Impact Of Pesticides On The Morphological Characteristics And The Diversity Of Earthworm Species From District Okara Punjab Pakistan

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Abstract

Current study was conducted to check the morphological changes and diversity of different species of earthworms in district Okara Punjab, Pakistan. One way anova was used to compare the mean vale and stranded deviation of length and weight. Weight and length were the basic parameters that were used to evaluate morphological characters of earthworms. Shannan veneer index was used to check out the diversity of different species of earthworms that were present in district Okara Punjab, Pakistan. The low values of length and weight in experimental site indicate that pesticides have a great impact on morphological characters and Shannon veneer index also indicate that pesticides also effect the diversity of earthworms. Our study gives base line data about the morphological characters and diversity of earthworms in Okara Punjab, Pakistan. Collaboration with experts in the field of soil ecology and entomology can also provide the valuable insight and guidance throughout the study.

Keywords: Pesticides, Morphological Characters, Earthworm, Diversity, Earthworm

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Introduction

Earthworms belong to the kingdom Animalia, Phylum Annelida, Class Oligochaeta and include 3 orders, 4 suborders, 7 superfamilies, 27 families, and 8 subfamilies.(Sial et al., 2017). They cover 1800 species.(Ansari & Saywack, 2011). They are invertebrates that are found all over the world. There are over 5,500 identified earthworm species, with 590 of them thriving in South Asia, including Pakistan.(Ansari & Saywack, 2011). There are 3627 earthworm species documented globally, with an average annual addition of 68 new species The main source of food for earthworms is organic debris in various states of degradation.(Curry & Schmidt, 2007). In many terrestrial ecosystems, earthworms comprise up to 40–90 percent of the soil macrofaunal biomass, making them important soil creatures. Earthworms are also called night crawlers Earthworms are one of the most significant groups of creatures, serving as both a biofertilizer and a composting, biological, aerator, moisture retention, and crusher. Earthworms are used as a food source for birds and other organisms (Rao, Pavan, & Madhavendra, 2003). Earthworms are in direct touch with the soil solution and play a critical role in many soil processes.(Capowiez, Rault, Mazzia, & Belzunces, 2003). In agro-ecosystems, the number of species and ecological categories (e.g. epigeic, endogeic, and anecic) is preferred as critical indicator. (Kousar & Akhter, 2020). Epigeic species, such as *Lumbricus castaneus*, live and eat on or near the soil surface. Anecic earthworms, such as Lumbricus terrestris, dwell in permanent vertical burrows under the soil and may emerge to feed on surface trash. Endogeic species, like Allolobophora chlorotica, live in horizontal burrows and eat soil (Pélosi et al., 2013). Earthworms play a vital part in the nutritional cycle of terrestrial ecosystems as the main decomposers of organic materials and play a crucial part in the nutritional cycle. (Yu et al., 2006) .Earthworms act as ecosystem engineers (Denoyelle, Rault, Mazzia, Mascle, & Capowiez, 2007). Eisenia foetida, a kind of earthworm, is thought to be a good bio monitor for determining the level of ecological risk.(Reddy & Rao, 2008). Earthworms are known as "the bowels of the earth," according to Aristotle, and Charles Darwin emphasized their role in breaking down dead plants and animal debris that enters the soil, as well as in the ongoing maintenance of soil structure.(Dureja, Patra, Johnson, & Tomar, 1999). Earthworms come into direct touch with both the solid and watery phases of the soil (Schreck, Geret, Gontier, & Treilhou, 2008). Earthworms are able to detect a variety of external stimuli, including pollutants, mostly through chemoreceptor tubercles in the prostomium and anterior segments, as well as sensory cells in the mouth.(Pereira, Antunes, Ferreira, Goncalves, & Pereira, 2010). Earthworms have both positive and negative consequences. They're used for a variety of things, including fishing bait, fish food, and medication. They are also utilized to improve soil fertility, pH, soil texture, porosity, and amendments of soil organic matter content, as well as plant performance.(Faheem, Ali, Alam, & Khan, 2014). Earthworms are one of the many animal species that make up the intricate soil food chain. They function as an early warning indicator for the impacts of soil pollution .(Sousa & Andréa, 2011). The earthworm lives in a variety of environments. They are generally found where there is a lot of organic debris, such as manure, litter, compost, and so on. They can be found in both fresh and brackish water. They also live in a hydrophilic habitat near both fresh and brackish water, and some species can even tolerate winter .(Malik & Afsheen, 2021).Earthworms are regarded as "one of the most important entities in environmental toxicity. (Pelosi, Joimel, &Makowski, 2013). Earthworm populations can provide information about a soil's structural, microclimatic, nutritional, and toxic condition.(Pfiffner & Mäder, 1997). The aim of study was to evaluate the impact of pesticides on the morphological characteristics and diversity of different species of earthworm in district Okara, Punjab, Pakistan.

Material and methods

Study area

This study was done in Okara district, Punjab Pakistan which is known for its fertile

agricultural land during. Okara district is famous for its fertile lands, peaceful natural environment and green fields of potato, tomato, sugarcane, wheat, rice, and maize crops. The subsoil water is sweet and good for agriculture. 0kara is the capital city of okara district in Punjab Pakistan. It is the 23rd largest city of Pakistan by population.

Sampling

Earthworms **w**ere collected from different fields on monthly bases which were sprayed with different pesticides. Sampling was done during April, May, June 2022. These fields included sorghum, maize, sugar cane, *Trifolium axelendrium* guava garden, and sunflower respectively. There were six control sites in every sampling to check the comparison of earthworms' morphology between the control and experimental sites

Earthworms were obtained by using quadrate and hand sort method.(Khan, Andleeb, Khan, & Mustafa, 2021). After collection, these earthworms were kept in cloth bags containing mother soil from sampling sites and sprinkling with water.(Jeyaprakasam, Muniyandi, James, Karmegam, & Ponnuchamy, 2021) Every bag was labeled according to the site with permanent marker. (Khan et al., 2021) Google earth locations were noted. (Singh, Singh, & Vig, 2016) After collection, earthworms were taken to the General lab of Zoology University of Okara where the length of each individual was measured with a foot rule (Khan et al., 2021) and the weight was measured by a digital weight balance.

Soil samples were taken from the sampling sites. Soil texture was checked by physical observation of the soil as loamy, clay, moist, dry, and sandy(Khan et al., 2021). The pH of the soil was measured by a Ph meter (Tripathi & Bhardwaj, 2004). The earthworm species were identified with the help of identifications keys provided by Stephenson (Khan et al., 2021).

Results

Species Identified:

Lumbricus terrestris

Description of the Habitat

Except for coarse sands, bare rock, and acidic peat, *Lumbricus terrestris* may live in any soil type the -15 °C isotherm has been discovered to restrict it. It may grow in a variety of soils with varying pH levels. *L. terrestris* cannot survive in temperature fluctuations, with values as low as 3.5–3.7 and as high as around 8. During the winter, it hibernates in deep soil layers. **(**Addison, 2009; Tiunov *et al*., 2006)

Reproduction

Lumbricus terrestris is a sexually reproducing reciprocally mating simultaneous hermaphrodite with individuals sharing sperm. *L. terrestris* emerges from its burrow to copulate on the surface of the earth. Mated individuals make cocoons for up to 12 months after mating, and sperm is preserved. According to a study on cocoon hatchability, 76 to 62 percent of cocoons hatched in the first five months after mating, but that number dropped to approximately 11 percent in the sixth month, and cocoons after that failed to hatch. The median total number of viable cocoons produced per person was 5, with a range of 0 to 21. Sperm can be kept for up to 8 months.(Butt & Nuutinen, 1998).

Nutrition:

Lumbricus Terrestris is a soil-dwelling detritivore that feeds on dead leaves. L. Terrestris emerges from its burrow to devour leaf litter quickly. High Ca and maybe N concentrations are linked to preference. As a result, basswood, ash, and aspen are the most popular, followed by sugar maple and other maple species. Oak is less appealing due to its low calcium content, but if no other leaves with a higher calcium content are present, it will be ingested. (Frelich et al., 2006; Suarez et al., 2006; Belote & Jones, 2009). Despite its widespread presence in agricultural fields, it suffers from herbicide resistance. mechanical wear and tear, as well as a shortage of leaf litter (L. Frelich, pers. comm.).

Scientific Classification:
- Kingdom: Animalia
- Phylum: Annelida
- Class: Clitellata
- Order: Opisthopora
- Family: Lumbricidae
- Genus: Lumbricus **(**Linnaeus, C. 1758)

Identification marks
Setae are paired widely at both ends of the body. These are rich in pigmentation. They have brown-red color on the dorsal and yellow on the ventral side. They have a length of 90-300 mm. They have 11o-160 body segments.

Benefits of *Lumbricus terrestris*:
L.terrestris is an anecic earthworm that catches litter from the soil, and draws it down into the mineral layer, leaving casts of mixed organic and mineral material on the soil surface.it plays a vital role in nitrogen cycle for agriculture. (Addison, 2009).

Image 1: *Lumbricus terrestris*

Pheretima californica
Morphological characters:
Theses have two pairs of spermathecal pores at segment number 7/8. They have 70-170 mm length. They have 10-150 segments. They have Reddish - brown, clitellum creamy to dark gray

Description of Habitat
These earthworms live in damp, moist soil. In dry weather these go deeper in soil to protect their body

Classification
- Kingdom: Animalia
- Phylum: Annelid
- Class: Oligochaeta
- Order: Ophisthopora
- Family: Megascolecidae
- Genus: Pheretima
- Species: californica

Image 2: *Pheretima californica*

Aporrectodea longa
Anecic species are found primarily on grassland and arable settings with little soil organic matter (Jänsch et al., 2013). This species is reported from a wide range of soils, with pH values ranging from 4.5 to 8.0, where it can be found in lawns, peat bogs, greenhouses, cultivated soil, gardens, pastures, and woodlands. It is also documented from caves (Reynolds 1977). It has been discovered in garden soil, under old growth Douglas-fir (Marshal & Fender 2007).

Morphological characters
These earthworms have black head. The number of body segments differ from 95-120. These are 25-105 mm in length (Stephen son). They are dark-brown color

Classification:
- Kingdom: Animalia
- Phylum: Annelid
- Class: Clitellata
- Order: Opisthopora
- Family: Lumbricidae
- Genus: Aporrectodea
- Species: Aporrectodea *longa*

Image 3: *Aporrectodea longa*

The mean value and stranded deviation of weight of all the samples that were taken from six experimental sites during April month are shown in table 1. The data was collected from different time intervals of the month. There is no statically significant difference between all sites except site 2 and site 6 because the weight of all the samples that were taken from site 2 and 6 has the different weight values as compared to others. Others have low weight.

The mean value and stranded deviation of weight of all the samples that were taken from six experimental sites during May month are shown in table 2. There is a statically significant difference between all except site3, 4,5 because the weight of all the samples that were taken from site3, 4,5 has the same weight as compared to others while other has low weight.

The mean value and stranded deviation of weight of all the samples that were taken from six experimental sites during June month at different time hours are shown in table 3. There is no significant difference between all except site 3,4 because the weight of all the samples that were taken from site3, 4 has the same weight as compared to others while the others have low weight.

The mean value and stranded deviation of length of all the samples that were taken from six experimental sites during April month are shown in table 4. There is no significant difference between all except site 2,3,4,5 because the length of all the samples that were taken from site 2,3, 5 has the same weight as compared to others while the others have low length.

The Shannon diversity index was measured from different study sites. For Shannon diversity index three sites were selected for sampling. Diversity index from the site 1 was measured for three species i.e., *Lumbricus terrestris, Aporrectodea longa,* and *Pheretima californica* and measured Diversity index for these species was 1.08, 1.09 and 0.938 respectively (Table 5).

Diversity index from the site 2 was measured for three species i.e., *Lumbricus terrestris, Aporrectodea longa,* and *Pheretima californica* and measured Diversity index for these species was 0.927, 1.05 and 1.09 respectively (Table 6).

Diversity index from the site 3 was measured for three species i.e., *Lumbricus terrestris, Aporrectodea longa,* and *Pheretima californica* and measured Diversity index for these species was 1.05, 1.05 and 1.08 respectively (Table 7).

Comparison between weight of control groups

The mean value and stranded deviation of weight of all the samples that were taken from six control sites during April month are shown in Figure 1. There is no significant difference between all except site 2,4 and 5 because the weight of all the samples that were taken from site 2 ,4 and 5 the same weight as compared to others. Others have low weight.

Comparison between length of control groups

The mean value and stranded deviation of length of all the samples that were taken from six control sites during April month are shown in Figure 2. There is no significant difference between all except site 2, 3,5,6 because the length of all the samples that were taken from site 2,3, 5,6 has the same weight as compared to others while the others have low length.

The mean value and stranded deviation of length of all the samples that were taken from six control sites during May month are shown in Figure 3. There is no significant difference between all except site 2, 4,5, because the length of all the samples that were taken from site 2,4, 5, has the same weight as compared to others while the others have low length.

The mean value and stranded deviation of length of all the samples that were taken from six control sites during June month Figure 4. There is no significant difference between all except site 2, 3,5, because the length of all the samples that were taken from site 2,3, 5, has

the same weight as compared to others while the others have low length.

Comparison between weight of experimental group and control group.

The mean value and stranded deviation for weight of earthworms collected from control site is more than experimental sites for the first two samplings .The mean value and stranded deviation is not significant for weight of earthworms collected from both control sites and experimental sites during third sampling During the sampling 1 weight measured from control sites is higher than the experimental sites. and during the 2nd sampling weight measured from control site is higher than the experimental site sampling it means in first two sampling from control and experimental site, weight of control site is high as shown in table 1. This showed that pesticides have impact the earthworm's morphology.

Impact of pesticides on earthworm species length.

The mean valve and stranded deviation for the length of earthworm specimens collected from control site is more than the experimental sites during the first sampling. The mean valve and stranded deviation for the length of earthworm specimens collected from control sites is less than the experimental sites during second and third sampling. Results indicate that the pesticides have less impacts on the length of earthworm species Table 2 showed the value of mean and stranded deviation.

Table 1: Mean length and weight of earthworms recorded during April at sampling sites.

No of sites	Mean Wt. ± S.D.		
	Morning	**Noon**	**Evening**
site 1	0.75±0.23	0.60±0.20	0.65±0.28
site2	1.079±0.29	0.60±0.20	0.677±0.24
site3	0.57±0.23	0.65±0.30	0.74±0.29
site 4	0.70±0.22	0.81±0.27	0.77±0.28
site5	0.63±0.30	0.57±0.26	0.61±0.17
site6	0.29±0.09	0.58±0.28	0.62±0.16

Table 2: Mean length and weight of earthworms recorded during May at sampling sites.

No of sites	Mean Wt. ± S.D.		
	Morning	**Noon**	**Evening**
site 1	6.1±0.71	6.83±1.81	6.68±2.26
site2	7.11±2.3	7.12±1.64	7.36±0.14
site3	7.11±2.2	5.8±1.81	7.58±0.13
site 4	6.27±2.1	8.22±3.27	6.30±0.01
site5	8.41±3.4	9.15±2.71	7.11±0.02
site6	8.9±2.7	6.82±2.46	5.8±0.13

Table 3: Mean length and weight of earthworms recorded during June at sampling sites.

No of sites	Mean Wt. ± S.D.		
	Morning	**Noon**	**Evening**
site 1	6.3±0.69	6.60±2.01	6.12±1.06
site2	6.99±2.1	6.89±0.98	6.95±0.11
site3	7.13±2.0	5.99±1.11	7.10±0.11
site 4	6.35±1.9	7.97±2.14	5.96±0.01
site5	8.21±2.4	8.79±1.99	7.32±0.01
site6	9.1±2.1	6.41±1.03	5.9±0.16

Table 4: Mean length and weight of earthworms recorded during different months at sampling sites.

No of sites	Mean Wt. ± S.D.		
	April	**May**	**June**
site 1	0.85±0.25	0.69±0.19	0.67±0.20
site2	2.01±0.19	0.63±0.22	0.71±0.22
site3	0.63±0.18	0.59±0.21	0.73±0.21
site 4	0.79±0.28	0.79±0.21	0.71±0.21

site5	0.69±0.27	0.51±0.21	0.72±0.10
site6	0.30±0.07	0.61±0.24	0.64±0.19

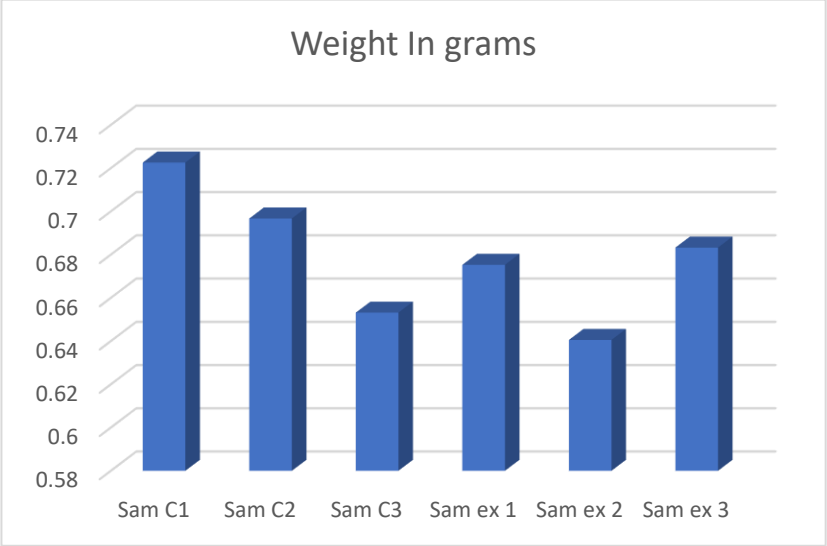

Figure 1: Comparison of weight between different sampling sites and control group.

Sam C1: sampling site 1
Sam C2: sampling site 2
Sam C3: sampling site 3
Sam ex 1: sampling experiment 1
Sam ex 2: sampling experiment 2
Sam ex 3: sampling experiment 3

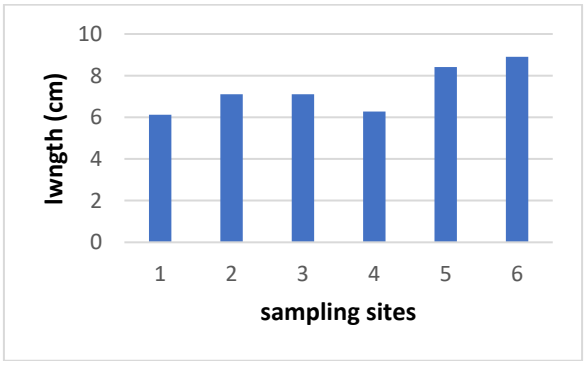

Figure 2: Comparison of length (cm) between different study sites of experiment group 1 (April)

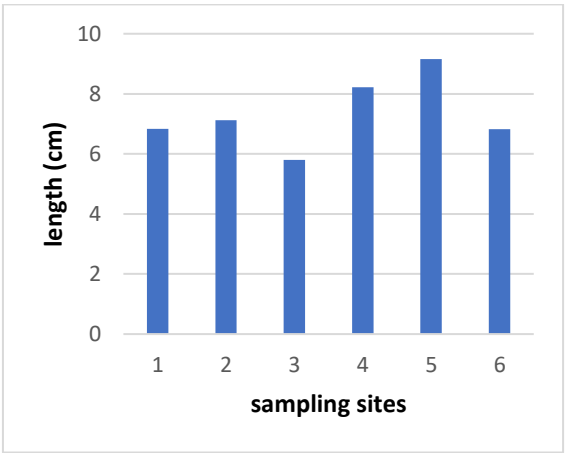

Figure 3: Comparison of length (cm) between different study sites of experiment group 2 (May)

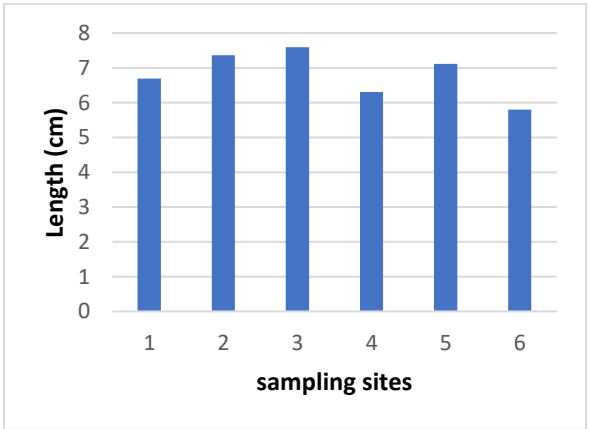

Figure 4: Comparison of length (cm) between different study sites of experiment group 3 (June)

Diversity of earthworm species (*Lumbricus terrestris, Apporrectodea longa, Pheretima californica*)

In the present study attempt have been made to know the distribution of earthworms in okara district Punjab Pakistan during the April, May and June. A total of 643 specimens of earthworms (*Lumbricus terrestris, Aporrectodea longa, Pheretima californica*) were collected during present study from the sites included Sorghum, Maize, Sun flower, Sugarcane, Guava, and Berseem. Total 176 *Lumbricus terrestris* specimens were collected from site 1 with diversity index 1.08 and average population size 58.7. There were 43 specimens of earthworm (*Apporrectodea longa*)

collected from site with diversity index 1.09 and population size is 14.3. Total 20 *Pheretima californica* species were collected during sampling 1 with diversity index 6.67 and population size is 6.67. During second sampling 151 specimens 0f *Lumbricus terrestris* were collected with diversity index0.927 and average population size 50.3. There were 25 specimens of *Apporrectodea longa* with diversity index 1.05 and 8.33 average population size. There were 10 specimens of *Pheretima californica* with diversity index 1.09and 3.3 average population size

During third sampling 181 specimens of *Lumbricus terrestris* were collected with diversity index 1.05 and average population size 60.3. Total 25 specimens of *Aporrectodea longa* were collected with diversity index 1.05 and average population size 8.33 and 12 specimens of *Pheretima californica* were collected with diversity index 1.09 and average population size. Reported data indicate that the earthworm collected during second sampling have low density as compared to other sampling data. Our results showed that pesticides affected the diversity of earthworms.

Table 5: Shannon diversity index of earthworm collected from sampling site 1

Sampling 1						
Sr.No	**Spp. Name**	**Sp. No**	**Diversity index**	**Species richness**	**Evenness**	**Average population size**
1	*Lumbricus terrestris*	176	1.08	3	0.982	58.7
2	*Aporrectodea longa*	43	1.09	3	0.997	14.3
3	*Pheretima californica*	20	0.938	3	0.853	6.67

Table 6: Shannon diversity index of earthworms collected from sampling site 2

Sampling 2						
Sr. No	**Spp. Name**	**Sp. No**	**Diversity Index**	**Species Richness**	**Evenness**	**Average Population Size**
1	*Lumbricus terrestris*	151	0.927	3	0.844	50.3
2	*Aporrectodea longa*	25	1.05	3	0.957	8.33
3	*Pheretima californica*	10	1.09	3	0.991	3.33

Table 7: Shannon diversity index of earthworms collected from sampling site 3

Sampling 3						
Sr.No	**Sp. Name**	**Sp. No**	**Diversity Index**	**Species Richness**	**Evenness**	**Average Population Size**
1	*Lumbricus terrestris*	181	1.05	3	0.958	60.3
2	*Aporrectodea longa*	25	1.05	3	0.957	8.33
3	*Pheretima californica*	12	1.08	3	0.981	4

Discussion

Our results showed that there is significant difference between the weight of control sites and experimental sites. The control sites have more weight as compared to experimental site. This shows that pesticides impact the morphological characters of earthworms According to.(Bustos-Obregón & Goicochea,

2002) pesticides also impact on earthworm weight loss and our results also support to this work .(Van Gestel, Van Dis, & Soils, 1988) Organochlorine pesticides also reduce the weight of earthworms and our results also support to this work .(Choo & Baker, 1998) also studied that use of pesticides reduce the weight of earthworms.

The length of earthworms that were collected from the experimental site and control site has compatibly less significant value so our results show that pesticides has comparatively less impact on the length of earthworms. Our study gives the baseline data about the impact of pesticides on the length of Earthworm. Further study is required to check the impact of pesticides on the body length of Earthworm.

Reported data indicates that the earthworm collected during second sampling have low density as compared to other sampling data collected during first and second sampling. There was more use of pesticides before second because crops were young and there were more pests' attacks. Our results showed that pesticides affected the diversity of earthworms .According to(Jeyaprakasam et al., 2021) diversity is more at unpolluted area. According to (Singh, D., Singh, S., Sahu, J., Srivastava, S., & Singh, M. R. (2016) The excessive. use of pesticides results in earthworm extinction ion. This work is also in line with our results. According to(Sial et al., 2017)Harsh environmental conditions are limiting factor for some s species. According(Singh et al., 2016)The diversity is also affected by abiotic factors of the soil.

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