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Study On Diversity And Abundance Of Earthworms Fauna In Cotton And Sugarcane Crops Of District Bhakkar Punjab – Pakistan

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Abstract
The diversity and abundance of earthworm fauna are essential for maintaining soil health and maximizing agricultural productivity, particularly in crop systems such as cotton and sugarcane. This study aims to assess the abundance and variety of earthworm species in the sugarcane and cotton fields of District Bhakkar, Punjab, Pakistan. A collection of 651 earthworms from six different species was made from the Kacha and Razai Shah crop fields in District Bhakkar, which consisted of cotton and sugarcane crops. Earthworm populations in cotton fields were found to be significantly higher than those in sugar cane fields at both sampling sites. From a total of 651 specimens, the majority, 525, were collected from Cotton fields, while the remaining 126 were collected from Sugarcane fields. The specimens recovered in this study were recognised as six species: *Pheretima posthuma*, *Pheretima hawayana*, *Pheretima elongata*, *Pheretima heterochaeta*, *Aporrectodea calignosa*, and *Aporrectodea longa*. They belong to two genera, two families (Megascolecidae and Lumbricidae), and one order (Haplotaxida). Among the six species, *Pheretima posthuma* and *Pheretima hawayana* showed a broad range of tolerance to edaphic variables. These two species were not only the most abundant but they were also found at the highest number of sample sites. Data confirmation was conducted using the Shannon diversity index. The index analysis revealed that the cotton field had a higher level of diversity (H' = 1.253 & 1.091) compared to the sugarcane region (H' = 0.1&0.585) in Razi Shah and Kacha, respectively.

Key Words: Earthworm, Diversity and Abundance, Cotton Crop, Sugarcane Crop

Introduction

Pakistan is a fertile country with a strong agriculture sector. The soil fertility is diminishing due to intensive agricultural practices, which primarily impact the soil's porosity [1]. The primary factors contributing to soil deterioration in the agricultural industry are expansion and intensification [2]. Macro-organisms, particularly earthworms, play a crucial role in maintaining the health and fertility of soil. The presence of a well-developed and functioning soil food web is considered a key sign of the overall health of an ecosystem [3]. Fertilisers generally increase the diversity and abundance of soil organisms [4]. The soil biota significantly contributes to the functioning of agro-ecosystems. The ecology is adversely impacted by alterations in soil biota [5]. Earthworms play a crucial role in productive soils worldwide and serve as prominent indicators of soil characteristics and physical conditions [6]. Earthworms, as members of the soil fauna, play a crucial role in decomposing organic matter. This process helps in the recycling of different nutrients and contributes to the development of soil structure [7]. Earthworms possess variable data on diverse factors, nevertheless, there are still numerous breaches that want attention [8-11]. In 1981 District Bhakkar was upgraded as capital. The weather in District Bhakkar is predominantly hot throughout most months of the year, with temperatures reaching up to 50°C. The area consists of plains and deserts. The primary agricultural products in District Bhakkar consist of wheat and sugarcane. In addition to these crops, cotton, bajra, guar seed, moong, jawar, mash, masoor, ground nut, oil seed, and sunflower are also cultivated in small quantities [12, 13]. Cotton holds a distinctive role in the agricultural economy of Pakistan. The government is actively promoting both quantitative and qualitative research on cotton. The quality of cotton has been diminished due to factors such as mixed seed and mixed grades, as well as incorrect picking. Pakistan cultivates two primary types of cotton, namely American cotton (*Gossypium hirsutum*) and Desi cotton (*Gossypium arborium*) [14]. Desi cotton is less susceptible to pest infestations compared to American-type [15, 16].

Sugarcane is a valuable cash crop and very important for sugar production[17]. Earthworms are hermaphrodites, and in ideal conditions of temperature, moisture and food, they can double their population [18]. Specialized digesting enzymes like chitinases, cellulases, amylases, lipases and proteinases are secreted by earthworms, which rapidly bio-transform cellulosic and proteinaceous material in organic wastes from anthropogenic and natural sources [19]. Earthworms are also used as food sources in tribes in Australia and New Zealand. Animal feed is commercially enhanced by adding dry and powdered forms of

linoleic and arachidonic acid to improve reproductive and growth processes. Earthworm is a rich source of these acids, so are used by animal food industries [20]. In dry body weight of earthworm, 8-20 % carbohydrates, 60-70% proteins, 2-3% minerals and 7-10% fat is present [21]. Earthworms have a vital role due to their nutritional values, which have been used as part of poultry feed [22], food for bait, and food supplements for ornamental and other fishes in North America since 1940 [22]. Earthworms play a vital role in soil fertility and biology. Earthworms constitute a big part of the invertebrate fauna of the soil. They are wet soil, soil-dwelling living beings. They change organic waste into organic manure. They are useful in managing organic waste, soil development and land renovation [18].

Solid waste, water pollution and urban wastes could be recycled through earthworms [23]. Earthworms play a major role in the formation and aeration of soil and play a vital role in the cycling of nutrients by decomposing organic matters [7]. Earthworms are considered a major group of soil fauna in forest ecosystems and its functioning [24] and are recognized as important ecosystem engineers [25]. Earthworms play a major role in developing soil structure [26]. For analyzing polluted urban sites, earthworms have been used [27]. Earthworms act as crushers and aerate the soil [6]. The importance of Earthworms in soil fertility has been confirmed by a number of biologists [28]. In Australia, application of superphosphates in pastures and pastoral farming has good effect on the population of giant Gipps land earthworm classified as unprotected species [23]. Earthworm belongs to Phylum Annelida and class Oligochaeta. The shape of earthworms is cylindrical, having internal and external segmentation instead of appendages or suckers. They have few hooks like chaetae and so called as oligochaeta. Sexually mature worms have gland cells and ring shape clitellum. Glands secrete material that is used for cocoon formation [29]. Earthworms which are present in aquatic habitat are called microdrilli and terrestrial worms are known as megadrilli. They prefer material like manures, compost and litter that is also present in hydrophilic environment. Some species of earthworms can live under snow [30]. The main aim of the present research was to gain scientific information about kinds of earthworms, their diversity, relative abundance and seasonal fluctuation of population in cotton and sugarcane crop fields of District Bhakkar. The information collected from this research can be used to increase agricultural production and soil fertility.

Material and Methodology

10 % ethyl alcohol was used to anesthetize the earthworms, and 10 % formalin was used to wash and store the earthworms.

Study area:

Bhakkar is situated in the Punjab province on the river Indus left bank and the boundary of Dera Ismail Khan, Khyber Pakhton Khawa (KPK). Its geography coordinates are 31.6230°N- 71.0626°E.

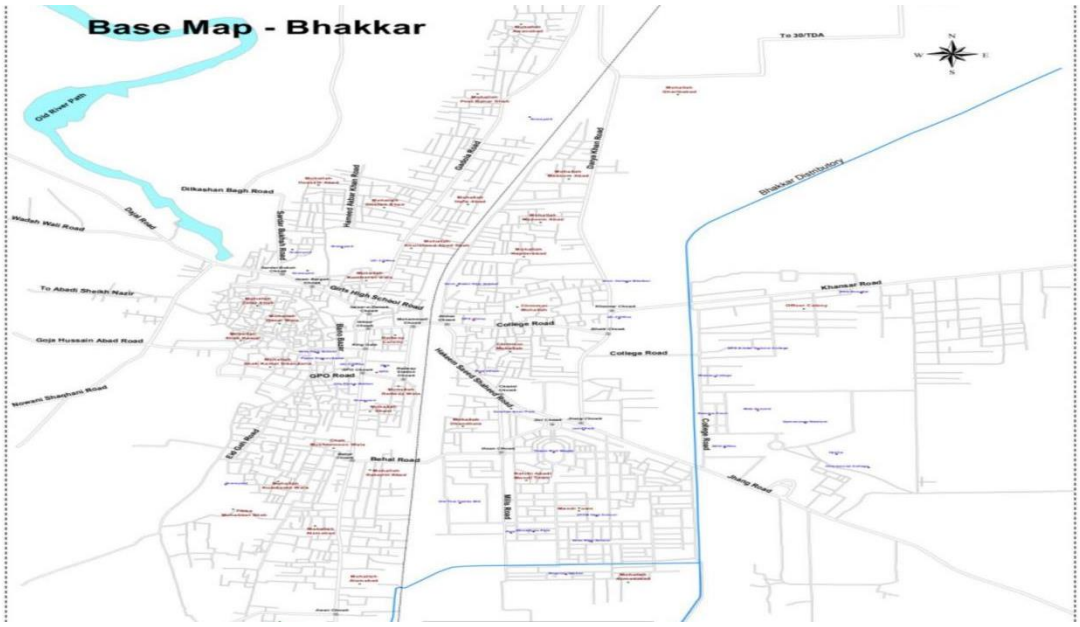


Figure 1: Study area Bhakkar Punjab-Pakistan.

Sampling sites:

The sampling of earthworm fauna was continued to six months from two sampling sites, i.e., Kacha (7 km) and Razai Shah (5 km) from District Bhakkar was made. Sampling was carried out from cotton and sugarcane fields every month.

Sampling protocol:

Earthworms were collected from cotton and sugarcane fields by removing an equal amount of soil from the 4-5 hectare area by digging a quadrate of 1 m^2 up to a depth of 1.5 m^2 in the soil (Qazi, 2015). Hoe was used for soil digging, and the standard was maintained throughout sampling. A simple hand-sorting method was used for earthworm collection. In each field of one

acre, two quadrangles were fitted. Soap water was used on the quadrangle for an assemblage of earthworms and then washed with simple water to proceed for preservation.

Preservation:

The earthworms were subjected to a 10% ethyl alcohol solution for a duration of 15 minutes to induce anesthesia. The anesthesia was considered successful when the earthworms exhibited no response to mild probing. The specimens were euthanized using formalin solution and then placed on a moist blotting paper in a dish until they hardened, which took around 24 hours. The items were cleansed and placed in a solution of 10% formalin for preservation. Numbered glass bottles were utilized to store them.

Species analysis:

A binocular microscope with 20X to 30X magnification was used for earthworm species analysis. Different characteristics were analyzed for earthworm identification, as given by Stephenson [31] and Bhatti [32].

Identification

The identification and classification of earthworm specimens were done using the data obtained and the keys provided by Stephenson [31] and Bhatti [32]. According to Stephenson [31] and Bhatti [32], the immature specimens were identified by the following characteristics.

1.Incomplete and unclear annulation.
2.Small in length with less width than adults.
3.Less number of total segments.
4.Male and female genital aperture, genital papillae, and spermathecal pores were not prominent.
5.No clitellum.

Statistical analysis

Data was statistically analyzed using the Shannon-Weinee diversity index (Shannon, 1948). Parameters used for analysis were species diversity (H'), species richness, and species evenness. Species diversity (H') was calculated by using the following equation (i):

$$H' = (NInN - \sum nInn)/N \text{.....(i)}$$

Distribution of data affected the extent of H'. N = number of individual species, and I = total number of samples
The number of categories and evenness is calculated by using equation (ii)

$$E = \text{evenness} = H/H\ max \text{.....(ii)}$$

E has been termed evenness and can be considered homogeneity or relative diversity. Based on the sample, E estimates the population evenness. A measure of heterogeneity of dominance was measured using equation (iii).

$$D = I - E \text{.....(iii)}$$

Where D is the measure of heterogeneity of dominance. Then, statistics were applied to the data to determine the faunal diversity and abundance of the earthworms with the Shannon-Weiner diversity index.

RESULT AND DISSCUSION

Different characteristics of earthworm specimens were analyzed (Table 1). Ventral and dorsal view of different genus of two families Megascolecidae and Lumbricidae (Figre 2).

Table 1: Different characteristics of family Megascolecidae and Lumbricidae

Sr No.	Characteristics	MEGASCOLECIDAE				LUMBRICIDAE	
		Pheritima posthuma	***Pheretima hawayana***	***Pheretima elongate***	***Pheretima heterochaeta***	***Aporrectodea Orely***	***Aporrectodea longa***
1	Color	Varied frdark brown to blackish gray dorsally, gray ventrally	Grayish brown	Grayish yellow	Yellowish grey	Grey dark reddish brown or unpigmented pale pink.	darkest reddish brown
2	Prostomium	Tanylobous.	Combined pro and epilobous.	Without dorsal process.	Epilobous	Epilobous	Epilobous
3	Segments	i-iv with single annulus, vii and viii biannulate, rest were triannulate	i-iv consisted single annulus, vi was biannulate, rest were triannulate.	i-iv with single annuli, vii and viii biannulate, rest triannulate.	i-iv simple, vii biannulate, rest are triannulate.	i-vi simple, vi and vii biannular, rest was triannular	i-x simple, xi-xviii biannulate, rest triannulate
4	Number of segments	Immature specimens 40-124, Adult specimens 34-134.	Immature specimens 42-110, Adult specimens 52-120.	95-120	Immature 42-87, Adults 48-120	90	110-190

5	Length	Immature specimens 40-106 mm, Adult specimens 41-167.	Immature specimens 45-90 mm, Adult specimens 50-120 mm	90-130 mm.	Immature 45-90 mm, Adult 50-110	100 mm.	Immature 60-100 mm, Adult 88-130 mm
6	Breadth	Immature specimens 2.0-4.5 mm, Adult 1.0-6.5 mm.	Immature specimens 2.25-4.0 mm, Adult specimens 3.5-5.25 mm.	2.00 – 4.25 mm.	Immature 2.5-4.5mm, Adult specimen 3.25-5.25 mm	3-5 mm	Immature 2.75 – 5.25 mm, Adult specimen 5.5-6.9 mm
7	Clitellum:	Annular and ring-shaped	Ring and saddle-shaped,	Usually without setae,	Ring shaped	Saddle shaped	NIL
8	Setae:	Perichaetine, unbroken ring, clitellar region absent	Perichaetine with dorsal and ventral break.	Perichaetine	Perichaetine	Lumbricine, closely paired	Lumbricine, Close paired
9	Dorsal pores:	Start from 12/13 segment	NIL	Starts from 12/13 segment	Starts from 10/11 segment	Starts from 9/10 segment	started from 12/13 segment
10	Male pore:	A pair on segment no. xviii, at the setal line on prominent porophore	One pair in disc shaped porophore on segment no. xviii	A pair on segment no. xviii, at the setal line. One quarter of circumference	On xviii and elevated papilla	On xv as transverse slits above setal line	Male pores on segment xv
11	Female pore:	Single pore present in the center of segment xiv.	Single in an oval depression, midventral in position on segment xiv.	Single pore on segment no. xiv.	On segment no. xvi.	On xiv segments.	Present just above the male pores on xiv
12	Genital papillae:	Present on segment no. vi, vii and viii	Present on segment no. xvii, xviii and xix.	Three to seven pairs on xix, one pair on anterior part of each segment.	Present	5/6, 9/10 and 7/8.	Present on xiv and xvi segments. Present on the clitellum.
13	Spermathecal pore:	Four pairs in 5/6-8/9.	Present in three pairs on segment 5/6-7/8.	Mostly two pairs in 5/6-6/7.	Four pairs from 5/6-8/9.	Two pairs, in 9/10 and 10/11.	Two pairs in furrows 9/10 – 10/11
	Localities:	**District Bhakkar**					
14	Habitats:	Cotton field of District Bhakkar				Sugarcane fields of District Bhakkar	
15	Total number of specimens:	192	158	92	83	95	31
16	Total number of adult specimens:	138	141	83	78	81	20
17	Total number of immature specimens:	54	17	9	5	14	11

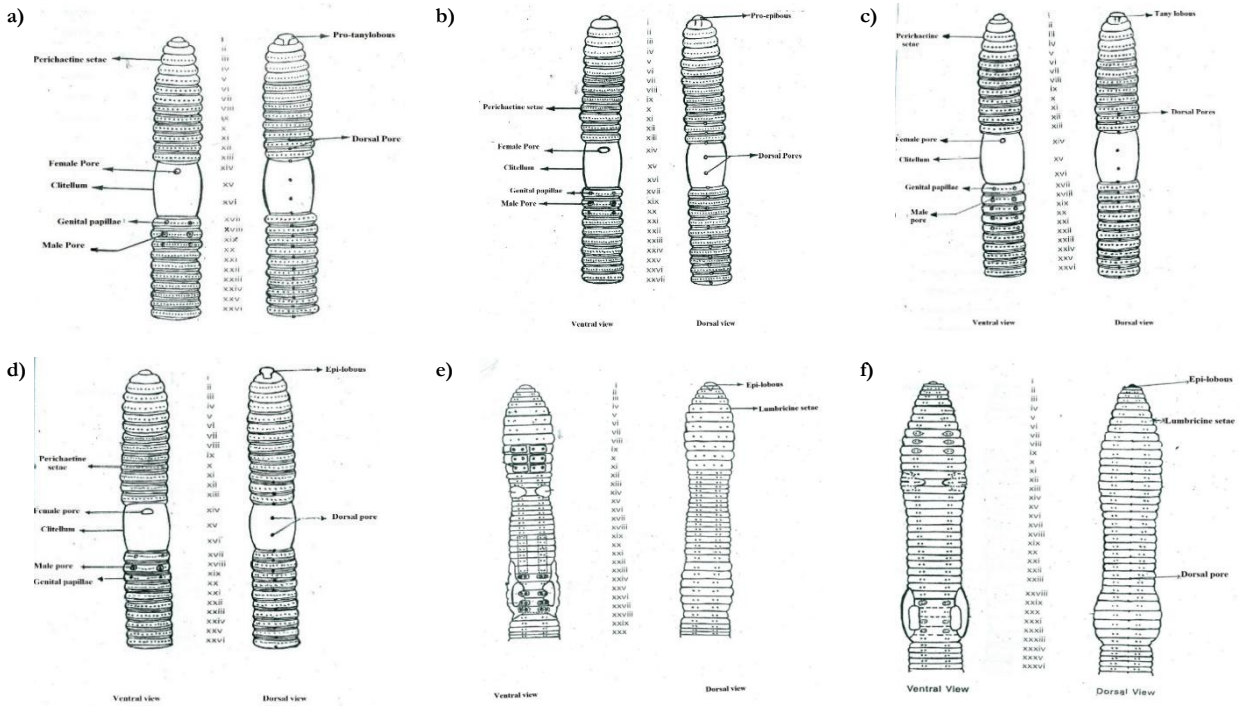


Figure 2: Ventral and Dorsal view of different earthworm genus. **a)** *Pheretima posthuma* (Vaillant), **b)** *Pheritima hawayana* (Rosa), **c)** *Pheretima elongate* (E.Perr), **d)** *Pheretima heterochaeta* (Michaelsen)**, e)** *Aporrectodea calignosa* (Savigny), **f)** *Aporrectodea longa* (Ude)

DIVERSITY AND RELATIVE ABUNDANCE

During the sampling period, 651 total adult specimens were collected (cotton =525 and sugarcane = 126) with a prevalence of 377 from the Kacha site and 274 from the Razai Shah site. According to a Key by Stephenson (1923) and Bhatti (1962), adult specimens were found to be a members of two families i.e. *Megascolecidae* that was restricted in cotton fields of both sampling sites, and *Lumbricidae* of the same order *Haplotarida,* class *Oligochaeta* was found in the sugarcane fields of both sampling sites. Total of six species, 4 from the family *Megascolecidae* named *Pheretima posthuma, Pheretina hawayana, Pheretima elongata, Pheretima heterochaeta,* whereas 2 species from the family *Lumbricidae* named *Aporrectodea calignosa and Aporrectodea longa* were identified with the help of key. Statistically, earthworms in the Megascolecidae family showed abundance compared to those in the Lumbricidae family (Figure 3).

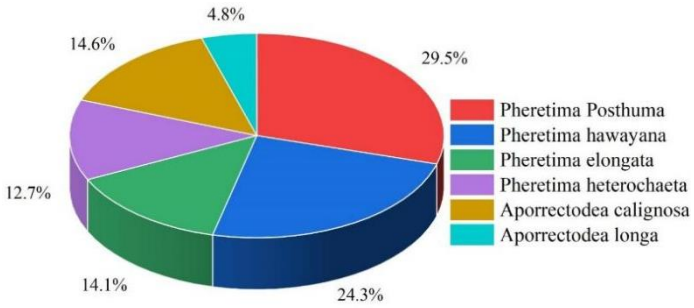


Figure 3: Diversity and Abundance in (%) of different earthworm species of order Haplotaxida present in District Bhakkar

Earthworms of species *Pheretima posthuma* were highest in number i.e. 192 (29.49%) of total specimens, *Pheretima hawayana* was second in abundance i.e. 158 in number (24.27%), *Pheritima elongata* was 92 in number (14.13%) and members of *Pheretima heterochaeta* were 83 in number i.e. 12.74%. In contrast, in the family *Lumbricidae* members of species *Aporrectodea calignosa* were highest in this family i.e. 95 in number (14.59%) and *Aporrectodea longa* was 31 in number (4.761%) (Table 2).

Table 2: Diversity and Abundance in (%) of different earthworm species of order Haplotaxida present in District Bhakkar

Order	Family	Species	Total%
Haplotaxida	Megascolecidae	*Pheretima Posthuma*	192 (29.49%)
		Pheretima hawayana	158 (24.27%)
		Pheretima elongata	92 (14.13%)
		Pheretima heterochaeta	83 (12.74%)
	Lumbricidae	*Aporrectodea calignosa*	95 (14.59%)
		Aporrectodea longa	31 (4.761%)
	Total		651 (100%)

In cotton field diversity, the index showed that the highest number of earthworms were 192 (36.57%) belonging to the family *Megascolecidae*, species *Pheretima posthuma* while the second highest number was 158 (30.09%) of species *Pheretima hawayana.* The third highest number was 92 (17.52%) belonging to species *Pheretima elongate* and the fourth one species was *Pheretima heterochaeta* i.e. 83 in number (15.8%) lowest in count. Overall, 525 earthworms of four species of family Megascolecidae were found in the Cotton field of both sampling sites (Table 3 and Figure 4).

Table 3: Diversity and abundance in (%) of different earthworm species of order Haplotaxida in cotton crop of District Bhakkar

Order	Family	Species	Cotton	Total%
Haplotaxida	Megascolecidae	*P. Posthuma*	192	36.57
		P. hawayana	158	30.09
		P. elongata	92	17.52
		P. heterochaeta	83	15.8
	Total		525	100

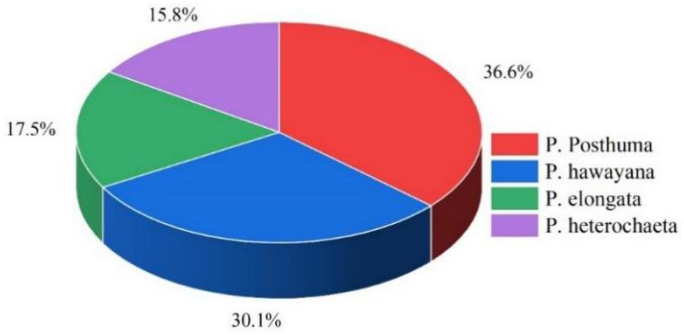


Figure 4: Diversity and abundance in (%) of different earthworm species of order Haplotaxida in cotton crop of District Bhakkar

Diversity and abundance of earthworm species of the family *Lumbricidae* in the Sugarcane field from the Kacha and Razai Shah sites was presented in Table 4. In this field, two species were found: one was *Aporrectoda calignosa,* which was 95 in number (75.39%), and the second one was *Aporrectodea longa*, which was 31 in number (24.6%). Richness of *Apporrectodea calignosa* species as compared to *Aporrectodea longa* was noticed in the sugarcane field (Table 4, Figure 5).

Table 4: Diversity and abundance in (%) of different earthworm species of order Haplotaxida in sugarcane crop of District Bhakkar

Order	Family	Species	Sugarcane	Total%
Haplotaxida	**Lumbricidae**	***A. calignosa***	**95**	**75.39**
		A. longa	31	24.6
	Total		126	100

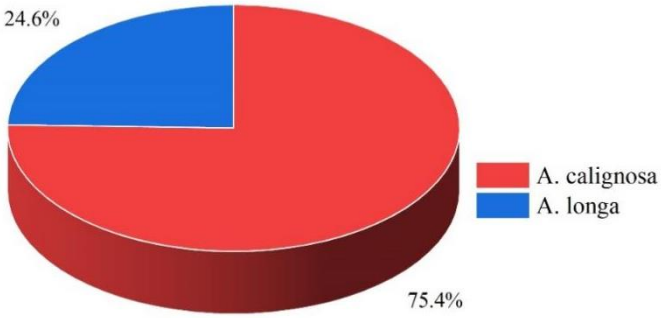


Figure 5: Diversity and abundance in (%) of different earthworm species of order Haplotaxida in sugarcane crop of District Bhakkar

A comparison of the dominant species from the cotton and sugarcane fields in different sampling sites is given in Table 4. Earthworms of species *Pheretima posthuma* were dominant as compared to other species i.e. 192 (29.49%) in cotton fields of Kacha (103) and Razai Shah (89). However, this species' abundance was higher in the cotton field of Kacha site (39.6%) when compared to the Razai Shah (33.96%) sampling site. The total number of an earthworm of *Pheretima hawayana* species was found as 158 (24.27%) in the cotton field of both sampling sites; however, abundance was observed in the cotton field of Kacha site (31.55%) in comparison to that in Razai Shah (28.62%) and members of species *Pheretima elongata* were 92 in number (14.13%) with more abundance in cotton field of Kacha site (29.27%) as compared to Razai Shah (5.72%). No earthworm of the species *Pheretima heterochaeta* was found in the cotton field of the Kacha sampling site, whereas the Razai Shah site carried 83 (12.74%) earthworms of this species. Cotton field of both sampling sites showed no member of the family *Lumbricidae,* while no member of the family *Megascolecidae* was observed in the soil of the sugarcane fields of both sampling sites. Total members of species *Apporrectodea calignosa* in the sugarcane field of Razai Shah and Kacha sampling sites were 95 (14.59%), with more abundance in the sugarcane fields of the Kach site (72.80%) than in Razai Shah (12.63%). Earthworm of species *Apprrectodea longa* was 31 in number (4.761%) localized in the sugarcane field of the Kacha sampling site with no appearance in the sugarcane field of the Razai Shah site (Table 5).

Table 5: Diversity and relative abundance (%) of order Haplotaxida in cotton and sugarcane crops from different sampling sites of District Bhakkar

Species	Kacha		Razai Shah		Total
	Cotton	Sugarcane	Cotton	Sugarcane	
P. Posthuma	103 (39.16%)	-	89 (33.96%)	-	192 (29.49%)
P. hawayana	83 (31.55%)	-	75 (28.62%)	-	158 (24.27%)
P. elongata	77 (29.27%)	-	15 (5.725%)	-	92 (14.13%)
P. heterochaeta	-	-	83 (31.67%)	-	83 (12.74%)

A. calignosa	-	83 (72.80%)	-	12 (100%)	95 (14.59%)
A. longa	-	31 (27.19%)	-	-	31 (4.761%)
Total %	263 (40.39%)	114 (17.51%)	262 (40.24%)	12 (1.843%)	
	377 (57.91%)		274 (42.08%)		651 (100%)

Information about the numerically dominant species on a monthly basis in cotton and sugarcane fields is presented in (Table 6).

Table 6: Diversity and relative abundance (%) of monthly variations of earthworm species in cotton and sugarcane crops of District Bhakkar

Species	Months						
	Oct	**Nov**	**Dec**	**Jan**	**Feb**	**Mar**	**Total**
P. Posthuma	21	35	11	7	46	72	192 (29.49%)
P. hawayana	33	19	13	9	31	53	158 (24.27%)
P. elongata	9	17	11	-	21	34	92 (14.13%)
P. heterochaeta	31	27	12	-	2	11	83 (12.74%)
A. calignosa	17	14	-	6	32	26	95 (14.59%)
A. longa	2	3	-	-	9	17	31 (4.761%)
Total %	113	115	47	22	141	213	651 (100%)

In March, *Pheretima posthuma* was the most dominant species (72/192) in the cotton fields of both sampling sites. Similarly, *Pheretima hawayana* was also highest in number in March (53/158) from the cotton field of both sites. Number of earthworms of *Pheretima elongata* showed great fluctuation with highest sampling number in March (34/92) and 0/92 in January. *Pheretima heterochaeta* was highest 27/83 in number in November and totally vanished in January from Razai Shah sampling site while it was already absent from Kacha site. *Aporrectodea calignosa* was highest in number in March (26/95) and vanished in December from sugarcane fields of both sampling sites. *Aporrectodea longa* attained highest number (17/31) during sampling in March and was missing in samples of December and January. Total earthworm belonging to all species *Pheretima posthuma, Pheretima hawayana, Pheretima elongate, Pheretima heterochaeta, Aporrectodea calignosa and Aporrectodea longa* were respectively found 113 in Oct, 115 in Nov, 47 in Dec, 22 in Jan, 141 in Feb and 213 in Mar with total number of 651.

Shannon- Weiner index

Shannon – Wiener index of diversity of earthworm species of two families (Megascolecidae and lumbricidae) of order *Haplotaxida* in cotton and sugarcane crop showed that in cotton field, number of species was 4, abundance was 525, diversity 1.326, evenness 00.957 and dominance was 00.28 while sugarcane field showed number of species 2, abundance was 126, diversity 00.558, evenness 00.805 and dominance 00.629. A comparison of both fields showed that the cotton crop was richer in the number of species, abundance, diversity and dominance than the sugarcane crop (Table 7).

Table 7: Shannon- Weiner index of diversity of earthworm species of order Haplotaxida in cotton and sugarcane crops of District Bhakkar

Crop	Number of species (S)	Abundance (N)	Diversity (H')	Evenness (E)	Dominance (D)
Cotton	4	525	1.326	0.957	0.28
Sugarcane	2	126	0.558	0.805	0.629
Total	6	651	1.884	1.762	0.909

Shannon – Wiener index of the diversity of earthworm species of order *Haplotaxida* in two sampling sites of Kacha and Razai Shah showed the same number of species was present in both, but abundance was higher in the Razai Shah site (Table 8).

Table 8: Shannon- Weiner index of the diversity of earthworm species of order Haplotaxida in two sampling sites of District Bhakkar

Site	Number of species (S)	Abundance (N)	Diversity (H')	Evenness (E)	Dominance (D)
Kacha	5	377	1.551	0.964	0.22
Razai Shah	5	274	1.378	0.856	0.277
Total	10	651	2.929	1.82	0.497

Shannon – Wiener index of diversity of earthworm species of order *Haplotaxida* in cotton and sugarcane crops in two sampling sites revealed that diversity was more in the cotton fields of Razai Shah, evenness was more in sugarcane fields of the Kacha site and dominance was more in the cotton crop of Kacha site. In Razai Shah sampling site, diversity of earthworms in cotton was more than in sugarcane, evenness was more in sugarcane and more dominance was observed in sugarcane than cotton (Table 9).

Table 9: Shannon- Weiner index of diversity of earthworm species of order Haplotaxida of cotton and sugarcane crops in two sampling sites of District Bhakkar

Sites	Crop	Diversity (H')	Evenness (E)	Dominance (D)

Kacha	**Cotton**	1.091	0.993	0.339
	Sugarcane	0.585	0.844	0.604
Razai Shah	**Cotton**	1.253	0.904	0.301
	Sugarcane	0.1	0.1	1

4.2.2. Comparative analysis of cotton and sugarcane fields based on Shannon- Weiner index

A t-test comparison of several earthworm species in cotton and sugarcane crops from the Razai Shah sampling site showed that cotton fields had significantly more species abundance and diversity than sugarcane crops. The T value was 2.312 (Table 10).

Table 10: Comparison (t-test) between cotton and sugarcane crops of earthworm species for Razai Shah

Crop	**Number of species (S)**	**Abundance (N)**	**Diversity (H')**
Cotton	4	262	1.253
Sugarcane	1	12	0.1
T. Value	2.31242		
Probability	*0.040907		
	*=significant (P<0.05)		

A T-test between some earthworm species in cotton and sugarcane crops from the Kacha sampling site revealed that no significantly high abundance and diversity was found in cotton crops than in sugarcane. T value was 1.4019 (Table 11).

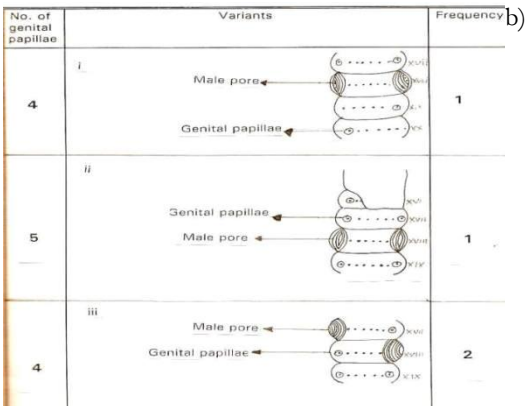
Table 11: Comparison (t-test) between cotton and sugarcane crops of earthworm species for Kacha.

Crop	**Number of species (S)**	**Abundance (N)**	**Diversity (H')**
Cotton	3	263	1.091
Sugarcane	2	114	00.585
T. Value	1.4019		
Probability	0.127748		
	* = significant (P<0.05)		

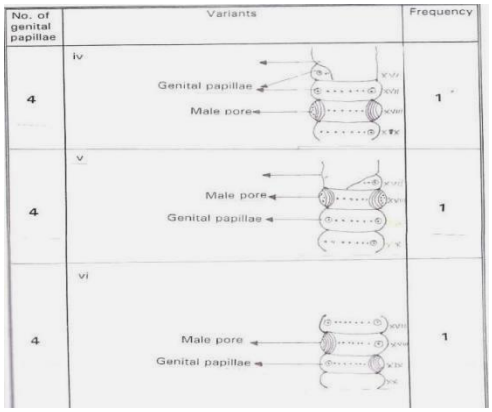
GENITAL PAPILLAE VARIATIONS

Most earthworms hold various markings on the anterior ventral surface at sexual maturity, such as tubercles, ridges, and papillae [6]. These markings are said to be the openings of accessory glands. Their secretion perhaps assists in copulation, but the exact purpose of these glands is still unidentified. In the present study, most of the earthworms were found to have genital papillae on pre-clitellar and post-colitellar regions on ventral surface. These genital papillae varied greatly in number and position. These variants have already been reported for *Pheretima posthuma, Pheretima hawayana* [32]. In the present study, the variation in the number and position of genital papillae were also found in addition to the above in following species viz., Pheretima heterochaeta. (Figure 6) displays frequency of variants of different earthworms concerning the position and number of their genital papillae.

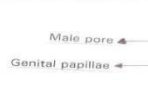
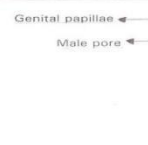
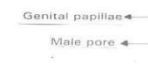
a)



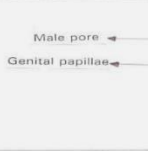
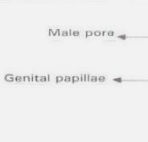
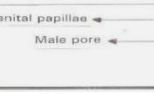
b)



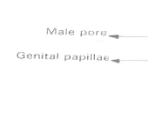
c)

No. of genital papillae	Variants	Frequency
2	vii 	1
7	viii 	1
3	ix 	1

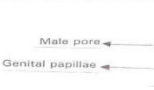
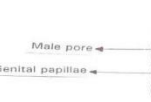
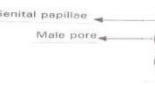
d)

No. of genital papillae	Variants	Frequency
8	x 	1
9	xi 	1
3	xii 	1

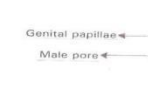
e)

No. of genital papillae	Variants	Frequency
5	i 	1
5	ii 	1
5	iii 	1

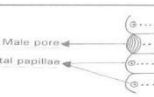
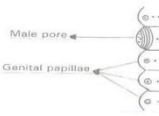
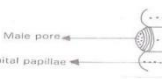
f)

No. of genital papillae	Variants	Frequency
3	iv 	1
5	v 	1
3	vi 	2

g)

No. of genital papillae	Variants	Frequency
7	vii 	1
6	viii 	1

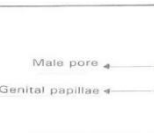
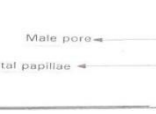
h)

No. of genital papillae	Variants	Frequency
6	i 	1
7	ii 	1
2	iii 	1

i)

No. of genital papillae	Variants	Frequency
4	iv 	1
2	v 	1
3	vi 	1

j)

No. of genital papillae	Variants	Frequency
8	vii 	1
3	viii 	1
7	ix 	1

k)

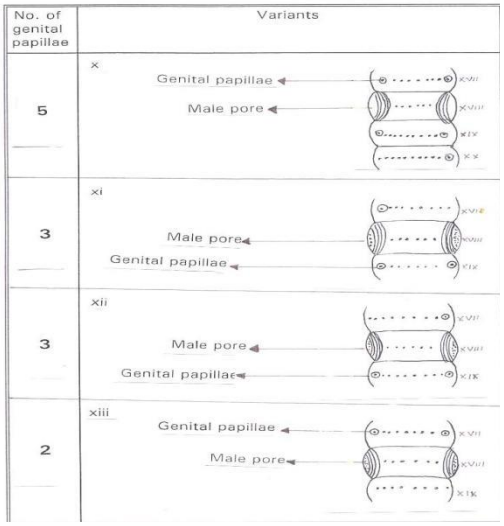


Figure. 6 (a-d):

Post-clitellar segments showing variable positions and number of genital papillae, and male porophore in *Pheretima heterochaeta, (e-g):* Post-clitellar segments showing variable positions and number of genital papillae, and male porophore in *Pheretima hawayana*, (h-k): Post-clitellar segments showing variable positions and number of genital papillae, and male porophore in *Pheretima posthuman.* gments showing variable positions and number of genital papillae, and male porophore in *Pheretima posthuma*. This study was conducted to discover the biodiversity of earthworms in Bhakkar District due to their prime importance in increasing soil fertility and crop quality, respectively serving as farmer friends by cycling the organic matter and nutrients. Wheat and sugarcane crops are Pakistan significant and valuable cash crop and a good source of income [33]. Earthworms can change the chemical properties of soil through ingestion and fragmentation [34]. This was in accordance with Jalal *et al*., 2014 who reported *Pheretima posthuma* and *Pheretima hawayana* as most abundant species of crop fields *(*cotton, wheat sugarcane and maize) as we found these species to be the most abundant among all species under study [35].

Out of six species *Pheretima posthuma* and *Pheretima hawayana* were exhibiting wide range of tolerance to edphaic factors as they were not only highest in number but also present at the maximum number of sampling sites. Confirmation of data was carried out through Shannon diversity index. This index confirmed that the cotton field was most diversified (H' = 1.253 & 1.091) compared to the sugarcane area (H' = 0.1&0.585) of Razi Shah and Kacha, respectively. Contrarily, Jalal *et al*., 2014, reported that the sugarcane field is more diversified than the cotton field. If concerned with the interspecies diversity in different sampling sites, 3 species were found in Cotton field Kacha side and 4 species were found in Cotton in Razai Shah, whereas 2 species were found in sugarcane on Kacha Side, and 1 species was found on Razai Shah. *Aporrectodea longa* was found to be the least abundant overall The total number of species Kacha side is 377 and the number of Razai Shah species is 274. Diversity and relative abundance of monthly variation of earthworms species in cotton and sugarcane crops was 29.49% in October, 24.27% in November, 14.13% in December, 12.74% in January, 14.59% in February, and 4.76% in March, respectively found. Pheretima posthuma and Pheretima hawayana were present in cotton fields during the six months of the study period, while the abundance of species *Pheretima elongata, Pheretima heterochaeta* declined in January. Two species of earthworms belonging to the family Lumbricidae, identified from the Sugarcane crop, namely *Aporrectodea calignosa and Aporrectodea longa* showed a decline in December. Our results are in consent with Pelosi *et al.,* (2009), who reported the absence of most earthworm species during cold and dry months.

Monthly variations of earthworm fauna for one year were also described by Goswani (2015). In his study the monthly sampling, diversity and relative abundance % of earthworms revealed that the diverse fauna was present in February and March, while the fauna decline in December and in months of April and May. In this study, the cold and hot seasons seem limiting for various earthworm species. Our study is in accordance with Goswani (2015), as we found a decline in abundance and diversity during colder months of December and January [36].
Earthworm communities nearly always include species that pursue different ecological strategies; familiarity with these strategies is essential to understanding the structure of earthworm communities. *Pheretima posthuma* and its congeners in most abundant of all species in cultivated crops. These findings also aligned with Mannan *et al*., (1994), who studied the effect of different environmental factors and vegetation on abundance, morphometry, and distribution of *Pheretima posthuman* [37].

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