The Journal of Basic & Applied Zoology (2016) 74, 41–50

HOSTED BY
ELSEVIER

The Egyptian German Society for Zoology
The Journal of Basic & Applied Zoology
www.egsz.org
www.sciencedirect.com

Effect of abiotic factors on the distribution of earthworms in different land use patterns

CrossMark

Sharanpreet Singh [a], Jaswinder Singh [a,*], Adarsh Pal Vig [b]

[a] *Department of Zoology, Khalsa College, Amritsar, Punjab, India*
[b] *Department of Botanical and Environmental Sciences, Guru Nanak Dev University, Amritsar, Punjab, India*

Received 27 February 2016; revised 24 April 2016; accepted 2 June 2016

KEYWORDS
Abiotic factors;
Biodiversity;
Earthworms;
Principal Component Analysis (PCA);
Shannon–Wiener index

Abstract The distribution of earthworms is usually diverse and their numbers fluctuate in relation to the different abiotic factors and land use patterns of the soil. The aim of the present study is to determine the biodiversity, distribution and relative abundance of earthworms under different land use pattern and its relation to abiotic factors (physico-chemical properties) of the soil. Earthworms were collected from different sites on the basis of various environment niches like agriculture fields, gardens, nurseries, along the river and road side etc. by hand sorting method. Physico-chemical analysis of the soil was also done to know the important factors affecting earthworm biodiversity and distribution. Total five species of earthworms belonging to the families Megascolecidae and Octochaetidae were identified: *Metaphire posthuma*, *Lampito mauritti*, *Amynthas morissi*, *Eutyphoeus waltoni* and *Eutyphoeus incommodus*. *M. posthuma* was the most abundant species and found in all the collection sites while other four species were abundantly found in gardens and nurseries. Shannon–Wiener diversity index, Margalef species richness and Pielou's evenness was ranged from 0.11 to 0.37, 0 to 0.6 and 0 to 0.53 respectively. Principal component analysis also proved that the abiotic factors like pH, moisture, soil texture and OC has strong positive effect on the distribution of earthworm. Earthworm biodiversity and distribution have been found to be positively correlated with type of vegetation and moisture content at the different collection sites and also varied according to soil habitat, soil tillage and land used pattern.

Introduction

The fertility of soil depends on the biological diversity and soil faunal biomass. Earthworms (belonging to the Phylum Annelida, Order Oligochaeta, Class Clitellata) are known to be the most important soil fauna biomass in humid soils of temperate and tropical regions (Lee, 1985). The beneficial role of earthworms in the breakdown of dead plant material in the forest litter was first documented by Darwin (1881). For a long time, earthworms have been known as the farmer's friend, natural ploughmen, soil ecosystem engineers and intestines of earth. Earthworms can significantly influence soil physical, chemical and biological properties, hence improving the fertility and structure of soil (Doan et al., 2013; Singh et al., 2016). Earthworms also play an important role in mixing of mineral soils and plant materials. Various studies reported that the disturbance and degradation of natural forest affect the number of

* Corresponding author.
E-mail address: singhjassi75@yahoo.co.in (J. Singh).
Peer review under responsibility of The Egyptian German Society for Zoology.

http://dx.doi.org/10.1016/j.jobaz.2016.06.001
2090-9896 © 2016 The Egyptian German Society for Zoology. Production and hosting by Elsevier B.V.

earthworms and their distribution (Baretta et al., 2007; Chandran et al., 2012). The distribution of earthworm is usually heterogeneous (Guild, 1952; Satchell, 1955; Svendsen, 1957) and their numbers fluctuate in relation to the abiotic factors of the soil (Evans and Guild, 1947). Environmental factors like moisture, temperature, pH and soil texture also affect the distribution of earthworms. Bhadauria and Ramakrishnan (1989) determined that regional earthworm biodiversity and species dispersal pattern was influenced by a variety of biotic and abiotic forces such as soil properties, surface litter inputs, surface vegetation type, dynamic land management history, local or regional climate and human pressure. The significance of diverse soil habitats is one of the most influencing factors affecting the overall earthworm distribution (Rajkhowa et al., 2014). Changes in land use patterns have also directly affected the composition and population structure of earthworm communities in different agro-climatic regions (Blanchart and Julka, 1997; Behera et al., 1999; Bhadauria et al., 2000; Lalthanzara et al. 2011). Endogeic earthworm appears a key feature of soil functioning in the urban context through their roles on organic matter transformation, the formation and maintenance of soil structure (Amosse et al., 2015).

There are about 1800 species of earthworm widely dispersed all over the world (Edwards and Bohlen, 1996) and constitute 80% of the total soil invertebrates biomass (Nainawate and Nagendra, 2001). In recent study, 3627 species are known worldwide (Kooch and Jalilvand, 2008). India is one of the important mega biodiversity countries and only 11.1% of earthworm diversity is available out of total global earthworm's diversity (Chaudhuri and Nath, 2011; Suthar, 2011). It includes about 408 species placed in 10 families and 69 genera (Dash, 2012). Michaelsen (1909) described the Indian Oligochaetes and produced taxonomic keys for all known species of earthworm in India. Stephenson (1923) and Gates (1972) documented the earlier work on earthworms in the Fauna of British India and compiled a monograph, which included species from Andaman and Nicobar Islands and North Eastern India while Julka (1988) further authenticated the work on Oligochaetes. Indian earthworm fauna is predominantly composed of native species, which constitute about 89% of total earthworm diversity in the country (Julka and Paliwal, 2005).

Despite varied habitat, good moisture content and intensive farming there have been fewer studies on earthworm diversity in the agro ecosystem of the northwestern part of Punjab. The present study is the first report to know the effect of different abiotic factors of soil on the distribution and relative abundance of earthworms collected from different habitat of this region.

Materials and methods

Study site

The study was conducted at different sites of northwestern part of Punjab, India (Fig. 1). Most of the northwestern part of Punjab lies in a fertile, alluvial plain with two rivers viz. Ravi and Beas. This area has an extensive irrigation canal system and is influenced by three seasons: summer, monsoon and winter. In summer (April to June) temperature typically rise as high as 43 °C, in monsoon season (July to September) a majority of rainfall occurs, and in winter (December to February) temperatures typically fall as low as 4 °C. There is a transitional period between winter and summer in March and early April, as well as a transitional season between monsoon season and winter in October and November. The average annual rainfall is 541.9 mm. Relative humidity generally exceeds 70% in the mornings except during the summer season when the humidity in the afternoon is about 25% or less. The available flora in northwestern part of Punjab is patches of grass, small bushes, and shrubs. Paddy, wheat, sugarcane and vegetables are the most important crops of this region during summer, winter and transitional periods.

Sampling and identification of earthworms

An extensive survey of the northwestern part of the Punjab was done in various environmental niches such as agricultural fields, irrigation channels, gardens, plant nurseries, urban ornamental gardens, waste and grasslands, kitchen gardens, canal sites and wastage drains. The characteristics of survey sites are shown in Table 1. Earthworms were sampled from 21 different sites for three consecutive seasons (Table 2). Earthworms were sampled by the hand-sorting method up to 30 cm deep using quadrats ($30 \times 30\ cm^2$ area) for each sampling site. A global positioning system (GPS) (Garmin, Gpsmap 78 s) was used to mark the latitude and longitude of each site. Moisture content was measured with a digital soil moisture meter (Micro make). The collected samples of earthworms with appropriate amount of soil were placed in polythene bags labeled with place name, date of collection, surrounding soil biota etc and brought to the lab for further study. Earthworms were washed in fresh water and sorted on the presence or absence of clitellum. Clitellated earthworms were narcotized in 70% ethyl alcohol and fixed in 5% formalin for 6–8 h and finally preserved in 5% formalin. The preserved samples were studied morphologically and dissected for study diagnostic taxonomic character such as spermathecae (number and location), prostate gland (location and shape), prostomium shape, and clitellum position.

Physico-chemical analysis of soil

Soil was taken from sites for its physico-chemical analysis. Soil was analyzed for texture, pH, electrical conductivity (EC), total dissolved salts (TDS), nitrogen (N), phosphorus (P), potassium (K), organic carbon (OC), ash, sodium (Na), calcium (Ca), lithium (Li) and heavy metals. Soil texture was measured using method of Bouyoucos (1962). EC, pH and TDS were measured using a digital meter (Eutech Instruments, PCSTestr 35 series). The method of Bremner and Mulvaney (1982) was used for estimation of Total Kjeldhal Nitrogen. Content of organic carbon and ash was measured by the method of Nelson and Sommers (1996). Phosphorus was estimated by the method of John (1970) using Systronics UV/Visible spectrophotometer-117. Sodium, potassium, calcium and lithium were analyzed by Systronics Flame Photometer-128.

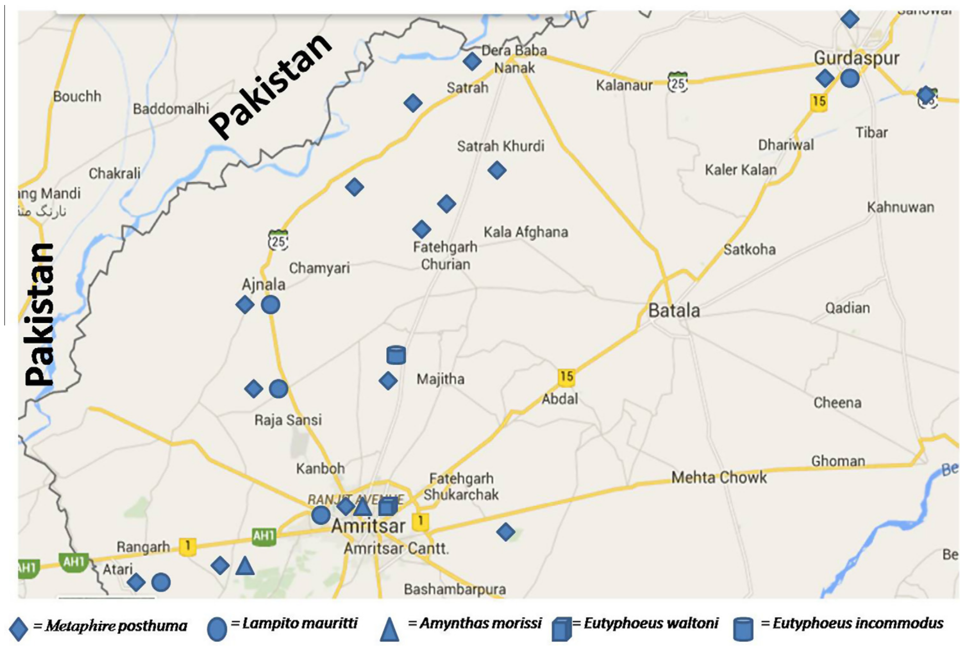

Figure 1 Map of Northwestern areas of Punjab showing earthworm collection sites and locations of different species of earthworms.

Table 1 Major Sampling sites and their characteristics.

Sampling sites	Characteristics
Agriculture land	Standing crop field, zero tillage agriculture plots etc
Irrigation channels	Soils was moist due to regular water supply for the fields (Tubewell)
Gardens	Gardens with fruit tress (Guava, Citrus etc) or gardens with intercropping system
Plant nurseries	Nurseries with different varieties of flowers
Urban ornamental garden	Different lawns, grass cover, city parks and garden at public places like school, colleges, universities, public parks etc
Waste and grasslands	Barren lands, forest land, land not being used for agriculture purposes
Kitchen gardens	Vegetable gardens in rural and urban localities with different horticulture crops (Brinjal, potatoes, chilly, cucumber etc)
Canal sites	Different lands around rivers and canals, along the sides of rivers and canals, different waterlogged sites in rural and urban areas
Waste drain	Moist soils around wastewater drain and channels in urban and sub urban areas, waste water collection sites near drinking water resources like hand pump, pond, water tanks etc

Statistical analysis

Principal component analysis (PCA)

Principal component analysis was used to characterize the effect of different abiotic components of soil on the distribution of earthworms. Analyses were done with the help of SPSS 16 software program.

Species diversity index

a) The diversity index of earthworm species was calculated using Shannon–Wiener index (1949).

$$\text{Shannon–Wiener index (H)} = -\sum \mathrm{p}_i \times \ln \mathrm{p}_i, \quad (1)$$

where p_i is the proportion of total sample represented by species and ln is logarithm to base *e*.

b) Margalef species richness index was used to measure the species richness (Margalef, 1958).

$$\text{Margalef species richness index } (\mathrm{D_{Mg}}) = S - 1/\ln N \quad (2)$$

where S is number of species, N is number of individuals and ln is natural logarithm.

c) Pielou's evenness index (E) was used to measure the species evenness (Pielou, 1966)

$$\text{Pielou Evenness (E)} = H/\ln S \quad (3)$$

where H is Shannon–Wiener diversity index and S is number of species.

Results

Community structure

Total five species of earthworms were found: *Metaphire posthuma* (Vaillant), *Lampito mauritti* (Kinberg), *Amynthas morissi*

Table 2 Description of study sites with latitude, longitude and type of vegetation.

Sample Name	Site	GPS coordinates	Altitude	Vegetation
Government College, Ajnala	I.	N 31° 50′ 26.2″, E 074° 44′ 14.2″	229	Garden
Nursery, Khalsa College, Amritsar	II.	N 31° 63′ 22.6″, E 074° 87′ 15.1″	219	Flower
Botanical Garden, Khalsa College, Amritsar	III.	N 31° 63′ 22.3″, E 074° 87′ 15.2″	220	Garden
Village Othiyan	IV.	N 31° 38′ 44.3″, E 074° 57′ 20.2″	244	Wheat
Company Bag, Amritsar	V.	N 31° 38′ 19.6″, E 074° 51′ 15.7″	226	Garden
Nursery, Guru Nanak Dev University, Amritsar	VI.	N 31° 62′ 92″, E 074° 82′ 67″	221	Nursery
Ramdass	VII.	N 31° 57′ 32.6″ E 074° 54′ 59.8″	242	Paddy field
Village Chinna	VIII.	N 31° 45′ 32.0″, E 074° 46′ 56.7″	229	Paddy field
Village Ghrinda	IX.	N 31° 36′ 50.1″, E 074° 39′ 47.5	213	Guava Garden
Rainbow Resort, Attari	X.	N 31° 36′ 5.56″, E 074° 41′ 09.6″	211	Garden
Village Sangatpura	XI.	N 31° 76′ 69.6″, E 074° 90′ 96.8″	216	Paddy
Government College, Gurdaspur	XII.	N 32° 04′ 087.6″ E 075° 37′ 83.72″	225	Garden
Pandit Mohan Lal College, Gurdaspur	XIII.	N 32° 04′ 083.6″ E 075° 37′ 84.72″	221	Garden
Mango Garden, Keshopur	XIV.	N 32°06′ 089.2″ E 075° 39′ 86.65″	222	Mango Garden
Village Talwandi Rama	XV.	N 31° 56′ 53.3, E 074 59′ 29.2″	235	Vegetables
Fatehgarh Churian	XVI.	N 31° 51′ 33.8″ E 074 56′ 50.4″	241	Garden
Beas River, Purana Shala	XVII.	N 31°59′42″ E 75°30′35″	225	Along the river side
Village Dhanda	XVIII.	N 31° 53′ 0.98″, E 074 57′ 49.0″	243	Paddy
Indo Pak Border, Dera Baba Nanak	XIX.	N 32° 02′ 48.8″, E 075 01′ 39.8″	263	Vegetable
Village Dharamkot	XX.	N 32° 01′ 22.7″ E 074 58′ 06.5″	257	Sugarcane
Village Shangewali	XXI.	N 31° 53′ 57.1″, E 074 55′ 54.1″	270	Eucalyptus field

(Beddard), *Eutyphoeus waltoni* (Michaelsen) and *Eutyphoeus incommodus* (Beddard) belonging to two families Megascolecidae and Octochaetidae. All the species were abundantly found in rainy season (from July to September) and minimum in winter season (December to February) due to cold and dry weather with less rain fall. During winter season the temperature fall upto 2 °C to 4 °C and epigeic worms cannot survive in this adverse condition. The abundance of *M. posthuma* has been found in all types of soil and widespread in cultivated (agriculture field) and non-cultivated land (garden and nurseries) due to its endogeic nature. But highest density of endogeic worms were found in agriculture field having vegetables and fodder cultivation. Other four species *L. mauritti*, *A. morissi*, *E. waltoni* and *E. incommodus* were more abundant in non-cultivated areas free from pesticides and fertilizers application (Table 3).

Effect of abiotic factors on distribution of earthworm

The distribution of earthworm species with respect to different physico-chemical parameters of soil is given in Table 4.

Physico-chemical analysis of the soil

Earthworms were present within the a range of soil pH of 8.03–9.36, EC of 63.9–417 μS/cm, TDS of 45.4–296.5 mg/L, N of 0.03–0.5 g/kg, P of 0.13–22.76 g/kg, K of 0.03–5.75

Table 3 Diversity of earthworm species in different study sites.

Sites	*M. posthuma*	*L. mauritti*	*A. morissi*	*E. waltoni*	*E. incommodus*
I.	+	+ +	–	–	–
II.	+ +	–	–	–	–
III.	+ +	+	+ +	–	–
IV.	+ +	–	–	–	–
V.	+ +	–	–	+	–
VI.	+	+	+ +	–	–
VII.	+ +	–	–	–	–
VIII.	+ +	+	–	–	–
IX.	+	–	+ +	–	–
X.	+	+ +	–	–	–
XI.	+	–	–	–	+ +
XII.	+ +	+	–	–	–
XIII.	+	+ +	–	–	–
XIV.	+ +	–	–	–	–
XV.	+ +	–	–	–	–
XVI.	+ +	–	–	–	–
XVII.	+ +	–	–	–	–
XVIII.	+ +	–	–	–	–
XIX.	+ +	–	–	–	–
XX.	+ +	–	–	–	–
XXI.	+ +	–	–	–	–

+ = abundance, + + = most abundance, – = absent

Table 4 Physico-chemical analysis of the soil collected from different study sites.

Sample Site	Texture	Moisture (%)	pH	EC (μS/cm)	TDS (mg/L)	N (g/Kg)	P (g/Kg)	K (g/Kg)	OC (%)	Ash (%)	Na (g/Kg)	Li (g/kg)	Ca (g/kg)	Species found
I.	Loam	83	8.90	82.21	57.85	0.06	4.86	1.34	2.66	95.80	1.02	1.35	1.12	Mp, Lm
II.	Sandy clay loam	82	9.36	197.89	140.84	0.08	0.26	2.92	1.62	97.20	1.40	2.01	0.86	Mp
III.	Sandy loam	94	9.15	163.23	98.19	0.15	0.61	3.93	1.28	96.93	1.47	2.39	40.72	Am, Lm
IV.	Sandy loam	92	8.80	133.25	93.95	0.21	0.56	4.38	3.59	95.60	1.31	2.63	2.56	Mp
V.	Sandy loam	90	8.59	163.60	116.50	0.06	0.93	1.23	2.08	96.40	0.91	0.11	4.21	Mp, Ew
VI.	Loam	95	8.03	417.00	296.50	0.08	1.56	0.67	2.20	96.20	0.93	1.42	2.40	Am
VII.	Loam	91	8.97	63.90	45.40	0.03	3.25	5.73	2.08	96.40	1.38	2.44	1.07	Mp
VIII.	Loam	89	9.12	121.65	86.92	0.17	0.22	2.40	3.36	94.20	1.74	1.47	0.40	Mp, Lm
IX.	Sandy loam	84	8.77	98.00	70.35	0.25	0.30	3.41	2.43	95.80	2.71	1.21	0.98	Am, Mp
X.	Sandy loam	86	9.33	109.72	77.38	0.36	0.56	4.21	3.24	95.90	2.76	1.98	0.86	Mp, Lm
XI.	Loam	83	8.81	132.40	93.20	0.22	13.46	1.35	8.46	85.40	1.30	1.30	0.21	Ei, Mp
XII.	Loam	82	8.31	206.34	151.23	0.28	3.18	3.95	4.17	92.80	1.50	4.17	3.18	Lm, Mp
XIII.	Loam	80	8.75	141.85	99.25	0.06	9.84	0.21	11.60	80.00	0.95	5.68	0.14	Lm, Mp
XIV.	Clay loam	83	8.45	190.17	136.27	0.42	0.45	4.96	3.24	94.40	1.53	0.47	0.21	Mp
XV.	Sandy loam	88	8.88	86.80	61.65	0.11	0.43	0.26	0.92	98.40	0.64	4.79	1.66	Mp
XVI.	Sandy loam	79	8.90	91.80	64.90	0.17	0.84	4.22	2.78	95.20	1.22	0.39	1.91	Mp
XVII.	Sandy loam	88	9.30	93.75	65.00	0.20	11.81	0.33	0.69	98.80	0.62	1.15	0.43	Mp
XVIII.	Sandy loam	86	8.79	134.65	95.65	0.28	22.76	0.03	1.85	96.80	0.76	1.12	0.23	Mp
XIX.	Silt loam	89	8.87	98.35	69.80	0.50	0.70	5.75	1.62	97.20	1.08	3.75	1.18	Mp
XX.	Sandy loam	82	8.81	113.85	80.80	0.03	0.46	5.40	3.01	94.80	1.09	0.46	0.96	Mp
XXI.	Loam	81	8.99	96.85	102.85	0.14	0.13	5.66	3.94	93.20	1.45	1.17	14.66	Mp

Mp = *Metaphire posthuma*, Am = *Amynthas morissi*, Lm = *Lampito mauritti*, Ei = *Eutyphoeus incommodus*, Ew = *Eutyphoeus waltoni*.

Table 5 Principal components and eigenvalues with total and cumulative variance of soil factors.

Parameters	PC1	PC2	PC3	PC4	PC5
Sand	−0.814	−0.272	−0.247	−0.122	0.304
Silt	0.727	0.13	0.225	0.325	−0.437
OC	0.625	0.127	−0.619	−0.02	0.291
Ash	−0.618	−0.078	0.561	−0.233	−0.364
pH	−0.579	0.551	−0.155	0.129	0.039
Clay	0.497	0.428	0.142	−0.428	0.197
EC	0.437	−0.742	0.363	−0.163	0.211
TDS	0.47	−0.715	0.361	−0.18	0.215
K	0.069	0.613	0.542	0.159	0.142
Na	0.102	0.594	0.367	−0.245	0.376
P	−0.056	−0.239	−0.635	−0.299	−0.386
Moisture	−0.235	−0.459	0.501	0.353	−0.162
Li	0.321	0.015	−0.335	0.656	−0.05
Ca	−0.231	−0.033	0.317	0.458	0.422
N	0.222	0.388	0.314	−0.219	−0.473
Eigenvalues	3.23	2.81	2.51	1.42	1.37
Total variance (%)	21.574	18.731	16.731	9.481	9.148
Cumulative Variance (%)	21.574	40.304	57.035	66.516	75.663

g/kg, OC of 0.69–11.60%, Ash of 80–98.8%, Na of 0.62–2.76 g/kg, Ca of 0.14–40.72 g/kg and Li of 0.11–5.67 g/kg. Moisture levels at all the collection sites was in the range of 80–95% with highest moisture level found in gardens and nurseries. It was also observed that availability and distribution of earthworm was less in soil having moisture below 60%.

Principal component analysis

Principal component analysis (PCA) was used on 15 physico-chemical parameters of soil for 21 sites to identify the most important factors affecting earthworm distribution. Eigenvalues greater than 1 were considered as standard for extraction of the principal components analysis. PCA resulted in five principal components viz PC1, PC2, PC3, PC4 and PC5, contributing variances of 21.57, 18.73, 16.73, 9.48 and 9.14 respectively. The different factors, respective eigenvalues, total variance (%), cumulative variance (%) and component loadings for the each component are given in Table 5. The scree plot (Fig. 2) for five principal components clarifies the method of extraction of different components. Variance in PC1 is due to sand, silt, clay, OC and Ash; in PC2 it is due to pH, EC, TDS, K and Na; in PC3 it is due to P, and moisture; in PC4 it is due to Li and in PC5 it is due to Ca and N.

Species diversity index

Species diversity index of earthworms in different sites is given in Table 6. The value of Shannon–Wiener index usually ranges from 0 to 4. In the present study it ranged from 0.11 at site VII to 0.37 at site III. The Shannon–Wiener diversity index was 0.37 and 0.34 at sites III and VI respectively with 3 species of earthworm and only single species of earthworm was identified from site VII, which has a lowest diversity index (0.11). The value of Margalef species and Pielou's evenness richness ranged from 0 to 0.6 and 0 to1 respectively. The species richness 0.60 was in site VI i.e garden and nurseries and low (0)

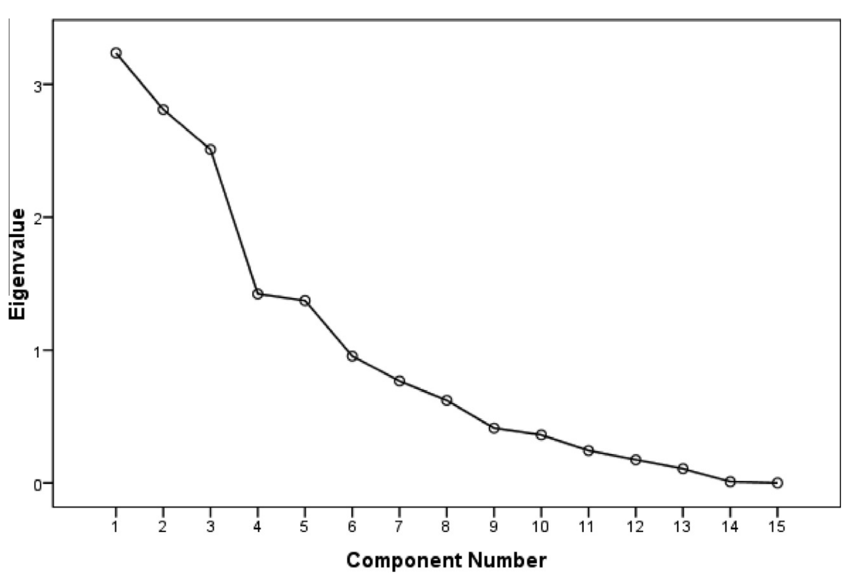

Figure 2 Scree plot of eigenvalue of principal components.

Table 6 Number of species, Diversity index, Evenness and species richness at different study sites.

Site	Species no	Diversity index (H)	Species richness (D_{Mg})	Evenness (E)
I.	2	0.24	0.33	0.34
II.	1	0.14	0	0
III.	3	0.37	0.58	0.33
IV.	1	0.12	0	0
V.	2	0.21	0.35	0.30
VI.	3	0.34	0.60	0.30
VII.	1	0.11	0	0
VIII.	2	0.33	0.28	0.47
IX.	2	0.26	0.31	0.37
X.	2	0.36	0.27	0.53
XI.	2	0.23	0.33	0.33
XII.	2	0.22	0.34	0.31
XIII.	2	0.25	0.32	0.36
XIV.	1	0.18	0	0
XV.	1	0.2	0	0
XVI.	1	0.18	0	0
XVII.	1	0.16	0	0
XVIII.	1	0.16	0	0
XIX.	1	0.21	0	0
XX.	1	0.2	0	0
XXI.	1	0.15	0	0

in agriculture field. In this study Pielou's evenness value was maximum (0.53) in site X and zero at those sites where only one species of earthworm was found in different sites.

Discussion

Effect of abiotic factors on earthworm distribution

The number of earthworm species and relative significance of different ecological categories were determined by abiotic factors, type of vegetation and soil characteristics (Lavelle and Spain, 2001). The physico-chemical characteristics of soil, presence of organic matter and climatic condition of a particular site determined the earthworm diversity of that area (Hackenberger and Hackenberger, 2014). In this study, total five principal components were identified which explains 75.49% of the total variance. According to Liu et al (2003), factor loadings are classified as strong, moderate and weak corresponding to absolute loading values >0.75, 0.75–0.50 and 0.50–0.30. PC1 explaining the 21.57% of total variance, has strong positive loading of silt and OC, moderate loading of clay and strong negative loading of sand and ash. Capowiez et al. (2012) also reported that the variables such as soil bulk density or soil texture may influence earthworm growth and activity. Nordstrom and Rundgren (1974) reported that the abundance of *Aporrectodea rosea* in alluvial soil was due to higher clay content. Soil organic carbon is the critical factor in the distribution and abundance of earthworms at a particular site (Chan and Barchia, 2007). Thus PC1 indicated the soil texture. PC2 explained 18.73% of total variance with moderate positive loading of pH and K and strong negative loading of EC and TDS. This corroborates the findings of Sanchez et al. (1997) on earthworm preference toward salt concentrations. Thus, PC2 represents chemical factors. PC3 explains 16.37% of total variance with positive loading of moisture and negative loading of phosphorus which are significant in the distribution of earthworms (Curry 2004). Thus PC3 represents growth factors. PC4 explains the 9.48% of the total variance with positive loading of Lithium and PC5 explains 9.14% of the total variance with positive loading of Ca as it is used by earthworms for cocoon formation and negative loading of N. Sabrina et al. (2009) reported Ca as significant in influencing earthworms' population in soils. Thus PC4 and PC5 represent edaphic factors. Soil moisture content plays an important role in the occurrence and distribution of earthworm species (Bhaduria and Ramakrishnan, 1989; Dash and Senapati, 1980). Total earthworm density and biomass were strongly correlated with each other and positively associated with soil moisture (Crusmey et al., 2014). The present study also showed that occurrence of maximum number of species at sites VI and III is due to high moisture content i.e. more than 90% at these sites.

Earthworm distribution

Total five earthworm species were found: *M. posthuma*, *L. mauritti*, *A. morissi*, *E. waltoni* and *E. incommodus* from 21 different sites in northwestern Punjab. Mohan et al. (2013) also reported *M. posthuma*, *A. morissi*, *L. mauritti* and *E. incommodus* from the campus of Guru Nanak Dev University, Amritsar but *E. waltoni* has been reported for first time in this region. Earthworm populations are very sensitive to land use practices, which directly affects the distribution of earthworms. *M. posthuma* was present in all types of lands like vegetable lands, agricultural land and garden soil while the highest population density of *M. posthuma* was recorded in agricultural fields having vegetable and fodder crops. This high abundance of *M. posthuma* in agriculture field is due to its endogeic ecological category. During adverse condition and agricultural practices it can go upto 15–20 cm deep into the soil. The differences in agricultural management practices affect the population density and biomass of earthworm (Amador et al. 2013). Diversity of epigeic species in agricultural field was low due to physical disturbance of the soil during ploughing and intensive use of insecticide and pesticide. Our results are corroborated with the findings of Lagerlof et al. (2002) and documented that endogeic species are more affected by heavy soil cultivation, which damage their burrows but can grow best in moderately cultivated soil with sufficient amount of food. The lowest population density of epigeic worms during winter season may be due to its superficial nature. The epigeic worms were more affected by dry and warm summer having less moisture. Their population declined in the uncultivated area. They do not undergo aestivation during adverse condition and therefore mortality is high during warm and dry period (Lagerlof et al. 2002). The earthworm can restored population during rainy season which may be due to its high reproduction rate and more availability of food. The remaining four species were present only in garden and nursery soil having moisture level more than 80%. Agriculture with manure, fertilizers, moderate soil cultivation and varying crop in general is favourable for earthworm except for certain species (Lagerlof et al. 2002). It was observed that *M. posthuma* has been completely adapted to physical disturbance, intensive use of insecticide and pesticide and human intrusion. *M. posthuma* is a burrowing worm which belongs to the endogeic ecological category (Suthar, 2009). Jouquet et al (2010) also reported that endogeic earthworms are the most resistant earthworm recorded in disturbed soil. This burrowing nature of earthworm protects it directly from effects of insecticides and pesticides and mechanical disturbance produced during agriculture management practices. Our results are corroborated with findings of Ernst and Emmerling (2009), who determined that ploughed fields contain fewer anecic earthworms but higher abundance of endogeic earthworm. They also found that ploughing increased availability of organic matter to earthworms which can positively influence endogeic species. Chan (2001) suggested that ploughing resulted in destruction of burrows, burying of surface organic matter and change in soil physical properties which reduced the quantity of large anecic species but small endogeic species were able to survive better in ploughed field. Hackenberger and Hackenberger (2014) resulted that endogeic species were dominant in all seasons while the anecic category was only represented by one species per location or was completely absent. Crittenden et al (2014) also observed that ploughing decreased earthworm abundance and continued to decrease at subsequent samplings. Ploughing disturbs anecic species because it damages the burrows or tunnels made by earthworm, which may be the reason for why anecic species are abundant in gardens and nurseries. Soil with organic inputs also supports earthworm colonization (Suthar, 2009).

Species diversity index

The value of Shannon–Wiener index usually ranges from 0 to 4. A value near 0 indicates that every species in the sample is the same, whereas a value near 4 indicates that the numbers of individuals are evenly distributed between all the species. Our values lie between 0.11 and 0.37, which means that most sites had the same species of earthworm, i.e., *M. posthuma*. Our results are consistent with the findings of Holland (2004) and showed the relationship between soil structure and earthworm biodiversity. Higher earthworm diversity was recorded in gardens and nurseries due to low usage of inorganic pesticides and insecticide. Mohan et al (2013) collected earthworms from Guru Nanak Dev University Campus, Amritsar during different seasons and the Shannon–Wiener index was 1.08. Sharma and Bharadwaj (2014) studied Shannon–Wiener diversity index at agricultural fields and Gardens, which was 1.19 and 1.33 respectively. The majority of earthworm diversity reports showed the presence of two to five species at any single location (Edwards and Bohlen, 1996; Lee, 1985). High species richness and evenness was observed at site VI (0.60) and site X (0.53) respectively, which may be due to use of organic manure. This clearly supports the hypothesis that organic farming systems promote biological diversity (Suthar, 2009). Crittenden et al. (2014) also observed that organic farming has higher abundance of earthworms, biomass and Shannon diversity than conventional farming. Tripathi and Bharadwaj (2004) reported high species richness in agricultural lands but in our study species richness was zero in cultivated lands. The difference may be due to type of soil and agricultural practices. Mohan et al (2013) also determined species richness index (D_{Mg}) and Pielou evenness at a nursery at Guru Nanak Dev University, Amritsar, which are 0.29 and 0.98 respectively. Sharma and Bharadwaj (2014) showed species richness index (0.75) and Pielou evenness (0.45) in agricultural fields and in the gardens 0.18 and 0.20 respectively. Najar and Khan (2011) also reported high diversity index and evenness in vegetable garden soil. Crittenden et al. (2014) studied that mean species richness was significantly reduced from 4 to 2 after ploughing. The value of Pielou evenness ranged between 0 and 1 while species richness index has no limit value and shows variation depending upon the number of species. The closer the value to 1 means the more even is the distribution of species. Species richness index and Pielou evenness were zero at those sites where only one species of earthworm was found. Blanchart and Julka (1997) also found that flora in a particular area determined the relative abundance of earthworm species.

Conclusion

This study has provided new information regarding the effects of abiotic factors of soil on diversity and distribution of earthworm species. In this study, 5 species of earthworm have been identified i.e. *M. posthuma*, *L. mauritti*, *A. morissi*, *E. incommodus and E. waltoni*. *M. posthuma* is most abundant in agricultural fields, while the other four species are mostly found in gardens and nurseries. Principal component analysis also proved that the abiotic factors (pH, moisture, soil texture and OC) have strong positive effects on the distribution of earthworms. This study also revealed that diversity of earthworm was higher in gardens and nurseries having high doses of organic manure and minimum supply of chemical fertilizers and pesticide. Distribution of earthworm is also positively correlated with the type of crop sown and moisture content in the field.

Acknowledgement

We are thankful to Science and Engineering Research Board (SERB) Department of Science and Technology, GOI, New Delhi, India for financial assistance in the form of a Major research project letter No SR/SO/AS-030/ 2013. We are also thankful to Dr J. M. Julka, Shoolini University, Solan (Himachal Pradesh) for identification and confirmation of earthworm species.

References

Amador, J.A., Winiarski, K., Sotomayor-Ramirez, D., 2013. Earthworm communities along a forest–coffee agroecosystem gradient: preliminary evidences supporting the habitat-dependent feeding hypothesis. Trop. Ecol. 54, 365–374.

Amosse, J., Turberg, P., Kohler-Milleret, R., Gobat, J., Le Bayon, R., 2015. Effects of endogeic earthworms on the soil organic matter dynamics and the soil structure in urban and alluvial soil materials. Geoderma 243–244, 50–57.

Baretta, D., Brown, G.G., James, S.W., Bran, E.J., Cardoso, N., 2007. Earthworm populations sampled using collection methods in Atlantic forests with *Araucaria angustifolia*. Sci. Agric. 64, 384–392.

Behera, B., Giri, S., Dash, N.C., Senapati, B.K., 1999. Earthworm bioindicators of forest land use pattern. Indian. Forest. 124, 273–281.

Bhadauria, T., Ramakrishnan, P.S., 1989. Earthworm population dynamics and contribution to nutrient cycling during cropping and fallow phases of shifting agriculture in north east. Ind. J. Appl. Ecol. 26, 505–520.

Bhadauria, T., Ramakrishnan, P.S., Srivastava, K.N., 2000. Diversity and distribution of endemic and exotic earthworms in natural and regeneration ecosystems in the central Himalaya. India. Soil Biol. Biochem. 32, 2045–2054.

Blanchart, E., Julka, J.M., 1997. Influences of forest disturbance on earthworm communities in Western Ghat, South India. Soil Biol. Biochem. 29, 303–306.

Bouyoucos, G.J., 1962. Hydrometer method improved for making particle size analysis of soils. Agron. J. 54, 464–465.

Bremner, J.M., Mulvaney, R.G., 1982. Nitrogen total. In: Page, A.L., Miller, R.H., Keeney, D.R. (Eds.), Methods of Soil Analysis. Am Sco Agron, Madison, Wisconsin, pp. 575–624.

Capowiez, Y., Samartino, S., Cadoux, S., Bouchant, P., Richard, G., Boizard, H., 2012. Role of earthworms in regenerating soil structure after compaction in reduced tillage system. Soil. Biol. Biochem. 55, 93–103.

Chan, K.Y., 2001. An overview of some tillage impacts on earthworm population abundance and diversity- implications for functioning in soils. Soil Till. Res. 57, 179–191.

Chan, K.Y., Barchia, I., 2007. Soil compaction controls the abundance, biomass and distribution of earthworms in a single dairy farm in south-eastern Australia. Soil Till. Res. 94, 75–82.

Chandran, M.S.S., Sujatha, S., Mohan, M., Julka, J.M., Ramasamy, E.V., 2012. Earthworm diversity at Nilgiri biosphere reserve, Western Ghat, India. Biodivers. Conserv. 21, 3342–3353.

Chaudhuri, P.S., Nath, S., 2011. Community structure of earthworms under rubber plantations and mixed forests in Tripura. India J. Environ. Biol. 32, 537–541.

Crittenden, S.J., Eswaramurthy, T., de Goede, R.G.M., Brussaard, L., Pulleman, M.M., 2014. Effect of tillage on earthworms over short and medium term in conventional and organic farming. Appl. Soil Ecol. 83, 140–148.

Crusmey, J.M., Moine, J.M..Le., Vogel, C.S., Nadelhoffer, K.J., 2014. Historical patterns of exotic earthworm distribution inform contemporary associations with soil physical and chemical factors across a northern temperate forest. Soil Biol. Biochem. 68, 503–514.

Curry, J.P., 2004. Factor affecting the abundance of earthworms in soils. In: Edwards, C.A. (Ed.), Earthworm Ecology, second ed. St Lucie Press, Boca, Raton, pp. 91–113.

Darwin, C., 1881. The formation of vegetable mould through the action of worms, with observation on their habits Murray, p. 326, London.

Dash, M.C., 2012. Charles Darwin's Plough: Tools for Vermitechnology I K International, p. 185, New Delhi.

Dash, M.C., Senapati, B.K., 1980. Cocoon morphology, hatching and emergence pattern in tropical earthworms. Pedobiologia 20, 316–324.

Doan, T.T., Ngo, P.T., Rumpel, C., Nguyen, B.V., Jouquet, P., 2013. Interactions between compost, vermicompost and earthworms influence plant growth and yield: a one year greenhouse experiment. Sci. Hortic. Amsterdam 160, 148–154.

Edwards, C.A., Bohlen, P.J., 1996. Biology and Ecology of Earthworm. Chapman & Hall, London.

Ernst, G., Emmerling, C., 2009. Impact of five different tillage system on soil organic carbon content and density, biomass and community composition of earthworms after a ten year period. Eur. J. Soil Biol. 45, 247–251.

Evans, A.C., Guild, W.J., Mc, L., 1947. Studies on the relationship between earthworm and soil fertility I biological studies in the field. Ann. Appl. Biol. 34 (3), 307–330.

Gates, G.E., 1972. Burmese earthworms an introduction to the systematics and biology of megadrile oligochaetes with special reference to South East Asia Transactions of the American. Philos. Soc. NS 62, 1–326.

Guild, W.J.Mc.L., 1952. Variation in earthworm number within field population. J. Anim. Ecol. 21, 169–181.

Hackenberger, D.K., Hackenberger, B.K., 2014. Earthworm community structure in grassland habitats differentiated by climate type during two consecutive seasons. Eur. J. Soil Biol. 61, 27–34.

Holland, J.M., 2004. The environmental consequences of adopting conservation tillage in Europe: reviewing the evidences. Agric. Ecosyst. Environ. 103, 1–25.

John, M.K., 1970. Colorimetric determination of phosphorus in soil and plant material with ascorbic acid. Soil Sci. 109, 214–220.

Jouquet, P., Plumere, T., DoanThu, T., Rumpel, C., TranDuc, T., Orange, D., 2010. The rehabilitation of tropical soils using compost and vermicompost is affected by the presence of endogeic earthworms. Appl. Soil. Ecol. 46, 125–133.

Julka, J.M., 1988. The Fauna of India and the adjacent countries Megadrile Oligochaeta (Earthworms) Haplotaxida: Lumbricina: Megascolecoidea: Octochaetidae. Zoological Survey of India, Calcutta, 400pp + xiv.

Julka, J.M., Paliwal, R., 2005. Distribution of earthworms in different agro-climatic region of India. In: Ramakrishnan, P.S., Saxena, K. G., Swift, M.J., Raoks, Maikhuri, R.K. (Eds.), Soil Biodiversity, Ecological Processes and Landscape. Oxford and ABH Publications Co Pvt Ltd, New Delhi, pp. 3–13.

Kooch, Y., Jalilvand, H., 2008. Earthworm as ecosystem engineers and the most important detritivores in forest soil. Pak. J. Biol. Sci. 11, 819–825.

Lalthanzara, H., Ramanujam, S.N., Jha, L.K., 2011. Population dynamics of earthworm in relation to soil physico-chemical parameters in agroforestry systems if Mizoram, India. J. Environ. Biol. 32, 599–605.

Lavelle, P., Spain, A., 2001. Soil Ecology. Kluwer Academic Publishers, Dordrecht.

Lagerlof, J., Goffre, B., Vincent, C., 2002. The importance of field boundaries for earthworms (Lumbricidae) in the Swedish agricultural landscape. Agric. Ecosyst. Environ. 89, 91–103.

Lee, K.E., 1985. Earthworms Their Ecology and Relationship with Soils and Land Use. Academic press, Sydney.

Liu, C.W., Lin, K.H., Kuo, Y.M., 2003. Application of factor analysis in the assessment of groundwater quality in a blackfoot disease area in Taiwan. Sci. Total Environ. 313 (1–3), 77–89.

Margalef, R., 1958. Temporal succession and spatial heterogeneity in phytoplankton. In: Buzzati-Traverso (Ed.), Perspectives in Marine Biology. Univ Calif Press, Berkeley, pp. 323–347.

Michaelsen, W., 1909. The Oligochaeta of India, Nepal, Ceylon, Burma and the Andaman Islands. Memoirs of Indian Museum I.

Mohan, V.C., Watts, P., Kaur, A., 2013. Diversity and distribution of earthworm species in Guru Nanak Dev University Campus, Amritsar, Punjab, India. Res. J. Agric. Sci. 1 (2), 35–40.

Nainawat, R., Nagendra, B., 2001. Density and distribution of earthworm in different localities of Jaipur. J. Eco-Physiol. 4, 9–13.

Najar, I.A., Khan, A.B., 2011. Earthworm communities of Kashmir Valley, India. Trop. Ecol. 52, 151–162.

Nelson, D.W., Sommer, s.L.E., 1996. Total carbon and organic carbon and organic matter. In: Page, A.L., Miller, R.H., Keeney, D.R. (Eds.), Method of Soil Analysis. American Society of Agronomy, Madison, pp. 539–579.

Nordstrom, S., Rundgren, S., 1974. Environmental factors and lumbricid association in southern Sweden. Pedobiologia 14, 1–27.

Pielou, E.C., 1966. The measurement of diversity in different types of biological collections. J. Theor. Biol. 13, 131–144.

Rajkhowa, D.J., Bhattacharyya, P.N., Sarma, A.K., Mahanta, K., 2014. Diversity and distribution of Earthworms in different soil habitats of Assam, North-East India, an Indo-Burma Biodiversity Hotspots. Proc. Natl. Acad. Sci., India, Sect. B Biol. Sci.

Sabrina, D.T., Hanafi, M.M., Azwady, N.A.A., Mahmud, T.M.M., 2009. Earthworm population and cast properties in the soils of oil palm plantations. Malay. J. Soil. Sci. 13, 29–42.

Sanchez, E.G., Munoz, B., Garvin, M.H., Jesus, J.B., Cosin, D.J.D., 1997. Ecological preference of some earthworm species in southwest Spain. Soil Biol. Biochem. 29 (3/4), 313–316.

Satchell, J.E., 1955. Some aspects of earthworm ecology. In: Kevan, D. K., Butterworths, Mc E. (Eds.), Soil Zoology, pp. 180–201, London.

Shannon, C.E., Wiener, W., 1949. The Mathematical Theory of Communication Urbana. University of Illinois Press, 177.

Sharma, R.K., Bharadwaj, P., 2014. Earthworm diversity in trans-gangetic habitats of Haryana, India. Res. J. Agric. Sci. 2 (2), 1–7.

Singh, S.P., Singh, J., Vig, A.P., 2016. Earthworm as ecological engineers to change the physico-chemical properties of soil: Soil vs vermicast. Ecol. Eng. 90, 1–5.

Stephenson, J., 1923. Oligochaeta. In: The Fauna of British India. Fran Tay, London.

Suthar, S., 2009. Earthworm communities a bioindicator of arable land management practices: a case study in semiarid region of India. Ecol. Ind. 9, 588–594.

Suthar, S., 2011. Earthworm biodiversity in western and semiarid land of India. Environmentalist 31, 74–86.

Svendsen, J.A., 1957. The distribution of Lumbricidae in an area of Penine moorland (moor House, Nature reserve). J. Anim. Ecol. 26 (2), 411–421.

Tripathi, G., Bhardwaj, P., 2004. Comparative studies on biomass production, life cycles and composting efficiency of *Eisenia fetida* (Savigny) and *Lampito mauritii* (Kinberg). Bioresour. Technol. 92, 275–278.