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Diversity and distribution of earthworms in a natural reserved and disturbed sub-tropical forest ecosystem of Imphal-West, Manipur, India.

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Abstract
A comparative analysis of earthworm communities was carried out in sub-tropical forest ecosystem to understand the impact of biotic disturbances. Three sites selected were: natural mixed reserved forest, disturbed oak dominated sub-tropical forest ecosystem and managed oak plantation sector. We evaluated the presence and diversity of various earthworm genera and species in selected forests sites. Our investigation revealed the presence of at least 7 species of earthworms belonging to 5 genera and 4 families: Megascolecidae (*Metaphire houlleti*, *Metaphire anomala*, *Amynthas corticis,* and *Amynthas morrisi*.), Glossoscolecidae (*Pontoscolex corethrurus*) and Moniligastridae (*Drawida* sp.), Octochaetidae (*Eutyphoeu*s sp.). Glossoscolecidae and Megascolecidae were generally found in disturbed forest ecosystem. *Drawida* sp. had a mean density of 65 ind. m^{-2} in a natural reserved forest whereas in the disturbed oak dominated forest it showed maximum mean densities of only 15 ind. m^{-2}. Our results showed the great adaptability of Glossoscolicidae to various levels of disturbances and suggested the possibility of a large occurrence of this worm species in sub-tropical forest ecosystem of Imphal, Manipur.

Keywords: Native species, natural forest, disturbed forest, population density, diversity.

INTRODUCTION

Earthworms are the members of the class Oligochaeta of phylum Annelida. These are one of the major macrofauna of soil and have important roles in soil physical, chemical and biological properties [1]. Earthworms consume soil organic matter and litter thereby increasing availability of plant nutrients in their casts [2] and have been used as indicator of the health of soil ecosystems [3] due to their role in soil fertility through fragmentation and mixing of soil with mineral particles, promoting microbial activity and in breakdown of plant organic matter. Incorporation of litter, casting, burrowing and other activities of earthworm greatly alter soil physical structure, soil nutrients, biological soil organisms and ultimately to plants. But there is scarcity of data on the study of the basic biology and ecology of earthworms, especially in Manipur. Therefore, this study will provide new insight on earthworms in forest ecosystem of Imphal West, Manipur and will be valuable resources (species richness, population distributions, diversity) for future basic and applied earthworm research and also increase knowledge and understanding about earthworm for people who work in the field of ecology, agriculture and environmental science.

In the present paper, an attempt has been made to know the species composition, richness and their distribution in relation to some physico-chemical parameters of soil in sub-tropical forest ecosystem of Imphal - West. The results of the study are given in the present communication.

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MATERIAL AND METHODS

The survey of the present study was conducted during January to December 2009 in three study sites. The site I was located at natural mixed reserved sub-tropical forest of Koirengei about 10 km away from Imphal city, having an area of 25 hectares and it is protected from various biotic interferences. The site II is located at oak dominated Langol hills with frequent biotic interferences situated at a distance of 4 km away from site I. Site III is the managed oak plantation sector and is located on the valley area at Mantripukhri about 7-8 km away from Imphal city. These sites lie at $24^0 45'$ north latitude and $93^0 55'$ east longitudes and altitude ranges from 780 to 1100 above MSL. The soil of Manipur was developed from shale and sand stones on gentle sloping narrow valleys to steep slopes of hills and is heterogeneous in nature [4]. The climate of the area is monsoonic with warm moist summer and cool dry winter. The year is divisible into three season's *viz*. summer, rainy and winter. The month of March and October are the transitional ones between winter and summer and rainy and winter season respectively. The mean maximum air temperature varied from 25.64^0C (January) to 30.8^0C (May) and mean minimum air temperature varied from 5.68^0C (January) to 23.03^0C (July). Minimum monthly rainfall occurred in January (22.5mm) and maximum in July (203mm). The area received an average annual rainfall of 1001.6 mm. Relative humidity was recorded to be maximum in the month of August (83.21%).

Sampling of soil and earthworms

Sampling of earthworms and soil were done by using the tropical soil biology methodology [5]. At each site a plot of 10 × 10 m^2 were randomly selected for earthworm sampling as well as soil sampling. Six sub-plots of 1 × 1 m^2 are again selected. Eighteen 25 × 25 × 30 cm^3 soil monoliths were randomly sampled from each replicate plot at regular monthly intervals in all the study sites. The soil samples collected from 0-10 cm depth of the soil monolith were brought in the laboratories, air dried (bigger lumps crushed) and sieved through 2 mm sieve and stored for subsequent chemical analysis. Soil temperature was recorded every month at 0-10 cm depth using soil thermometer. Bulk density was measured using 10 × 5 cm^3 diameter corer at 0-10 cm depth. Moisture was determined by gravimetric method monthly at 0-10 cm depth and was expressed as a percentage of the weight of the sample after oven drying at 105^{0}C for 24 hours. Soil pH was measured in 1:2 soil water solutions using pH meter. Organic carbon was determined after modified Walkley and Black Method [6] and soil total nitrogen (N) by acid digestion Kjeldahl procedure as given by Anderson and Ingram [5]. Available phosphorus by ammonium molybdate stannous chloride method by Sparling et al. [7] and potassium by AAS 200 Atomic Absorption Spectrophotometer (Perkin-Elmer). Earthworms were sampled using the tropical soil biology methodology [5]. Earthworms were extracted by hand sorting after digging a trench up to 30cm deep around 25 × 25 cm^2 area at each sampling point to get soil monolith. Worms were counted and preserved in 4% formalin for taxonomic identification. Evenness, Margalef's and Shannon-Wiener index were used to compare the richness and diversity of earthworms among sites. The Pearson correlation was employed to find relationship among soil parameters and earthworm density.

RESULTS

The total earthworms recorded during one year study period were 12,520, 8670 and 4860 individuals in the forest sites I, II and III respectively. Altogether seven species belonging to five genera and four families of the class Oligochaeta were collected from the study sites (Table 1). The four species of Megascolecidae included *Metaphire houlleti*, Perrier, 1872 [8], *M. anomala*, Michaelsen, 1907 [9], *Amynthas morrisi*, Beddard, 1892 [10], and *A. corticis*, Kinberg, 1867[11]. Moniligastridae was represented by *Drawida* sp., Octochaetidae by *Eutyphoeus* sp. and Glossoscolecidae by *Pontoscolex corethrurus*, Müller, 1856 [12]. *Drawida* sp. exhibited the most dominant earthworm group contributing 38.3% in site I. In site II, its contribution was only 12.5% and was absent in site III. The next dominant earthworm in site I was *Eutyphoeus* sp. with 23.6%, *Eutyphoeus* sp. was absent in site II and III. In site II and III, *P. corethrurus* exhibited the most dominant earthworm group with 45.1% and 47.9 % respectively while site I has only 7.2%. *M. houlleti* was next dominant species in site II contributing 42.4%, 14.7% only in site I and was absent in site III. *M. anomala* was recorded from site I only with 16.3% contribution and were absent in site II and III. *A. morrisi* and *A. corticis* was found only in site III contributing 33.46% and 18.58% respectively to the total earthworms collected of the site.

All the species exhibited maximum population density during the rainy season and gradually decreases in summer and winter (Table 3). Mean value of *Drawida* sp. during rainy season showed 97 m^{-2}, 22.80 m^{-2} in site I and II respectively. *Eutyphoeus* sp. exhibited 65.20 m^{-2} in site I. *P. corethrurus* showed 20 m^{-2}, 80 m^{-2} and 54 m^{-2} in site I, II and III respectively. The physico-chemical variables of all the sites are given in Table 2. The maximum soil temperatures were recorded during rainy season and exhibited a decreasing trend till winter season. The maximum temperature recorded were 23.89^{0}C, 23.78^{0}C and 28.55^{0}C in site I, II, and III respectively. The minimum soil temperatures as recorded during winter season were 16.15^{0}C, 13.03^{0}C and 19.41^{0}C in site I, II and III respectively. The percentage of soil moisture of the site I range from 21.11 % (winter season) to 32.24 %(rainy seasons). In site II and III the soil moisture ranges from 18.17 % to 33.77% and 16.78 % to 28.42 % respectively. Bulk density ranges from 1.18 g cm^{-3} to 1.26 g cm^{-3} in site I. In site II and III bulk density ranges from 1.21 g cm^{-3} to 1.28 g cm^{-3} and 1.35 g cm^{-3} to 1.42 g cm^{-3} respectively. The soil pH showed slightly acidic in nature ranging from 5.09 (minimum) to 5.9 (maximum). Soil organic carbon was recorded highest in site I during rainy season (4.72 %). Site II and III showed 3.50 % and 3.46 % respectively. The soil nitrogen was also recorded highest in site I with a value of 0.69 % during rainy season, followed by site II (0.61 %) and least in site III (0.44 %). Soil phosphorus ranges from 0.06 % to 0.09 % in site I. In site II and III the value ranges from 0.05 % to 0.08 % and 0.01 % to 0.05 % respectively. Soil potassium was also recorded highest in site I (0.51 %) in rainy season followed by site II (0.413 %) and least in site III (0.343 %).

Table 1. Distribution of earthworms in three study sites of sub-tropical forest ecosystem.

Earthworm species	SITE I	SITE II	SITE III
Drawida sp.	++	+	–
Eutyphoeus sp.	++	–	–
Pontoscolex corethrurus	+	++	++
Metaphire houlleti	++	++	–
Metaphire anomala	++	–	–
Amynthas morrisi	–	–	+
Amynthas corticis	–	–	+

- = Absent
++ = High population density
+ = Low population density

Table 2. Abiotic variables and physico chemical characteristics of soils in forest Site I, II and III (0 - 10cm depth).

Sites	Seasons	Temp (°C)	Moisture (%)	Bulk density (g cm^{-3})	pH	Organic Carbon (%)	Nitrogen (%)	Phosphorus(%)	Potassium (%)
I	Summer	20.58	24.92	1.260	5.56	3.67	0.590	0.046	0.390
	Rainy	23.89	32.24	1.180	5.09	4.72	0.690	0.090	0.510
	Winter	16.15	21.11	1.260	5.51	3.19	0.520	0.060	0.350
II	Summer	22.62	29.73	1.287	5.67	3.13	0.563	0.034	0.346
	Rainy	23.78	33.77	1.207	5.54	3.50	0.614	0.081	0.413
	Winter	13.03	18.17	1.285	5.86	2.26	0.452	0.055	0.273
III	Summer	27.39	18.50	1.437	5.8	2.23	0.344	0.028	0.255
	Rainy	28.55	28.42	1.348	5.48	3.46	0.440	0.055	0.343
	Winter	19.41	16.78	1.417	5.90	1.92	0.277	0.013	0.205

Table 3.Seasonal variation of population density of earthworms m^{-2} in Site I, II and III. (Mean ± S.D).

Earthworm Species	Sites	Seasons			
		Summer	Rainy	Winter	Annual
Drawida sp.	I	46.00 ± 3.33	97.00 ± 5.87	39.20 ± 4.53	65.00 ± 4.37
	II	10.33 ± 0.80	22.80 ± 1.47	8.70 ± 0.60	15.00 ± 1.03
	III	-	-	-	-
Eutyphoeus sp.	I	25.33 ± 1.50	65.20 ± 4.70	22.50 ± 2.78	41.00 ± 3.26
	II	-	-	-	-
	III	-	-	-	-
Pontoscolex corethrurus	I	8.33 ± 1.15	20.00 ± 2.08	6.00 ± 0.96	12.42 ± 1.23
	II	44.33 ± 7.30	80.20 ± 8.41	29.50 ± 5.46	54.33 ± 5.06
	III	23.67 ± 2.37	54.00 ± 5.22	11.75 ± 1.96	32.33 ± 3.21
Metaphire anomala	I	20.0 ± 2.14	45.80 ± 2.94	12.75 ± 1.96	28.33 ± 2.31
	II	-	-	-	-
	III	-	-	-	-
Metaphire houlleti	I	12.67 ± 2.23	42.80 ± 3.86	13.50 ± 2.67	25.50 ± 2..57
	II	31.67 ± 3.48	78.60 ± 4.38	31.25 ± 5.70	51.08 ± 3.89
	III	-	-	-	-
Amynthas morrisi	I	-	-	-	-
	II	-	-	-	-
	III	16.67 ± 1.67	30.20 ± 2.65	17.50 ± 1.80	22.58 ± 1.52
Amynthas corticis	I	-	-	-	-
	II	-	-	-	-
	III	7.67 ± 1.01	19.20 ± 1.62	8.00 ± 1.26	12.58 ± 1.06

Table 4. Correlation studies between population density of earthworms (m^{-2}) and abiotic factors in Site I, II and III.

Earthworm species	Sites	Temperature (°C)	Moisture (%)	Bulk Density (g cm^{-3})	pH	Organic carbon (%)	Nitrogen (%)	Phosphorus (%)	Potassium (%)
Drawida sp.	I	0.746**	0.850**	-0.958**	-0.678*	0.812**	0.906**	0.850**	0.879**
	II	0.677*	0.771**	-0.850**	-0.680*	0.705*	0.863**	0.600*	0.829**
	III	0.401	0.837**	-0.837**	-0.656*	0.837**	0.811**	0.817**	0.628*
Eutyphoeus sp.	I	0.636*	0.796**	-0.940**	-0.662*	0.732**	0.827**	0.824**	0.791*
	II	-	-	-	-	-	-	-	-
	III	-	-	-	-	-	-	-	-
Pontoscolex corethrurus	I	0.676*	0.772**	-0.940**	-0.670*	0.769**	0.905**	0.789**	0.817**
	II	0.753**	0.805**	-0.940**	-0635*	0.633*	0.719**	0.701*	0.804**
	III	0.625*	0.907**	-0.780**	-0.670*	0.890**	0.895**	0.957**	0.849**
Metaphire anomala	I	0.791**	0.839**	-0.932**	-0.797**	0.869**	0.888**	0.799**	0.902**
	II	-	-	-	-	-	-	-	-
	III	-	-	-	-	-	-	-	-
Metaphire houlleti	I	0.657*	0.746**	-0.955**	-0.716**	0.758**	0.817**	0.881**	0.788**
	II	-	-	-	-	-	-	-	-
	III	0.238	0.952**	-0.967**	-0.910**	0.855**	0.889**	0.780**	0.505*
Amynthas morrisi	I	-	-	-	-	-	-	-	-
	II	-	-	-	-	-	-	-	-
	III	0.154	0.908**	-0.952**	-0.837**	0.729**	0.803**	0.698*	0.373
Amynthas corticis	I	-	-	-	-	-	-	-	-
	II	-	-	-	-	-	-	-	-
	III	0.347	0.945**	-0.958**	-829**	0.865**	0.938**	0.845**	0.609*

N.S. - not significant, *P <0.05, ** P< 0.01.

Table 5. Showing the total number of earthworms in different seasons of the year in the three sites.

Earthworm species	Seasons	Site I	Site II	Site III
Drawida sp.	Summer	828	234	0
	Rainy	3032	684	0
	Winter	942	210	0
Eutyphoeus sp.	Summer	456	0	0
	Rainy	1956	0	0
	Winter	540	0	0
Pontoscolex corethrurus	Summer	150	798	426
	Rainy	600	2406	1620
	Winter	110	708	282
Metaphire anomala	Summer	360	0	0
	Rainy	1374	0	0
	Winter	306	0	0
Metaphire houlleti	Summer	228	570	0
	Rainy	1284	2358	0
	Winter	324	750	0
Amynthas morrisi	Summer	0	0	300
	Rainy	0	0	906
	Winter	0	0	420
Amynthas corticis	Summer	0	0	138
	Rainy	0	0	576
	Winter	0	0	192

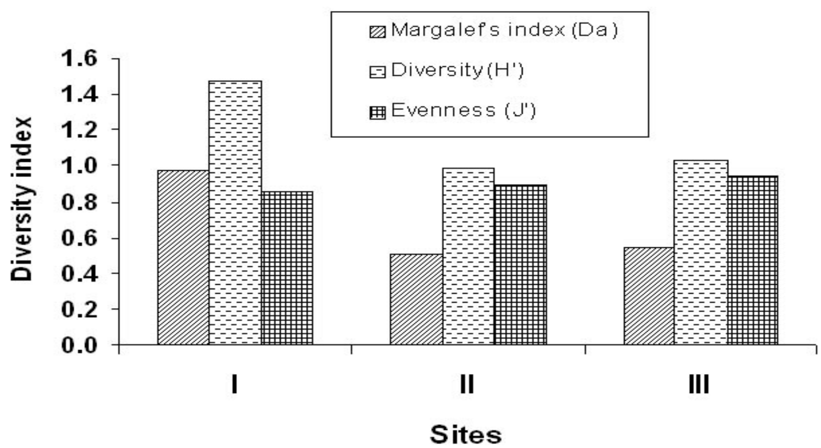

Fig1. Showing species diversity and evenness of the three sites during one year study period.

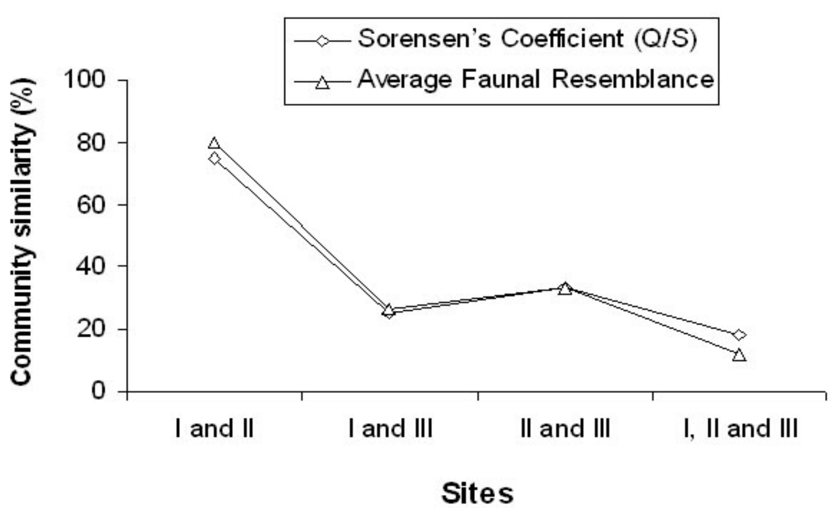

Fig 2. Showing community similarity of earthworms between the study sites.

Correlation studies between earthworm species and physico-chemical factors such as temperature, moisture, bulk density, pH, organic carbon, nitrogen, phosphorus and potassium were carried out and given in (Table- 4). Correlation studies between populations of earthworm species showed positive significant relationship with moisture, organic carbon, nitrogen, and phosphorus in all the study sites. However, with temperature and potassium it exhibited positive but not significant correlationship with some species in some of the sites. All the species recorded showed negative correlation with bulk density and pH in all the study sites.

The species diversities, richness and similarities of communities were analyzed using the following indices of Shannon – Wiener Index (H') [13], Margalef's index (Da) [14], Sorensen's index (Q/S) of similarity [15], and evenness or equitability index of Pielou [16]. Analysis of data's of earthworm species by using Shannon - Wiener diversity index (H') and Margalef's index (Da) have shown the maximum value of diversity and richness in Site I, followed by site II and III (Figure -1). The community similarity of earthworm species of the reserved forest and disturbed sites were compared using Sorensen's coefficient of community similarity index (Quotient of similarity) and average faunal resemblance (Figure- 2). Sorensen's Quotient of community similarity (Q/S) and average faunal resemblance of the earthworm species between site I and II was highest (75% and 80% respectively), followed by Site II and III (33.33%), site I and III (26% and 26.67% respectively) and site I, II and III (12.22% and 18.18% respectively). A value of evenness index (J') was highest in site III, followed by site II and I (Figure- 1).

DISCUSSION

A total of 26,050 individuals of 7 earthworm species were found in this study. They belonged to the following four families: Monoligastridae, Octochaetidae, Glossoscolecidae, and Megascolecidae. Five species of earthworms distributed in four families in site I, three species belonging to three genera and three families in site II, and three species belonging to two families in site III were found throughout the study period (Table 1). Seven species were *Drawida* sp, *Eutyphoeus* sp., *Pontoscolex corethrurus, Metaphire houlleti, Metaphire anomala, Amynthas morrisi* and *Amynthas corticis.* The most abundant was *P. corethrurus* and present in all the three sites. The rarest were *Eutyphoeus* sp, found only in site I*, A. morrisi* and *A. corticis* species group found only in site III.

It was also observed a variation of earthworm density and diversity in all the three study sites which were found to affect by several microclimatic or abiotic factors of the soil ecosystem. Among the abiotic factors the soil physico-chemical factors such as soil moisture content, soil temperature, bulk density and nutrient content viz. organic carbon, and total nitrogen have been found to play an important role in the variation of population structure and species diversity of earthworms of the study sites. In the present investigation, the maximum soil moisture content were found during rainy season and gradually decreased during summer and winter season (Table - 2), the population density also showed the similar trend with highest population density during rainy season (Table 3) where moisture was also highest. Among the different edaphic factors studied soil moisture content was found to play the most important role in the fluctuation patterns of the earthworm population. The importances of soil moisture content in relation to population of earthworm in India were reported by Dash and Senapati, [17] and by others Julka, [18, 19], Bhadauria and Ramakrishnan, [20, 21]. Blanchart and Julka, [22] have also recorded higher number of earthworm during wet periods. In the present investigation the maximum soil moisture content recorded during the rainy seasons exhibited high significant positive correlation with the total population in all the three sites (Table- 4). Rainfall together with relative humidity during rainy season leads to the increased in earthworm's population. Low rainfall and moisture content in winter season probably decreased the population of earthworm which was clearly revealed from the result of the present investigation. Earthworm's population density is the result of the interaction of a number of factors of which moisture is of greater importance [23]. The soil organic C, N, P and K were recorded maximum during rainy season followed by summer and winter season in all the study sites. High status of soil organic carbon, N, P, and K may be due to higher decomposition rate of litter and availability of all favorable micro-climatic conditions which enhanced the decomposition during rainy season. The higher population density of earthworms in reserved forest as compared to disturbed forests may also be attributed to the higher organic C, N, P and K content in the reserved site which had a direct influence on the availability of food sources of earthworms.

The highest species diversity and species richness of earthworm was found in natural mixed reserved forest, followed by disturbed oak dominated and managed oak plantation site.(Figure 1). The evenness of earthworm was highest in site III (Figure- 1). The high species diversity and richness of earthworm in site I may be due to absence of biotic interference, good canopy cover of mature varieties of tree species, good litter fall, under sub-tropical condition of Imphal probably these factors provided congenial physical habitat and trophic resource for earthworms. The greater litter accumulation on the ground of forests can lead to higher biodiversity of macrofauna likely due to the availability of territory space, food, shelter and protection from predation by other animals [24]. The lesser species diversity and richness of earthworm in site II and III may be due to various biotic interference such as cutting of woods, removal of litter by burning of forest floor, open canopy as such less organic matter may have cause some of the species unable to thrive in the said sites. The soil organic carbon, nitrogen, phosphorus and potassium was comparatively more in the natural mixed reserved forest than disturbed oak dominated forest and managed oak plantation sites. This may be due to the higher accumulation of litter and higher decomposition rate of organic matter in the reserved site than that of disturbed sites. Our findings were in agreement with Bhadauria and Ramakrishnan [21], who reported that large scale destruction of natural forests has severely affected the diversity of earthworms. Similarly the slash and burn system in the forest of Meghalaya has been responsible for reduction of original forest species of earthworms [25]. The absence of native species such as *Eutyphoeus* sp. in site II and III and negligible number of *Drawida* sp. in site II and absence in III supported the above findings. *Eutyphoeus* sp. showed restricted distribution, found only in site I due to narrow range of ecological tolerances. *Eutyphoeus* is endemic species in Burma, Eastern Himalaya and North-East ranges [26]. The presence of a native species is generally associated with well preserved natural ecosystem. Endemic peregrine species *Drawida* sp. dominated natural mixed reserve sub-tropical forest though few numbers was found in site II may be due to less disturbances compared to site III where it was totally absent. Exotic worms like *P.*

corethrurus were quite good in number in site II and III where biotic interference was quite high. *P. corethrurus* are adapted to wide range of environmental condition. Besides environmental plasticity, parthenogenetic mode of reproduction, anthropogenic influence, efficient assimilation of low quality soil organic matter and outstanding abilities to colonize due to their demographic profile could explain the wider distribution of *P. corethrurus* and absence of native species in other regions [27]. The present investigation on various soil parameters showed more favorable conditions for worms in mixed reserved forest ecosystem as compared to disturbed oak dominated and managed oak plantation forest (Table 2) this is the reason that a higher value of density and diversity was recorded from this area. Phillipson et. al. [28] and Baker et. al. [29] have also been reported that difference in various chemical properties, viz. organic C, N, P, K etc. are factors responsible for the distribution and abundance of earthworms in soil of an area. Therefore the present investigation concludes that effect of various climatic and edaphic factors rather than single was responsible for diversity, distribution and abundance of earthworms.

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