Evaluation of physico-chemical parameters of soil in different cropping systems and their co-relation with earthworm diversity

Abstract

Aim: The present study is an attempt to evaluate the impact of earthworm diversity on physico-chemical parameters of soil in different cropping systems (i.e. basmati-wheat, basmati-chickpea, soybean-wheat, moong-wheat) under organic and conventional farming systems

Place and duration of study: The study was conducted at the School of Organic Farming, Punjab Agricultural University, Ludhiana, Punjab, India from June, 2020 to March 2021.

Methodology: The four earthworm species found during the study period are *Metaphire posthuma, Lampito mauritti, Amynthas morissi*a and *Travoscolides chengannure,* which belong to two families i.e. Megascolicidae and Octochateidae. Out of these *Travoscolides chengannure* was reported for the first time in Punjab.

Results: The results indicate that richer earthworm diversity was found in the organic farming systems as compared to the conventional farming systems. The correlation analysis of earthworm abundance with the physicochemical parameters of soil in different farming systems revealed that the abundance of earthworms in organic farming system shows positive but non-significant correlation with pH, nitrogen and potassium levels. In conventional farming system, significant positive correlation (p=0.01) was found for organic carbon, electric conductivity and nitrogen.

Conclusion: The findings of this encourage switching from conventional to organic farming practices. These practices not only increase earthworm diversity, but enrich the soil with many major and micro-nutrients. The agriculture practices which are earthworm-friendly should be adopted for long-term soil productivity.

Keywords: Cropping system, earthworms, soil, physico-chemical

Introduction

Soil structure is the fundamental property of soil that affects the soil physical properties like water infiltration, plant nutrient uptake, soil porosity, hydraulic conductivity, water holding capacity etc. Earthworm populations significantly improve the soil structure by various mechanisms like provision of adequate aeration to the soil, creating deep burrows and mixing the organic residues well with the soil particles (Ahmed and Al-Mutairi, 2022). They actively participate in nutrient cycling thus increasing the overall fertility and productivity of agricultural soils (Ahmed and Al-Mutairi, 2022). Therefore, it is right to say that the abundance or even the presence of earthworms in the soil profile indicates a positive sign for the soil health. Not only this, they also play a vital role in the biological degradation of organic fuel which consequently leads to nutrient rotation. The earthworms feeding only on litter have massive amounts of plant nutrients inside their castings. The greater availability of nitrogen, phosphorus, potassium and calcium in the casts of earthworms enriches the soil with substantial amounts of major nutrients than the surrounding soil (Tiwari *et al.*, 1989).

Also, the soil management practices heavily intervene the activities of earthworms within the soil. Any modification in their activity thus indicates the fertility and quality aspects of the soil. Furthermore, there are some earthworms that feed on toxic soil nematodes. Such species of earthworms help in reducing the population of poisonous nematodes from the soil (Arancon *et al.* 2005; Marhan and Scheu, 2005). The scientists show considerable interest to exploit earthworms for breaking down complex organic waste into simpler forms (Karmegam and Daniel, 2009; Alagesan and Dheeba, 2010). Via decomposing organic materials, the earthworms run the energy transformation cycles through mineralization of organically bound nutrients such as lignocellulose (Bhadauria and Ramakrishnan 1989). This deterioration of organic materials by earthworms is due to the presence of various degrading microorganisms in their guts (Edwards and Bohlen, 1996). Many species of earthworms are also bio-indicators for detecting chemical toxicity in the soil. This is because the earthworms tend to accumulate considerable amounts of such chemicals inside their tissues. Therefore, the present investigation was carried out to evaluate the physico-chemical parameters of soil in different cropping systems in relation to the earthworm diversity.

Materials and Methods

Collection and Identification of Earthworms

Earthworms were hand-sorted up to 50 cm deep (25 cm x 25 cm) at each study site under organic and conventional farming systems. The earthworms were separated from the above-mentioned block. The same area was also dug deeper with a spade to collect the deep burrowing earthworm individuals. The extracted earthworms were washed thoroughly with tap water and dried on filter paper. Further, the earthworms were disinfected using 70% ethanol and preserved in a 5% formalin solution. Then, using a stereomicroscope, all the preserved earthworms were examined for various morpho-anatomical characteristics such as total number of segments, prostomium shape, position and type of clitellum, position and number of spermathecae, position of male pore, and total length (in cm) using measuring scale. These external characteristics were investigated by examining earthworm samples under a microscope. Eventually, a pointed needle was used to count the number of segments from prostomium to anus.

Molecular Characterisation of Earthworms

Different species of earthworms were collected from four different conventional and organic cropping systems: basmati-wheat, basmati-chickpea, soybean-wheat, moong-wheat. The collected earthworm species were preserved in 100% ethanol. Extraction of DNA and their amplification for mtCO- I gene was carried out. Earthworms total genomic DNA was isolated with modified CTAB method and the quantity was measured using NanoDrop Spectrophotometer. Further, the quality was determined using 0.8% agarose gel. Again, the PCR" of the mtCO- I gene with "universal primers namely" **LCO 1490 (5′-GCTCAACAAATCATAAAGATATTGG-3′)** and **HCO 2198 (5′-TTTCAGGAAACGTGACCAAAAAATCA -3′)** was carried out.

The amplifications of PCR were done in an **Veriti™ 96-Well Thermal Cycler** (Applied Biosystem, USA) with the following parameters: three minutes of denaturation at a temperature of 94°C, 38 cycles at 94°C for 30 seconds, annealing at 52°C for 45 seconds, and 1 minute at 72°C, followed by an ultimate elongation step of 10 minutes at 72°C, and held at 4°C. A single discrete PCR amplicon band of 750 bp was observed when resolved on agarose gel (1.5%). The 'barcode fragment'/ mtDNA COI 3' fragment gene PCR amplicon was eluted and purified using NucleoSpin Gel and PCR Clean-up kit according to instructions of the producer. The LCO1490 forward and HCO 2198 reverse DNA sequencing reaction of PCR amplicon was done using a forward and a reverse primer respectively. BDT v3.1 was also used along with cycle sequencing kit on ABI 3730xl Genetic Analyzer which brought out the consensus sequence of barcode fragment gene.

Finally, the insect DNA barcode fragment gene sequence was exploited to perform the NCBI-Basic Local Alignment Search Tool database. Based on maximum identity score, the first ten sequences were selected and aligned using multiple alignment software program i.e. ClustalW multiple alignment. Distance matrix was generated and the phylogenetic tree was constructed by using MEGAX.

Physico-Chemical Analysis of Soil

The analysis of soil temperature, moisture content and other physic-chemical attributes of soil such as N, P, K, OC, EC and pH of both organic and conventional farming systems were done. After burning the soil samples in a muffler furnace at a temperature of 550 °C, the organic carbon was computed using the Walkely and Black method (1934). The N content in soil was calculated using Kjeldahl assembly. The soil sample was digested with concentrated H2SO4, it was then made to run through the Kjeldahl assembly, and eventually the titration was carried out using 0.01 N HCl, according to the Subbiah and Asija (1956). Further, the soil phosphorous was measured by spectrophotometer method. It included the digestion the soil using perchloric acid as well as nitric acid in a ratio of 1:4, respectively. Then with a colorimeter, Olsen's method was used to estimate phosphorus. The assessment of soil temperature and moisture are given below:

Soil temperature

Soil temperature in the field was computed by an apparatus known as digital thermometer. The thermometer was inserted directly in the soil and was kept for one minute before the temperature was noted. Soil temperature was recorded at monthly interval after sowing of each crop.

Soil moisture

Soil moisture was measured by gravimetric method. In this method, a fresh sample of soil was sieved and core weight was taken. It was then oven dried and reweighed.

Statistical analysis

The t-test analysis was done to analyze the soil parameters of the two selected farming systems i.e. organic and conventional farming systems at $p \leq 0.01$ and 0.001 using SPSS ver. 16.0.

Results and Discussion

Molecular characterization of earthworms

Identification of Earthworms using 'barcode fragment'/mtDNA COI 3' fragment based on DNA barcoding Technique

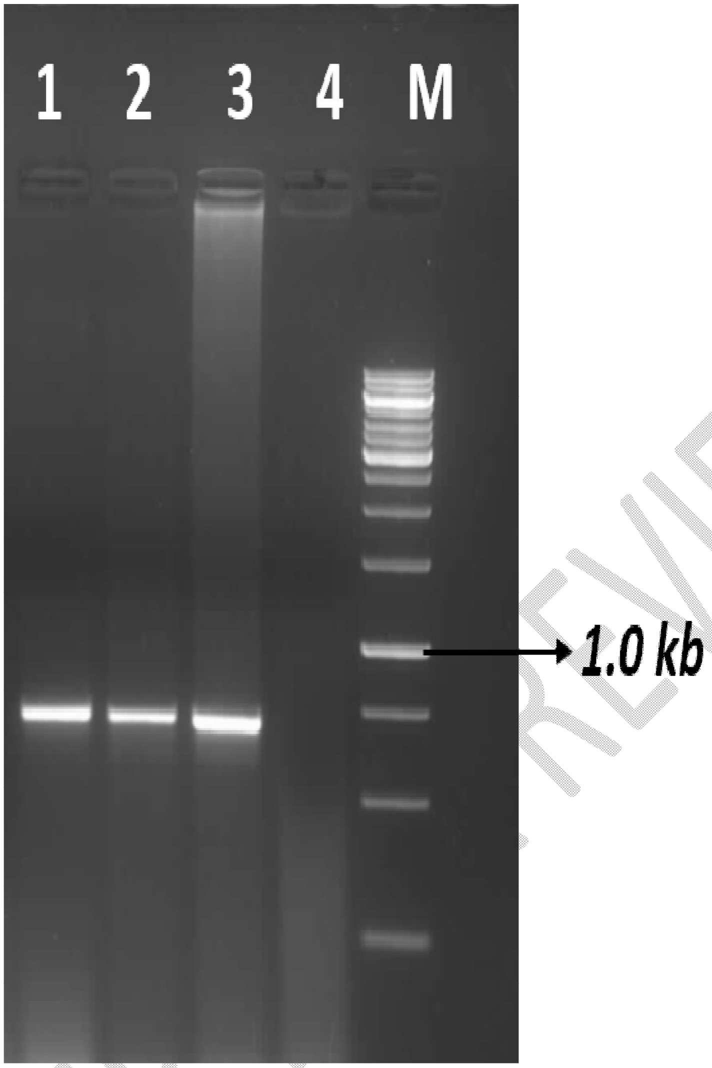

Figure1. Earthworm gDNA sample agarose (1.5%) gel electrophoresis of PCR using LCO1490 and HCO2198 primers. Legends:

M-Molecular Weight Marker- 1.0 kb Thermo Scientific, USA,

1- E1 gDNA sample,

2- E2 gDNA sample,

3- E3 gDNA sample,

Based on sequence homology and phylogenetic analysis, the earthworm sample 1 (E1) and 2 (E2) was found to be *Metaphire posthuma* while the 3 (E3) was found to be *Travoscolides chengannure.* (Figure 1).

Insect DNA barcode fragment gene sequence was used to carry out NCBI-Basic Local Alignment Search Tool database. Based on maximum identity score first ten sequences were selected and aligned using multiple alignment software program ClustalW multiple alignment. Distance matrix was generated and the phylogenetic tree (Fig 2) was constructed by using MEGAX

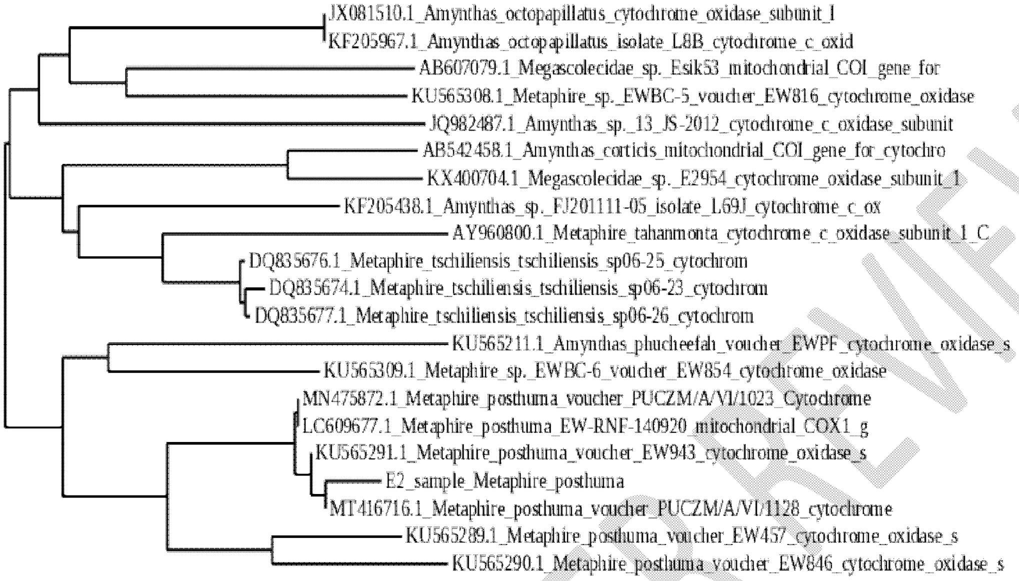

Fig.2 *Evolutionary relationships of earthworm E2 sample with other taxa.*

According to Panjgotra (2016) and Ahmed *et al* (2020), Megascolecidae is the most diverse family in Punjab, Haryana, and Himachal Pradesh. The Megascolecidae family has a perichaetine arrangement of setae arranged in a ring around the segment, whereas the Octochaetidae family has a lumbricine arrangement with 8 setae closely paired around the segment. The earthworm species were identified using the steps in the keys that corresponded to the setae arrangement. The spermathecae pores differed according to species and family. The Megascolecidae family has 2 to 4 spermathecae pores, while the Octochaetidae family has only 1 to 2 spermathecae pores. Except for *L. mauritii*, whose clitellum was located at segments 14-17, the clitellum was annular in all reported earthworm species with positions at segments 14-16 in the Megascolecidae family. The clitellum position in Octochaetidae family species, on the other hand, was located at 13-17 segment. The male pore was located at segment 18 in the Megascolecidae family and varied from 17 to 18 in the Octochaetidae family. Several researchers have reported similar morphological features in earthworm specimens from the Megascolecidae and Octochaetidae families (Kumar and Singh 2013; Sharma and Garg, 2018).

Physico-chemical properties of soil

The statistical analysis of pH, Electrical Conductivity (EC), nitrogen (N), phosphorous (P), potassium (K), organic carbon (OC) in soils of different cropping patterns were given. The pH was found in the range of 7.12-7.80 with an average value of 7.5 in organic soils. In conventional soils, pH ranged from 7.11-7.56. On the other hand, EC (mS) ranged from 0.22-0.36 and 0.19 to 0.22 in organic and conventional farming systems, respectively. The mean content of EC among organic and conventional fields was 0.19 and 0.36, respectively. The OC ranged from 0.54 to 0.63 in organic fields and 0.41 to 0.46 in conventional soils. In contrast, the content of nitrogen was higher in organic soils (362.40 g/kg) as compared to conventional soils (339.40g/kg). Hackenberger and Hackenberger (2014) also revealed that the physico-chemical attributes of soil such as organic matter as well as type of climatic influences the earthworm diversity. Further, Singh *et al* (2016) performed the principal component analysis and demonstrated that the physico-chemical parameters of soil such as pH, moisture content as well as organic carbon are firmly associated with the distribution of earthworm communities.

The highest pH in B-W was recorded in organic farming (7.7±0.05) followed by conventional farming (7.1±0.09), but was not significant in variation between the organic and conventional farming for B-W. The highest pH in B-C was observed in organic farming (7.6±0.03) followed by conventional farming (7.2±0.05), but no significant variation was observed between the organic and conventional farming for B-C. The highest pH in S-W was recorded in organic farming (7.8±0.05) followed by conventional farming (7.1±0.05) and was found to be significant. The highest pH in M-W was recorded in organic farming (7.1±0.05) followed by conventional farming (7.3±0.03), and was significant.

The highest EC in B-W was recorded in organic farming (0.26±0.005) followed by conventional farming (0.22±0.005) and the variation was found significant between the organic and conventional farming for B-W. The highest EC in B-C was observed in organic farming (0.22±0.005) followed by conventional farming (0.21±0.005) and there was no significant difference between the organic and conventional farming for B-C. The highest EC in S-W was recorded in organic farming (0.22±0.005) followed by conventional farming (0.19±0.05), and were significantly different. The highest EC in M-W was recorded in organic farming (0.36±0.005) followed by conventional farming (0.20±0.005), and the variation was found to be significant between the organic and conventional farming for M-W.

The highest OC in B-W was recorded in organic farming (0.63±0.005) followed by conventional farming (0.46±0.005), which was found to be significant between the organic and conventional farming for B-W. The highest OC in B-C was observed in organic farming (0.54±0.005) followed by conventional farming (0.44±0.005), but no significant variation was observed. The highest OC in S-W was recorded in organic farming (0.59±0.005) followed by conventional farming (0.41±0.005), and was Significant for S-W. The highest OC in M-W was recorded in organic farming (0.62±0.005) followed by conventional farming (0.0±0.005), which was significant. Further, Chan and Barchia (2007) studied that the OC in soil is the most influential and critical parameter that affects not only the abundance, but also the distribution of earthworm communities at any specific area.

The highest N in B-W was recorded in organic farming (356.7±2.1) followed by conventional farming (339.4±3.25), and was found to be significant between the organic and conventional farming for B-W. The highest N in B-C was observed in organic farming (362.4±1.35) followed by conventional farming (317.2±1.47), but no significant variation was observed between the organic and conventional farming of B-C. The highest N in S-W was recorded in organic farming (360.6±0.7) followed by conventional farming (314.8±1.49), and was found to be significant in variation between the organic and conventional farming for S-W. The highest N in M-W was recorded in organic farming (324.1±1.9) followed by conventional farming (311.5±0.7), and was found to be significant in variation between the organic and conventional farming for M-W.

The highest P in B-W was recorded in organic farming (50.3±0.7) followed by conventional farming (45.5±1.65).and was found to be significant in variation between the organic and conventional farming for B-W. The highest P in B-C was observed in organic farming (44.2±0.7) followed by conventional farming (41.4±0.56) but no significant variation was observed between the organic and conventional farming of B-C. The highest P in S-W was recorded in organic farming (48.2±1.46) followed by conventional farming (42.6±1.6) and was found to be significant in variation between the organic and conventional farming of S-W. The highest P in M-W was recorded in organic farming (50±0.75) followed by conventional farming (44.2±0.81) and found to be significant in variation between the organic and conventional farming of M-W.

The highest K in B-W was recorded in organic farming (151.8±0.7) followed by conventional farming (145.4±2.33) and found to be significant in variation between the organic and conventional farming of B-W. The highest K in B-C was observed in organic farming (152.3±1.19) followed by conventional farming (145.4±2.33) but no significant variation was observed between the organic and conventional farming of B-C. The highest K in S-W was recorded in organic farming (143±1.95) followed by conventional farming (137.3±1.51) and found to be significant in variation between the organic and conventional farming of S-W. The highest K in M-W was recorded in organic farming (151.8 ±0.7) followed by conventional farming (144.9±0.79) and Found to be significant in variation between the organic and conventional farming of M-W

Table 1: Correlational analysis of earthworm abundance with the soil parameters in different farming systems

	Cropping System		pH	EC	OC	N	P	K
Organic	B-W	Mean	7.7	0.26	0.63	356.7	50.3	157.80
		S.E.	0.15	0.03	0.02	9.02	1.4	3.06
	B-C	Mean	7.6	0.22	0.54	362.4	44.2	152.3
		S.E.	0.18	0.04	0.02	10.8	1.4	2.61
	S-W	Mean	7.8	0.22	0.59	360.6	48.2	143
		S.E.	0.2	0.04	0.08	9.16	1.1	2.27
	M-W	Mean	7.1	0.36	0.62	324.1	50	151.8
		S.E.	0.02	0.04	0.04	5.68	2.1	3.76
		R	0.318	-0.388	-0.171	0.462	-0.21	0.562
		P	0.312	0.211	0.594	0.13	0.51	0.056
Conventional	B-W	Mean	7.1	0.22	0.46	339.4	45.5	145.4
		S.E.	0.09	0	0	6.33	0.89	2.34
	B-C	Mean	7.2	0.21	0.44	320.1	41.4	136.8

		S.E.	0.1	0.005	0.005	1.43	0.7	2.26
	S-W	Mean	7.1	0.19	0.41	316.9	42.6	137.3
		S.E.	0.14	0	0	1.16	0.56	2.68
	M-W	Mean	7.5	0.2	0.42	311.5	44.2	144.9
		S.E.	0.1	0.07	0.14	110.13	15.6	2.22
		R	-0.466	0.733	0.808	0.813	0.1	0.147
		P	0.145	0.006**	0.001**	0.001**	0.75	0.648

**$p<0.01$(significant at 0.01 level)

The correlational analysis of earthworm abundance with the soil parameters in different farming systems i.e. organic and conventional farming systems has revealed that, the abundance of earthworms in organic farming system showed marginal positive correlation (r = 0.31, p = 0.32) with pH of the soil; marginal negative correlation (r = -0.38,p = 0.21) with EC; very marginal negative correlation (r= -0.17, p=0.59); moderate positive correlation (r = 0.46, p = 0.13) with N; marginal negative correlation (r= -0.21, p=0.51) with P; moderate positive correlation (r=0.56, p=0.05) with K. For the conventional farming system the abundance of earthworms showed a moderate negative correlation (r= -0.46, p=0.14) with pH; strong positive correlation which was statistically significant (r = 0.73, $p\leq 0.01$) with EC; strong positive correlation which was statistically significant (r = 0.808, $p\leq 0.001$) with OC; strong positive correlation which is statistically significant (r = 0.81, $p\leq 0.001$) with N; very nominal positive correlation (r = 0.1, p =0.75); very nominal positive correlation (r = 0.14, p=0.64) with K was also found.

Table 2. Analysis of soil parameters of organic and conventional farming systems

	Mean		SD		T	P
	Organic	Conventional	Organic	Conventional		
pH	7.51±0.08	7.19±0.04	0.29	0.13	3.48	0.002**
EC	0.265±0.017	0.205±0.004	0.604	0.014	3.347	0.002**
OC	0.595±0.01	0.43±0.006	0.037	0.021	12.968	0.0001**
N	350.9±4.07	320.7±2.9	16.27	11.6	6.048	0.0001**
P	48.17±0.84	43.42±0.71	2.91	2.46	4.31	0.0003**
K	151.22±1.73	141.1±1.47	6.002	5.11	4.44	0.0002**

** $p\leq 0.01$(significant at 0.01 level) ** $p\leq 0.001$ (significant at 0.001 level)

Table 3. Soil Temperature and moisture of different crop systems in both organic and conventional cropping system.

Sowing		
CROPPING SYSTEMS	Soil temperature (°C)	Soil moisture (%)
organic basmati	23.9	37
organic soybean	23.5	18.9
organic moong	23.7	19.2
organic wheat	21.8	19.3
organic chickpea	23.1	16.4
conventional wheat	21.2	18
conventional chickpea	22.1	16
conventional basmati	23.5	37.5
conventional soybean	23.1	18.2
conventional moong	23.5	17.5

The t-test analysis between different soil parameters of the two selected farming systems i.e. organic and conventional farming systems has revealed that, there is a significant difference in the pH of soil (t = 3,48, p ≤ 00.001), EC of the soils (t = 3.34, p≤ 0.001), OC of the soil (t = 12.96, p≤ 0,001), N in the soil (t = 6.04, p≤ 0.001), P in the soil (t = 4.31, p≤0.01) and K levels of the soil (t = 4.44, p≤ 0.001) between two farming systems. Therefore, there was a significant difference in the soil parameters of organic farming systems compared to the conventional farming systems.

Conclusion

In this study, the highest soil temperature among organic crops was recorded in organic basmati. The correlation analysis of earthworm abundance with the physico- chemical parameter of soil in different farming systems revealed that the abundance of earthworms in organic farming system showed positive but non-significant correlation with pH, nitrogen and potassium levels Therefore, it can be concluded that organic farming systems are more appropriate to improve the soil health and earthworm diversity.

References

Ahmed S, Julka JM and Kumar H (2020) Earthworms (Annelida: Clitellata: Megadrili) of Solan a constituent of Himalayan Biodiversity Hotspot India Travaux du Muséum. National d'Histoire Naturelle Grigore Antipa ***63***:19

Ahmed N and Al-Mutairi KA (2022) Earthworms effect on microbial population and soil fertility as well as their interaction with agriculture practices. *Sustainability* 14(13): 7803.

Alagesaran P and Dheeba R (2010) November Utilization of earthworms in organic waste management. In *Proceedings of International Forestry and Environment Symposium* (Vol 15)

Arancon NQ, Edwards CA Bierman P Metzger JD and Lucht C (2005) Effects of vermicomposts produced from cattle manure food waste and paper waste on the growth and yield of peers in the field. *Pedobiologia **49***:297-306

Bhadauria T and Ramakrishnan PS (1989) Earthworm population dynamics and contribution to nutrient cycling during croing and fallow phases of shifting agriculture (jhum) in north-east India. *Journal of Alied Ecology* 505-520

Chan KY and Barchia I (2007) Soil compaction controls the abundance biomass and distribution of earthworms in a single dairy farm in south-eastern Australia *Soil and Tillage Research 94*: 75-82

Edwards CA and Bohlen PJ (1996) *Biology and ecology of earthworms* (Vol 3) Springer Science & Business Media

Hackenberger DK and Hackenberger BK (2014) Earthworm community structure in grassland habitats differentiated by climate type during two consecutive seasons. European Journal of Soil Biology, 61, 27-34.

Karmegam N and Daniel T (2009) Growth reproductive biology and life cycle of the vermicomposting earthworm Perionyx ceylanensis Mich (Oligochaeta: Megascolecidae) *Bioresource Technology **100***: 4790-4796

Kumar Y and Singh K (2013) Distribution of earthworm in different block of Gorakhpur district in eastern Uttar Pradesh *World Al Sci J **21***: 1379-1385

Marhan S and Scheu S (2005) Effects of sand and litter availability on organic matter decomposition in soil and in casts of *Lumbricus terrestris* L Geoderma ***128***: 155-166

Panjgotra S (2016) Diversity of earthworms in sugarcane and wheat crop fields and vermicomposting of crop waste (Doctoral dissertation Punjab Agricultural University Ludhiana)

Sharma K and Garg VK (2018) Comparative analysis of vermicompost quality produced from rice straw and paper waste employing earthworm Eisenia fetida (Sav) *Bioresource technology **250***: 708-715

Singh S, Singh J and Vig AP (2016) Earthworm as ecological engineers to change the physico-chemical properties of soil: soil vs vermicast *Ecological Engineering **90***:1-5

Tiwari SC, Tiwari BK and Mishra RR (1989) Microbial populations enzyme activities and nitrogen-phosphorus-potassium enrichment in earthworm casts and in the surrounding soil of a pineale plantation *Biology and Fertility of Soils **8***: 178-182