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Comparative study on Population of Earthworms in Different Habitat Types along altitude in Tsholingkhar gewog, Tsirang district, Bhutan

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Abstract— *Earthworms are one of the very diverse organisms in the environment. The abundance of the earthworms relates to the different land use, human activity, biotic and abiotic factors on nature. The diversity and abundance of earthworms was studied in different habitats; broadleaved forest, chirpine forest, residential area and agriculture land with the aim to understand the variation in earthworm species in those habitats. Between the altitude 650-1450masl. a total of 20 major plots and 100 sub-plots was made to assess the earthworm diversity in selectedhabitat. Physio-chemical analysis of soil was done to know the diversity, abundance and density of earthworms. The result of study does find two orders, five families and seven species of earthworms. They were Amynthasalexandri, Metaphirehoulleti, Perionyx excavatus, Aporrectodeacalciginosa, Dichogastersp., Pontoscolexcorethrurus and Darwidasp. Broadleaved had the highest diversity with Shannon index of 2.04 and the lowest diversity was found in chirpine forest with Shannon index of 1.6. The highest richness was in the broadleaved forest with index of 0.827. Amynthasalexandri was present in all the habitats and it had the highest relative abundance of 28.12%, relative density of 32.80 per m2 and frequency of 25%. The lowest relative density, abundance and frequency was found in Darwida sp. The analysis of variance showed thatthe NPK content in the soil has effect on the density of earthworm along the altitude. In lower altitude at 650 masl. The density of earthworms was more with a high amount of NPK in soil and in higher altitude at 1450masl. the decrease in NPK showed low earthworm density. Pearson correlation showed a positive correlation with soil Physico-chemical parameters and an abundance of earthworms.*

Keywords*— *Altitude, Diversity, Earthworms, Habitats, Soil parameters.

I. INTRODUCTION

Earthworms are burrowing saprozoic and geophagic organisms living with different types of microorganisms in the environment. They are known for their cross-fertilizing ability though they are hermaphrodite's oligochaete (Zhenjun, 2011). Earthworms provide a supportive role in maintaining abundance, biomass, species composition and diversity of plants (Lazcano*et al*., 2008). Therefore, the earthworms are widely accepted as organisms that perform ecosystem services (Millennium Ecosystem Services [MEA], 2005).

In worldwide 6,200species of earthworms are present (Csuzd, 2012). The neighboring country India has 505 species in 10 families have been identified (Kathireswari, 2016). Meanwhile there is limited record and scientific investigation of this important creature in Bhutan. Moreover, with rampant developmental activities and land-use systems in Bhutan, there are chances of the disappearance of these earthworms from the country. Therefore, the present study aims to investigate the earthworm diversity and abundance in different habitats and to determine the relationship between the soil Physico-chemical characters and earthworm diversity and abundance. Also, to analyze the variation between the soil nutrients and earthworm density along the altitude.

II.

III. METHODS AND MATERIALS

Study area

The study on the diversity of earthworm was carried out in Tsholingkhar gewog of Tsirang district which is located in the southern part of Bhutan. The district is located at 26°49' to 27°11' latitude and 90°00' to 90° 20' longitude. The altitude is between 300 meters to 4200 meters above sea level (MoAF, 2017). The dzongkhag has forest coverage of 87.50%. The forest is dominated by broadleaf with very few areas with chirpine and mixed conifer. The annual rainfall ranges from 1000mm to 3000mm per year. The agriculture land-use types are kamzhing and chhuzhing. The forest and agriculture land have sandy loamy, black soil and clay loamy soil with some red soil (MoAF, 2017).

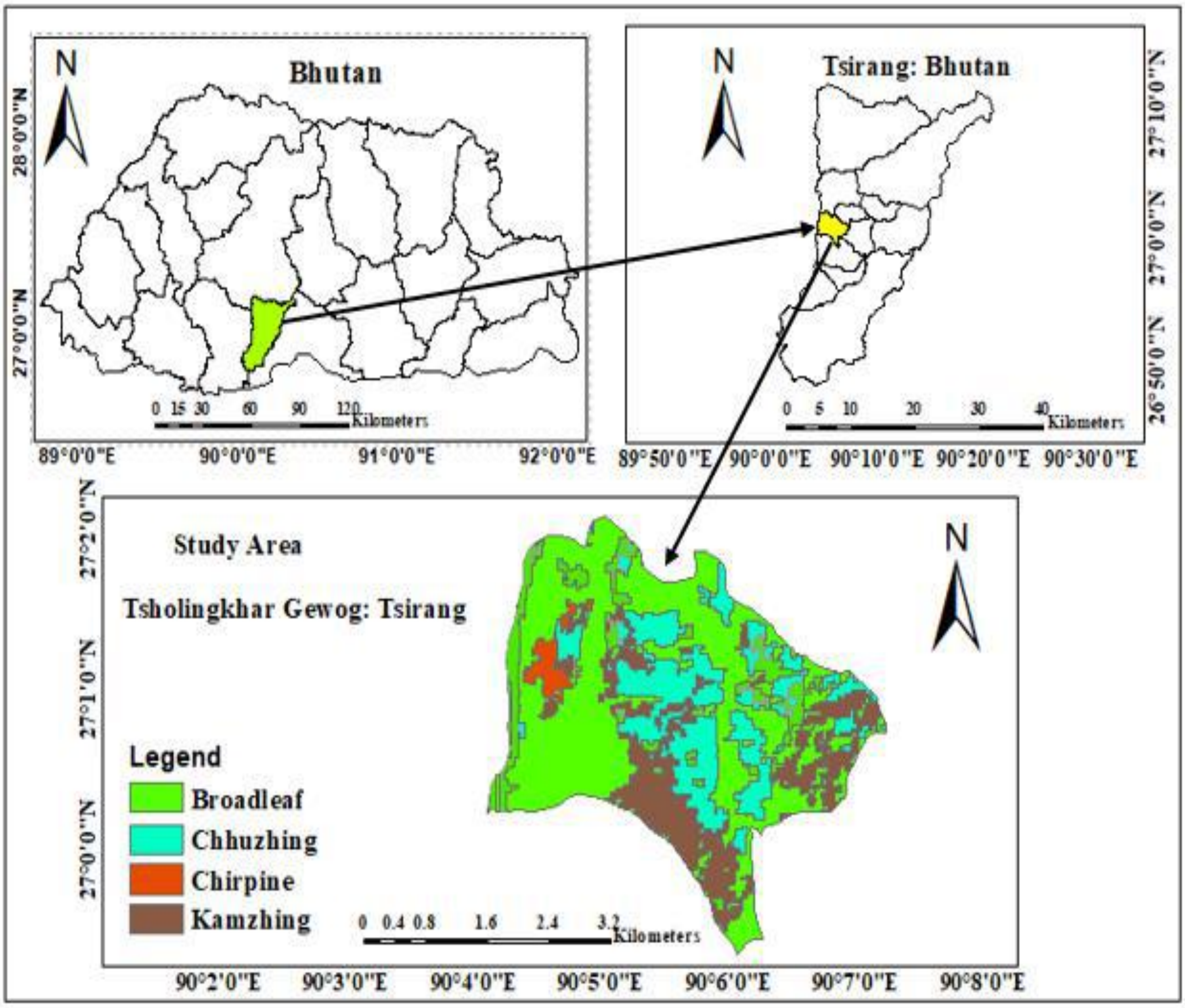

Fig.1: Map showing study area

Sampling method

The study was done in four different habitats 1) Broadleaved forest 2) Chirpine forest 3) Residential area and 4) Agriculture land along 650-1450masl.The difference between the altitudes was kept 200m. In each altitude, 4 different major plots were allocated in four habitats. The plot size for the major plot was 10*10m^2 and in each major plot, 5 subplots was randomly selected. The size of the subplot was 1*1m^2 and a minimum distance of 1.5m was kept from one subplot to another. In total 100 subplotswere studied within 20 major plots between altitude 650-1450masl.

A combination of passive and behavioral techniques was used to collect earthworms (Bouche, 1969). Passive involves hand sorting of earthworms from the soil, litter and other habitats (Bouche, 1969). Behavioral captures earthworms after they move out of the soil (Lee, 1985). In each quadrate depth of 10cm was dug to find earthworms (Dickey andKladivko, 1989).

In total 20 soil samples were collected from each habitat for soil analysis. Soil moisture was calculated using the Gravimetric method (ZiadatandTaimeh, 2013). Soil organic matter and organic carbon by loss of the ignition method. The power of hydrogen was measured calibrating with the buffer solution of known pH. Soil Phosphorus was calculated using Olsen's method. Soil Nitrogen was calculated using Kjeldahl method and Potassium with Flame photo-metric method (Karltun*et al*., 2013).

Data analysis

The data collected from the field was compiled in excel. The species diversity of earthworms in different habitat was calculated with various indices. The Shannon diversity, Pielou evenness Margalef richness, Berger Parker dominance was calculated (Ludwig*et al*., 1988; Morris *et al*., 2014).

Equation 1: Shanon-Wiener Diversity Index

$H' = -\Sigma pi \ln pi$, [where pi is the relative abundance of the species (pi = ni/N; ni stands for the number of individual species and N stands for the total number of individual earthworms.)]

Equation 2: Margalef richness

$M = (S-1) / \ln N$, [where S: Total number of species; N: Total number of individuals.]

Equation 3: Pielou evenness

$H/\ln S$, (S: number of species in a community; H: Shannon index.)

Equation 4: Berger Parker dominance

$d = Nmax/N$, (Nmax: number of individuals in the most abundant species; N: Total number of individuals in a sample.)

Also, the Relative abundance, Density, Relative frequency and Relative density was calculated with the various formula (Ghavzan*et al*., 2006; Miléo*et al*., 2016).

1. Relative abundance = Abundance of individuals of a species/ Total abundance of all species * 100.
2. Abundance= Total number of individuals of a species in all quadrates/ Total number of quadrates in which the species occurred.
3. Relative frequency= Number of quadrates in which species occurred/ Total number of quadrate occupied by all species * 100.
4. Density = Total number of individuals of species/ Total number of quadrates used in sampling.
5. Relative density= Total number of individuals of species/ Sum of all individuals of all species *100.

The correlation was analyzed between soil physio-chemical characters and abundance of earthworms. One-way analysis of variance performed between NPK and density of earthworms along altitude. Bray-Curtis cluster analysis was performed to find habitat similarity of earthworm with soil physio-chemical parameters.The earthworm identification was done looking at the morphological and anatomical characteristics (Gates, 1972;Julka, 1988; Sims and Gerard, 1985;Stephenson, 1923)and the species confirmation was sought from earthworm taxonomists.

Materials

GPS was used to record altitude and coordinates, plastic bag, test tubes and stationery to record field data.

IV. RESULT AND DISCUSSION

Earthworm composition in different habitat

A total of 375 earthworms was found in various study habitat with 198 juveniles and 177 mature earthworms. The seven different species of earthworms (Table1) was found in different habitat belonging to two order and five families.In order, Haplotaxida, *Amynthasalexandri*, *Metaphirehoulleti* and *Perionyx excavates* species was found in family Megascolecidae. *Aporrectodeacalciginosa* in family Lumbricidae. *Dichogaster*sp. in family Octochaetdae and *Pontoscolexcorethrurus* in family Glossoscolecidae. In order Moniligastrida, *Darwida*sp. was the only species found in family Moniligastridae.

Table 1: Earthworm species found in different habitat

Order	Family	Earthworm species	CP	BP	AP	RP
Haplotaxida	Megascolecidae	*Amynthasalexandri*	+	+	+	+
Haplotaxida	Megascolecidae	*Metaphirehoulleti*	+	+	+	—
Haplotaxida	Megascolecidae	*Perionyx excavatus*	—	—	+	+

Haplotaxida	Lumbricidae	*Aporrectodeacalciginosa*	—	+	—	—
Haplotaxida	Octochaetdae	*Dichogaster*sp.	—	+	—	—
Haplotaxida	Glossoscolecidae	*Pontoscolexcorethrurus*	—	+	—	+
Moniligastrida	Moniligastridae	*Darwida*sp.	—	—	—	+

(—)Absent, (+)Present, CP:Chirpine, BP:Broadleaved, AP:Agriculture, RP:Resident

Variation in indices among different habitat

The number and type of species found in different habitat showed variation in diversity, evenness, richness and dominance of earthworm species (Figure 2).Shannon diversity (*H*) was highest in broadleaved forest (*H*=2.04) since it was foundthat the broadleaved habitat had the good ratio of soil physico-chemical parameters and more species of earthworms. Lowest diversity in chirpine forest (*H*=1.6) was due to low amount of soil physico-chemical parameters and only two species was found in chirpine habitat. The agriculture and residential area had diversity index 1.79 and 1.97 respectively with four species of earthworm in residential habitat and three species of earthworm in agriculture habitat.

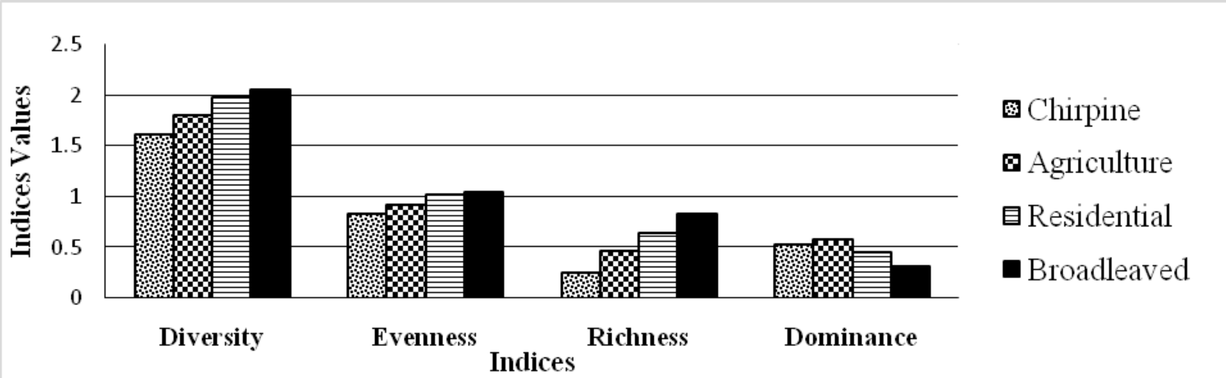

Fig.2: Indices comparison for different habitats

On an average Poulie evenness was high in all the habitats. Where Broadleaved and residential had index of 1.04 and 1.01 respectively. Agriculture habitat had evenness of 0.91 and chirpine habitat had 0.82. All the habitat showed a narrow species count making the species evenness high in all the habitat. The species diversity and richness will be high in those habitats where the amount of soil physico-chemical parameters such as soil moisture, organic carbon and organic matter are high which favors the earthworm to live in the environment (Lee, 1985; Makin *et al*., 2014).

The broadleaved forest had the highest Margalef richness of 0.827 with five different species due to sufficient amount of feed for earthworms and it is natural forest with high litter content. Similarly,Tripathi and Bhardwaj (2004) reported higher diversity in a stable ecosystem than an unstable ecosystem having low litter contents. The chirpine forest had the lowest species richness of 0.243 with two species present in the habitat.

The residential area showed the richness of 0.635 with four species of earthworms and agriculture habitat richness was 0.463 with three species. Since this habitat are constantly added with organic manure and watered by farmers to increase the crop productivity that attracted earthworms to live in the area. The earthworms are diverse in areas with more intensive land management than less intensive management (Bullock *et al*., 2008; Najarand Khan, 2011).

Berger Parker dominance was highest in Agriculture land with value 0.573 followed by chirpine forest with value 0.525. In the residential area dominance value was 0.442 and in broadleaved forest dominance value was 0.317 (Figure 2). The dominance was high in chirpine forest because only two species was found in the habitat and low dominance was found in the broadleaved forest as there was five species having average individual count. A maximum of two to five species of earthworms found in particular site where the earthworm studies were carried out (Edward and Bohlen, 1996; Fragoso and Lavelle, 1992; Lee, 1985; Najarand Khan, 2011).

Population structure of individual earthworm species in habitats

Among all the earthworm species *Amynthasalexandri* showed the highest relative abundance with 21.82%

(Figure 3) with a total count of 123 individuals in 38 quadrates. It had the highest relative density of 32.80% andfrequency of 25% as it was found in all the habitats (Table 2). The study showed that the *Amynthasalexandri* can resist to change in environment conditions as the species was found in changing soil physico-chemical parameters in various habitat. Also, other studies showed*Amynthasalexandri* can adapt and live in various kinds of environments. The species can be found in managed land and undisturbed land. The species can adapt in places with low temperature and moisture respectively (Bhadauriaand Ramakrishnan, 1991; Bhadauria*et al*., 2000).

Perionyx excavates also showed high relative abundance (17.92%) with a relative density of 24.80% and had a relative frequency of 23.03% with 93 individuals in 35 quadrates on agriculture and residential habitat. The lowest relative abundance was shown by *Darwida*sp. with 8.99% and relative density of 2.13% and relative frequency 3.95% in six quadrates with 8 individuals present only in a residential area**.**

Both the species *Perionyx excavates* and *Darwida*sp. was found in residential habitat where there was enormous number of degradable wastes thrown by farmers. The species *Perionyx excavates* was found in some of the agriculture plots with low count where there was manure. The earthworm species are attracted in a man-made environment with presence of suitable temperature, moisture and feed for the earthworm (Bhadauriaand Ramakrishnan, 1991; Makin et al. 2014).

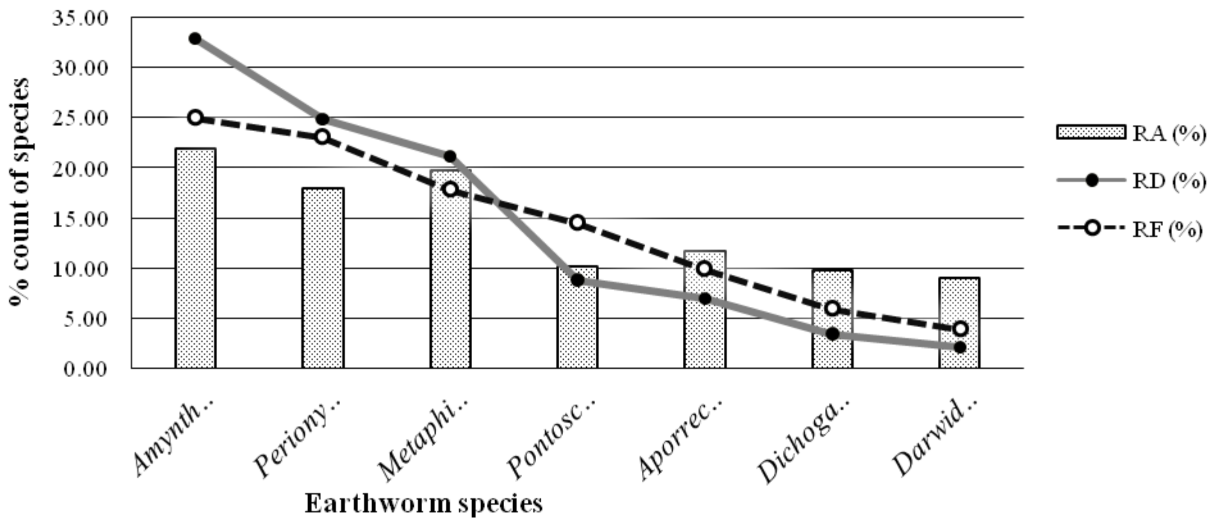

Fig.3: Individual earthworm species RA (Relative Abundance), RD (Relative Density) and RF (Relative Frequency)

In chirpine, broadleaved and agriculture habitat, *Metaphirehoulleti* had individual count of 79 in 27 quadrates making the relative abundance 19.73%, relative density 21.07% and frequency of 17.76%. In 15 quadrates *Aporrectodeacalciginosa* has individual count of 26 with relative abundance 11.69%, relative density 6.93% and relative frequency of 9.87% only in the broadleaved forest. It showed that the species prefer to live in different habitat base on the species-specific characteristics and the feed it gets from the habitat. Fragoso *et al*.,(1999) also reported that structural composition in earthwormvaries depending on the type of agro-ecosystem in which the species are living and the nutrition they get from the habitat.

Diochogaster sp. had individual count of 13 in nine quadrates with its presence only in broadleaved habitat. It showed low relative abundance of 9.74%, relative density of 3.47% and relative frequency with 5.92%. *Pontoscolexcorethrurus* also showed low relative abundance 10.11%,the relative density of 8.80%and relative frequency of14.47% in 22 quadrates with 33 individuals. It was present only in broadleaved and residential habitat and observed the species adapt to different habitat base on the living and feeding characteristics. Earthworms do not migrate or changes the habitat unless the habitat in not disturbed and feeds are sufficient for the earthworms(Najar, and Khan, 2011; Satchell, 1983; Singh, 1997; Tripathi and Bhardwaj, 2004).

Table 2: Habitat preference of individual earthworm species

Species	RA(%)	RD(%)	RF(%)	Species count	Sp. in quadrate
Amynthas alexandri	21.82	32.8	25	123	38
Perionyx excavatus	17.92	24.8	23.03	93	35
Metaphire houlleti	19.73	21.07	17.76	79	27
Aporrectodea calciginosa	11.69	6.93	9.87	26	15
Pontoscolex corethrurus	10.11	8.8	14.47	33	22
Dichogaster sp.	9.74	3.47	5.92	13	9
Darwida sp.	8.99	2.13	3.95	8	6

Relationship between earthworm density and soil nutrient along altitude

The one-way ANOVA showed that the NPK content in the soil was related to change in earthworm density at different altitudes. A significant difference was found with NPK and the density of earthworms $F(4, 15) = 20.946$, $p = .001$ at various altitudes (Table 3). The mean density of earthworm (Figure 4) at 650 m asl. was high (29.41 ± 4.31) since metabolic activity in the soil was found high, a faster rate of decomposition and higher nutrient content in the soil. Letting the earthworm density increase with nutrient availability. The mean density at 1450 masl. was low (12.38 ± 3.08) as the trees were scattered with little litters on ground making it hard for earthworms to survive with low nutrients and it makes a possibility for getting low density of earthworms in high altitude.

The nitrogen content in the soil of 1450m asl. was low (2.97 ± 1.78) comparing to the altitude at 650m asl. (5.87 ± 2.25). It was found that the density of earthworm increases with increase in nitrogen content in the soil mainly in lower altitude due to warmer and good texture soil addnitrogen to soil. And during ingestion the nitrogen are taken by the earthworms. The microbial activity decreases in high altitude preventing decomposition of litters and averting nutrients to the soil (McNown & Sullivan, 2013). Other studies also found low nitrogen in high elevation and dependence of earthworm with nitrogen for growth and survival (Curry, 2004; Huber *et al.*, 2007; Kale, 1998; Mubeen and Hatti, 2018; Tripathi and Bhardwaj, 2004).

Similarly, mean and standard deviation showed low phosphorus content in 1450 m asl. (27.40 ± 12.00) and high in 650 m asl. (64.15 ± 35.63). The potassium content was high in 650 m asl. (64.15 ± 35.63) and low in high altitude 1450 m asl. (15.14 ± 6.41). Potassium and phosphorus are influenced by density of earthworm for the plants (Ramanujamand Jha, 2011). The high altitude has harsh climate conditions such as frost and earthworm density decrease with altitude (Hopp and Linder, 1947; Rożen*et al.*, 2013). Recycling of litter and supply of nutrients to soil affected by low temperature and soil moisture condition comparing to lower altitude (Drollinger*et al.*, 2017; Holtmeier, 2009; Körnerand Paulsen, 2004). The intense change in soil nutrient supply from low to high altitude coincides with changes in vegetation composition and growth parameters (Schickhoff*et al.*, 2016).

The earthworm abundance has a positive correlation with the soil components (Table 4). The earthworm abundance highly depends on the amount of soil moisture. Earthworm abundance was high where the moisture content was high $r(18) = .806$, $p = .001$ and $R^2 = 0.649$ (Figure 5). The residential and broadleaf habitat had the highest moisture content 1.69 ± 0.07 and 1.68 ± 0.10 respectively with high abundance of earthworms since litters retain the moisture in soil and watering in plants increases moisture in residental area The lowest earthworm abundance was found in chirpine forest with moisture content 1.24 ± 0.06 and the agriculture habitat showed moisture content of 1.26 ± 0.11. Dewi and Senge (2015) stated that earthworms highly depend on moisture as their respiration rate depends on gas diffusion through body wall. Low soil moisture cause moisture stress and earthworms have to stay hydrated for survival and fecundity (Najar and Khan, 2011; Smetak*et al.*, 2007). Positive correlation was found between earthworm abundance and soil moisture (Bhadauria*et al.*, 2000; Schmidt and Curry, 2001).

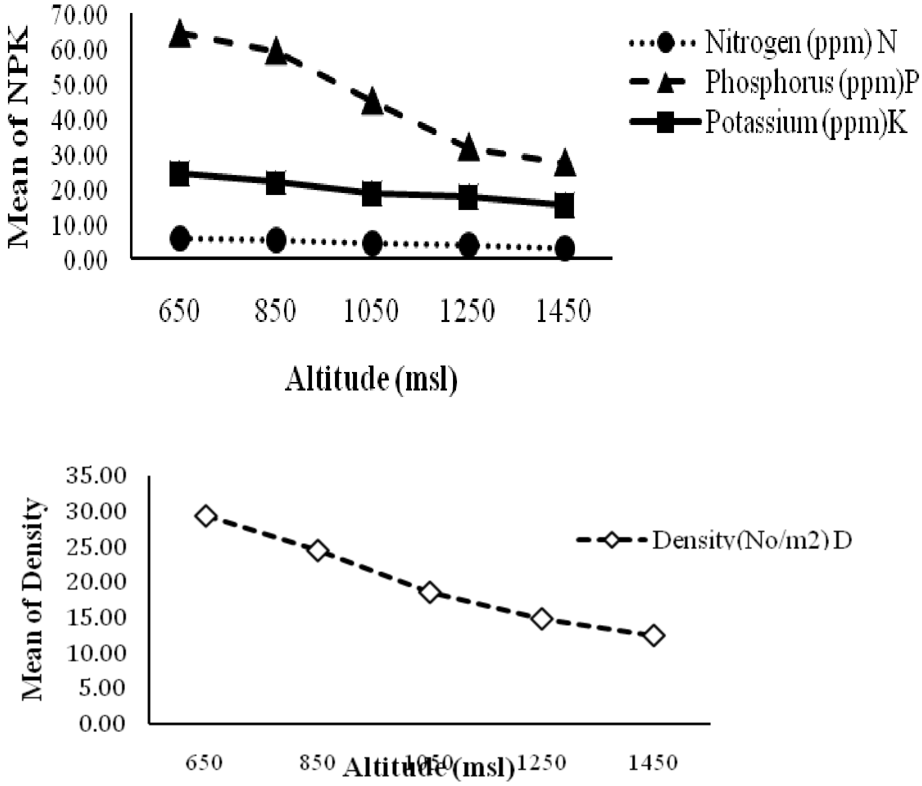

Fig.4: Relationship between NPK and density of earthworms in different altitude

Earthworm abundance in correspondence with soil components

Table 3: Mean density of earthworm and soil nutrient in different altitude

	Altitude	Mean ± SD	*F*	*P*
D(no/m^2)	650	29.41 ± 4.31		
	850	24.48 ± 3.37		
	1050	18.53 ± 1.46	20.95	0.01
	1250	14.75 ± 2.29		
	1450	12.38 ± 3.08		
N	650	5.87 ± 2.25		
	850	5.48 ± 2.08		
	1050	4.60 ± 1.96	5.24	0.023
	1250	4.00 ± 1.82		
	1450	2.97 ± 1.78		
P	650	64.15 ± 35.63		
	850	59.20 ± 33.61		
	1050	44.95 ± 26.20	9.91	0.048
	1250	31.80 ± 14.43		
	1450	27.40 ± 12.00		
K	650	24.28 ± 7.66		
	850	21.68 ± 7.21		
	1050	18.55 ± 7.37	14.11	0.027
	1250	17.71 ± 6.92		
	1450	15.14 ± 6.41		

P <.05; D:Density, N:Nitrogen, P:Phosphorus, K:Potassium, SD:Standard Devation

Table 4: Correlation with abundance and soil component

	Variables	1	2	3	4
1	**Abundance**				
2	**Moisturre**	.806**			
3	**OM**	.490*	.626**		
4	**CNratio**	.489*	.625**	1.000**	
5	**pH**	.507*	.466*	0.381	0.382

* *p*< .05. ** *p*< .01

The earthworm also depends on soil organic matter *r*(18) = .490, *p* = .028, R^2= 0.240. The organic matter was high in residential (1.15 ± 0.12) and broadleaf habitat (1.28 ± 0.50) with high numbers of earthworms.The different tree species litter fall in broadleaved habitat and farmers throwing biodegradable waste and adding manure in residential plots can be the possibility for higher abundance of earthworms in the two habitats.The agriculture habitat had organic matter 0.95 ± 0.41 and the lowest organic matter was in chirpine 0.60 ± 0.16 with lower abundance. The low abundance of earthworms in agriculture habitat and chirpine was due low amount of organic matter. The earthworms were found abundantly inhigh organic matters. It prefers to live in soil with rich organic matters (Brown *et al*., 2003; Mubeen and Hatti, 2018; Scullion and Malik, 2000). Tripathi and Bhardwaj (2004) also reported a positive correlation between earthworm abundance with soil organic matter.

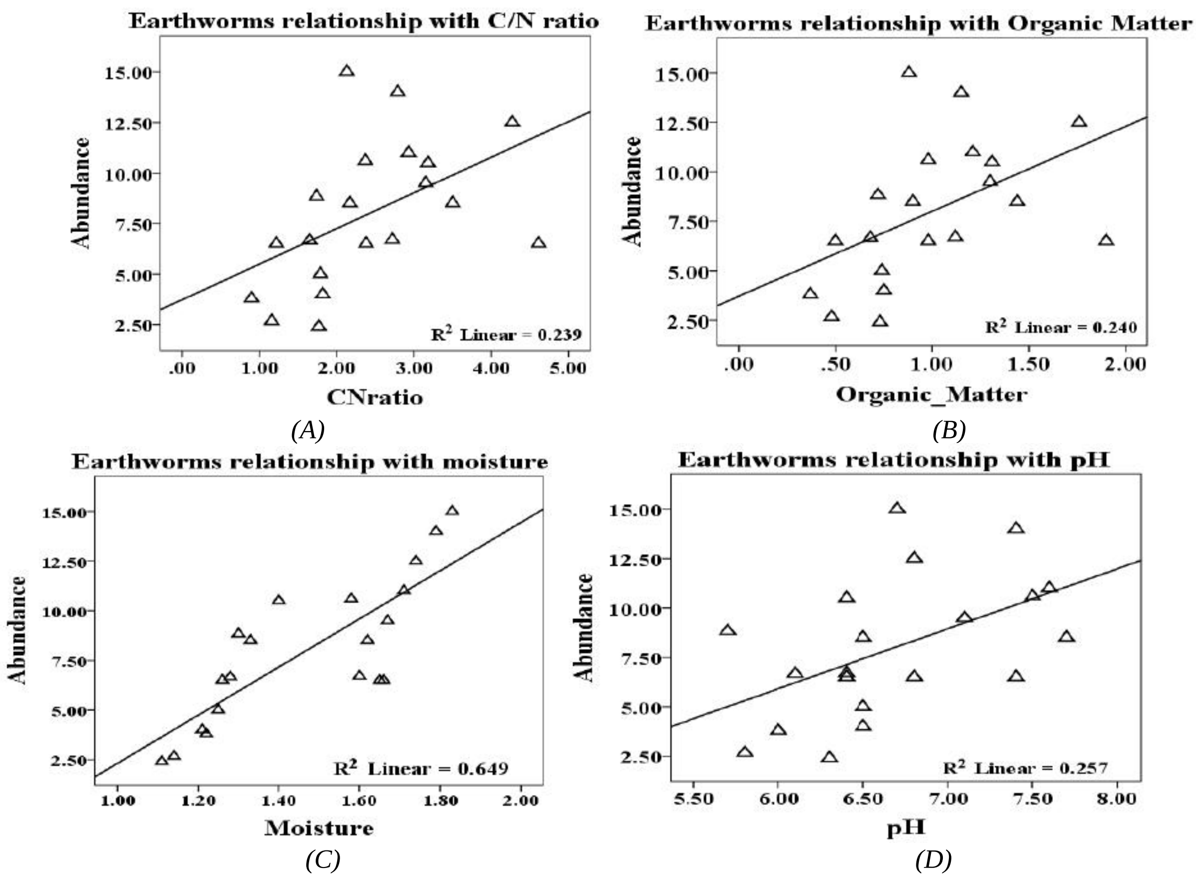

Fig.5: Relationship between earthworm abundance and soil components

The correlation (Table 4) showed that the earthworms significantly depend on C/N ratio*r*(18) = .489, *p* = .029, R^2= 0.239. Earthworm's abundance was high in the broadleaved forests with mean C/N of 3.11 ± 1.22 and low in chirpine forest (1.45 ± 1.45). The residential and agriculture habitat had a mean C/N ratio of 2.79 ± 0.28 and 2.30 ± 0.99 respectively. Earthworm act as carbon sink decreasing carbon content in soil and increasing nitrogen to soil for themselves and plants. (Aira*et al*.,2006; Hau*etal*., 2005; McLeanand Parkinson, 2000). The earthworm abundance was affected by carbon content in the soil (Kale, 1998) with a decrease in carbon there was an increase in nitrogen in the soil. The carbon has important role in earthworms and shows positive correlation (Decaëns*et al*.,2003; RamanujamandJha,2011).

The soil pH showed positive correlation with earthworm abundance $r(18) = .507$, $p = .022$, $R^2 = 0.257$. The mean pH was neutral in the residential area (7.06 ± 0.48), broadleaved forest (6.78 ± 0.43) and in agriculture habitat (6.86 ± 0.64). The chirpine forest showed slightly acidic soil (6.02 ± 0.31). The earthworm's prefer neutral pH for their growth and development. The earthworms prefer pH of 6.0-7.0 and above pH 7.0 the earthworm abundance and diversity are reduced. Earthworms are not found where the pH exceeds 9.0 as it is unfavorable for earthworms (Reynolds, 1994 and Sathianarayananand Khan, 2006). Also,RamanujamandJha(2011) found a positive correlation between earthworm's abundance and soil pH.

V. CONCLUSION

The earthworms are beneficial in the human-managed land and in the natural forest was revealed by the present study. The seven different species found in different habitats showed the earthworms have the habit of living in specific habitats until the habitat provides sufficient nutrition to it. The high relative density, frequency and abundance of *Amynthasalexandri* and its presence in all the habitat showed some earthworms are not habitat-specific. They can adapt to all kinds of environments.The study discovered that the habitat having less impact on soil can have a greater diversity of earthworms. It was foundgreater diversity and richness in the broadleaved forests. Density of earthworm depend on NPK. The nutrients and density of earthworms are found more in lower altitude than in higher altitudes. But more studies are required in larger areas, different habitat in different places to get better diversity of earthworms. Distribution patterns of different species are needed to know more on the benefits of earthworms in organic farming of the country to enhance crop productivity and income to farmers compared to what the present study found in small areas.

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