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Comparative Diversity, Abundance, and Community Pattern of Nematodes in Natural and Disturbed Habitats

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Abstract: This work evaluates the diversity, and abundance of nematodes and their use as indicators of soil health in an area strongly influenced by industrial wastes (food, metal and paper industries). The relationships between trophic groups, coloniser-persister scale and nematode community indices as well as nematode indicators of soil elements and the relationships of soil elements with different habitats were investigated. Nematodes were recovered from the soil samples of fifty sites from five different habitats. The trophic groups, colonizer-persister scale, and nematode community indices were analysed and compared. To test the significance of the dataset, bivariate linear regression; several samples repeated measure test of Analysis of Variance (ANOVA) have been performed. The Canonical Correspond Analysis (CCA); Principal Component Analysis (PCA), and clustering of habitats were performed to know the relationships between such variables among different habitats. Bacterial feeders with 15,582 individuals were found to be a highly diverse and most abundant group. The results indicated that the nematode diversity and abundance, trophic groups and coloniser-persister ratio were adversely affected by organically enriched habitats to food, metal and paper industries as compared to natural habitats. The habitats contaminated by industrial wastes were mainly dominated by bacterivores and fungivores of *c-p*2 class. Few colonizer genera were observed to be cosmopolitan and prevalent in all habitats. However, some genera showed specificity towards a particular set of conditions and were more or less endemic for specific habitats.

Keywords: Correlation, Diversity, Habitats, Nematode Community, Soil Elements

1. Introduction

Soil provides an environment to support all living organisms and maintains Earth's belowground biodiversity. Besides promoting the health and productivity of biota, the soil maintains environmental quality by recycling large quantities of piled up pollutants produced by industrial processes or other anthropogenic activities. Nematodes are the group of metazoans which show adverse effects on their community composition in disturbed or changing environments. The stress induced by contaminants/ pollutants usually decreases species diversity and increases the dominance of few species; eliminates K-strategists (persisters) and increases the short-lived, relatively small-sized, r-strategists (colonisers). These effects were reported by a number of workers [24, 40-42]. Due to their ubiquity, abundance and diversity, they serve as excellent bio-indicators to assess the quality of the environment [14, 38, 39, 45], conceive all the habitats on the earth where life exists. The nematodes are an important component of soil ecosystem that play a crucial role in the decomposition of organic matter, nutrients and minerals cycling, sequestration of carbon, and detoxification of pollutants [6, 16]. For instance, most of the free-living bacterial feeder nematode groups (*Oscheius, Metarhabditis, Pelodera, oryctonema, Eudronema Teratorhabditis, Xylorhabditis* and *Acrostichus, Allodiplogaster, Fictor, Mononchoides, Sudhausia, Teratodiplogaster* etc. belonging to infraorder Rhabditomorpha and Diplogastromorpha respectively) associated with insects in the dauer form for dissemination/ dispersal, however, they become active after natural death of insects, grow rapidly and help in the decomposition of carcasses along with bacterial communities [32-34]. They are highly diverse and directly or indirectly affect soil nutrient and mineral cycling through their participation in material decomposition with the help of symbiotically associated bacteria

and through predation on other organisms [11, 13, 17, 25, 46]. To understand the influence of soil properties and disturbances on the diversity and abundance of nematodes and the resulting impact on material decomposition and nutrient cycling, several studies [1, 20, 22, 23, 27, 28, 30, 35, 43, 44] were conducted on belowground diversity and ecology in different types of ecosystems.

The present work aims to evaluate the diversity, abundance of nematodes and their use as the indicators of soil health in an area strongly influenced by industrial wastes (food, metal and paper industries) and compares them to natural and organically enriched habitats. The present study also analyses the differences between the abundance of trophic groups and coloniser-persister classes as well as changes in the nematode community indices in five different habitats using Principal Component Analysis (PCA) and significantly correlated using Spearman's rank correlation method. The Canonical Correspond Analysis (CCA) has been done to investigate the nematode indicators of soil elements and the relationships of soil elements with different habitats, whereas clustering of habitats based on the abundance of the genera has been found to be a remarkable tool to study similarities among habitats.

2. Material and Methods

2.1. Study Area and Soil Sampling

Nematodes were recovered from the soil sample collected from fifty sites of five different habitats *viz.,* natural habitat, organically enriched habitat, and the soil contaminated with industrial (food industry, metal industry, and paper industry) wastes. Four districts *viz*., Aligarh, Ghaziabad, Matura, and Siddharthnagar were marked for the collection of samples based on the type of habitats *viz*., natural environment and industries. Ten composite samples (each formed of three replicate samples taken from 10-20 cm depth) of 300g soil were collected from each habitat. The selection of habitats was based on the substrate and vegetation type which led to differences in diversity, abundance, and food web complexity of nematodes in disturbed habitats in contrast to the natural environment. The soil samples were placed in polythene bags and brought to the laboratory and processed within 24 hours.

2.2. Extraction, Identification, and Counting of Nematode

The samples of 300g soil were processed using sieving and decantation method [10] and the nematodes were extracted using modified Baermann's funnel technique [5] after 24 hours. The nematode suspension was poured into a large-sized cavity block (55 X 55 mm) and the nematodes were identified up to generic level under compound microscope (Olympus CX 31) with the help of various literature [2-4, 18, 26]. The identified genera were assigned to different trophic groups [8, 13]. The identification of the genera was done by making a temporary mount in a drop of water or formalin. The trophic groups were assigned according to their feeding habits, feeding apparatus and pharynx morphology [47] into bacterivores (BF), fungivores (FF), plant parasites (PP), and omnivores-predators (OP).

For the counting of nematodes, water was added to nematode suspension to make its volume 100 ml. The suspension was stirred thoroughly, and then 5ml volume was sucked by a syringe to pour into a Syracuse dish. Counting was done thrice for each sample, and finally, the mean was calculated. The, representative nematodes from each genus were counted and later processed for dehydration in glycerol alcohol, placed in a desiccator containing calcium carbonate ($CaCO_3$) for 15 days and finally mounted in anhydrous glycerol. The formula used for calculating the final population of nematodes in each sample (250g) was: total volume of nematode suspension (100 ml) x means of counted nematode /quantity of suspension used for counting (5 ml).

2.3. Nematode Community Analyses

For calculation of nematode community indices, the abundance of nematodes (individual count) of the genera was estimated and entered on an excel spreadsheet. The abundance of nematodes and trophic groups was considered as a total number of individuals in 250 g of dry soil. Maturity indices were calculated based on a *c-p* scaling assigned to different genera of nematodes [7, 16] ranging from extremely r-strategist colonizers (*c-p*1) to extremely K-strategist persisters (*c-p*5). Maturity Index (MI), Maturity Index (MI2-5) with excluding nematodes of *c-p*1 value, Plant Parasite Index (PPI), Enrichment index (EI), Structure index (SI), Basal Index (BI), Channel Index (CI), and Nematode Channel Ratio (NCR) were calculated according [15]. For the calculation of Enrichment index (EI), Structure index (SI), and Basal Index (BI), the basal (b) ($s = \Sigma\ K_b n_b$), enrichment (e) ($e = \Sigma\ K_e n_e$), and structure (s) ($s = \Sigma\ K_s n_s$), components were calculated [15].

2.4. Soil Element Analyses

The 50g of soil from each 300g of soil sample out of fifty samples from different habitats of the districts Aligarh, Ghaziabad, Mathura, and Siddharthnagar was separated to analyzed the available soil elements using Energy Dispersal X-ray (EDX)/ Energy Dispersal Spectroscopy (EDS). The soil was mixed properly to make a homogenous mixture and dried in an oven at 110–120 ºC. The dried soil was crushed with the help of a pestle and mortar to make it fine powder and sieved it using a 0.5 mm sieve followed by a <0.5 mm. A small amount of powder was placed over double adhesive tape mounted on a stub. To separate the particles adhering to the adhesive tape on the stub, the surface of the tape was pressed firmly onto the bottom surface of a 0.5 mm sieve and gently rotated on the tape to separate the soil particles. The stubs were observed under Scanning Electron Microscope (model JEOL XL30 FEG), equipped with Energy Dispersal X-Ray (EDX) spectrometer to determine the elemental compositions.

2.5. Data Analysis

All the analyses were performed in PAST version 4.03. A matrix of trophic groups, abundance of colonizer-persister groups, nematode community indices, soil elements, and abundance of genera (counts) as per sites of habitats was compiled. Various testing methods including Bivariate linear

regression, Analysis of Variance (ANOVA), Spearman's rank correlation coefficient, One-way Analysis of Similarity (ANOSIM), Permutation Multivariate Analysis of Variance (PERMANOVA) were used to test the significance of the dataset. For the Canonical Correspondence Analysis (CCA), Principal Component Analysis (PCA), and clustering of habitats, data were transformed as square roots to normalize the influence of the higher values of the data. The variables including total nematode abundance, abundance of trophic groups and colonizer-persister groups among habitats were compared using Bivariate linear relationships at the significant difference (P= < 0.01). The repeated measure test of Analysis of Variance (ANOVA) was carried out between nematode community indices among different habitats with significant differences (P= 0.0001). The values of Spearman's rank correlation coefficient between various nematode trophic groups, colonizer-persister groups, and nematode community indices were calculated. To study the relationships between trophic groups, colonizer-persister groups, and nematode community indices, the Principal Component Analysis (PCA) was performed while Permutation Multivariate Analysis of Variance (PERMANOVA) was done to investigate the correlation of variables at significance level P= 0.0001. However, Canonical Correspondence Analysis (CCA) was performed to investigate the responses of nematodes to soil nutrients and their habitat preferences. One-way Analysis of Similarity (ANOSIM) was performed using the Bray-Curtis similarity index to test the similarity between variables among different habitats at level of significance, P= 0.0001. The clustering of habitats was done based on the abundance of the genera to investigate similarities in habitats. The tree was inferred with 10000 bootstrap pseudo-replicates using paired group (UPGMA) algorithm under the correlation similarity index.

3. Results

3.1. Diversity, Abundance, Prevalence, and Specificity of Nematodes

Fifty sites of five different habitats yielded a total of 24,796 nematodes belonging to 54 genera and 30 families. The nematodes were categorized into five trophic groups based on their feeding habits (bacterial feeders, fungal feeders, omnivores, plant-parasitic, and predators). However, bacterial feeders with 15,582 individuals were found to be a highly diverse and most abundant group, followed by plant-parasitic with 2,954 individuals, omnivores with 2,345 individuals, and fungal feeders with 2,282 individuals, and predators were found to be the least abundant group with 1,633 individuals. Four genera (*Mesorhabditis, Panagrolaimus, Cephalobus,* and *Aphelenchoides*) were commonly found in all habitats (figures 1, 2; table 1) and were recovered from 38-46 sites, however, *Trischistoma, Achromadora, Chrysonema,* and *Xiphinema* were the least abundant and least diverse taxa (table 1) which occurred only in few sites (figures 1, 2). Few genera showed specificity towards a particular habitat (figure 3) such as *Oscheius* (0.09 ± 0.02), *Dorylaimus* (0.37 ± 0.07), *Criconemoides* (0.23 ± 0.05), *Hemicriconemoides* (0.12 ± 0.03), *Aporcelaimus* (0.39 ± 0.08), *Ironus* (0.10 ± 0.09) and *Mylonchulus* (0.16 ± 0.17) belonging to bacterivore, omnivore, plant-parasitic, and predator's trophic groups respectively were frequent in natural habitats. However, *Aspidonema* (0.03 ± 0.01), *Distolabrellus* (0.04 ± 0.01), *Metarhabditis* (0.06 ± 0.02), *Pterygorhabditis* (0.04 ± 0.01), *Teratorhabditis* (0.11 ± 0.02), *Halicaphalobus* (0.12 ± 0.02), *Acrostichus* (0.03 ± 0.01), *Fictor* (0.03 ± 0.01), *Mononchoides* (0.03 ± 0.01), and *Paroigolaimella* (0.02 ± 0.00) were most prevalent in organically enriched habitats. Few genera, *Pelodera* (0.15 ± 0.01) and *Prismatolaimus* (0.01 ± 0.00) were found in samples from food industry, *Pseudoacrobeles* (0.16 ± 0.03) *Acrobeloides* (0.19 ± 0.03), *Cervidellus* (0.09 ± 0.02), *Dorylaimellus* (0.12 ± 0.04), *Rotylenchus* (0.33 ± 0.13) and *Achromadora* (0.06 ± 0.05) were the most common groups occurring in the vicinity of the metal industries, while *Poikilolaimus* (0.24 ± 0.04), *Eucephalobus* (0.29 ± 0.03), *Ditylenchus* (0.11 ± 0.04), and *Tylenchorhynchus* (0.24 ± 0.07) were predominantly found in the substrate of the paper industries (figure 3; table 1).

Table 1. *Relative abundance (proportion) of nematode genera (mean ± standard error) in different habitats.*

Sr./ No.	Family	Genus	Generic code	Trophic group	cp Scale	Natural Hab.	Enriched Hab.	Food Ind.	Metal Ind.	Paper Ind.
1	Bunonematidae	*Aspidonema*	*Aspido*	Bacterial feeder	Ba1	–	0.03 ± 0.01	–	–	–
2	Cephalobidae	*Acrobeles*	*Acrob*	Bacterial feeder	Ba2	0.14 ± 0.03	0.08 ± 0.01	0.17 ± 0.01	–	–
3	Cephalobidae	*Acrobeloides*	*Acrobel*	Bacterial feeder	Ba2	–	–	–	0.19 ± 0.03	–
4	Diplogastridae	*Acrosticus*	*Acrost*	Bacterial feeder	Ba1	–	0.03 ± 0.01	–	–	–
5	Cephalobidae	*Cephalobus*	*Cephal*	Bacterial feeder	Ba2	0.11 ± 0.03	0.06 ± 0.01	0.07 ± 0.02	0.08 ± 0.02	0.31 ± 0.02
6	Plectodae	*Ceratoplectus*	*Ceratop*	Bacterial feeder	Ba2	0.04 ± 0.03	–	–	–	–
7	Cephalobidae	*Cervidellus*	*Cervid*	Bacterial feeder	Ba2	–	–	–	0.09 ± 0.02	–
8	Rhabditidae	*Crustorhabditis*	*Crustor*	Bacterial feeder	Ba1	–	–	0.02 ± 0.01	0.03 ± 0.01	–
9	Cephalobidae	*Chiloplacus*	*Chilopl*	Bacterial feeder	Ba2	0.16 ± 0.04	0.03 ± 0.01	0.06 ± 0.01	0.21 ± 0.03	
10	Rhabditidae	*Diploscapter*	*Diplosc*	Bacterial feeder	Ba1	–	–	0.19 ± 0.02	0.06 ± 0.01	–
11	Rhabditidae	*Distolabrellus*	*Distolabr*	Bacterial feeder	Ba1	–	0.04 ± 0.01	–	–	–
12	Cephalobidae	*Eucephalobus*	*Euceph*	Bacterial feeder	Ba2	–	–	–	–	0.29 ± 0.03
13	Neodiplogastridae	*Fictor*	*Fic*	Bacterial feeder	Ba1	–	0.03 ± 0.01	–	–	–
14	Monhysteridae	*Geomonhystera*	*Geomon*	Bacterial feeder	Ba2	–	–	0.19 ± 0.03	0.03 ± 0.01	–
15	Panagrolaimidae	*Halicephalobus*	*Hali*	Bacterial feeder	Ba1	–	0.12 ± 0.02	–	–	–
16	Rhabditidae	*Mesorhabditis*	*Mesor*	Bacterial feeder	Ba1	0.14 ± 0.03	0.20 ± 0.02	0.01 ± 0.00	0.08 ± 0.02	0.11 ± 0.03

Sr./ No.	Family	Genus	Generic code	Trophic group	cp Scale	Natural Hab.	Enriched Hab.	Food Ind.	Metal Ind.	Paper Ind.
17	Rhabditidae	*Metarhabditis*	*Metar*	Bacterial feeder	Ba1	–	0.06 ± 0.02	–	–	–
18	Monhysteridae	*Monhystera*	*Monh*	Bacterial feeder	Ba2	0.14 ± 0.03	–	0.07 ± 0.03	–	–
19	Neodiplogastridae	*Mononchoides*	*Mononch*	Bacterial feeder	Ba1		0.03 ± 0.01	–	–	–
20	Rhabditidae	*Oscheius*	*Osch*	Bacterial feeder	Ba1	0.09 ± 0.02	–	–	–	–
21	Panagrolaimidae	*Panagrolaimus*	*Panagrol*	Bacterial feeder	Ba1	0.12 ± 0.03	0.19 ± 0.02	0.15 ± 0.01	0.08 ± 0.02	0.04 ± 0.01
22	Diplogastridae	*Paroigolaimella*	*Paroigo*	Bacterial feeder	Ba1	–	0.02 ± 0.00	–	–	–
23	Rhabditidae	*Pelodera*	*Pelod*	Bacterial feeder	Ba1	–	–	0.15 ± 0.01	–	–
24	Plectodae	*Plectus*	*Plect*	Bacterial feeder	Ba2	0.05 ± 0.01	–	–	–	–
25	Rhabditidae	*Poikilolaimus*	*Poikilo*	Bacterial feeder	Ba1	–	–	–	–	0.24 ± 0.04
26	Prismatolaimidae	*Prismatolaimus*	*Prismato*	Bacterial feeder	Ba3	–	–	0.01 ± 0.00	–	–
27	Cephalobidae	*Pseudoacrobeles*	*Pseudoacro*	Bacterial feeder	Ba2	–	–	–	0.16 ± 0.03	–
28	Pterygorhabditidae	*Pterygorhabditis*	*Pterigor*	Bacterial feeder	Ba1	–	0.04 ± 0.01	–	–	–
29	Rhabditidae	*Teratorhabditis*	*Terator*	Bacterial feeder	Ba1	–	0.11 ± 0.02	–	–	–
30	Aphelenchidae	*Aphelenchus*	*Aphel*	Fungal feeder	Fu2		0.46 ± 0.20	0.41 ± 0.11	0.37 ± 0.06	0.22 ± 0.04
31	Aphelenchoididae	*Aphelenchoides*	*Aphelench*	Fungal feeder	Fu2	0.28 ± 0.09	0.01 ± 0.00	0.36 ± 0.11	0.33 ± 0.05	0.44 ± 0.05
32	Tylenchidae	*Ditylenchus*	*Dityl*	Fungal feeder	Fu2	–	–	–	–	0.11 ± 0.04
33	Dorylaimellidae	*Dorylaimellus*	*Doryla*	Fungal feeder	Fu2	–	–	–	0.12 ± 0.04	–
34	Tylenchidae	*Filenchus*	*Filen*	Fungal feeder	Fu2	0.29 ± 0.10	–	0.23 ± 0.06	–	0.23 ± 0.04
35	Leptonchidae	*Leptonchus*	*Lept*	Fungal feeder	Fu4	0.17 ± 0.07	–	–	–	–
36	Tylencholaimidae	*Tylencholaimus*	*Tylenchol*	Fungal feeder	Fu4	0.05 ± 0.05	–	–	0.18 ± 0.03	–
37	Chrysonematidae	*Chrysonema*	*Chryso*	Omnivores	Om4	0.17 ± 0.05	–	–	–	–
38	Dorylaimidae	*Dorylaimus*	*Doryl*	Omnivores	Om3	0.37 ± 0.07		0.02 ± 0.01	0.45 ± 0.13	0.80 ± 0.13
39	Dorylaimidae	*Mesodorylaimus*	*Mesodoryl*	Omnivores	Om4	0.36 ± 0.08	0.04 ± 0.01	–	0.55 ± 0.13	–
40	Criconematidae	*Criconemoides*	*Criconem*	Plant-parasitic	PP3	0.23 ± 0.05	–	–	–	–
41	Hoplolaimidae	*Helicotylenchus*	*Helicoty*	Plant-parasitic	PP3	0.23 ± 0.05	–	0.02 ± 0.01	–	–
42	Criconematidae	*Hemicriconemoides*	*Hemicrico*	Plant-parasitic	PP3	0.12 ± 0.03	–	–	–	–
43	Hoplolaimidae	*Hoplolaimus*	*Hoplol*	Plant-parasitic	PP3	0.21 ± 0.05	–	0.02 ± 0.00	0.30 ± 0.09	
44	Hoplolaimidae	*Rotylenchus*	*Rotyl*	Plant-parasitic	PP3	–	–	–	0.33 ± 0.13	–
45	Tylenchidae	*Tylenchus*	*Tyl*	Plant parasitic	PP3	0.12 ± 0.03	0.02 ± 0.01	–	0.21 ± 0.07	0.76 ± 0.07
46	Telotylenchidae	*Tylenchorynchus*	*Tylencho*	Plant-parasitic	PP3	–	–	–	–	0.24 ± 0.07
47	Longidoridae	*Xiphinema*	*Xiphi*	Plant-parasitic	PP5	–	–	0.09 ± 0.05	0.05 ± 0.04	–
48	Achromadoridae	*Achromadora*	Achrom	Predators	Pr3	–	–	–	0.06 ± 0.05	–
49	Aporcelaimidae	*Aporcelaimus*	Aporcel	Predators	Pr5	0.39 ± 0.08	0.02 ± 0.01	–	0.25 ± 0.13	–
50	Qudsianematidae	*Discolaimus*	Discol	Predators	Pr5	0.30 ± 0.07	–	–	0.48 ± 0.15	
51	Iotonchidae	*Iotonchus*	Ioton	Predators	Pr4	0.05 ± 0.06	–	–	–	–
52	Ironidae	*Ironus*	Iron	Predators	Pr4	0.10 ± 0.09	–	–	–	–
53	Mylonchulidae	*Mylonchulus*	Mylon	Predators	Pr4	0.16 ± 0.17	–	–	–	–
54	Tripylidae	*Trischistoma*	Trischi	Predators	Pr3	–	–	0.20 ± 0.13	–	0.20 ± 0.13

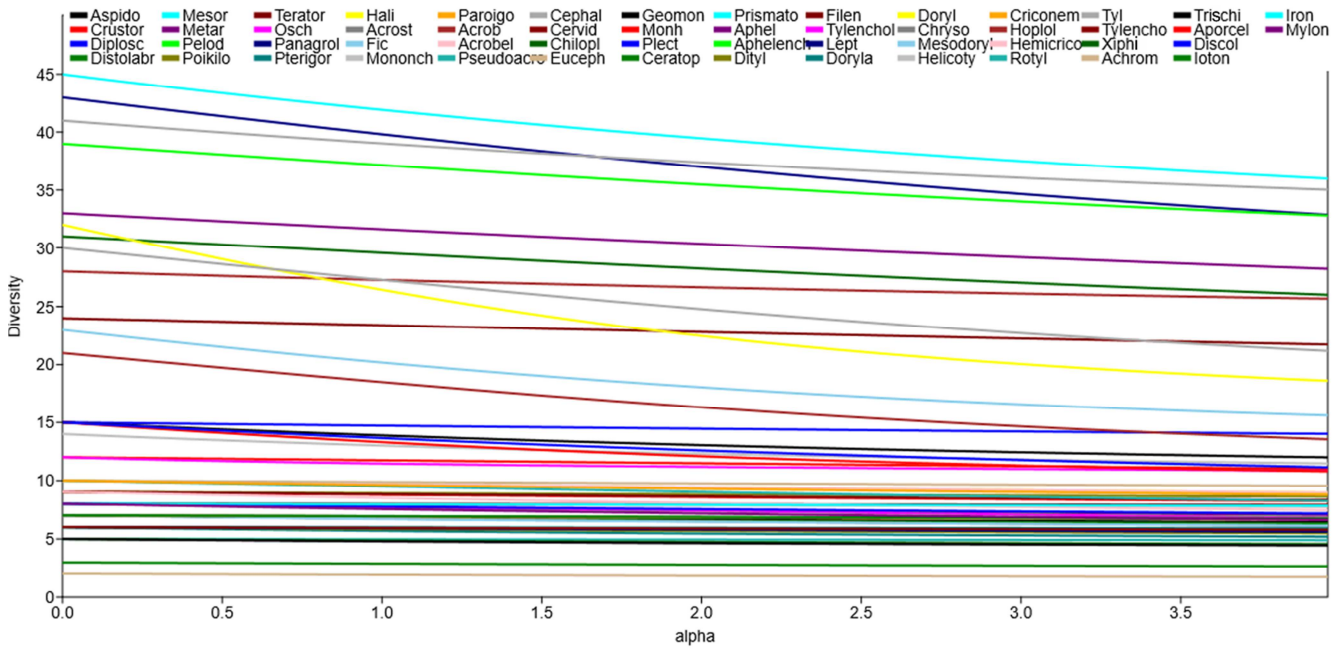

Figure 1. *A diversity profile of the nematode genera recovered from different habitats (natural habitat, enriched habitat, food industry, metal industry, and paper industry). The X-axis indicates the mean diversity of a species/ genus at different sites of habitats and the Y-axis shows the number of sites. The colored lines denote the diversity of a species/ genus.*

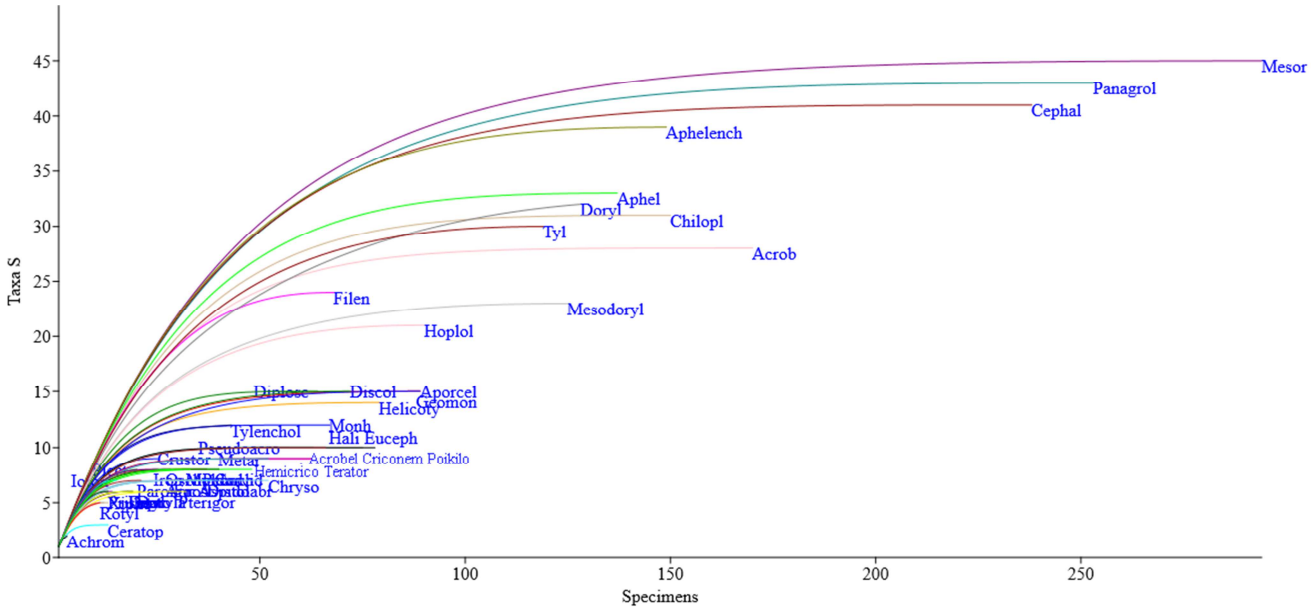

Figure 2. *Abundance and species/ generic richness among different habitats (natural habitat, enriched habitat, food industry, metal industry, and paper industry). The X-axis indicates the diversity of a species/ genus and the Y-axis reflects their abundance. The colored lines denote the diversity and abundance of a species/ genus.*

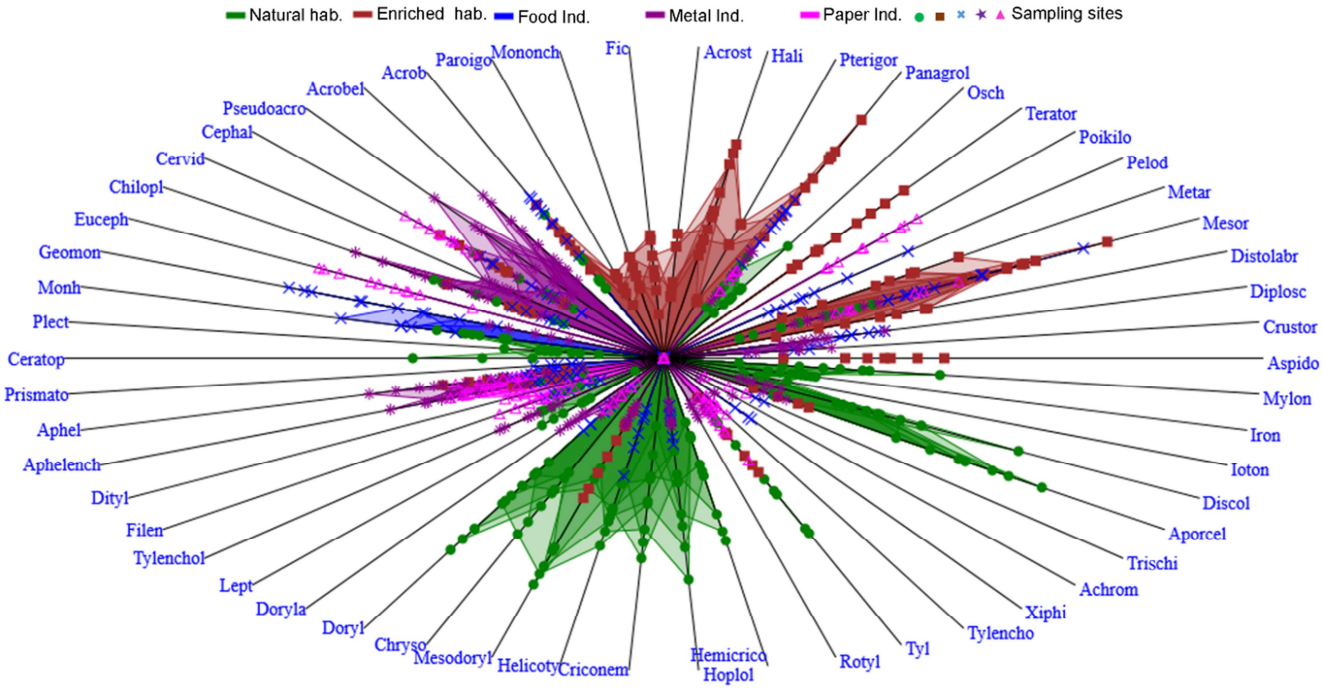

Figure 3. *The radar plot showed the prevalence and specificity of nematode with different habitats (natural habitat, enriched habitat, food industry, metal industry, and paper industry). The colored webs indicate different habitats and the points at the lines represent the sampling sites.*

3.2. Relative Abundance of Nematodes, Trophic Groups, and Coloniser-Persister Classes in Selected Habitats

A high nematode abundance was observed in natural and organically enriched habitats, most of the samples having about 500-950 individuals/ 250g of dry soil. The abundance was significantly decreased in the habitats like food, metal and paper industries respectively ranging from less than 500 individuals/ 250g of dry soil (*Slope*= 10.001, *Intercept*= 750.96, *r*= -0.83611, *r2*= 0.699, *t*= -10.56, *P*= < 0.01) (figure 4 A). Although, the proportion of bacterial feeders (*Slope*= -0.0007, *Intercept*= 1.0459, *r*= -0.638, *r2*= 0.407, *t*= -5.7406, *P*= < 0.01) and fungal feeders (*Slope*= -0.0003, *Intercept*= 0.2868, *r*= -0.83611, *r2*= 0.699, *t*= -10.56, *P*= < 0.01) was significantly higher in organically enriched habitats *viz*., paper, metal and food industries as compared to natural habitats. Abundant bacterial feeders were observed in organically-enriched habitats and food industry samples while fungal feeders could be abundantly found in metal

industry samples (figure 4 B, C). Furthermore, the proportion of omnivores (*Slope*= -0.0003, *Intercept*= -0.1153, *r*= 0.6552, *r2*= 0.4294, *t*= 6.0101, *P*= < 0.01), plant parasitic (*Slope*= 0.0004, *Intercept*= -0.1198, *r*= 0.6436, *r2*= 0.4142, *t*= 5.826, *P*= < 0.01), and predators (*Slope*= 0.0002, *Intercept*= -0.0982, *r*= 0.6337, *r2*= 0.4016, *t*= 5.6759, *P*= < 0.01) showed positive correlation, and were significantly higher in natural habitat and were lower in proportion in food, metal and paper industries (figure 4 D-F).

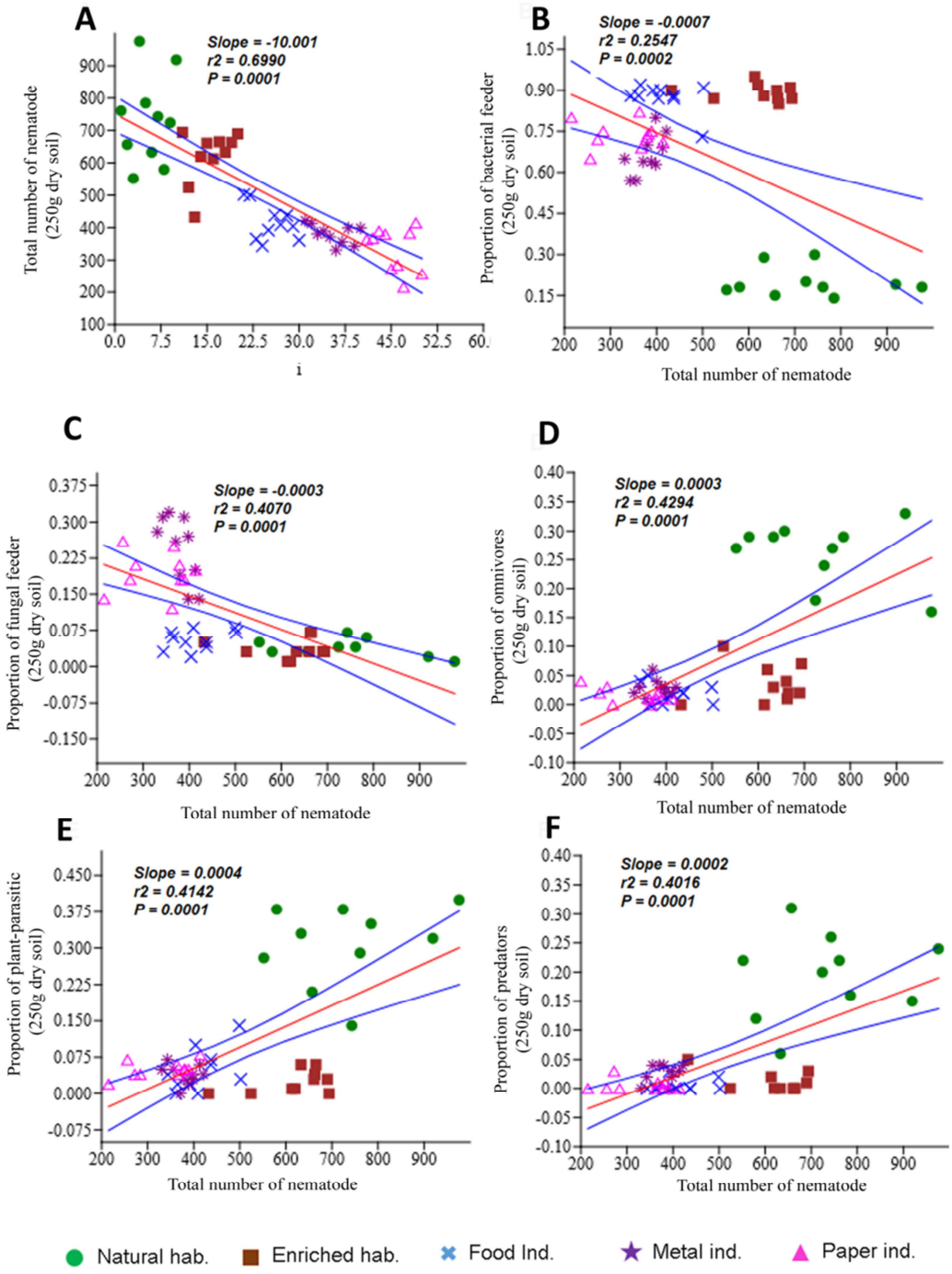

Figure 4. *Bivariate linear relationships showing the effect of habitats on (A) total number of nematodes, (B) bacterial feeders, (C) fungal feeders, (D) omnivores, (E) plant parasites, (F) predators. The x-axis represents the total number of nematodes and the y-axis represents the proportion of trophic groups. The sample points of different shapes belonging to five different habitats are shown. The red lines denote the predictions of the linear models and the blue lines represent 95% confidence intervals. r2= coefficient of determination, slope= regression; P= significance of the regression slope.*

In contrast to the abundance of coloniser-persister groups, most of the enrichment indicators (*c-p*1) were observed in the organically enriched habitats, however, r-strategists (*c-p*1) showed negative correlation with metal and natural industries (*Slope*= 0.0003, *Intercept*= 0.3179, *r*= 0.2487, *r2*= 0.0618, *t*= 1.7793, *P*= 0.08) (figure 5 A). Although, the basal

indicators (*c-p*2) were significantly higher in food, metal and paper industries, they indicated a negative correlation with natural and enriched habitats (*Slope*= -0.0011, *Intercept*= 2.0349, *r*= -0.346, *r2*= 0.1197, *t*= -2.5554, *P*= 0.01) (figure 5 B). K-strategists (*c-p*3-5), indicators of a stable environment, were significantly higher in natural habitats but negatively correlated with rest of the habitats (*Slope*= 0.0014, *Intercept*= 1.5371, *r*= 0.3532, *r2*= 0.1247, *t*= 2.6158, *P*= 0.01) (figure 5 C).

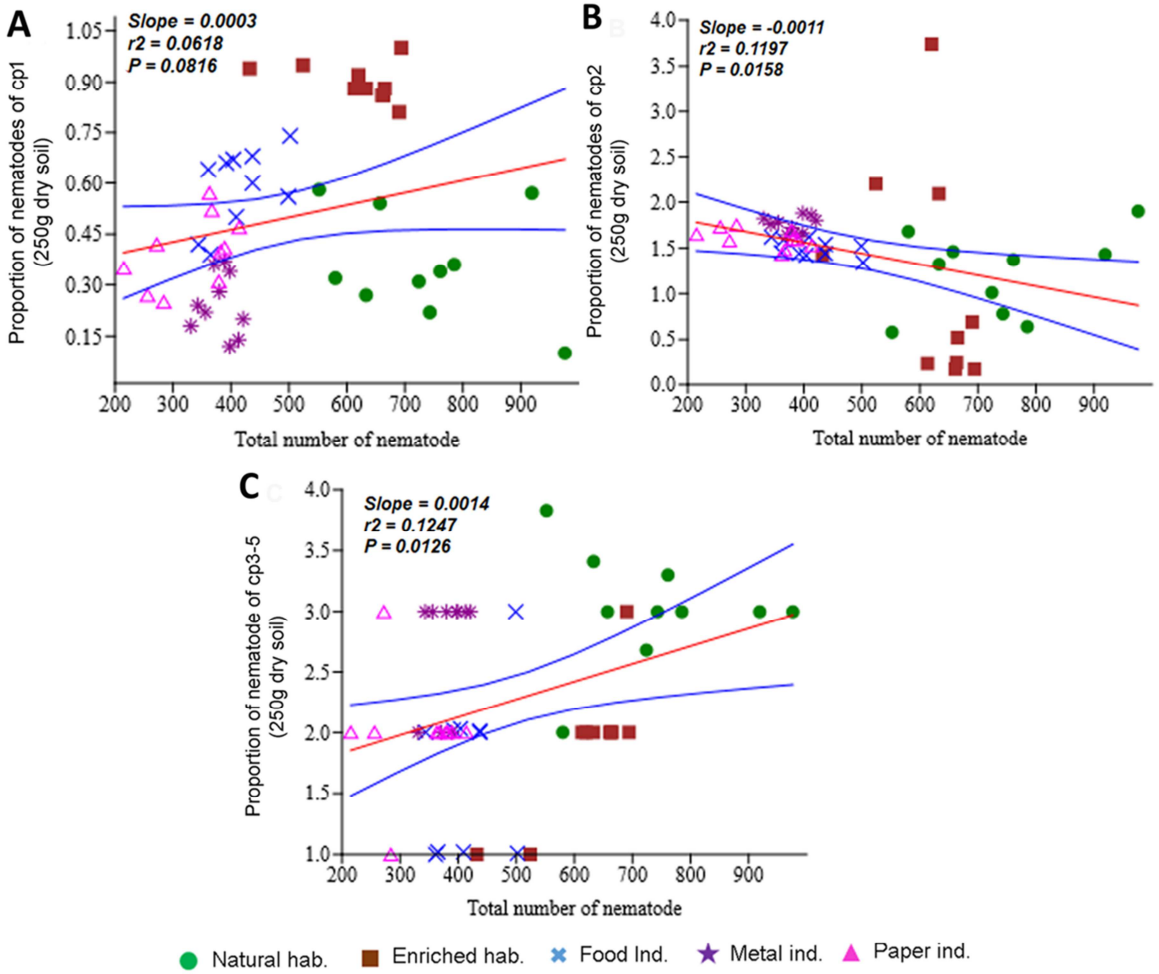

Figure 5. *Bivariate linear relationships showing the effect of habitats on (A) coloniser-persister (c-p1), (B) coloniser-persister (c-p2), (C) coloniser-persister (c-p3-5) classes. The x-axis represents the total number of nematodes and the y-axis represents the proportion of coloniser-persister classes. The sample points of different shapes belonging to five different habitats are shown. The red lines represent the predictions of the linear models and the blue lines represent 95% confidence intervals. r2= coefficient of determination, slope= regression; P= significance of the regression slope.*

3.3. Effect of Habitats on Nematode Community Structure

The nematode community structure was markedly affected in different habitats from natural to those contaminated by industrial wastes. The present data showed high variability and asymmetric distribution. Maturity index (MI, MI2-5) and trophic diversity indices (TDI) showed positive correlation and were significantly higher in natural habitats with all the points distributed at the maximum limit of whisker (figure 6A); however, these indices showed negative correlation with organically enriched habitats and all the points were dispersed at the lower limit of whisker while rest of the habitats showed points dispersed in quartile region. Although Nematode Channel Ratio (NCR) was relatively higher in habitats representing metal and paper industries with all the points dispersed at the quartile (Q1) and lower limit of whisker while negatively correlation was observed with TDI, MI2-5 and MI. The plant-parasitic index (PPI) did not show any significant pattern in the selected habitats (figure 6A).

Likewise, EI, SI, BI, and CI showed an asymmetric pattern of distribution. The enrichment index was high in organically-enriched habitats and lower in the metal industries as the sample points are shown at both extremities (minimum and maximum) and indicated a negative correlation with structure index (SI) while a positive correlation with basal index (BI). Structure index (SI) was exceptionally low in enriched or contaminated habitats with few samples showing greater deviation from mean value; on the other hand, SI was relatively higher in natural habitats. The basal index (BI) showed positive distribution with relatively higher values in paper and food industries and lower in enriched and natural habitats. However, the channel

index did not show any significant correlation (figure 6B).

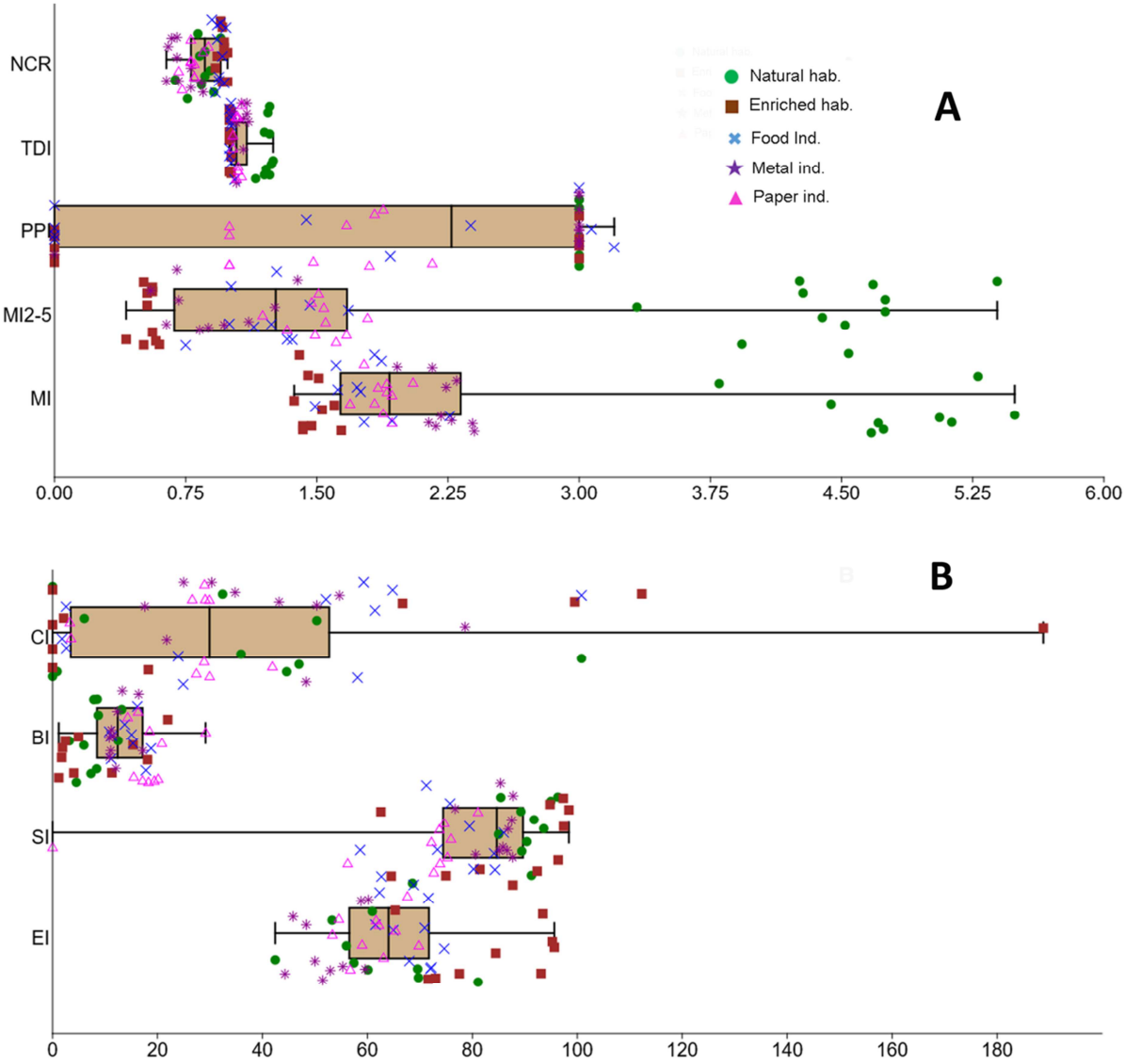

Figure 6. *Variations in nematode community indices in different habitats. (A) variation and distribution pattern between maturity indices (MI, MI2-5), plant-parasitic index (PPI), the trophic diversity index (TDI) and nematode channel ration (NCR); (B) variation and distribution pattern between enrichment index (EI), structure index (SI), basal index (BI), and channel index (CI). The sample points are marked with different shapes and colors for a particular habitat.*

3.4. Relationships Between Trophic Groups, Colonizers-Persisters, and Community Indices in Selected Habitats

In order to know the relationships of variables in different habitats, Principal Component Analysis (PCA) was performed with the fraction of variance of the first two components being 42.83% (PC1) and 23.65% (PC2). The results reflected variables responsible for the stability of an environment, food web complexity and high connectance *viz.,* SI, MI, MI2-5, PPI, TDI OM, PP, *c-p*3-5 lying on PC1 axis whereas the PC2 axis showed the variables (EI, BI, CI, NCR, BF, FF *c-p*1, and *c-p*2) that were responsible for indicators of enrichment and basal conditions. The variables of PC1 (SI, MI, MI2-5, PPI, TDI, OM, PP, *c-p*3-5) indicated a close relationship with the natural habitat, whereas, closely related EI, NCR, *c-p*1, indicated close relationships with the enriched habitats and a strong correlation was found between bacterial feeders (BF) and organically enriched habitats and food industries. On the other hand, indices including basal indicators (BI, CI, FF and *c-p*2) showed close relationships with the metal and paper industries (figure 7 A).

The significance of these correlations was assessed by Spearman's rank correlation coefficient (figure 7 B). A significantly positive correlation was observed between trophic groups (OM, PP and PRED); persisters (*c-p*3-5); and nematode community indices (MI, MI2-5, SI, TDI) whereas these variables were negatively correlated with nematode community indices (BI and EI), trophic group (BF) and colonisers (*c-p*1), the enrichment indicators. Interestingly, nematode community indices (BI and CI) and basal indicators of *c-p*2 values showed a positive correlation

together, whereas the trophic group (FF) indicated a positive correlation only with BI and *c-p*2 while no significant correlation was observed with CI.

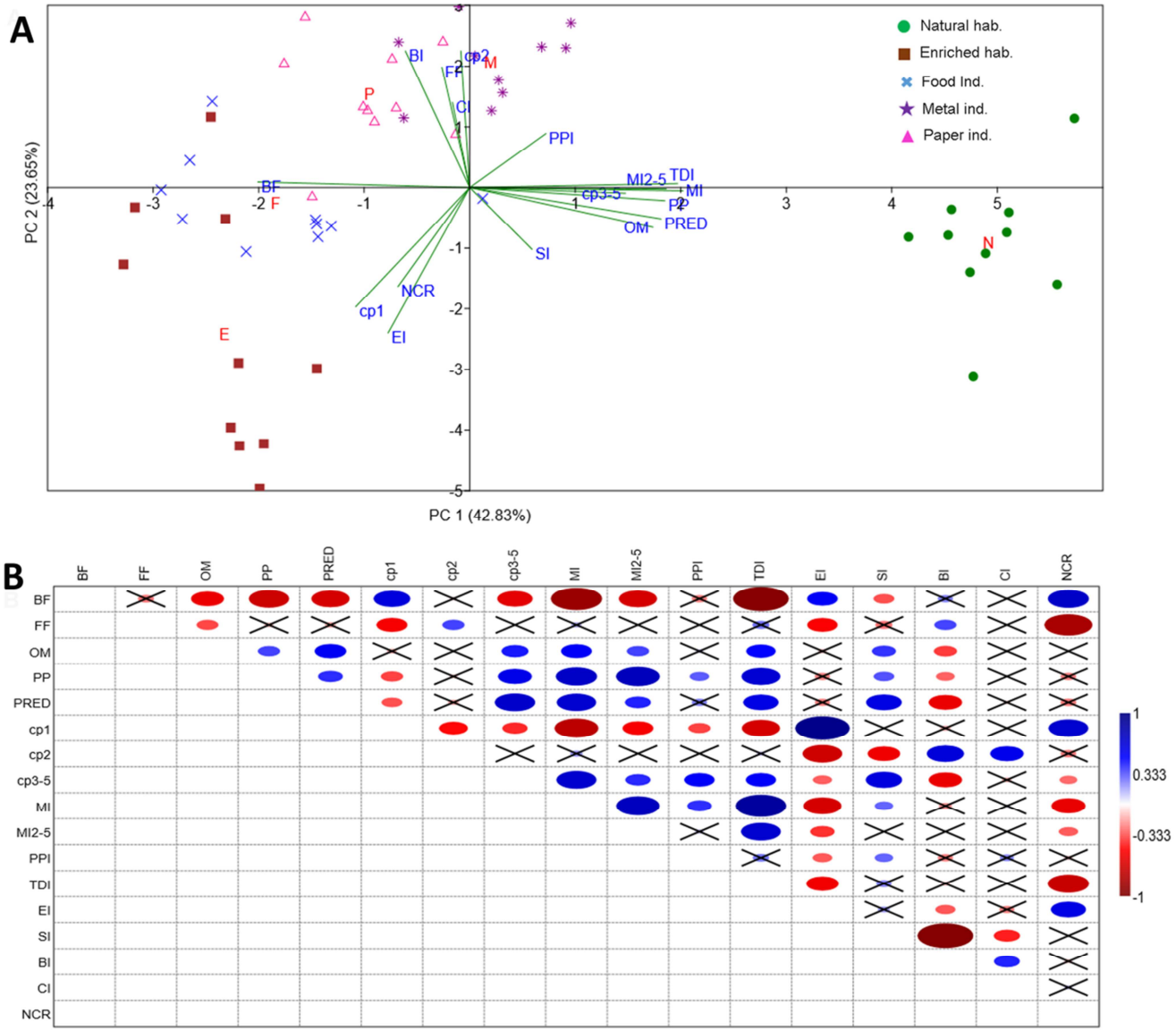

Figure 7. *Relationships between trophic groups, colonisers-persisters and nematode community indices in different habitats. (A) Principal Component Analysis (PCA) with a total variance of principal component (PC) 66.5%. The sample points of different shapes belonging to five different habitats are shown and the length of the green lines indicates the magnitude of the variables. (B) Spearman's rank correlation plot. The blue color indicates a significant positive and the red color negative correlation among variables, whereas, the significance values more than P= > 0.05 are marked with cross.*

3.5. Relationships Between Soil Elements and the Indicators of Different Habitats

The potential of nematodes as bio-indicators for effective soil health in different habitats was assessed using Canonical Corresponding Analysis (CCA). This analysis highlighted the relationship between nematode taxa and available soil elements in each habitat. The analysis was inferred based on the abundance of genera and % weight values of soil elements in the selected habitats such as natural, organically enriched habitats as well as habitats of food, metal and paper industries with Axis 1= 30.01% and Axis 2= 26.10%. The results indicated that among five habitats, negative relationship was observed between organically-enriched and natural habitats separated well, however, food, metal and paper industries clustered together showing closer relationship. The habitats were studied on the basis of available soil elements (explanatory environmental variable). The organically-enriched habitats corresponded to greater amount of Calcium (Ca) and Carbon (C), and the presence of Chlorine (Cl), Phosphorus (P), Sulphur (S), whereas as these elements were present in a very low amount or absent in other habitats. Although, the natural habitats mainly corresponded with presence of Sodium (Na) and Potassium (K) with the latter relatively higher in proportion. The other habitats (food, metal, and paper industries) were clustered together because of sharing common soil elements *viz.,* Iron

(Fe), Magnesium (Mg), Silicon (Si) and Aluminium (Al) in a more or less balanced amounts although these elements were relatively higher in few samples of natural habitats (table 2). The nematode communities' composition and their functional status indicated soil health condition. The ordination resembles that enrichment indicators/ r- strategists/ nematodes belonging to *c-p*1 class (*Aspidonema, Distolabrellus, Pterygorhabditis, Teratorhabditis, Acrostichus, Fictor, Mononchoides* and *Paroigolaimella*) grouped with organically-enriched habitats with high nutrient content and bacterial-dominated decomposition pathway suggesting disturbed food web condition. However, the indicators of stable environment/ k- strategists/ nematodes belonging to *c-p*3-5 scale (*Mylonchulus, Iotonchus, Criconemoides, Hemicriconemoides, Chrysonema, Helicotylenchus, Hoplolaimus, Discolaimus, Dorylaimus* and *Monhystera*) grouped together. Presence of some bacterial feeders of (*c-p*1 and *c-p*2) *viz., Plectus, Ceratoplectus,* and *Oscheius* in natural habitat indicated moderately disturbed to undisturbed, with C: N ratio low to moderate suggesting maturing to structured food web condition. Most of the basal indicator/ taxa representing *c-p*2 scale (*Aphelenchus, Aphelenchoides, Prismatolaimus, Geomonhystera, Tylencholaimus, Pelodera, Cephalobus* and *Chiloplacus*) grouped together with paper, metal and food industries indicating stressed condition, nutrient depletion and fungal-dominated decomposition pathway, high C: N ratio suggesting degraded or recovering food web conditions (figure 8).

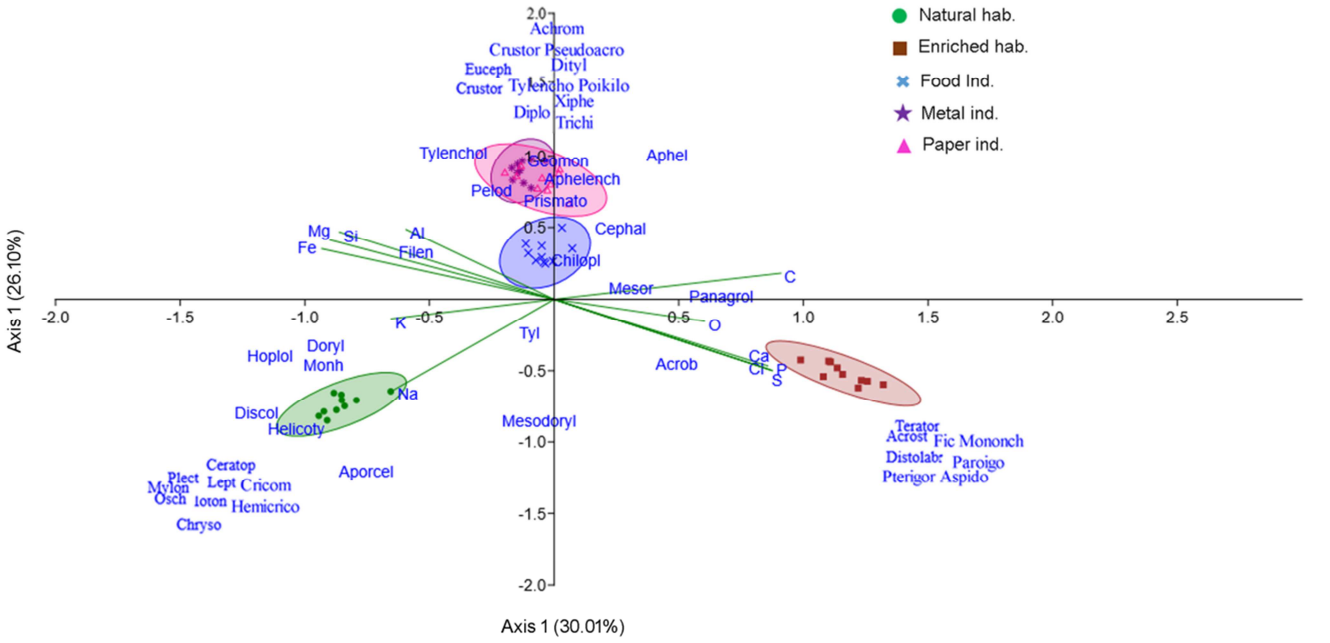

Figure 8. *Relationships between nematode communities and soil elements among different habitats. Canonical Corresponding Analysis (CCA) with a total variance of Axis (1 and 2) 56.11%. The sample points of different shapes are covered with ellipses belonging to five different habitats and the length of the green lines indicates explanatory environmental variables.*

Table 2. *Relative abundance (proportion) of trophic groups, nematode community indices and weight % of soil elements (mean ± standard error) of different habitats.*

Variables	Natural Hab.	Enriched Hab.	Food Ind.	Metal Ind.	Paper Ind.
Total nem.	733.00 ± 43.37	619.45 ± 25.96	415.00 ± 17.24	380.00 ± 9.38	332.10 ± 21.66
Trophic group					
BF	0.20 ± 0.02	0.89 ± 0.01	0.87 ± 0.02	0.66 ± 0.02	0.74 ± 0.02
FF	0.04 ± 0.01	0.04 ± 0.01	0.05 ± 0.01	0.24 ± 0.02	0.19 ± 0.01
OM	0.26 ± 0.02	0.04 ± 0.01	0.02 ± 0.01	0.03 ± 0.00	0.01 ± 0.00
PP	0.31 ± 0.03	0.02 ± 0.01	0.05 ± 0.01	0.04 ± 0.01	0.05 ± 0.00
Pred	0.19 ± 0.02	0.01 ± 0.01	0.00 ± 0.00	0.02 ± 0.00	0.00 ± 0.00
Nematode community index					
MI	4.79 ± 0.15	1.48 ± 0.03	1.79 ± 0.07	2.23 ± 0.04	1.87 ± 0.03
MI2-5	4.43 ± 0.17	0.53 ± 0.02	1.22 ± 0.08	0.90 ± 0.09	1.51 ± 0.05
PPI	2.40 ± 0.40	1.20 ± 0.49	1.50 ± 0.44	2.70 ± 0.30	1.48 ± 0.14
TDI	1.22 ± 0.01	1.01 ± 0.00	1.01 ± 0.00	1.07 ± 0.01	1.04 ± 0.01
NCR	0.84 ± 0.02	0.96 ± 0.01	0.94 ± 0.01	0.73 ± 0.02	0.79 ± 0.02
EI	61.91 ± 3.41	83.70 ± 3.52	68.70 ± 1.41	52.67 ± 1.80	52.67 ± 1.80
SI	90.73 ± 1.17	86.02 ± 4.46	75.57 ± 2.93	85.57 ± 1.14	61.34 ± 1.72
BI	8.01 ± 0.99	8.30 ± 2.45	15.50 ± 1.36	12.69 ± 0.73	18.95 ± 1.31
CI	31.79 ± 10.09	48.77 ± 20.86	42.75 ± 10.57	40.46 ± 5.84	24.95 ± 3.83

Variables	Natural Hab.	Enriched Hab.	Food Ind.	Metal Ind.	Paper Ind.
Soil element (Weight %)					
Al (%)	6.09 ± 0.24	3.71 ± 0.10	4.45 ± 0.08	7.76 ± 0.38	6.07 ± 0.19
Ca (%)	2.19 ± 0.10	14.18 ± 0.43	3.61 ± 0.12	3.14 ± 0.08	1.53 ± 0.24
C (%)	0.00 ± 0.00	25.48 ± 0.82	6.08 ± 0.82	10.74 ± 0.94	6.24 ± 0.94
Cl (%)	0.00 ± 0.00	0.88 ± 0.09	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Fe (%)	5.44 ± 0.22	0.00 ± 0.00	4.29 ± 0.08	3.81 ± 0.11	3.87 ± 0.17
Mg (%)	1.89 ± 0.13	0.00 ± 0.00	1.12 ± 0.04	1.94 ± 0.15	1.31 ± 0.07
O (%)	54.20 ± 0.62	59.34 ± 0.80	52.79 ± 0.58	56.32 ± 0.40	55.23 ± 0.31
P (%)	0.00 ± 0.00	3.37 ± 0.08	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
K (%)	2.50 ± 0.09	1.15 ± 0.09	2.18 ± 0.06	1.16 ± 0.04	2.19 ± 0.08
Na (%)	0.17 ± 0.03	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Si (%)	25.68 ± 0.19	11.40 ± 0.51	26.08 ± 0.26	26.08 ± 0.22	24.47 ± 0.86
S (%)	0.00 ± 0.00	1.13 ± 0.05	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00

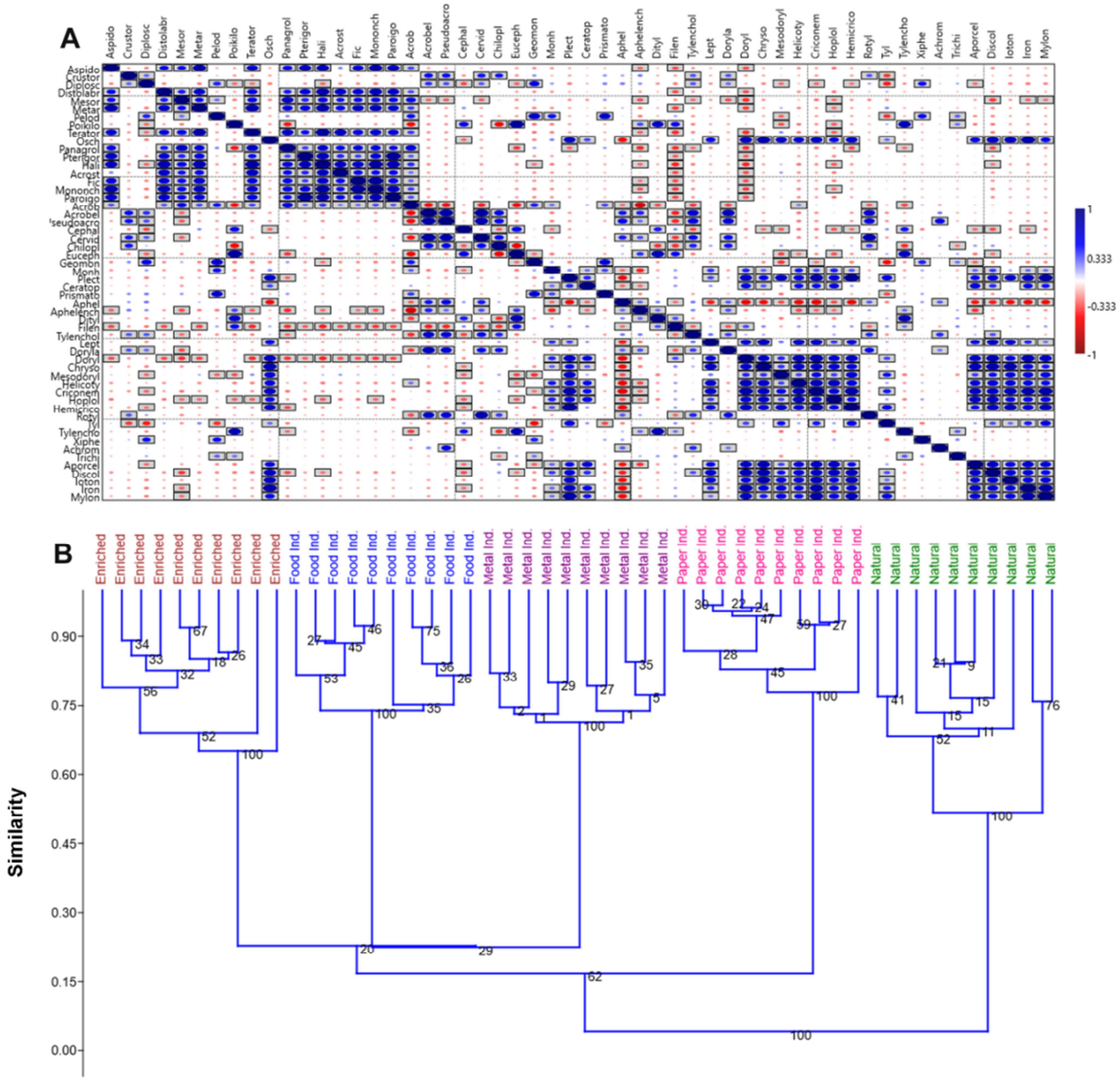

Figure 9. *Correlation of nematode communities among different habitats and clustering of habitats based on the nematode abundance. (A) Spearman's rank correlation plot. The blue color indicates a significant positive and the red color negative correlation among variables, whereas, the significance values more than P= > 0.05, are marked with a cross. (B) The tree was inferred with 10000 bootstrap pseudo-replicates using paired group (UPGMA) algorithm under the correlation similarity index.*

4. Discussion

It is a fact that the stability of ecosystem and its communities depend upon the interspecific interactions and the changes in environmental conditions. The present study provided insight into the substrate conditions that maintain soil biodiversity and biogeographic patterns of soil nematode community assemblage. The analyses showed that the diversity and abundance of nematodes among the habitats decreased from undisturbed (natural) to extremely disturbed habitats and well separated from each other by having a specific group of taxa, indicating the potential of the nematode communities as indicator of the ecological succession [12, 19, 21, 23, 29]. The spatial variation in nematode communities was found to be regulated by environmental processes and the drivers of nematode *β*-diversity were different among trophic levels. The habitats, dominated by a specific nematode community, clustered in a separate clade with a 100% branch support value (figure 9B). These communities have been categorized based on functional guilds, and coloniser-persister scales during the quality assessment of ecosystem. Some nematodes (Ba1 and Ba2 of *c-p*1 and *c-p*2 classes), tolerant or insensitive to pollutants, showed the capability to impede development, form dauer larvae and opt for some means of transport to reach a fresh habitat. Such nematodes dominated in organically rich (enriched habitats) and contaminated habitats (food and metal industries) and clustered together showing close relationship; likewise, the nematodes present in the paper industry possessed more or less similar characteristics but were clustered in a separate clade with abundance of *Eucephalobus, Poikilolaimus,* and *Tylenchorynchus.* However, the nematodes sensitive to pollutants, could not form dauer, and dominated natural habitats, clustered and positively correlated to each other. e.g. *Chrysonema, Criconemoides, Hemicriconemoides, Iotonchus, Ironus,* and *Mylonchulus* (figure 9A, B).

The present study revealed that a high proportion of bacterial-feeding nematodes in organically enriched habitats and in those areas contaminated by industrial wastes indicated rich nutrients, less competition and predation. On the other hand, the nematodes belonging to K- strategists (omnivore and carnivore nematodes) were most sensitive to contamination leading to a decrease in populations as the concentration of pollution or contamination in the soil increased [7, 37]. The decrease in the abundance of K-strategist genera with the increase in soil pollution contaminated by industrial wastes could reflect the loss of the most sensitive nematodes of coloniser-persister classes (*c-p*3-5) [36, 48].

However, the bacterial-feeding nematodes *Mesorhabditis* and *Panagrolaimus* belonging to r-strategist category, were the least affected, and dominated in nearly all the habitats. However, they were insensitive to pollutants showing significantly higher abundance in contaminated soils [9, 31]. Therefore, the genera *Cephalobus*, and *Aphelenchoides* mainly relying on the bacteria and fungi, belonging to *c-p*2 classes frequently occurred in most habitats but more abundant in those areas highly contaminated by industrial wastes.

In contrast, the abundance of coloniser-persister (*c-p*) classes, the abundance of the nematodes of *c-p* 3-5 classes were significantly decreased from natural and organically enriched habitats to food, metal and paper industries respectively. Although, the proportion of bacterial and fungal feeders was higher in contaminated areas and most abundant bacterial feeders of *c-p*1 class were observed in organically enriched habitat and food industries while most abundant fungal feeders and bacterial feeders of *c-p*2 classes in the metal and paper industries indicated nutrient depletion. The loss of the nematodes of higher *c-p* classes corresponded to stress condition or extremely very poor soil health.

5. Conclusions

Good soil health is necessary for the diversity and prevalence of soil microorganisms. In this study, we found that the diversity, abundance and higher *c-p* classes were drastically decreased from natural to organically enriched habitats and further to those contaminated by industrial wastes. The study also confirmed that nematodes with extremely lower *c-p* classes were tolerant to disturbed or highly polluted soil, whereas the nematodes with extremely higher *c-p* classes were more sensitive to the pollutant.

We also concluded that all the habitats are well separated from each other based on the nematode community structure, abundance, specificity, and coexistence. The abundance and diversity of k-strategists (persisters) nematodes were higher in natural habitats whereas, the organically enriched habitats were dominated by r-strategist (colonisers) bacterivores. However, the habitats influenced by industrial wastes were mainly dominated by bacterivores and fungivores of *c-p*2 classes. Four genera *Mesorhabditis, Panagrolaimus, Cephalobus,* and *Aphelenchoides* were considered cosmopolitan and commonly occurred in all the habitats, however, their very high abundance in contaminated/disturbed habitats may be a criterion to consider their bio-indicator properties. The genera *Oscheius, Criconemoides, Hemicriconemoides, Iotonchus, Ironus* and *Mylonchulus* in natural habitat; *Aspidonema, Distolabrellus, Metarhabditis, Pterygorhabditis, Teratorhabditis, Halicaphalobus, Acrostichus, Fictor, Mononchoides*, and *Paroigolaimella* in organically enriched; *Pelodera* and *Prismatolaimus* in the food industry and *Pseudacrobeles, Acrobeloides, Cervidellus, Dorylaimellus, Rotylenchus* and *Achromadora* in metal; *Poikilolaimus*, *Eucephalobus, Ditylenchus*, and *Tylenchorhynchus* in paper industries may be considered endemic taxa of such habitats.

Conflicts of Interest

The authors declare that they have no competing interests.

Ethics Approval

Not applicable.

Availability of Data and Material

The data collected and used in this study are available as supplementary material 1, 2.

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Authors' Contributions

QT originally conceived and formulated the idea and designed the experiments. MM performed the experiments and analyzed the data.

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