).**

The cumulative dominance curve for nematode species revealed a difference in all the stations where PM1 and PM2 showed much similarity

but PM5 showed a smaller curve ending abruptly because of very low species number (Figure 4).

### Nematode feeding types

The non-selective deposit feeders (1B) completely dominated the station PM1 with 94% abundance. PM2 showed a similar trend with 89% followed by PM3 with 84% dominance by 1B. The remarkable aspect of the study area was the absence of predatory/omnivores (2B) at the sewage disposal site station PM1 (mouth of *Nulla*). While, the dominance (41%) of selective deposit feeding nematodes (1A) increased at PM4 and non-selective deposit feeders (1B), became sub-dominant (34%), a complete change in feeding dominance was observed at PM5 with the dominance (93%) of predatory/omnivores (Figure 5).

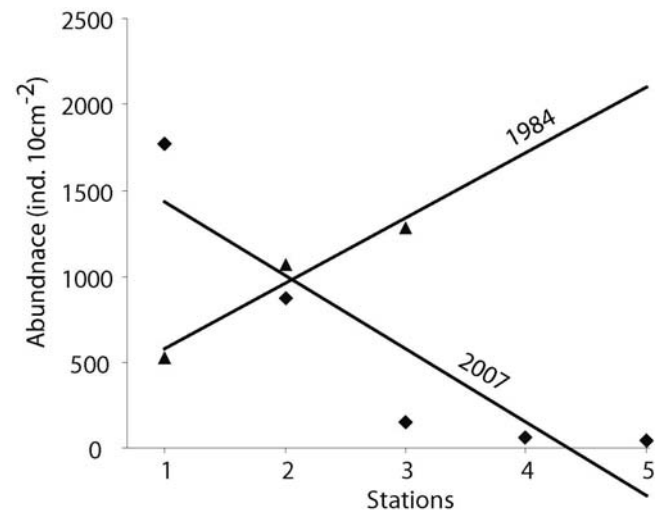


**Figure 6: Comparative trends in environmental parameters between 1984\* and 2007 (\*data source: Ansari *et al.*, 1984).**

*Daptonema* sp1 was the most dominant species, which is non-selective deposit feeder and known to consume heavy bacterial biomass (Heininger *et al.*, 2007; Singh and Ingole 2009). Reportedly high pathogenic bacteria such as *E. coli* and other coliform types are present in the study area (Ramaiah *et al.*, 2007). Bacterial consumption by nematodes can help in bio-remediation of the site at the domestic sewage outlet and the dominance of *Daptonema* sp1 can be considered a positive aspect from the perspective of environmental health.

Comparing the present results with a previous study (Ansari *et al.*, 1986) showed very similar trends for values of salinity, dissolved oxygen and sediment organic carbon (Figure 6) but exactly opposite trend was seen for temperature (Figure 6). The values for

nematode abundance from the present study showed contrasting values with the nematode abundance of 1984. The values in 1984, shows increasing trend away from the sewage discharge point whereas in 2007, the highest abundance was at the discharge point with a decreasing trend away from it (Figure 7). This reversing of trend in the gradient may indicate the reduction in the toxicants of sewage or the succession of the benthic community due to continuous organic waste (Ansari *et al.*, 1986). Nematode community is known to respond positively to decreasing level of organic pollution (Essink and Romeyn, 1994). The increase in nematode densities compared to earlier data (Figure 7), while no much change in other parameters indicates the succession of nematode species such as *Daptonema* sp1. Thus, the high dominance of enrichment opportunistic and bacterivorous nematodes can help in mitigating pathogenic bacteria harmful to human health.



**Figure 7: Comparative trends in nematode abundance between 1984\* and 2007 (\*data source: Ansari *et al.*, 1984).**

Further, detailed study is required on feeding selectivity of nematode species that can help in effluent treatment and bioremediation processes. The present findings will be helpful in monitoring the status of benthic environment and the response to changing environmental regulations. It will be interesting to observe future changes in the nematode community after the implementation of technically improved better sewage treatment plants as an environmental management strategy. Nevertheless, laboratory culture of opportunistic nematode species such as *Daptonema* sp could be a handy tool for pollution studies, especially for exploring the mitigating measures. Efforts are therefore being made to

develop appropriate methods to rear *Daptonema* spp. in laboratory.

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