

Diversity of fresh water nematode fauna from different locations of Sindh, Pakistan

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Abstract

Fresh water is natural source of water for drinking; domestic and industrial use which available primarily from melting of ice, rivers, streams ponds and ground. In Pakistan Indus River and its tributaries are the major sources of fresh water. Fresh water is clear from pollutants in upcountry, but as it flows downstream it becomes more pollutant. In the present study nematodes were selected as bio-indicators for these pollutants at eight localities of Sindh, which are Malir, Karachi; Kalri (Keenjhar) Lake, Thatta; Karachi Canal and Al-Manzar at River Indus near Jamshoro; Goth Detha Hyderabad; Khesana Mori near Tandojam; Mirpurkhas and Jamesabad (Kot Ghulam Mohammad). Samples from different sources of freshwater viz., river, lake, canal, tube well, pond and irrigation water chennal were examined for the prevalence of nematodes with an aim to disseminate the knowledge of fresh water nematodes as indicators in environmental monitoring. Detailed sample analysis revealed the presence of forty nematode species including three new species viz., *Bathyeurystomina minima* Nasira, Shahina & Shamim, 2014, *Belbolla longispiculata* Nasira, Shahina & Shamim, 2014 and *Metoncholaimus siddiqii* Shahina, Nasira & Shamim, 2015 while six species were reported as new records of Pakistan. These nematode species belong to 36 genera, 27 families and seven orders. Frequency of occurrence and distribution of nematode species varied from site to site. The overall occurrence (%) of fresh water nematode samples ranged from 3.22-32.25% at different sites. The highest number of positive samples was found at Malir, Karachi (32.25%) and the lowest was at Khesana Mori, Tandojam (3.22%). Free-living soil nematodes have more occurrences (55%) as compared to other group of nematodes. The occurrence (%) of nematode species at each site also varied considerably and ranged between 0.86-4.47% at Malir, Karachi; 1.93-5.79% at Kalri Lake, Thatta; 1.15-5.98% at Karachi Canal, Jamshoro; 1.58-5.93% at Al-Manzar, Jamshoro; 24-8.11% at Detha, Hyderabad; 2.33-8.84% at Khesana Mori, Tandojam; 1.71-5.57% at Mirpurkhas and 2.38-8.95% at Jamesabad. A similarity matrix based on the proportion of shared nematode community was used to establish the level of relatedness between the different sites. Two surveyed sites Karachi Canal and Al-Manzar at River Indus, Jamshoro were close to each other for the presence of fresh water nematodes as compared to other localities whereas, similarity for localities and nematode species ranged from 0.458 to 0.7 and 0.45 to 1.0, respectively. Ecological indices viz., Shannon's index, species richness, evenness and maturity index were used for monitoring environmental conditions of each sampling site. The two indices viz., diversity index and maturity index were lowest at sites, Jamesabad and Khesana Mori which can be considered as indications of stress at these sites.

Keywords: Fresh water nematodes, diversity, Sindh, Pakistan

Fresh water resources are diverse like glaciers, rivers, streams, lakes, ponds, springs, canals and even groundwater. Fresh water provides water for drinking, sanitation, agriculture, transport, electricity generation and recreation. It also provides habitats for diverse range of animals and plants. In fresh water environment nematodes occur in all sediments and are of great ecological

importance. They represent one of the most abundant, diverse and dominant taxa in fresh water habitats. They play key role in many environmental processes, as grazing on microbes, has the potential to regulate decomposition, predators and prey for other animals, symbiont of bacteria and other organisms. They are also effective for monitoring pollution and

environmental disturbance (Eyualet-Abebe *et al.*, 2006). Fresh water nematodes are aquatic pollution indicators in a community (Beier & Traunspurger, 2001; Eyualet-Abebe *et al.*, 2001).

Nematodes are found in all types of habitat whether lentic (Heip *et al.*, 1985; Traunspurger, 1996a, b) or lotic (Anderson, 1992; Traunspurger, 2000). Nematodes have several geological features that make them useful indicators; due to their permeable cuticle they well respond to pollutants and correspond with the restorative capability of soil ecosystem (Saly & Ragala, 1984; Wasilewska, 1979, 1989). Under unfavourable environmental conditions some nematodes show resistance having cryptobiosis or cysts formation to survive for their development. Members of the family Dorylaimidae have no resistant stages which make them more sensitive to environmental change (Bongers, 1999).

Nematodes in fresh water environment have great ecological importance as they occur in all types of sediments (Giere, 1993; Neher, 2001a, b). They are found in all depths of aquatic medium. Due to high potential of being bio-indicators then other organisms, researchers have found that Dorylamia are the most successful group of nematodes which belong to two thirds of all known fresh water nematodes belonging to the above sub-class (Eyualet-Abebe *et al.*, 2006).

Benthic types of nematodes are found at the bottom of lakes and streams and are non parasitic, they act as consumers and decomposers in the food chain in fresh water environment and are well adapted to river life, although accurate number of species is still unknown.

The Malir and Lyari rivers, situated around Karachi are highly polluted. Due to scarcity of water, people had to depend on ground water sources like wells, tube wells and hand pumps. The quality of water and specification of

nematode presence may be determined from their tolerance to a particular physico-chemical variation in a habitat.

Since, no information about the fresh water nematodes was available in the past; the present research study has undertaken to gather the information of nematode fauna in fresh water ecosystem for both lentic and lotic habitats. The present study is based on fresh water sources of lower plains of Sindh which covers the main rivers, lakes, streams, ponds, irrigation canals, channels and estuary, around Karachi, Thatta, Karachi Canal and Al-Manzar at River Indus, Jamshoro, Hyderabad, Tandojam, Mirpurkhas and Jamesabad.

Materials and Methods

Sites of sampling: Water samples were collected from eight different sites of Sindh province. The sites include Malir, Karachi; Kalri (Keenjhar) Lake, Thatta; Karachi Canal and Al-Manzar Restaurant at River Indus, Jamshoro; Detha, Hyderabad; Khesana Mori, Tandojam; Mirpurkhas and Jamesabad (Kot Ghulam Mohammad). There were altogether eight collecting sites; however, the source of fresh water was different at each site. Rivers, lakes, canals, tube wells, streams, ponds, tanks and irrigation channel were the source of sample collection for the study.

Collection of samples: About 620 samples were randomly collected from different areas from time to time during the course of study. Sampling from the catchment areas was based on both lentic and lotic waters. The sites had different pollution levels. These pollutants were added from faecal matters from animal breeding farms, pesticides, contaminated water from industrial and domestic discharge in rivers, streams, lakes and estuaries. Water and superficial sediments were collected from hand shovel and placed in plastic bags; tied with rubber bands and labeled with necessary information of sites and date of collection.

Samples were kept in cool boxes and brought to the laboratory for further process.

Processing of samples: Cobb's sieving and decanting method was used for water samples and sediments followed by Baermann funnel technique (Hooper *et al.*, 2005).

Extraction of nematodes: Nematodes of each sampling site were extracted from 100 g subsamples of sediments and 500 ml of fresh water. These samples were placed in plastic bucket and two litre of fresh water added and thoroughly stirred to allow homogeneity of water contents and allowed to stand for few minutes. The supernatant was passed through sieve (36 mesh) into the second bucket and again stirred and allowed to stand to get the optimum number of nematodes. This supernatant was then passed over the fine mesh (325 mesh). To avoid any loss of nematode, drained water was not discarded. Sieve was kept over 600 ml glass beaker and the contents were drained with gentle stream of water. The nematodes were then collected from the sieve with 25 ml of water in a cup. The suspension of the cup was allowed to remain undisturbed for 5 minutes, for the nematodes to settle down. The water suspension containing nematodes was gently poured over a double layered tissue paper placed in a glass funnel. The funnel was controlled with a screw clip attached with the tube. After 48 h, the nematodes were collected from the funnel with 100 ml of water from the bottom of the rubber tube. A stereoscopic binocular microscope was used for nematode observation. The nematodes were categorized into different ages (juveniles and adults), sexes (mature males and females) and identified later on.

Data analysis

Distribution of fresh water nematodes: Data was based on analysis with regard to

prevalence and distribution of fresh water nematodes in surveyed sites of Sindh, Pakistan.

Chi-square contingency: Analysis of different nematode taxa was subjected to Chi-square contingency.

Similarity index: The similarity between the localities and species of fresh water nematodes in Sindh province data based on the quantitative (presence/absence) of nematodes was calculated on the basis of Jacord's coefficient of similarity (Rohlf, 2005). All computations were carried out using the computer application JAVA Runtime Environment (JRE) supported software BioSim 2.0.03 Version B (Pearson *et al.*, 2005).

Diversity indices: Nematode species data were used to calculate the diversity as the number of species per sample (S), the Shannon Weiner diversity index (H^1 calculated using naperian logarithms). The Shannon Weiner index (H'), richness (d) and evenness (J) were calculated with the formulas.

Maturity index: Nematode families were assigned a c-p value from 1 to 5 based upon whether they were fast or slow reproducers (Bongers, 1990; Bongers & Bongers, 1998). Based on the colonizer-persister scale (Bongers, 1999) the nematode taxa are classified into following five classes as given by Ferris & Bongers (2009): c-p 1; c-p 2; c-p 3; c-p 4 and c-p 5.

Maturity index of nematode community was calculated as the weighted mean of the constituent nematode taxa values by the following formula:

$$\text{Maturity Index (MI)} = \sum v(i) \cdot f(i)$$

where:

v (i) was the c-p value of taxon i

f (i) was the frequency of taxon i in a sample.

Statistical analysis: Mean diversity indices (diversity index, species richness, species evenness and maturity index) were evaluated against eight different sites. Analysis of variance (ANOVA) was used and means separated by Duncan Multiple Range Test (DMRT) at the 5% level of significance.

Results and Discussion

Surveyed sites: Water samples were collected from eight different sites of Sindh province. Surveyed sites with their GPS presented in Table 1.

Inland water source: There were altogether eight collecting sites; however, the source of fresh water was different at each site. The surveyed sites (Fig. 1-8) have the following fresh water sources:

Samples were collected from tube well, tank, irrigation channel at agricultural field at Bajar Goth, Malir, Karachi, Sindh. Kalri Lake, also known as Keenjhar Lake, is the largest lake of fresh water in Pakistan situated near Thatta, Sindh. Lake water and sediment sample was collected for the study. Karachi Canal, an off canal of River Indus was the sample collection source situated near Jamshoro, Sindh. Water is supplied to Karachi from the tributaries of Indus River via Karachi Canal. Samples were collected from Indus River water at Al-Manzar restaurant, Jamshoro, Sindh. Irrigation channel was the source of fresh water collection at Sajam Goth Detha situated near Hyderabad, Sindh.

Samples of water from irrigation channel were collected from Khesana Mori, Tandojam, Sindh. Samples were collected from reservoir of ponds and tanks of purification plants supplying fresh water to Mirpurkhas, Sindh. Ponds and streams near agricultural lands of Jamesabad were the fresh water sources of collection.

Fresh water sample analysis: Fresh water samples collected from eight different sites resulted in the isolation and identification of forty nematode species from different water sources of Sindh, Pakistan (Table 2).

This includes three new species viz., *Bathyeurystomina minima* Nasira, Shahina & Shamim, 2014; *Belbolla longispiculata* Nasira, Shahina & Shamim, 2014 and *Metoncholaimus siddiqii* Shahina, Nasira and Shamim, 2015 while six new records viz., *Achromadora ruricola* (De Man, 1880) Micoletzky, 1925, *Basirotyleptus* (B.) *basiri* (Jairajpuri, 1964) Jairajpuri & Ahmad, 1992, *Eurystomina indica* Yoshimura, 1980, *Pareurystomina vaughtae* Keppner, 1989, *Prismatolaimus intermedius* (Bütschli, 1873) de Man, 1880 and *Propanagrolaimus hygrophilus* (Bassen, 1940) Andrassy, 2005 were reported for the first time from Pakistan.

All these nematode species belonged to thirty six genera, twenty seven families and seven orders.

Table 1. Surveyed sites of Sindh province and their G.P.S.

S. No.	Sites	Latitude	Longitude
1	Malir, Karachi	25° 03' 01" N	67° 16' 41" E
2	Kalri Lake, Thatta	24° 57' 53" N	68° 04' 9.6" E
3	Karachi Canal, Jamshoro	27° 04' 60" N	68° 10' 0" E
4	Al-Manzar, Jamshoro	25° 26' 38" N	68° 18' 40" E
5	Detha, Hyderabad	25° 24' 30" N	68° 18' 28" E
6	Khesana Mori, Tandojam	25° 26' 42" N	68° 35' 2.5" E
7	Mirpurkhas	25° 52' 51" N	69° 09' 59" E
8	Jamesabad	25° 17' 10" N	69° 15' 35" E



Fig. 1. Irrigation channel of Malir river, Karachi.



Fig. 2. Kalri (Keenjhar) Lake, Thatta.



Fig. 3. Karachi Canal at River Indus, Jamshoro.



Fig. 4. Al-Manzar Restaurant at River Indus, Jamshoro.



Fig. 5. Irrigation channel, Detha, Hyderabad.



Fig. 6. Irrigation canal, Khesana Mori, Tandojam.



Fig. 7. Purification plants supplying fresh water to Mirpurkhas



Fig. 8. Pond near agricultural area, Jamesabad.

Table 2. List of nematode species encountered during the study.

S. #	Nematode species
1	<i>Achromadora ruricola</i> (de Man, 1880) Micoletzky, 1925**
2	<i>Acrobeles geraerti</i> Rashid, Heyns & Coomans, 1990
3	<i>Alaimus minor</i> Cobb, 1893
4	<i>Allodorylaimus americanum</i> Andrassy, 1986
5	<i>Basitryleptus</i> (B.) <i>basiri</i> (Jairajpuri, 1964) Jairajpuri & Ahmad, 1992**
6	<i>Bathyrystomina minima</i> Nasira, Shahina & Shamim, 2014*
7	<i>Belbolla longispiculata</i> Nasira, Shahina & Shamim, 2014*
8	<i>Cephalobus nannus</i> de Man, 1880
9	<i>Clarkus papillatus</i> (Bastian, 1865) Jairajpuri, 1970
10	<i>Discolaimium brachyurum</i> Husain & Siddiqi, 1967
11	<i>Discolaimus pakistanensis</i> Nasira, Shahina & Erum, 2008
12	<i>Discolaimus texanus</i> Cobb, 1913
13	<i>Dorylaimellus</i> (D.) <i>vexator</i> Heyns, 1963
14	<i>Dorylaimoides parateres</i> Siddiqi, 1956
15	<i>Enchodelus macrodorus</i> (de Man, 1880) Thorne, 1939
16	<i>Eurystomina indica</i> Yoshimura, 1980**
17	<i>Halalaimus gidenensis</i> Nasira & Turpeenniemi, 2002
18	<i>Haliplectus dorsalis</i> Cobb in Chitwood, 1956
19	<i>Helicotylenchus dihystra</i> (Cobb, 1893) Sher, 1963
20	<i>Helicotylenchus falcatus</i> Eroshenko & Nguen Vu Thanh, 1981
21	<i>Labronema digitatum</i> Sakul, Das & Mitra, 1975
22	<i>Longidorella</i> (Saevadorella) <i>tharensis</i> Nasira, Shahina & Firoza, 2010
23	<i>Longidorus africanus</i> Merny, 1966
24	<i>Metoncholaimus siddiqii</i> Shahina, Nasira and Shamim, 2015*
25	<i>Mononchus aquaticus</i> Coetzee, 1968
26	<i>Mylonchulus contractus</i> Jairajpuri, 1970
27	<i>Mylonchulus minor</i> (Cobb, 1983) Andrassy, 1958
28	<i>Nygolaimus vulgaris</i> Thorne, 1930
29	<i>Pareurystomina vaughtae</i> Keppner, 1989**
30	<i>Plectus karachiensis</i> Shahina, Tabassum & Maqbool, 2001
31	<i>Pratylenchus coffeae</i> (Zimmermann, 1898) Filipjev & Schuurmans Stekhoven, 1941
32	<i>Prismatolaimus intermedius</i> (Bütschli, 1873) de Man, 1880**
33	<i>Propanagrolaimus hygrophilus</i> (Bassen, 1940) Andrassy, 2005**
34	<i>Psilenchus hilarulus</i> de Man, 1921
35	<i>Rhabditis producta</i> (Schneider, 1866) Linstow, 1878
36	<i>Rotylenchulus reniformis</i> Linford & Oliveira, 1940
37	<i>Spilophorella candida</i> Gerlach, 1951
38	<i>Tylencholaimus cynodonti</i> Nasira, Erum & Shahina, 2005
39	<i>Tylenchorhynchus annulatus</i> Cassidy, (1930) Golden, 1971
40	<i>Tylenchorhynchus elegans</i> Siddiqi, 1961

* = New species

** = New record species

Occurrence (%) of nematode taxa: The survey result showed that out of the total nematode species, maximum nematode species belonging to the order Dorylaimida, followed by the order Rhabditida. So the frequency of the order Dorylaimida was found highest (32.5%), followed by the order Rhabditida (27.5%) and the least frequency was of the order Triplonchida (2.5%) (Fig. 9).

Nematode faunal composition: A total of forty nematode species of fresh water belonging to 36 genera, 27 families and seven orders were found and identified from eight surveyed sites of Sindh, Pakistan (Fig.10). However, the nematode genera and species varied in number and abundance from locality to locality. Water analysis showed that the more or less similar species were encountered but their number varied at different sites. Maximum number of species (36) was recorded at Malir, Karachi whereas the minimum species (17) were extracted from Jamesabad, Sindh, Pakistan (Fig. 11).

Chi square analysis of different nematode taxa: The significance of higher taxon i.e., orders in relation to lower ranked taxa i.e.,

family, genus and species has been calculated by chi square (χ^2) analysis. The calculated value of chi-square was found 27.59 for 12 degrees of freedom at $p < 0.01$ level of significance and it exceeded the critical value i.e., 26.217. So it can be concluded that association of two variables i.e., family, genus and species against orders is significant.

Occurrence (%) of fresh water nematodes at surveyed sites of Sindh: Out of a total of 620 fresh water samples collected from eight sites of Sindh, the overall occurrence (%) of fresh water nematode samples ranged from 3.22-32.25%. The highest number of positive samples was found at Malir, Karachi (32.25%) and lowest was at Khesana Mori, Tandojam (3.22%). The occurrence (%) of fresh water nematode samples at other sites was found in descending orders as: Detha, Hyderabad (16.12%), Karachi Canal, Jamshoro (14.51%), Al-Manzar, Jamshoro (12.92%), Mirpurkhas (11.29%), Jamesabad (5.64%) and Kalri Lake, Thatta (4.03%) as shown in Fig. 12.

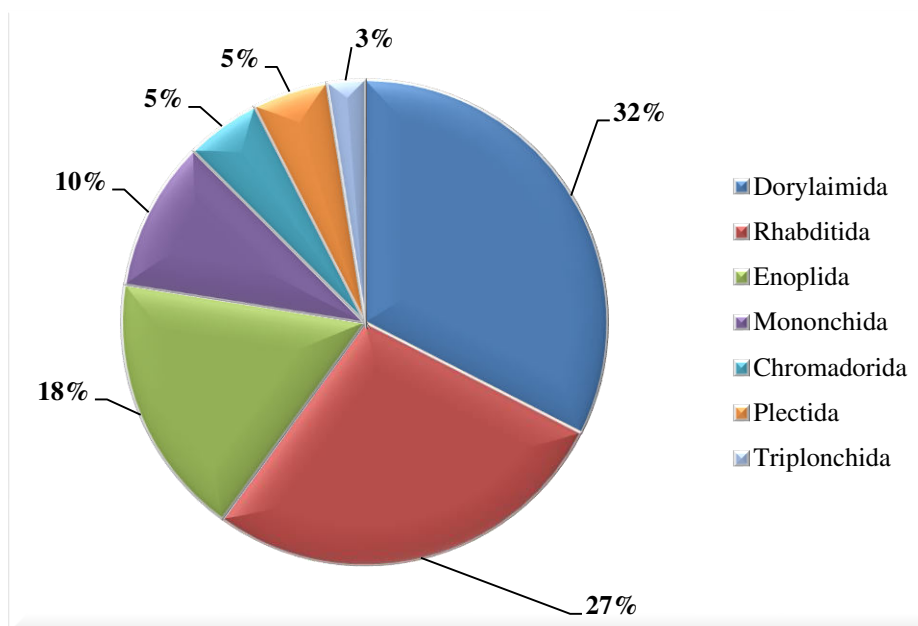


Fig. 9. Frequency (%) of fresh water nematode orders.

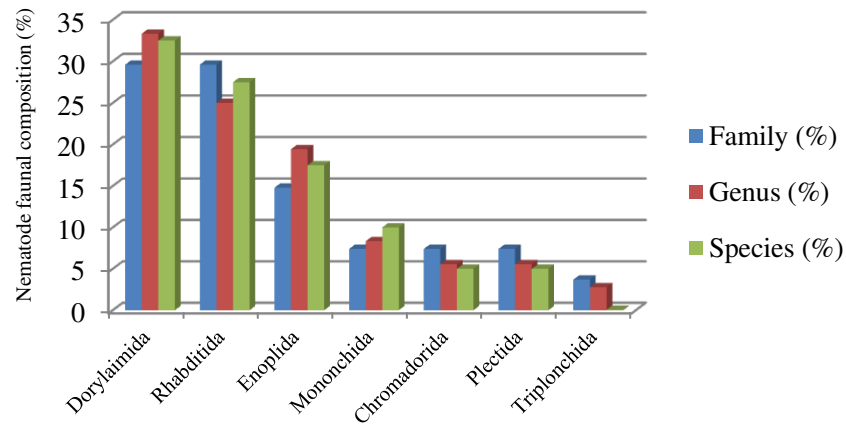


Fig. 10. Overall distribution of species, genera and families in seven different orders.

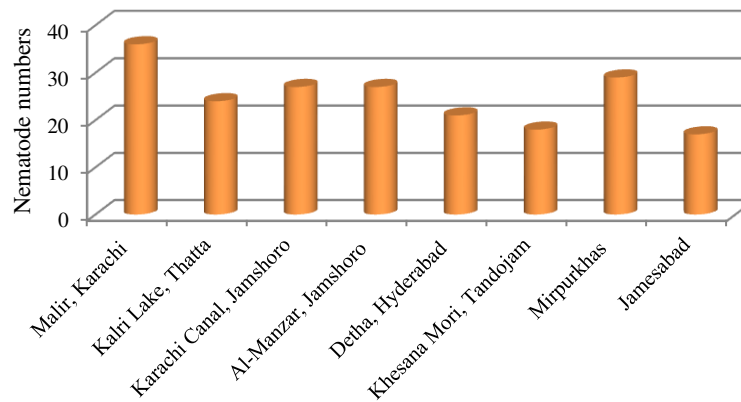


Fig. 11. Nematode species composition at eight surveyed sites of Sindh.

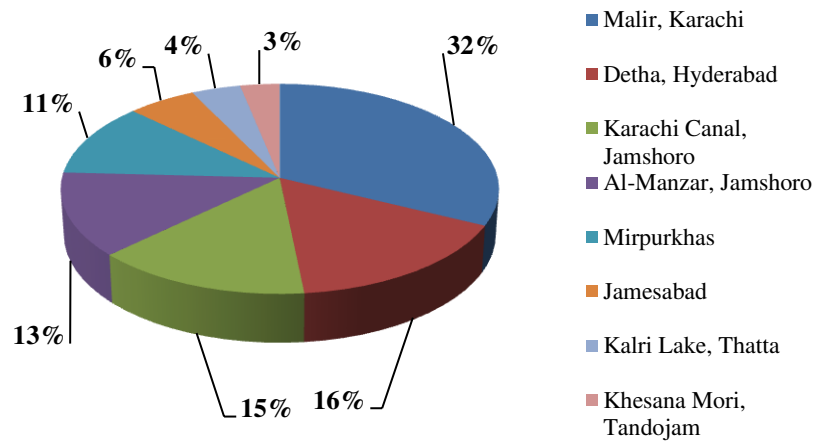


Fig. 12. Occurrence (%) of fresh water nematodes at surveyed sites of Sindh.

Occurrence percentage of different nematodes groups at surveyed sites of Sindh: Different groups of nematodes were encountered from fresh water sources of Sindh viz., plant parasitic nematodes, free-living soil nematodes and free-living marine nematodes. The overall highest occurrence (%) was of

free-living soil nematodes (55%) and it was more than two-folds as compared to plant parasitic nematodes (20%) and free-living marine nematodes (25%) as shown in Fig.13. The occurrence (%) of these fresh water nematode species at different surveyed sites varied considerably.

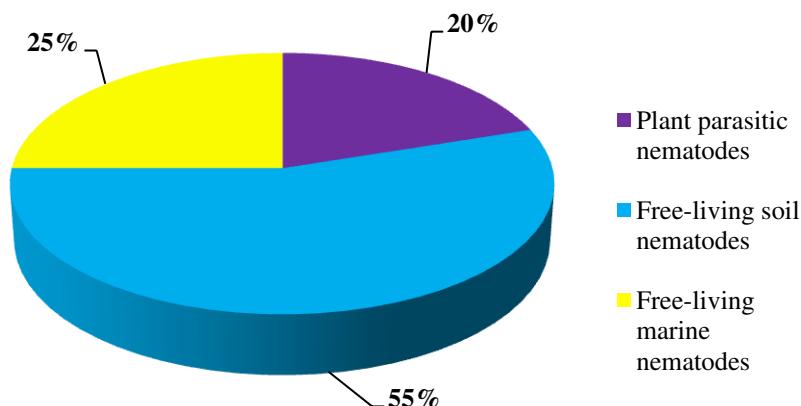


Fig. 13. Overall occurrence (%) of different nematode groups at surveyed sites of Sindh.

Malir, Karachi: Thirty six nematodes were recovered from 200 soil samples collected from Malir, Karachi. These nematodes include three groups of nematodes viz., plant parasitic nematodes, free-living soil nematodes and free-living marine nematodes in different occurrence (%). Plant parasitic nematodes recovered with 22.22% of the total nematodes, free-living soil nematodes found with 55.55% whereas the occurrence (%) of free-living marine nematodes 22.22% (Fig.14). The average occurrence (%) of thirty six nematode species at Malir, Karachi was ranged between 0.86- 4.47% (Fig.15).

Kalri Lake, Thatta: Twenty four nematodes were recovered from 25 soil samples collected from Kalri Lake, Thatta. These nematodes include three groups of nematodes viz., plant parasitic nematodes, free-living soil nematodes and free-living marine nematodes in different occurrence (%). Plant parasitic nematodes recovered with 25% of the total nematodes, free-living soil

nematodes found with 16.66% whereas the occurrence (%) of free-living marine nematodes 58.33% (Fig.16). The average occurrence (%) of twenty four nematode species at Kalri Lake, Thatta was ranged between 1.93-5.79% (Fig.17).

Karachi Canal, Jamshoro: Twenty seven nematodes were recovered from 90 soil samples collected from Karachi Canal, Jamshoro. These nematodes include three groups of nematodes viz., plant parasitic nematodes, free-living soil nematodes and free-living marine nematodes in different occurrence (%). Plant parasitic nematodes recovered with 22.22% of the total nematodes, free-living soil nematodes found with 55.55% whereas the occurrence (%) of free-living marine nematodes 22.22% (Fig. 18). The average occurrence (%) of twenty seven nematode species at Karachi Canal, Jamshoro was ranged between 1.15-5.98% (Fig. 19).

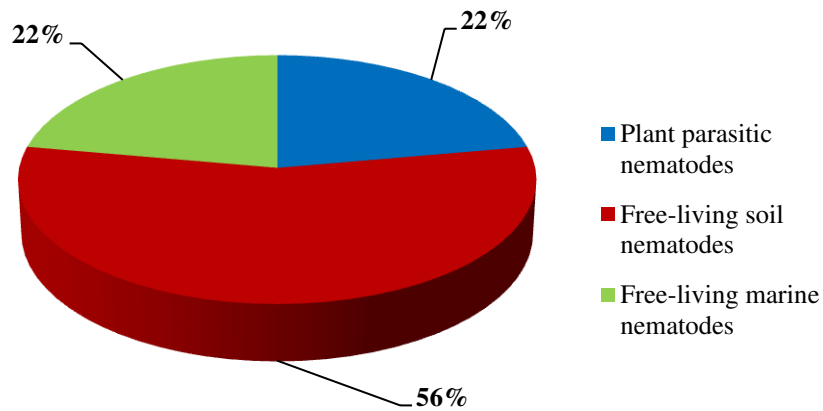


Fig. 14. Occurrence (%) of nematode groups at Malir, Karachi

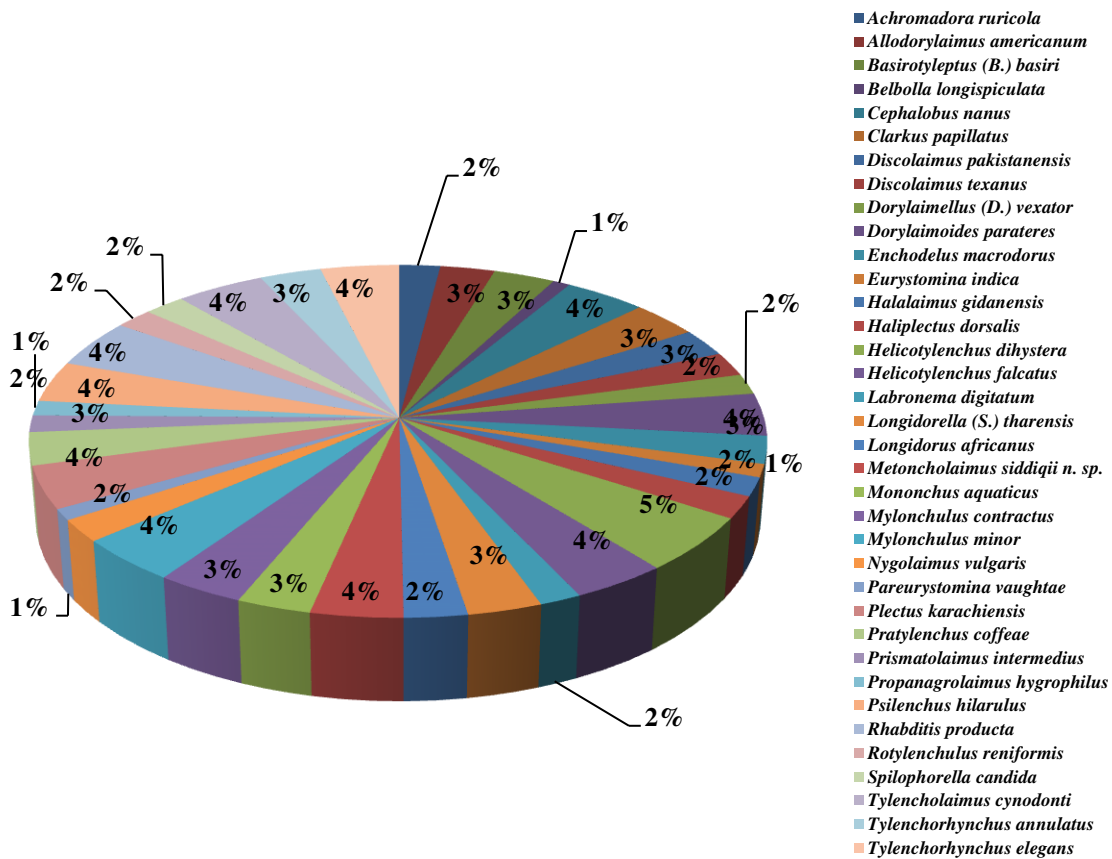


Fig. 15. Occurrence (%) of nematode species at Malir, Karachi

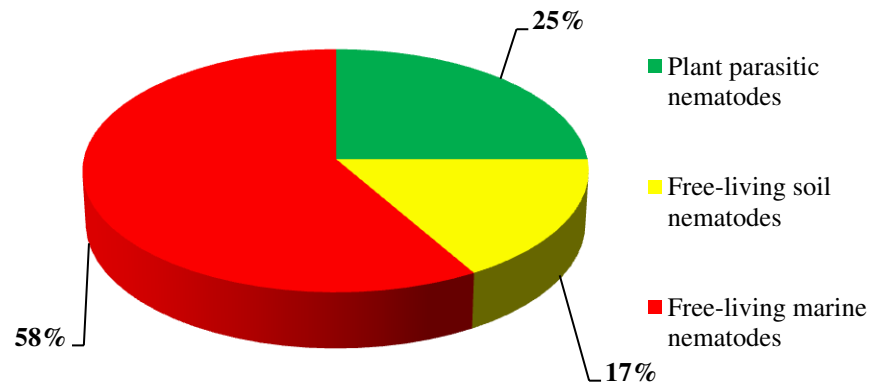


Fig. 16. Occurrence (%) of nematode groups at Kalri Lake, Thatta.

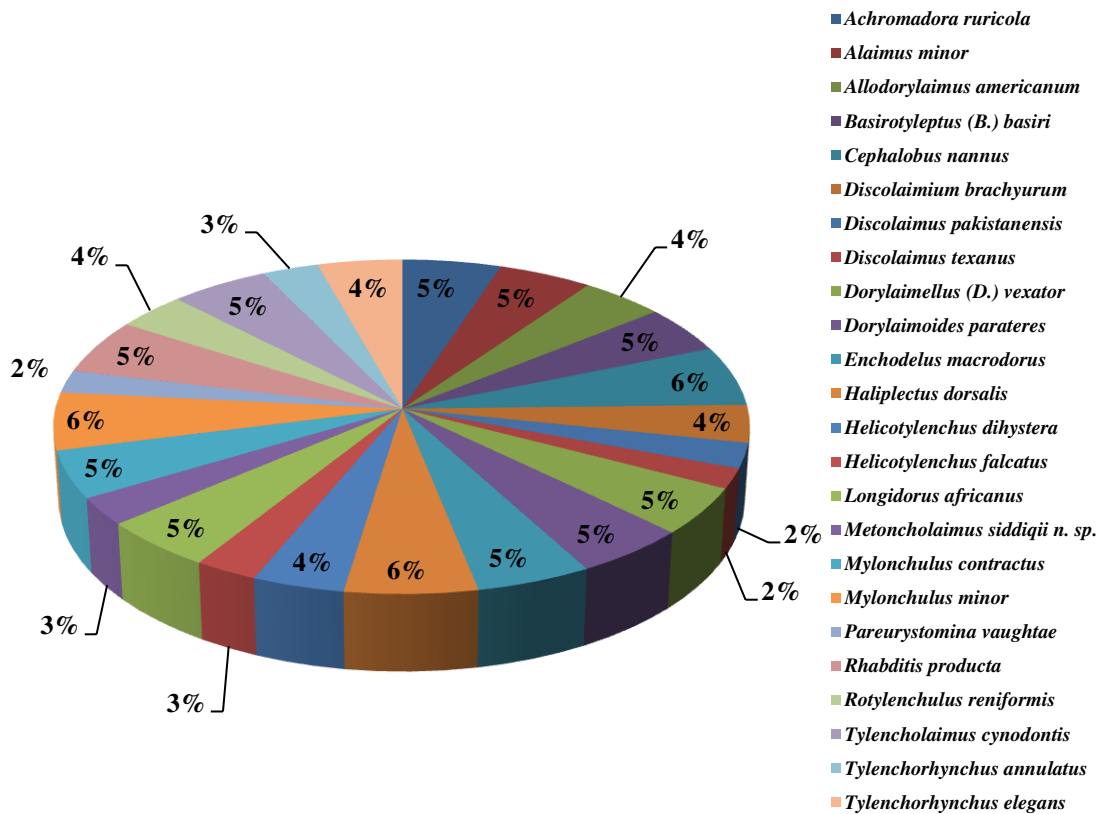


Fig. 17. Occurrence (%) of nematode species at Kalri Lake, Thatta.

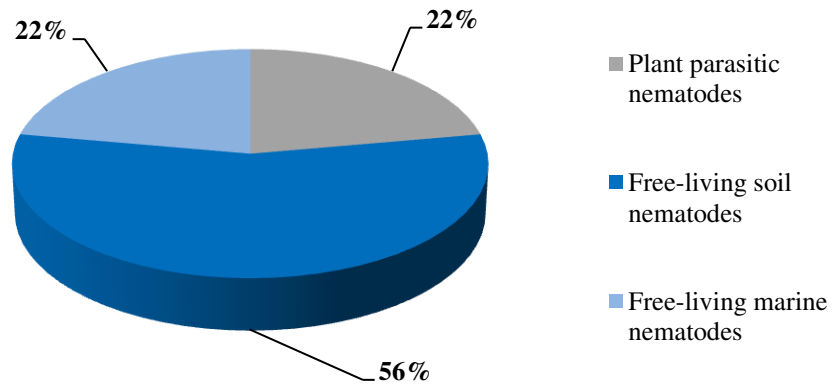


Fig. 18. Occurrence (%) of nematode groups at Karachi Canal, Jamshoro

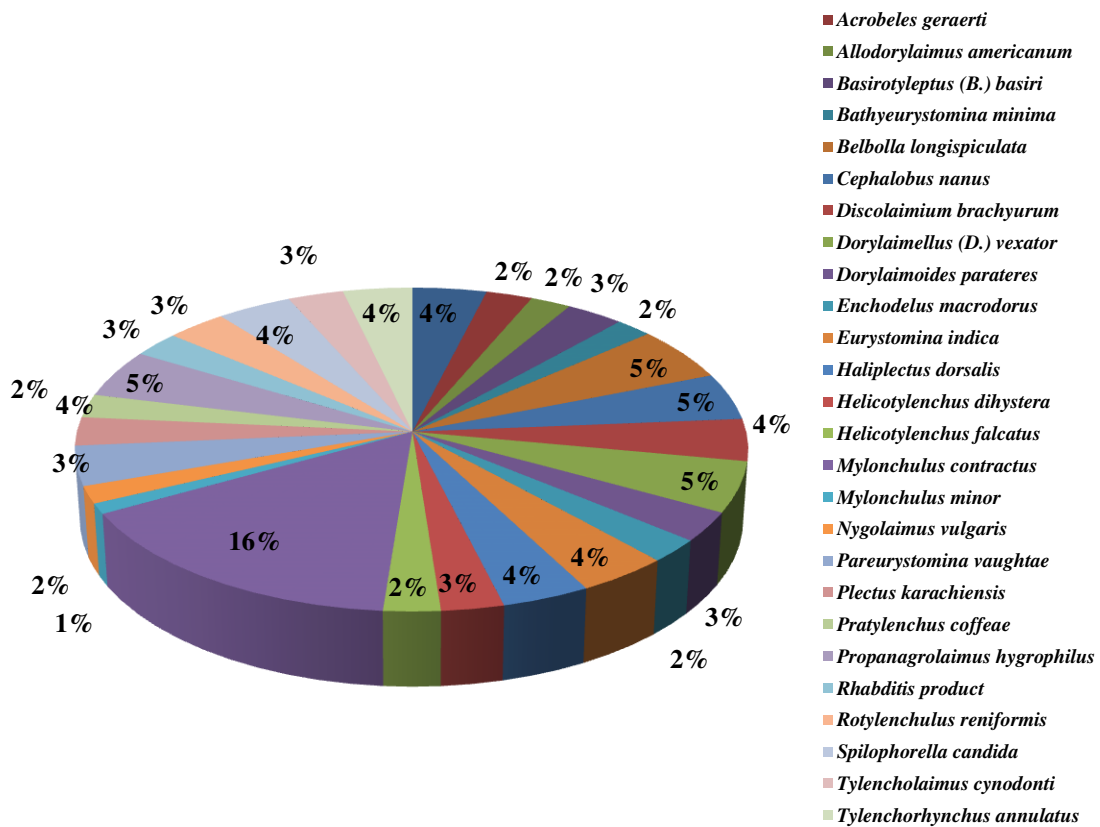


Fig. 19. Occurrence (%) of nematode species at Karachi Canal, Jamshoro.

Al-Manzar, Jamshoro: Twenty seven nematodes were recovered from 80 soil samples collected from Al-Manzar, Jamshoro. These nematodes include three groups of nematodes viz., plant parasitic nematodes, free-living soil nematodes and free-living marine nematodes in different occurrence (%). Plant parasitic nematodes recovered with 22.22% of the total nematodes, free-living soil nematodes found with 44.44% whereas the occurrence (%) of free-living marine nematodes 33.33% (Fig. 20). The average occurrence (%) of twenty seven nematode species at Al-Manzar, Jamshoro was ranged between 1.58-5.93% (Fig. 21).

Detha, Hyderabad: Twenty one nematodes were recovered from 100 soil samples collected from Detha, Hyderabad. These nematodes include three groups of nematodes viz., plant parasitic nematodes, free-living soil nematodes and free-living marine nematodes in different occurrence (%). Plant parasitic nematodes recovered with 28.57% of the total nematodes, free-living soil nematodes found with 52.38% whereas the occurrence (%) of free-living marine nematodes 19.04% (Fig. 22). The average occurrence (%) of twenty one nematode species at Detha, Hyderabad was ranged between 1.24-8.11% (Fig. 23).

Khesana Mori, Tandojam: Eighteen nematodes were recovered from 20 soil samples collected from Khesana Mori, Tandojam. These nematodes include three groups of nematodes viz., plant parasitic nematodes, free-living soil nematodes and free-living marine nematodes in different occurrence (%). Plant parasitic nematodes recovered with 38.88% of the total nematodes, free-living soil nematodes found with 50% whereas the occurrence (%) of free-living marine nematodes 11.11% (Fig. 24). The average occurrence (%) of eighteen nematode species at Khesana Mori, Tandojam was ranged between 2.33-8.84% (Fig. 25).

Mirpurkhas: Twenty nine nematodes were recovered from 70 soil samples collected from Mirpurkhas. These nematodes include three groups of nematodes viz., plant parasitic nematodes, free-living soil nematodes and free-living marine nematodes in different occurrence (%). Plant parasitic nematodes recovered with 24.13% of the total nematodes, free-living soil

nematodes found with 62.06% whereas the occurrence (%) of free-living marine nematodes 13.79% (Fig. 26). The average occurrence (%) of twenty nine nematode species at Mirpurkhas was ranged between 1.71-5.57% (Fig. 27).

Jamesabad: Seventeen nematodes were recovered from 35 soil samples collected from Jamesabad. These nematodes include three groups of nematodes viz., plant parasitic nematodes, free-living soil nematodes and free-living marine nematodes in different occurrence (%). Plant parasitic nematodes recovered with 23.52% of the total nematodes, free-living soil nematodes found with 64.70% whereas the occurrence (%) of free-living marine nematodes 11.76% (Fig. 28). The average occurrence (%) of seventeen nematode species at Jamesabad was ranged between 2.38-8.95% (Fig. 29).

Similarity index: A similarity matrix based on the proportion of shared nematode community was used to establish the level of relatedness between the different localities. There were 4 groups on the basis of localities and 9 groups on the basis of nematode species. Two fresh water surveyed sites viz., Al-Manzar and Karachi Canal at River Indus, Jamshoro were close to each other for the presence of fresh water nematodes as compared to other tested localities. Pair-wise estimates of similarity for localities and nematode species were ranged from 0.458 to 0.7 and 0.47 to 1.0 as given in Table 3, Fig. 30 and Table 4, Fig. 31, respectively.

Analysis of water samples of different sites showed that maximum nematode species belonged to the order Dorylaimida which is in conformity of the earlier research made by Eyualem-Abebe *et al.*, 2008; according to these researchers about two-thirds of all known freshwater nematodes belong to the subclass Dorylaimia of order Dorylaimida. Identification of fresh water nematode species indicates that Karachi water source site has a diverse nematode fauna than other sites of Sindh.

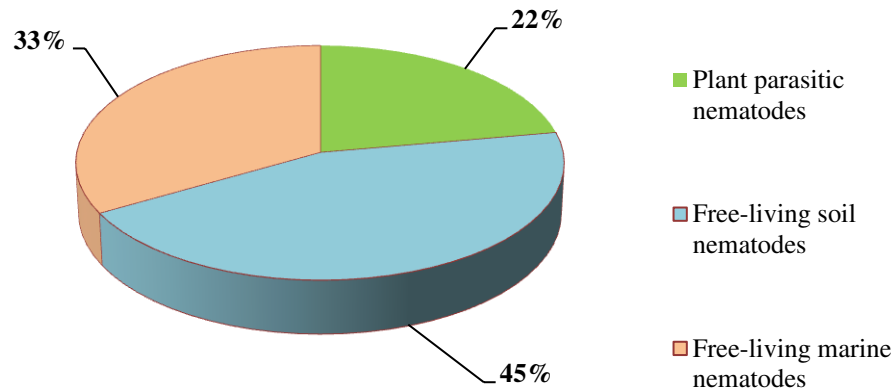


Fig. 20. Occurrence (%) of nematode groups at Al-Manzar, Jamshoro.

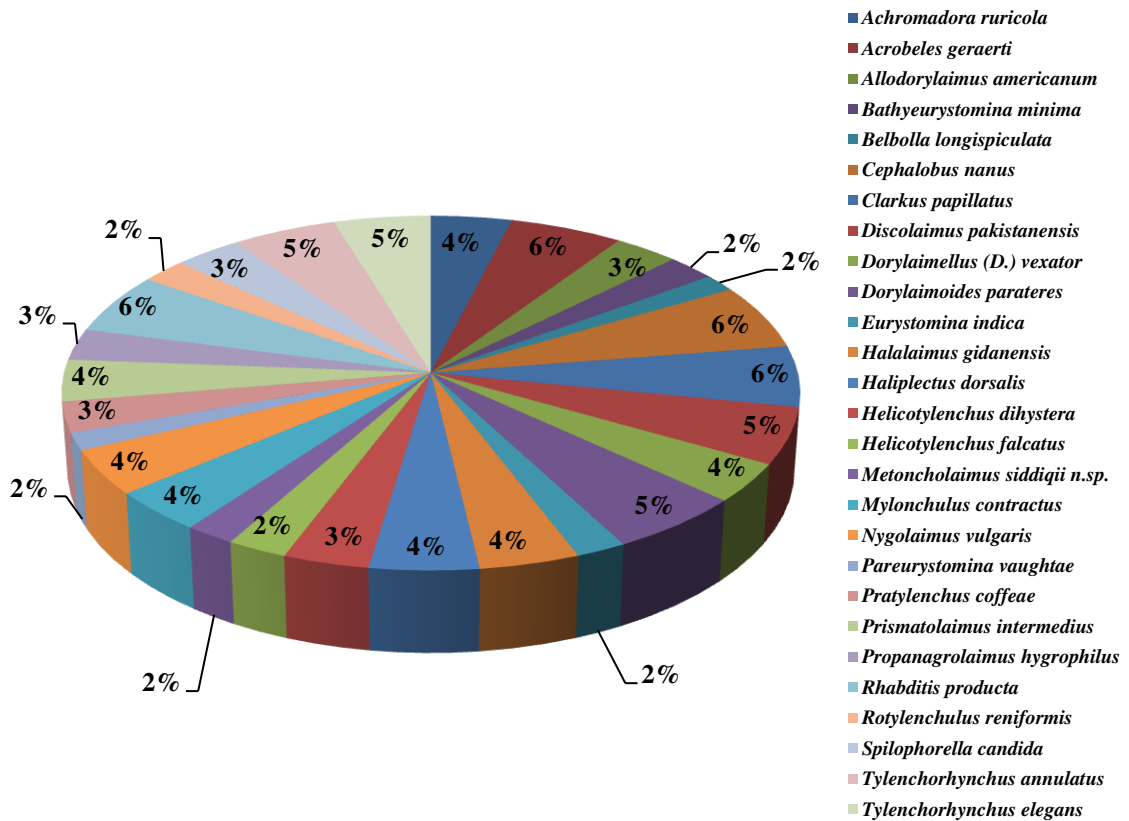


Fig. 21. Occurrence (%) of nematode species at Al-Manzar, Jamshoro.

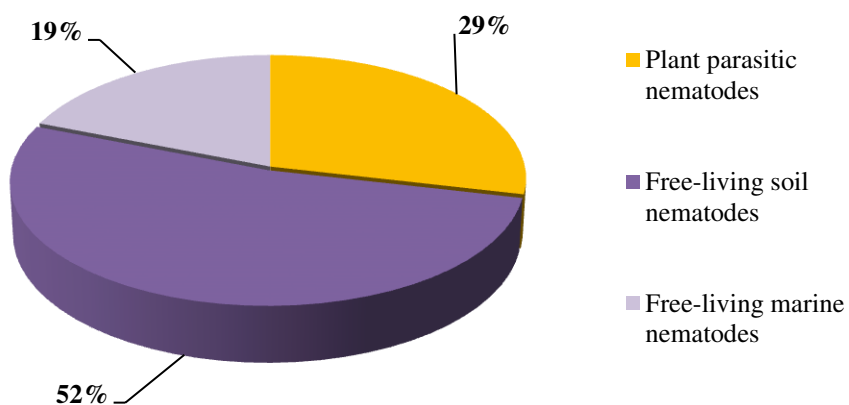


Fig.22. Occurrence (%) of nematode groups at Detha, Hyderabad.

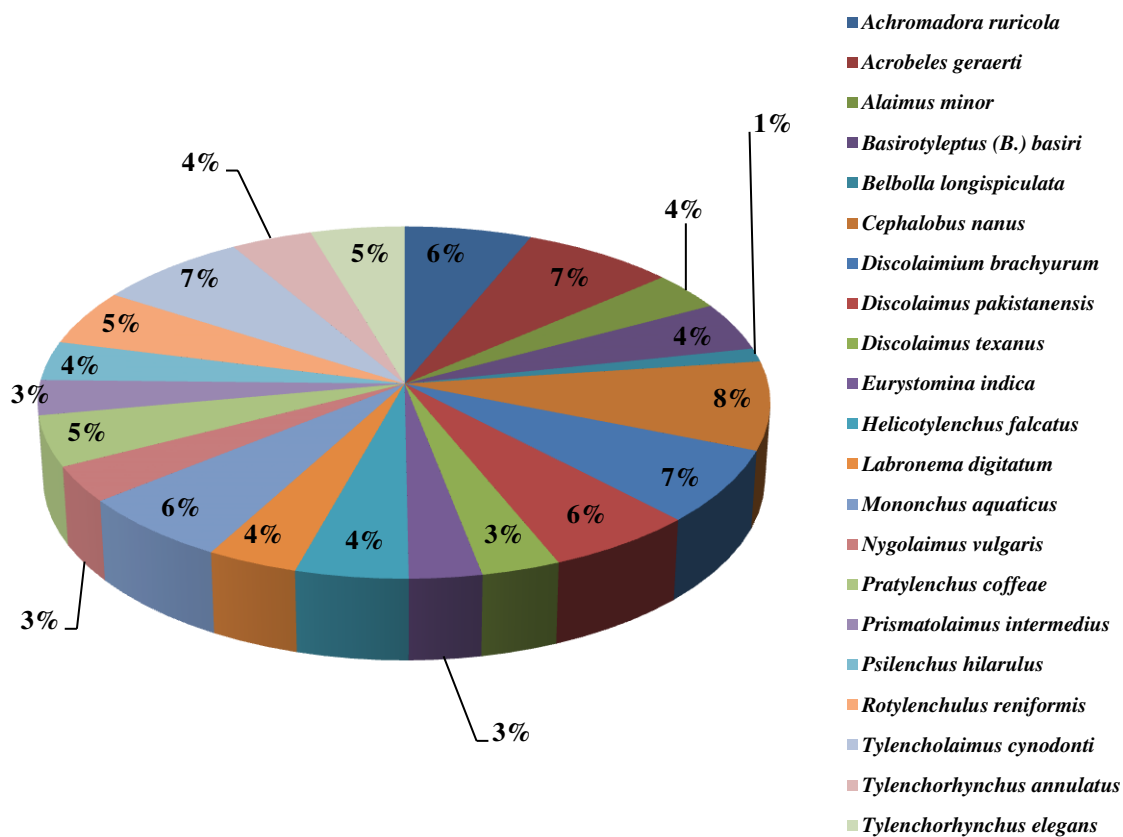


Fig. 23. Occurrence (%) of nematode species at Detha, Hyderabad.

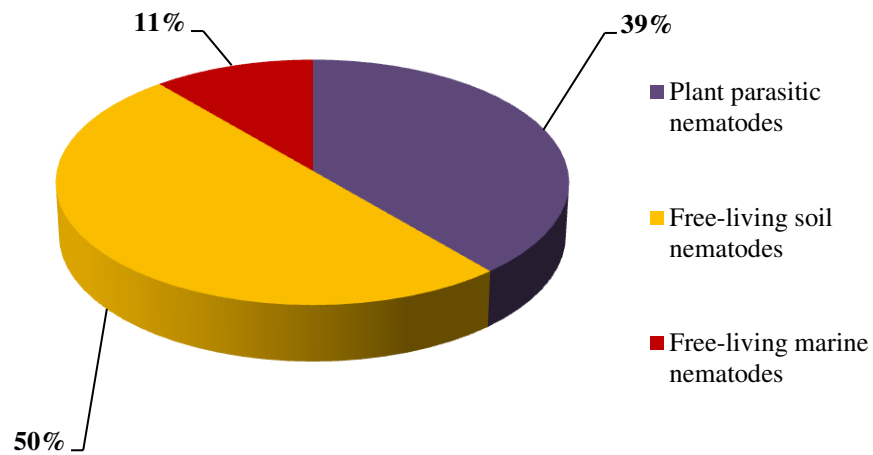


Fig. 24. Occurrence (%) of nematode groups at Khesana Mori, Tandojam.

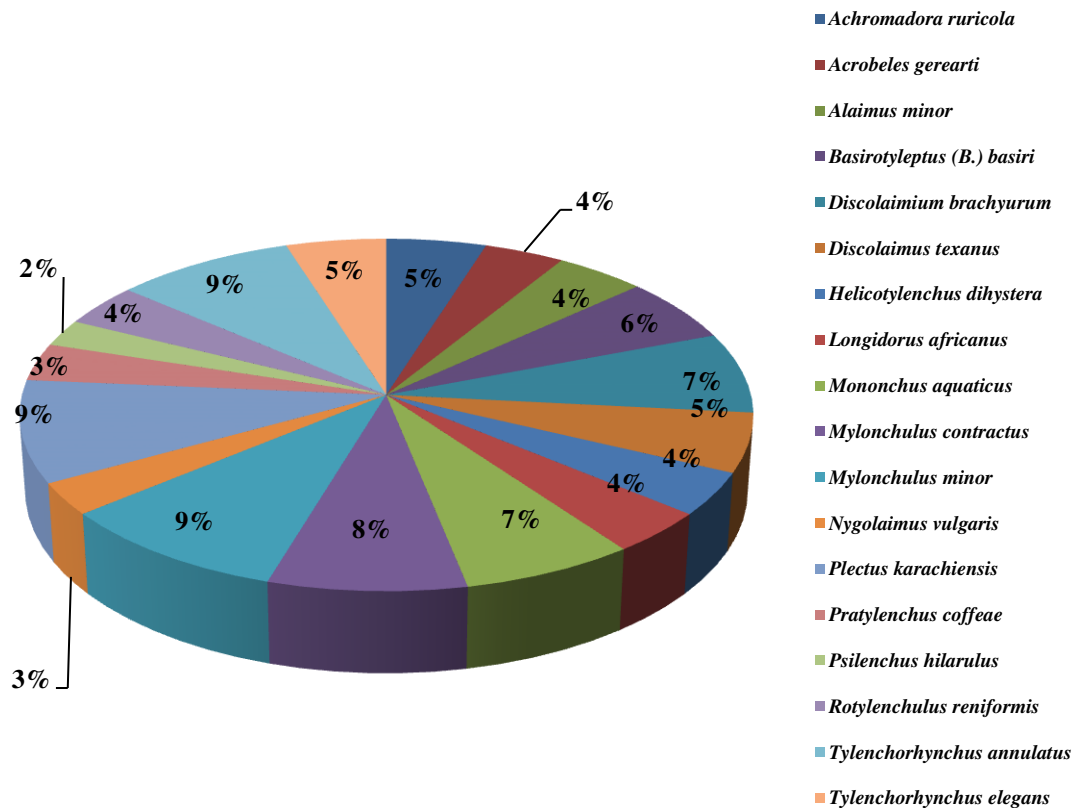


Fig. 25. Occurrence (%) of nematode species at Khesana Mori, Tandojam.

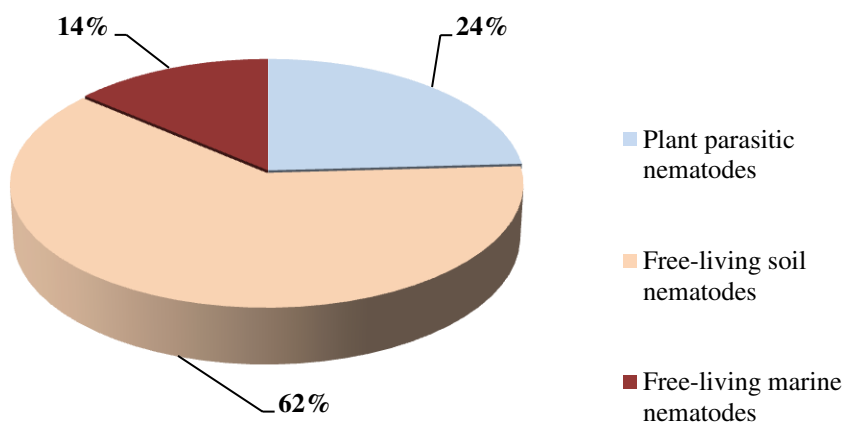


Fig. 26. Occurrence (%) of nematode groups at Mirpurkhas

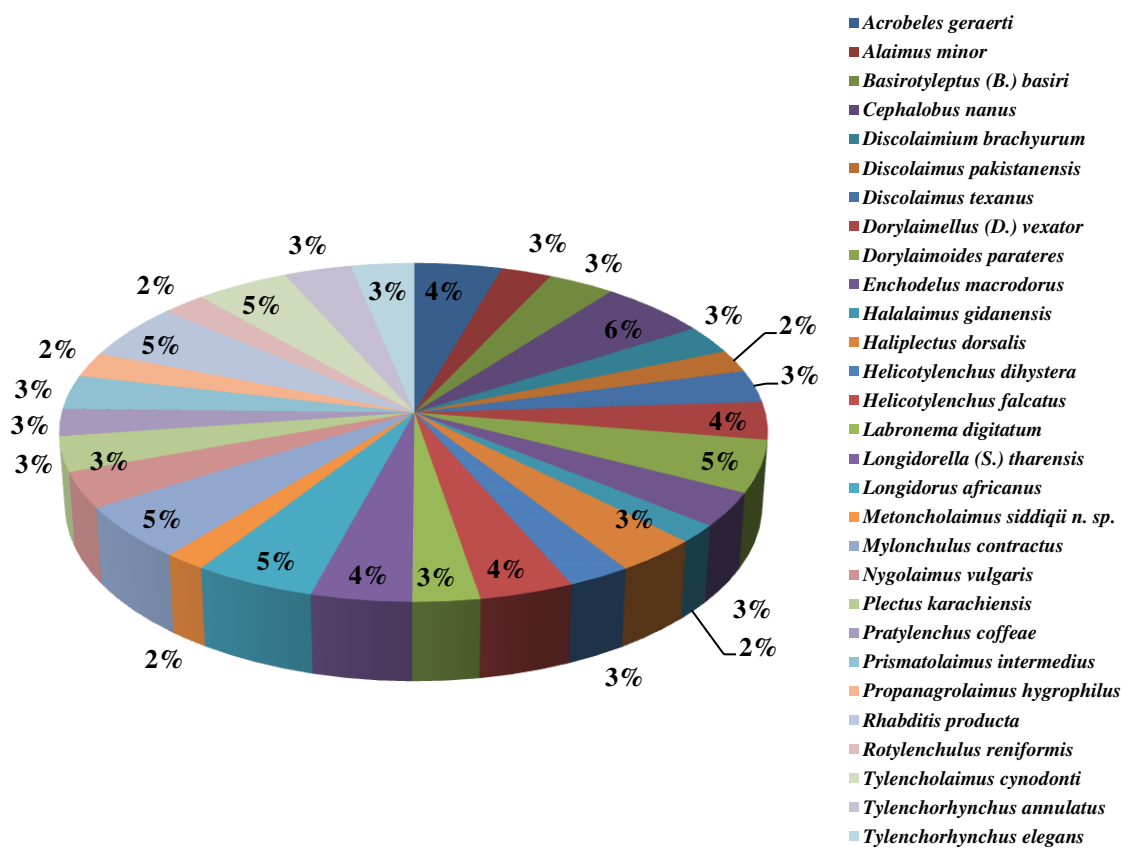


Fig. 27. Occurrence (%) of nematode species at Mirpurkhas.

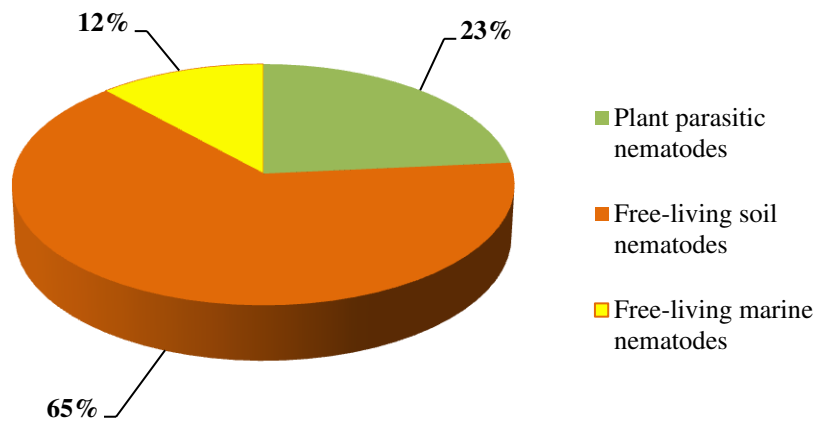


Fig. 28. Occurrence (%) of nematode groups at Jamesabad.

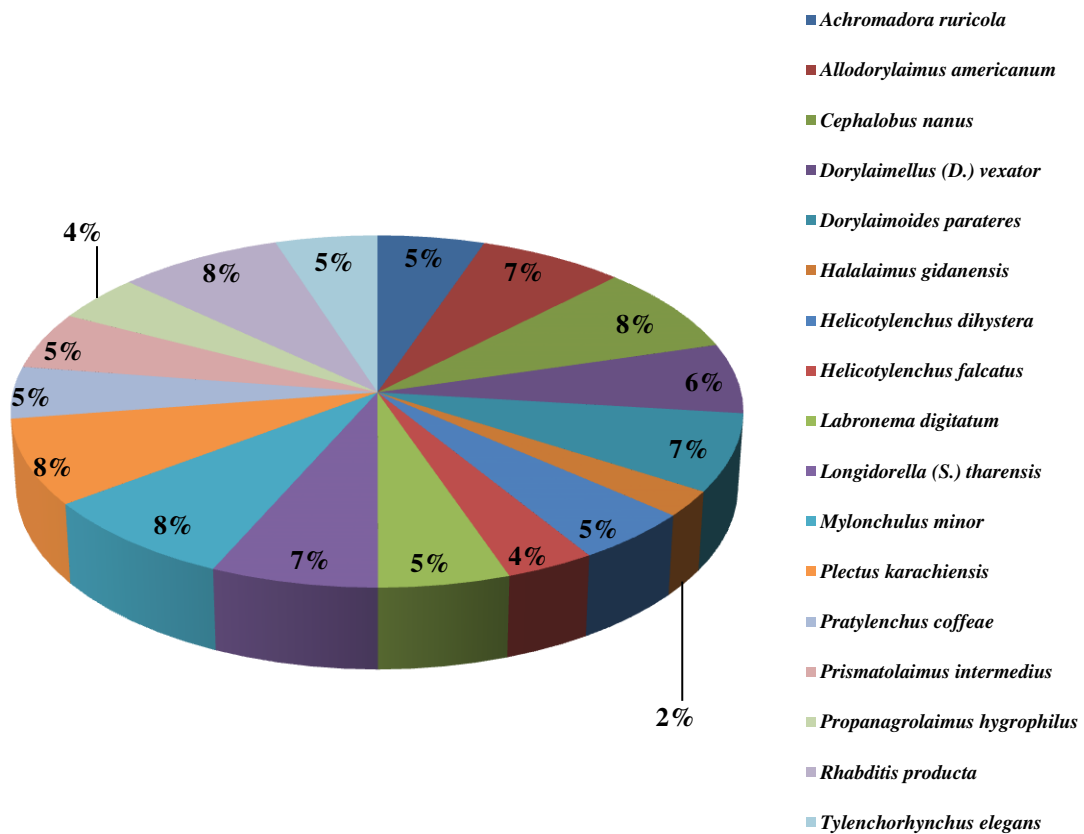
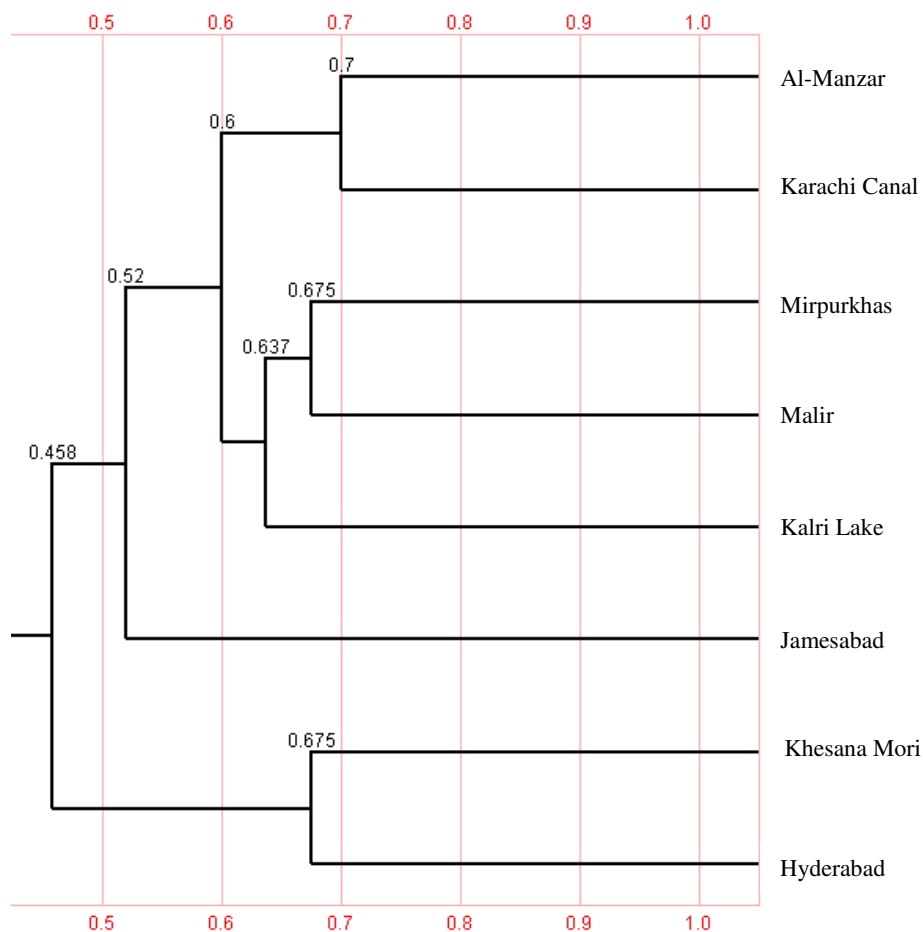


Fig. 29. Occurrence (%) of nematode species at Jamesabad.

Table 3. Similarity matrix for surveyed sites of Sindh.

Localities	Malir, Karachi	Kalri Lake, Thatta	Karachi Canal, Jamshoro	Al-Manzar, Jamshoro	Detha, Hyderabad	Khesana Mori, Tandojam	Mirpurkhas	Jamesabad
Malir, Karachi	1	0.6000	0.6250	0.6750	0.4750	0.4000	0.6750	0.5250
Kalri Lake, Thatta	0.6000	1	0.6250	0.5250	0.4750	0.5500	0.6750	0.4750
Karachi Canal, Jamshoro	0.6250	0.6250	1	0.7000	0.4500	0.4750	0.6000	0.5000
Al-Manzar, Jamshoro	0.6750	0.5250	0.7000	1	0.4500	0.3250	0.5500	0.5500
Detha, Hyderabad	0.4750	0.4750	0.4500	0.4500	1	0.6750	0.5500	0.4000
Khesana Mori, Tandojam	0.4000	0.5500	0.4750	0.3250	0.6750	1	0.5250	0.4250
Mirpurkhas	0.6750	0.6750	0.6000	0.5500	0.5500	0.5250	1	0.5500
Jamesabad	0.5250	0.4750	0.5000	0.5500	0.4000	0.4250	0.5500	1

**Fig. 30.** Dendrogram resulting from average linkage of eight localities of fresh water sites based on nematode communities from Sindh

[illegible]

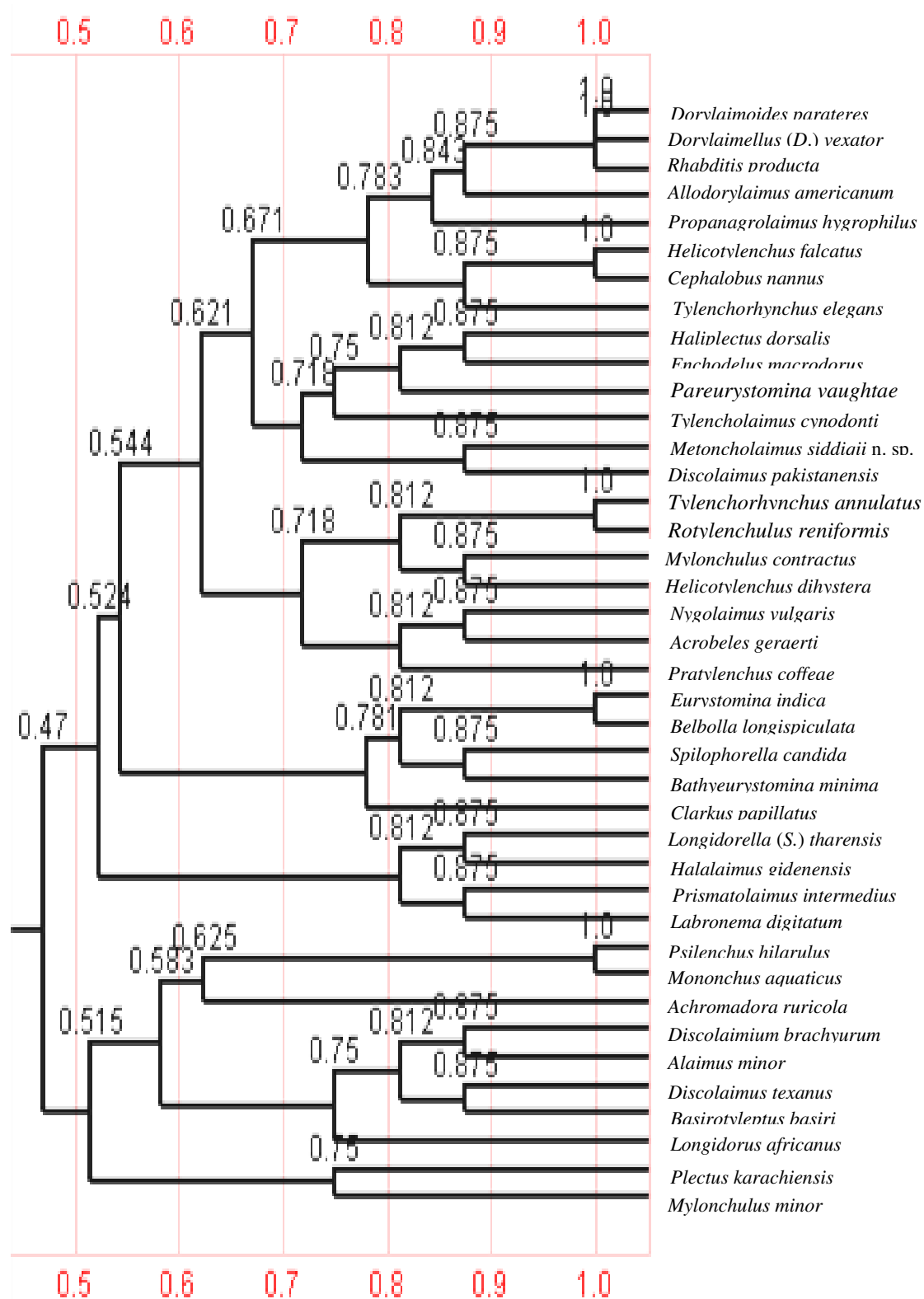


Fig. 31. Dendrogram for nematode species from 8 localities of fresh water sites of Sindh.

Ecological Indices for Biomonitoring

Freshwater nematode species diversity indices: The nematode species diversity indices viz., diversity index, species richness and evenness values for each site in relation to freshwater sources were computed

Results of the data of diversity parameters revealed that the highest value of diversity index (H') was found at Malir, Karachi (3.5078) followed by the second highest species diversity

at Mirpurkhas (3.31). Two sites viz., Al-Mazar and Karachi Canal at River Indus, Jamshoro have almost the same values (3.2899 and 3.2528), respectively. The lowest value was recorded at Jamesabad (2.7886).

Species richness as measured by Menhinick's index (d), was highest in Khesana Mori (1.26), second highest value was at Kalri Lake (1.17) and lowest at Detha, Hyderabad (0.6). Equitability or evenness (J) was quite similar at all sites (0.97-0.99) (Table 5, Fig. 32).

Table 5. Diversity (H'), species richness (d) and evenness (J), calculated at species level.

Localities	Species diversity (H')	Species richness (d)	Evenness (J)
Malir, Karachi	3.5078	0.66	0.97
Kalri Lake, Thatta	3.1364	1.17	0.98
Karachi Canal, Tandojam	3.2528	0.70	0.98
Al-Manzar, Tandojam	3.2899	0.75	0.99
Detha, Hyderabad	2.9752	0.60	0.97
Khesana Mori, Tandojam	2.8705	1.26	0.99
Mirpurkhas	3.3100	0.84	0.98
Jamesabad	2.7886	0.82	0.98

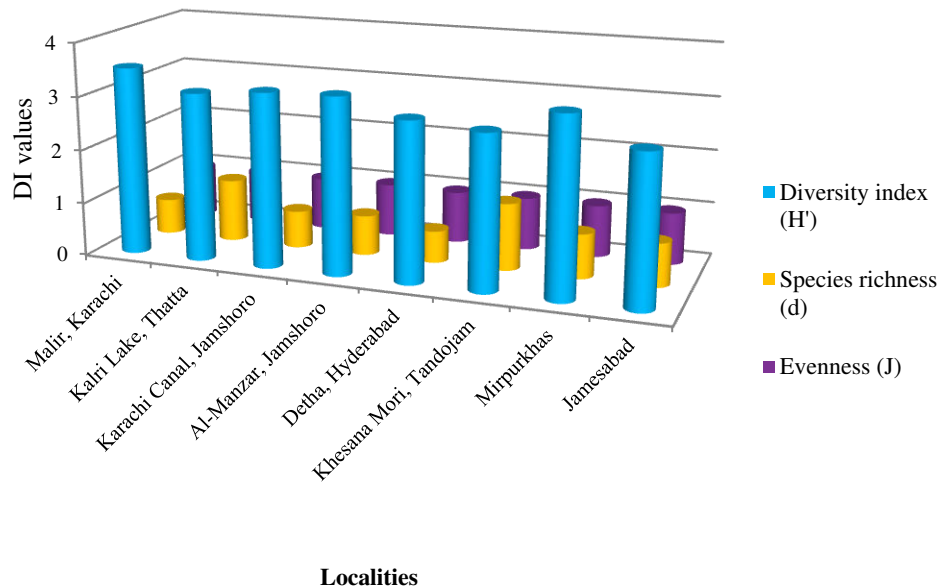


Fig. 32. Nematode diversity index, species richness and evenness.

A total of 40 species from 36 genera were recovered during the present studies (Table 2). Data obtained is in accordance with Traunspurger (2002) who reported that species richness in lakes and rivers tends to be between 30 and 70. The overall Shannon diversity index (H') for the nematode community in the present system was 3.12 (2.7-3.5).

The family Qudsianematidae was the most frequently occurred having the most species (5 species), Enchelidiidae was the second most occurred family with 4 species. All other families more or less have the same occurrence representing by one or two species. There are many environmental factors that affect species diversity. Some of these factors include seasonality, special heterogeneity, competition, predation, habitat type, environmental stability and productivity (Rosenzweig, 1995). Evenness was used by many ecologists; the evenness index was sensitive to species richness as it also considers even the rare taxa in the community.

Maturity Index

Maturity index of nematode families: The Maturity index (MI) was calculated on each sampling sites as the weighted mean frequency of individual colonizer–persister values (cp) after Bongers (1990). MI ranged from 1 to 5.

Nematode species with a $cp = 1$ were considered r-strategists (colonizers) with short-generation times, high fecundity and extreme population changes whereas those with a $cp = 5$ were defined as K-strategists (persisters) with lower breeding efficiency.

The MI is expected to decrease during disturbed periods, when opportunistic nematodes are favoured (Bongers & Bongers, 1998). The Maturity index which is a potential indicator of nematode communities under stress was calculated for each site during the study. Mean values computed are shown in Table 6 together with mean diversity index. MI showed variation within the range of 3.53 and 4.83 with average of 4.84.

When MI of each site was compared with average, most sites were not significantly different with the average.

However, significantly the highest MI value was found at Kalri Lake, Thatta (MI, 4.83); followed by Detha, Hyderabad (MI, 4.80); Karachi Canal, Jamshoro (MI, 4.78); Malir, Karachi (MI, 4.69); Al-Manzar, Jamshoro (MI, 4.55); Mirpurkhas (MI, 4.54) Khesana Mori, Tandojam (MI, 4.13) while the lowest value was found at Jamesabad (MI, 3.53).

Table 6. Mean values of Shannon-Wiener Diversity Index (H') and Maturity Index (MI) at the various surveyed sites of Sindh.

S. No.	Surveyed sites	Diversity index (H')	Maturity index (MI)
1	Malir, Karachi	3.50	4.69
2	Kalri Lake, Thatta	3.13	4.83
3	Karachi Canal, Jamshoro	3.25	4.78
4	Al-Manzar, Jamshoro	3.28	4.55
5	Detha, Hyderabad	2.97	4.80
6	Khesana Mori, Tandojam	2.87	4.13
7	Mirpurkhas	3.31	4.54
8	Jamesabad	2.78	3.53

The Maturity index is based on plant-feeding and non-plant-feeding taxa and considered a measure of environmental disturbance. The two indices viz., diversity index and maturity index, were lowest at sites, Jamesabad and Khesana Mori, which can be considered as indications of disturbance or stress at these sites. Bongers (1990) and Bongers & Ferris (1999) reported that low MI values (range from less than 2) indicate a disturbed and/or nutrient-enriched environment while high MI values (to about 4) show a stable, undisturbed and pristine environments environment. Nematode species characters, such as their c-p value and trophic level, seem to be related to environmental conditions indicating that nematodes are good

ecological indicators of pollution (Zullini *et al.*, 2011).

A comparison of different sites using diversity indices viz., Shannon-Weaver diversity index (H'), species richness (d) and species evenness (J) indicates that there were significant differences between different sites regarding the diversity index (ANOVA H' : $F=3.8$, $df= 28$, $p= 0.05$) and species richness (Anova d : $F=1.81$, $df= 28$, $p= <0.05$); however, species evenness showed no significant differences between sites (J : $F=4.47$, $df= 28$, $p= 0.05$). Comparison between eight different sites of Sindh using maturity index (MI) also resulted significant differences (MI: $F=8.09$, $df= 28$, $p= 0.05$) (Fig. 33).

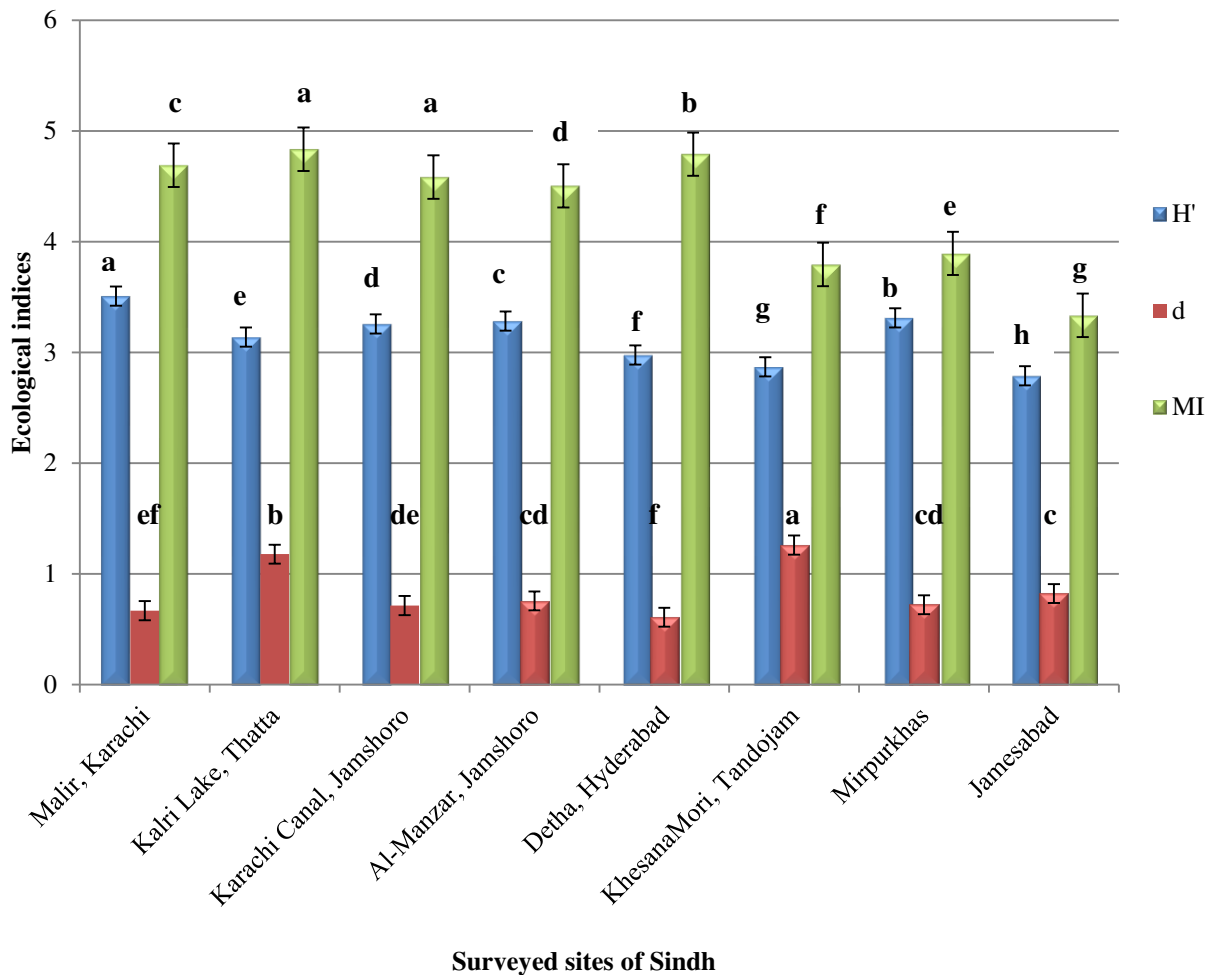


Fig. 33. Relationship of surveyed sites with ecological indices.

The present study revealed the diversity of freshwater nematodes at eight collecting sites of Sindh. A total of forty nematode species identified that include three new species viz., *Bathyeurystomina minima* Nasira, Shahina & Shamim, 2014, *Belbolla longispiculata* Nasira, Shahina & Shamim, 2014 and *Metoncholaimus siddiqii* Shahina, Nasira & Shamim, 2015 while six species were reported as new records of Pakistan from different water sources like rivers, streams, lakes, irrigation canals and reservoir of ponds and tanks. Results were emphasized on the knowledge obtained from the parameters of occurrence, prevalence, distribution and ecological indices of freshwater nematodes. The present study is a pioneer in the morphological and taxonomical variations obtained for the first time in freshwater from Sindh, Pakistan.

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