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Nematode Community Structure and Efficacy of the Free-Living Nematode *Metarhabditis andrassyana* as a Toxicological Assay Organism

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ABSTRACT: A survey of agricultural fields around Yamuna river in Faridabad, Haryana was conducted to study the community structure of the soil inhabiting nematodes. A total of 32 genera belonging to 8 orders and 21 families were recorded. In terms of number of genera, order Rhabditida was most frequent. The trophic diversity index (TDI) ranged from 1.2 – 1.4 (1.32 ± 1.11), Shanon's Diversity Index (H') varied from 1.1 – 2.9 (2.70 ± 0.15) and the maturity index (MI) ranged from 1.23 – 1.45 (1.36 ± 0.23). The low MI value indicated that it is a less stable and disturbed ecosystem. The efficacy of the free-living nematode *Metarhabditis andrassyana* as a toxicological assay organism was studied using lead, mercury, cadmium and copper as test substances. Testing of the toxic effect of the four heavy metals revealed that lead was the most toxic among all tested metals.

***Keywords*:** Nematode Community Structure, Agriculture, Maturity Index, Toxicity.

The functioning of soil ecosystem is dependent on biological processes that can be disrupted by soil contamination. Therefore, it is necessary to assess the toxicity of contaminated soils on soil dwelling organisms and combined effects of chemical substances that are usually present. Free-living, nonparasitic nematodes have an important role in the soil rich being the most abundant and species-richest metazoans (Andrassy, 1992; Yeates, 1981). By evolving various feeding types, these invertebrates have been able to occupy key positions in terrestrial food webs (Yeates *et al.,* 1993), thus influencing nutrient cycling in soils (Yeates *et al.*, 1982; Ingham *et al.*, 1985; Beare, 1997). The presence of nematodes and the structure of nematode communities are, therefore, important to agricultural production and sustainability (Fiscus and Neher, 2002; Ahalavat and Chaubey, 2017).

Accordingly, nematodes are suitable ecological indicators for monitoring and assessing agricultural areas (Neher, 2001). Nematodes are emerging organism group in the field of environmental sciences (Wilson and Kakouli-Duarte, 2009), offering a variety of molecular, ecotoxicological and ecological tools for an integrated risk assessment of soils. In environmental studies, the soil-dwelling bacterivorous nematode, *Caenorhabditis elegans*, has been successfully used as a test organism for investigating complex matrices,such as soils (Donkin and Dusenbery, 1993; Freeman *et al.*, 1999; Sochova *et al.*, 2007; Höss *et al.*, 2008) and freshwater sediments (Traunspurger *et al.*, 1997; Höss *et al.*, 2001; Comber *et al.*, 2008).

The aim of this study was to study the nematode community structure to assess the role of nematodes as indicators of soil conditions and evaluate the suitability of *Metarhabditis andrassyana* as a test organism for assessing the toxicity of potentially hazardous chemicals under standardized experimental conditions and the response is compared with control which has no effect on the viability of test organism.

MATERIALS AND METHODS

Soil samples from agriculture fields near Yamuna river area in Faridabad, Haryana were collected from a depth of 0-10 cm by using a hand spade. Samples were tagged, stored in sealed plastic bags and brought to laboratory for further processing. Nematodes were

extracted from 100 cc. of soil using Cobb's (1918) modified sieving and decantation and modified Bermann's funnel techniques. All the nematodes from each extracted sample were counted and identified to genus level. Trophic groups were allocated according to Yeates *et al.* (1993) and c-p groups were assigned following Bongers (1990).

Nematode diversity was described using the Shanon's diversity index calculated at generic level ($H^{'}$). Maturity index (MI) was calculated to estimate the relative state of two ecosystems studied (Bongers, 1990). Trophic diversity was calculated by the trophic diversity index (TDI) (Heip*et al.*, 1988). The channel index (CI) was calculated to indicate predominant decomposition pathways (Ferris *et al.*, 2001). Structure index (SI) and enrichment index (EI) were calculated to determine the relative stability of the ecosystem studied (Ferris *et al.*, 2001). All indices were calculated by using MS Excel.

The free living nematode, *Metarhabditis andrassyana* was extracted from manure collected from crop field near Yamuna river and cultured on nematode growth medium (NGM) supplemented with *E.coli* OP50 bacteria as food source. All the chemicals were obtained from Thermo Fisher Scientific (Powai, Mumbai, India). The studied metals were preferred because of their dominance in the environment. Five concentrations of four metals $HgCl_2$, $Pb(NO_3)_2$, $CuCl_2$, $CdCl_2$ solutions were used in the current investigation and were used at 10, 20, 50, 100, 150 ppm concentrations. For the lethality test, fresh nematodes were used in this experiment and bioassays were carried out *in vitro* in 12-well sterile polystyrene tissue culture plates (Cat. No. TPP12, HiMedia Laboratories, Mumbai, India).

From NGM, nematodes were washed twice with double-distilled water and then 20 ± 1 worms in 1ml sterile water were transferred in each well. One ml of double strength solution of chemical was added in each well, whereas distilled water was added to the control. The experiment was independently repeated thrice. The plates were kept in a Tupperware box and incubated at 25 ± 2^0C. Nematode mortality was recorded every 3 h till 100% mortality. Mortality data were used to calculate median lethal concentrations(LC_{50}) and median lethal time (LT_{50}) values.

Frequency (N): Frequency of nematode genus (i.e. the number of samples in which the genus was present).

Mean density (D): Number of nematode specimens of the genus counted in all samples / total number of the samples collected.

Shannon's diversity (H') = Σ (*pi* ln*pi*)

Trophic Diversity index (TDI) = 1 Σpi^2

Where pi^2 is the proportional contribution of ith trophic group.

Maturity Index (MI)

$$MI = \sum_{i=1}^{n} V(i).f(i)$$

Where Vi= cp value of the ith taxon. f(i) the frequency of that taxon in a sample. *Maturity index (MI) is calculated as the weighted mean of the individual c-p value.

Channel index (CI) = 100 x 0.8 Fu2 / (3.2Ba2 + Fu2)

Enrichment index (EI) = (e/e+b) x100

Structure index(SI) = (s/s+b) x100

Where e, b & s are sum products of assigned weights and number of individuals of all genera.

RESULTS

Nematode Diversity

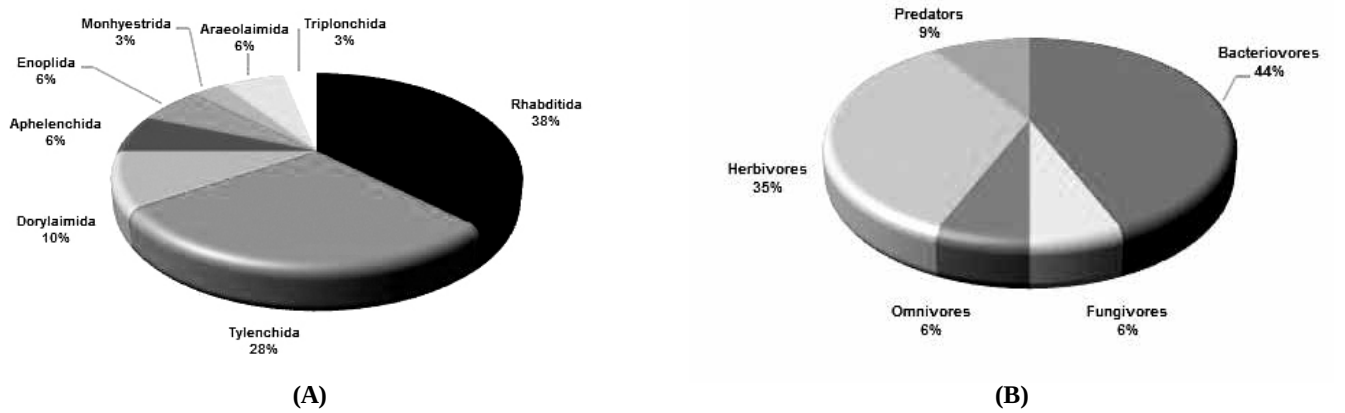
A total of 32 genera belonging to 8 orders and 21 families were recorded from the soil samples collected from agriculture fields near Yamuna river area in Faridabad, Haryana (Table 1). The number of genera varied from 3 to 11 per sample while in terms of abundance, the number varied from 142 to 1012 individuals per 100 cc of soil. *Meloidogyne* was the most abundant genus. In terms of number of genera (Fig. 1A), the Order Rhabditida was most frequent (38%) with 12 genera under 5 families, followed by Tylenchida (28%) with 9 genera under 7 families, Dorylaimida (10%) with 3

Table 1. Population structure of soil inhabiting nematodes, their mean abundance per 100 cc soil ± SD (N = 30).

Genera	c-pvalue	Order	N	Mean Abundance ± SD
Bacteriovores				
Bursilla	1	Rhabditida	4	2.6 ± 2.2
Mesorhabditis	1	Rhabditida	8	4.4 ± 5.3
Metarhabditis	1	Rhabditida	12	11.7 ± 3.2
Rhabditis	1	Rhabditida	1	0.30 ± 1.1
Acrobeles	2	Rhabditida	25	26.7 ± 21.5
Acrobeloides	2	Rhabditida	16	14.1 ± 10.1
Chiloplacus	2	Rhabditida	7	6.50 ± 7.87
Eucephalobus	2	Rhabditida	4	3.54 ± 13.7
Pseudacrobeles	2	Rhabditida	3	1.68 ± 5.98
Zeldia	2	Rhabditida	2	1.74 ± 3.78
Teratocephalus	2	Rhabditida	3	2.12 ± 2.87
Rhabdolaimus	2	Araeolaimida	2	1.34 ± 4.66
Chiloplectus	2	Araeolaimida	3	2.20 ± 3.88
Prismatolaimus	3	Monhysterida	6	5.54 ± 7.44
Fungivores				
Aphelenchoides	2	Aphelenchida	10	11.2 ± 19.40
Aphelenchus	2	Aphelenchida	12	14.9 ± 21.60
Omnivores				
Mesodorylaimus	4	Dorylaimida	4	2.1 ± 1.1
Minidorylaimus	4	Dorylaimida	3	1.7 ± 1.3
Herbivores				
Xiphinema	5	Dorylaimida	2	1.4 ± 0.7
Pratylenchus	3	Tylenchida	17	20.4 ± 12.46
Psilenchus	2	Tylenchida	3	5.50 ± 17.42
Helicotylenchus	3	Tylenchida	19	18.2 ± 21.24
Hemicriconemoides	3	Tylenchida	1	1.22 ± 3.12
Hoplolaimus	3	Tylenchida	21	20.2 ± 18.22
Meloidogyne	3	Tylenchida	27	29.5 + 22.1
Rotylenchulus	3	Tylenchida	22	20.9 ± 17.45
Tylenchorhynchus	3	Tylenchida	18	19.15 ± 19.5
Trichodorus	4	Triplonchida	2	1.40 ± 1.20
Basiria	2	Tylenchida	2	1.21 ± 3.45
Predators				
Tobrilus	3	Enoplida	3	3.2 ± 1.22
Mononchoides	1	Rhabditida	1	1.2 ± 0.4
Trypla	3	Enoplida	1	1.2 ± 4.2

genera under 2 families, Araeolaimida, Aphelenchida and Enoplida (6%) each with 2 genera under 2 families, while Monhysterida (3%) and Triplonchida (3%) were represented by 1 genus each.

In terms of trophic diversity, the bacteriovores (44%) constituted the most dominant group (Fig. 1, B) followed by herbivores (35%), predators (9%), omnivores (6%) and fungivores (6%). Among bacteriovores, *Acrobeles*



(A) **(B)**

Fig. 1. (A): Ordinal Diversity (Genera) and (B): Trophic Diversity of nematodes

was the most dominant genus while *Meloidogyne*, *Aphelenchus*, *Mesodorylaimus* and *Tobrilus* were most dominant genera among herbivores, fungivores, omnivores and predators, respectively.

Nematode Community Analysis

Shannon's diversity (H') and trophic diversity index (TDI) were calculated to assess diversity of nematode genera and trophic groups. The value of Shannon's diversity (H') was 2.70 ± 0.15 and trophic diversity index (TDI) was 1.32 ± 1.11 (Table 2). The Maturity index (MI) was calculated to assess the maturity of the agro-ecosystem and it was 1.36 ± 0.23 (Table 2). The structure index (SI) was calculated to assess the structure of the soil ecosystem. The values for SI was very low, indicating the polluted status of ecosystem (Table 2). The enrichment index (EI) gives the status of enrichment in the ecosystem due to contamination. The values of EI observed in the present study were very high at all the sites giving an idea of enriched ecosystem (Table 2). The higher values of channel index (CI) indicated a fungal dominated decomposition pathway while lower value indicated the bacteria based decomposition pathway. The values for CI in present work were mostly low (Table 2).

Table 2. Various ecological Indices for assessing the community dynamics.

Indices/Parameters	Values
Maturity Index (MI)	1.36 ± 0.23 (1.23 – 1.45)
Enrichment Index (EI)	71.65 ± 10.23 (63.21 – 73.26)
Structure Index (SI)	34.56 ± 14.24 (33.11 – 37.34)
Channel Index (CI)	16.66 ± 10.12 (10.17 – 16.98)
Trophic Diversity Index (TDI)	1.32 ± 1.11 (1.2 – 1.4)
Shannon's Diversity Index (H')	2.70 ± 0.15 (1.1 – 2.9)

Toxicological Assay

We calculated the median lethal concentration(LC_{50}) at 6 and 12h and the median lethal time(LT_{50}) for each concentration of the metal (Table 3 & 4). Only lead and mercury killed nematodes at 3h after inoculation at the lowest concentration. At later time points, mortality rate of *M. andrassyana* after treatment with various concentrations of lead, mercury, cadmium and copper were significantly different as compared with control as shown in Fig. 2 A & B. The effect of heavy metals (Hg, Pb, Cd and Cu) at 100 and 150ppm concentrations caused the severe ($p<0.05$) toxicity in nematodes as compared to control. Among these heavy metals lead was identified as the most lethal as it killed all the nematodes within 6h. At 6h, its LC_{50} was 12.65 ppm, significantly lower than all other tested metals (Fig. 2(A), Table 4). The least lethal metal was copper, showing

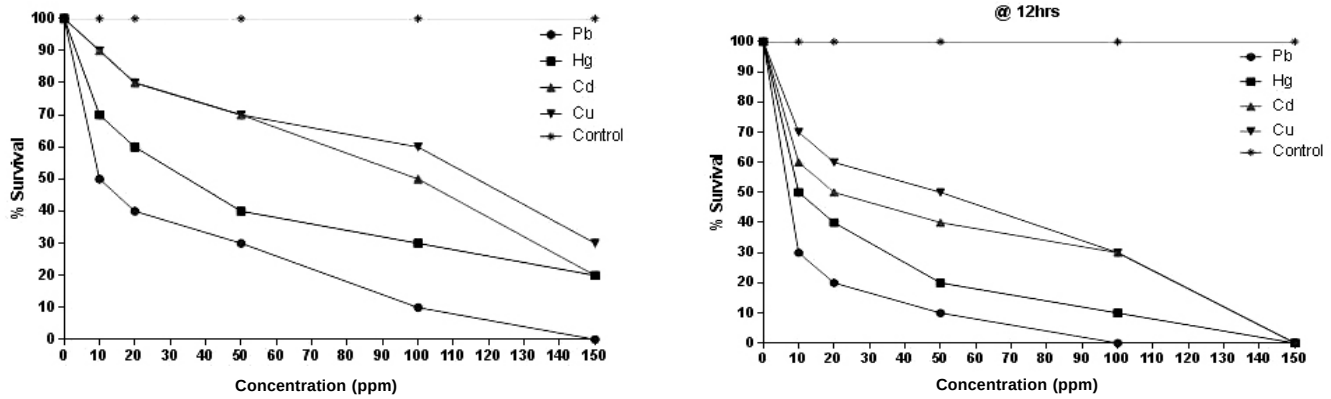


Fig. 2. Toxicity response curves of *Metarhabditis andrassyana* at (A) 6 h and (B) at 12 h to different concentrations of heavy metals

Table 3. Median lethal time (LT_{50}) values of heavy metals against the *Metarhabditis andrassyana.*

Dose (ppm)	LT_{50}			
	Cu	**Cd**	**Hg**	**Pb**
10	30	24	18	9
20	24	18	12	6
50	18	12	6	6
100	12	9	4.5	4.5
150	6	6	3	3
÷² (log-rank test)	42.97	49.45	44.87	43.40
p	< 0.0001	< 0.0001	< 0.0001	< 0.0001

higher LC_{50} value of 99.38 ppm @ 6h as compared to other metals (Fig. 2(A), Table 4).

The LT_{50} values for lead also showed that it was the quickest in killing *Metarhabditis andrassyana* with the lowest LT_{50} value of 9h at 10 ppm as compared to 30, 24 and 18h for copper, cadmium and mercury at 10 ppm (Table 3).

DISCUSSION

Soil organisms depend on each other for carbon and energy. The structure and function of below-ground food webs are disrupted by hydrocarbon and heavy-metal contaminants, mineral fertilizers and pesticides, and by physical disturbance. However, the results of such

Table 4. Median lethal concentration (LC_{50}) and LC_{90} values of heavy metals against the *Metarhabditis andrassyana*. (Numbers in parenthesis represent 95% confidence limits).

Metals	At 6h		At 12h	
	LC_{50}	LC_{90}	LC_{50}	LC_{90}
Cu	12.65 (1.33 – 24.53)	96.18 (47.37 – 1499.18)	5.55 (0.001 – 12.83)	33.00 (15.34 – 853.53)
Cd	30.86 (6.21 – 71.83)	410.39 (131.46 – 482981.13)	11.95 (1.51 – 22.65)	79.03 (40.93 – 758.40)
Pb	74.81 (42.72 – 193.75)	459.78 (182.67 – 12712.86)	20.33 (3.11 – 40.40)	208.05 (84.44 – 16749.12)
Hg	99.38 (51.68 – 652.52)	892.50 (250.24 – 797020.10)	29.64 (11.98 – 54.49)	205.65 (93.94 – 2980.04)

disruptions are unpredictable because they are influenced by the heterogeneity of the soil, fluctuations in abiotic conditions, chemical and physical buffering capacity, and by other biotic and abiotic interactions (Bongers and Ferris, 1999). Because nematodes occupy key positions as primary and intermediate consumers in soil food webs, evaluation and interpretation of the abundance and function of their faunal assemblages or community structure offers an *in situ* assessment of disruptive factors. The soil environment significantly impacts on soil dwelling nematode communities. Therefore, soil nematode communities and their structural changes were found to be one of the best biological tools for assessing soil processes and plant conditions in terrestrial ecosystems (Wang *et al*., 2009; Pen-Mouratov *et al*., 2010).

Recent research indicated that simple analyses of *in situ* nematode faunae at generic level provide a wealth of information on the nature of decomposition pathways and soil nutrient status (Ettema, 1998). The analyses also indicate effects of agricultural practices and contaminants on the functioning of the soil food web (Lau *et al.*, 1997). They provide a basis for environmental management, remediation and conservation decisions. Nematodes respond differently to soil disturbance and therefore changes the nematode community composition (Gupta and Yeates, 1997; Yeates and Pattison, 2006; Ahalavat and Chaubey, 2017).

A low percentage of dorylaims (c-p 4 &5) in the crop field (10 %) clearly indicated that the soil is more disturbed as cropping always involves ploughing and/or tilling together with addition of fertilizers, organic matter and pesticides/weedicides. The dorylaims appeared to be susceptible to these activities as also shown by Ahalavat and Chaubey (2017). Hence, the sensitivity of the dorylaims is a good indicator of soil disturbance (Neher, 2001). In this study *Acrobeles* was the most abundant genus and confirms with the work of Yeates and Bongers (1999) and Gomes *et al*. (2003) where it was found that cephalobids were the most abundant bacterial feeders present in cropping systems. Shannon's diversity index ($H^{'}$) reflected diversity of nematodes in an ecosystem. Higher values of $H^{'}$ showed highly diverse ecosystem while low values showed the contrary. Hanel (1995) found $H^{'}$ in crop fields to vary between 2.66-2.83. In present work, the value of $H^{'}$ was 2.70 ± 0.15. This is in perfect agreement to earlier records where crop fields are found to be highly diverse in comparison to other ecosystems (Ahalavat and Chaubey, 2017). The MI has been used successfully as indicators for disturbances (Gorgieva *et al*., 2002; Kumar and Ahmad, 2017). Various case studies (Bongers *et al.*, 2001; Kumar and Ahmad, 2017) suggested that the MI is decreased by disturbances but increases during the colonization process. The low value of MI in present study indicated a disturbed environment due to agricultural practices.

Food web indices like EI, SI and CI provide an excellent means for studying the stability of ecosystem, weather it is stressed, enriched or structured and provide information on the dynamics of the soil food web (Ferris *et al.*, 2001). EI is generally known to reflect availability of resources to the soil food web and response of primary decomposers to the resources (Ferris *et al.*, 2004). Present study, revealed that this region was highly enriched (EI 71.65 ± 10.23). SI describes whether soil ecosystem is structured / matured (high SI) or disturbed (low SI). The value of SI during present study was low (SI 34.56 ± 14.24) that agreed with earlier studies (Ferris *et al*., 2001; Berkelmans *et al.*, 2003) which argued that low values of SI indicated disturbed conditions of the ecosystems studied. The higher values of channel index (CI) indicate a fungal dominated decomposition pathway while lower value indicated the bacteria based decomposition pathway. The values for CI in present work were mostly low, which indicated that greater participation of bacteriovores in the breakdown of soil organic matter.

The efficacy of the free-living nematode *M. andrassyana* as a toxicological assay organism was studied using lead, mercury, cadmium and copper as test substances. Testing of the toxic effect of the four heavy metals revealed that lead was the most toxic metal among all tested metals that agreed with earlier studies (Freeman *et al.*, 1999; Peredney and Williams, 2000; Dayong and Xiaojuan, 2008).

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