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Impact of soil pollutants on diversity and abundance of earthworms in cauliflower crop

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Abstract
The present study was designed to elucidate the effect of soil pollutants on diversity and abundance of earthworms in a winter vegetable crop (cauliflower). Collection was done through November to January and samples were anesthetized in alcohol, preserved in 10% formalin solution. The soil and water analysis was performed by atomic absorption spectrophotometry. The Shannon-Weiner Diversity Index (H′) of sampling site (Chack 204) was 1.803, evenness was 0.302 and dominance was 0.831. A significant difference of abundance was observed (t=12.62, p=0.0066 at $p<0.05$). The pH of sewage water was recorded as 5.833 and sewage water irrigated soil was 6.28, whereas pH of canal water was 7.66 and canal water irrigated soil was 7.88. A significant difference of physico-chemical characteristics of sewage water and canal water was observed as following pH, p=0.0043), DO (p=0.0112), TDS (p=0.043), TSS (p=0.015), turbidity (p=0.0028), Pb (t=11.4 p=0.0076), Zn (p=0.0192) except temperature and heavy metals including Co, Cd, Cr, Mg and Ca. Similarly, significant difference of physico-chemical characteristics of sewage water irrigated soil and canal water irrigated soil was recorded as for Pb (p=0.0014), Zn (p=0.0001), Cr (p=0.0366), Mg (p=0.001) and Ca (p=0.0003) except temperature, Co and Cd. Significantly positive correlation occurred between earthworms abundance and temperature at site 1 (p=0.0246) and at sampling site 2 (p=0.0156). It is conceivable from the present data that earthworm population proves to be better and beneficial for non-polluted soil. Environmental factor, such as temperature is very important for earthworm management. The abundance of earthworms was low in cold months of winter. The harsh condition of winter seems to be limiting factor for abundance of earthworm. Acidic pH and high concentration of heavy metals badly affect the diversity and abundance of earthworms.

Keywords: Earthworms, diversity, abundance, soil pollutants

Introduction
Earthworms are the biggest component of creature biomass in soil and termed biological system engineers (Blouin *et al*., 2013) [1]. The nature of soil is demonstrated by earthworms (Rousseau *et al*., 2010) [2]. They rank among the most important soil biota of agro-ecosystem as an ‘ecosystem engineers’ where they assume a key part in controlling soil richness and advancing plant development through nutrient cycling and water infiltration and regulating greenhouse gas emission (Blouin et al., 2013; Lubbers *et al*., 2013) [1, 3].
Through tunneling, throwing and blending of soil and litter (bioturbation) they impact chemical and physical qualities of the upper layer of soil (Eisenhauer *et al*., 2007) [4] with critical outcomes for plant development and focused communications between plant practical gatherings and plant species (Eisenhauer and Scheu 2008a) [5].
Since earthworm’s wealth and dissemination are unequivocally impacted by the natural conditions and the biological status of the framework (Falco *et al*., 2015; Pelosi *et al*., 2015) [7, 6], the night crawler group structure can be effectively utilized as a natural marker of soil conditions (Momo *et al*., 2003) [8]. Worms can be utilized to screen land-use sorts with various levels of soil quality (Guei and Tondoh 2012) [9].
Sewage water is started from a mix of local, mechanical, business or farming exercises (Tilley *et al*., 2014) [11]. Populace, urbanization and enhanced living conditions are real wellsprings of waste water (Qadir *et al*., 2010) [10]. Untreated waste water is utilized for watering system as a part of less established nations, for example, in Africa, Asia, and Latin America. (Faruqui *et al*., 2004) [12].
Heavy metal accumulation in soil ecosystems interrupt the movements and activities of organisms living in them and results in decrease productivity from soil.

(Sharma and Dubey, 2005) [14]. Earthworms have the ability to separate heavy metals from humus in their digestive system which they later store in their bodies rather excreting out. Earthworms also used to remove pollutants and heavy metals from municipal waste (Patnaik and Reddy, 2011) [13].

Faisalabad is the third most populous city of Pakistan. The usual volume of waste water disposed off from the city was 5.28m^3/second. The analysis of wastewater showed mean maximum volume of biological oxygen demand and chemical oxygen demand. Sample analysis showed exceeding limits of biological oxygen demand and chemical oxygen demand. An excessive Ni content were found in soil irrigated with Madhuana drain and Cd in soil irrigated with Municipal drain. The vegetables grown with untreated wastewater were found highly contaminated with Cr, Pb, Cd and Fe. Wheat, sorghum, berseem, lettuce, mint and turnip were found to be more contaminated whereas sugarcane and barley showed least contamination (Kahlown *et al*., 2006) [15]. The study aimed the investigation of habitat preference of earthworms in different environmental conditions. Evaluation of diversity and abundance with references to soil pollution. As sewage water is use for irrigation without treatment. In sewage water industrial effluents are also added from industries present in city. This effluent has negative effect on the abundance of earthworms.

Material and Methods

Study Area

Faisalabad district lies between 30º 40′ to 31º 47′ N; 72º 42′ to 73º 40′ E, 605 feet above sea level (City District Govt. Faisalabad, 2010). The total area of Faisalabad District covers 58.56 km^2 (22.61 sq mi) (Mustafa 2009) [16]. A 2014 demographic profile shows the population count at 3.038 million (Pakistan Demographics Profile 2014). The climate of the district can see extremes, with a summer maximum temperature of 50 °C (122 °F), and a winter temperature of -2 °C (28 °F) (Climatic Normal of Pakistan 2013; Rasul and Mahmood. 2009; Imran *et al*., 2014) [18, 20, 19]. Two sites were selected for sampling. Sampling site 1 was located 14 Km away from city and known as 'Chowekra' where sewage water was used for irrigation. Sampling site 2 was located 10 Km away from city, known as 'Chak 204 RB' where canal water was used for irrigation. Sampling was carried out after one month from October 2015 through January 2016 in the fields of cauliflower.

Collection of Earthworms

The collection of earthworms was done in cauliflower fields in district Faisalabad. Samples were taken randomly selecting quadrangle with a standard of removing equal amount of soil for every sample by digging a quadrat of 50cm^2 up to the depth of 30 cm which were divided into three layers (0-10, 11-20, 21-30 cm) (Anderson and Ingram 1993; Moreira *et al*., 2008) [21, 22]. In each field of one hectare, two quadrangles were installed randomly. Hand sorting method was used for collection of earthworms described by Lewis and Taylor (1879).

Preservation of Earthworms

Every specimen of earthworms was washed with tap water and put in 5% alcohol for 15 min for anesthetizing until they became quiet, and did not respond to any gentle poking. Then they were put in a dish and killed by pouring 10% formalin solution. These specimens were put in this solution for 24 hours after which they were hardened. Then they were permanently preserved in 5% solution of formalin. Every sample was put in separate plastic bottle and given the number according to the month and site.

Identification

The collected samples were bought to the biocontrol laboratory, Department of Zoology, Wildlife and Fisheries**,** University of Agriculture, Faisalabad, Pakistan for estimation and identification. The stereomicroscope was used in the examination. Different morphological characters used for this study were adapted from Stephenson (1923) [24].

Collection of Water

Water samples were collected from the feeder's furrows at sampling points in each field where the grab samples from each sampling was taken to represent the water flowing into the fields. The water was preserved by adding few drops of HNO_3 and kept in freezer to inhibit the activity of microorganisms. Water was filtered through Whatman No. 42 filter paper and used for the estimation of metals recommended by AOAC (1990) [26]. The pH, total dissolved solids (TDS), total suspended solids (TSS) and alkalinity of the water was measured at the collection point by using digital meter (HANNA 8424).

Collection of Soil

Soil was collected at the place where the earthworms were collected. Soil was collected into polyethylene bags. The soil was air dried, crushed and sieved though 2mm mesh and used for different parameters. For the digestion of soil Nitric-perchloric acid digestion was performed, following the procedure recommended by the AOAC (1990) [26]. 0.5 gram soil was placed in a 250 ml conical flask and 10ml of nitric-perchloric acid 3:1 ratio was added and kept for overnight. The mixture was boiled at 150℃ until the color of solution was changed from brown to transparent or yellow. The solution was cooled, further filtered through Whatman No. 42 filter paper and transferred quantitatively to 25 ml volumetric flask by adding distilled water.

Determination of elements by Atomic Absorption Spectrophotometry

Elements in the prepared samples were determined by using Atomic Absorption Spectrophotometer following the conditions described in AOAC (1990) [26].

Soil Texture

The texture of the soil was determined following the procedure of Whiting *et al*. (2003) [27].

Statistical Analysis

Data were analyzed statistically to determine species diversity and abundance, species richness and species evenness with Shannon-Weiner diversity index (Shannon, 1948) [28]. The comparisons of booth sampling sites were done by using t-test.

Results

A total of 518 specimens were collected out of which 383 specimen were collected from Sampling site 2 and 135 specimens from sampling site 1 from crop of cauliflower. Only one order Haplotaxida and two families Megascoledidae and Lumbricidae were identified. A total of seven species *Pheretima hawayana*, *Pheretima postuma*, *Pheretima morrisi*, *Pheretima heterochaeta*, *Pheretima minima*, *Lampito willeyi* and *Aporrectode longa* were identified from these two families. The most abundant species from all other species was *Pheretima hawayana* with 85 specimens and abundance was 22.19%. Second most abundant species was also from family Megascoledidae *Pheretima posthuma* with 74 specimens and 19.32% abundance. Least abundant species were *Aporrectode longa* belonging to the family Lumbricidae with 3 specimens and abundance was 0.78% (Table 1).

Out of 518 specimens 135 individuals were collected from Sampling site 1. All specimens collected from Sampling site 1 were immature that could not be identified due to the lack of morphometric characteristics.

Shannon-Weiner diversity index (H′) for the species of earthworms at Chack 204RB was 1.803 while evenness was 0.302, dominance was 0.831. The relative abundance (%) of the earthworm was higher at ssampling site 2 (Chack 204RB) which was 73.93% while the relative abundance (%) of the site Sampling site 1 was lower (26.07%), whereas relative abundance of earthworms was higher up to the depth of 10 cm (43.05%), whereas the relative abundance at the depth of 11-20 cm was 35.72%. The least relative abundance (%) was observed at the depth of the 21-30 cm where it was 21.23% (Table 2). The t-value for earthworms abundance shows significant difference between both sites i.e. Sampling site 1 and sampling site 2. t-value for the abundance was 12.62 (p=0.006) that was statistically highly significant (Table 3).

The relative abundance of earthworms was different during the different months of sampling. During the month of November the relative abundance was higher (66.60%), while during the month of January the relative abundance was lower (8.49%) (Table 4). During the month of November relative abundance of earthworms at Sampling site 1 was higher 79.98% (Fig 1), whereas during the month of January abundance was 0%. Similarly during the month of November relative abundance of earthworms at sampling site 2 was higher 61.87% and during the month of January relative abundance was the least 11.48% (Fig 2).

Physico-chemical parameters

Following the guideline of Whiting *et al*. (2003) [27], the texture of the soil profile in Sampling site 1 was sand, silt and in sampling site was clay. While the mean pH of the sewage water was acidic 5.833 and pH of canal water was slight basic7.666 while the mean DO was 3.79 and 6.43mg/l, TDS was 34.13 and 60.67mg/l, TSS was 183.33 and 60.00 mg/l and turbidity was 191.67 and 105.67 NTU, respectively. The concentration of heavy metals was higher (Co=2.4167, Cd=1.500, Pb=2.876, Zn=71.92 and Cr=0.583 mg/kg) in sewage water while lower (Co=1.0833, Cd=0.250, Pb=2.367, Zn=30.32 and Cr=0.250 mg/kg) in canal water (Table 6). Similarly the concentration of heavy metals in sewage water irrigated soil was higher (Co=2.580, Cd=0.353, Pb=3.145, Zn=50.25 and Cr=4.248 mg/kg) and lower (Co=1.608, Cd=0.210, Pb= 0.248, Zn=20.58 and Cr=3.915 mg/kg) in canal water irrigated soil (Table 5).

The t-test of sewage water and canal water for temperature, Cd, Cr, Ca showed non-significant difference while pH, DO, TDS, TSS, turbidity, Co, Pb, Zn and Mg showed significant difference at (p<0.05) (Table 5). Similarly the t test for sewage water irrigated soil and canal water irrigated soil for Co, Cd and Moisture showed non-significant difference whereas the Cr, Pb, Zn, Mg and Ca showed significant difference (Table 5).

Correlation analysis was applied on the data of earthworms abundance and physico-chemical parameters. Results showed highly positive correlation between earthworm's abundance and temperature, at Sampling site 1, r=0.8778 p=0.318 and at Chack 204 r=0.946 p=0.210 as temperature decreased the abundance was also decreased.

Discussion

In the present study 383 individuals was identified 2 genera and seven species. In the present study the most abundant species was *Pheretima hawayana* with 85 specimens while least species was *Aporrectode longa* with only 3 specimens. Similarly Jalal *et al*. (2014) reported impact of earthworms to improve fertility in selected orchards of district Faisalabad. They identified 523 earthworms from the four orchards. 18 species belonging to 10 genera were identified. A fair number of *Pheretima posthuma* (n = 366, 69.98 %), *P. hawayana* (n = 43, 8.22 %) and *P. houleti* (n = 27, 5.16 %) were recorded. The least number of earthworms species were *P. suctoria*, *A. longa*, *E. waltoni*, *A. chlorotica* (n = 01, 0.20 %). Similarly Ghafoor *et al*. (2008) also described the biodiversity from district Narowal, Pakistan. They collected 2458.59 earthworm specimens, representing 5 families, 12 genera and 20 species. Three species of Megascolecidae viz. *Pheretima hawayana*, *Pheretima posthuma* and *Pheretima morrisi* were the most abundant species of earthworms.

In the present study the diversity and relative abundance (%) of species *Pheretima hawayana* was higher during the sampling period as compared to the other earthworm species. It is also observed that the biodiversity and relative abundance of earthworms was declined in December and January due to the harsh cold condition. Similarly monthly variations of earthworm fauna for a period of one year were described by Goswani in (2015). In this study the monthly sampling, diversity and relative abundance % of earthworms revealed that the diverse fauna was present in month of February and March while the fauna decline in December and also in months of April and May. In this study cold and hot season seems to be limiting for various earthworms species.

In the present study the physico-chemical parameters were also observed. Overall the concentration of pH of sewage water and sewage water irrigated soil was acidic, while the pH of canal water and canal water irrigated soil was basic. The concentration of EC, DO, TDS, alkalinity and turbidity of sewage water was lower than canal water. TSS of sewage water was higher the canal water. The concentration of heavy metals such as Co, Cd, Pb, Zn, Cr and Ca was higher in sewage water and sewage water irrigated soil then canal water and canal water irrigated soil. The concentration of Mg was higher in sewage water then canal water but its concentration was lower in sewage water irrigated soil than canal water irrigated soil. Increase in the concentration of heavy metals had highly negative affect on the earthworms as earthworms are sensitive bio-indicator of pollution in the soil. Bioaccumulation of metals in earthworms causes mortalities.

The effects of heavy metals lead (Pb), Cadmium (Cd) and Cromium (Cr) on nematode communities and earthworm density and biomass were studied in the wastewater irrigated farms of the Nairobi river basin by Karanja *et al*. 2010. The earthworms isolated from both sites were all epigeic with the metal content in Maili Saba suppressing their populations. This study has demonstrated that the use of untreated urban wastewater for irrigation has adverse effects on nematode and earthworm abundance and diversity and their potential as bioindicators of heavy metal presence. Similarly The Sivakumar and Subbhuraam, 2005 also described that organic matter content, clay, and hydroxides of Al and Fe and pH, generally called capacity controlling parameters, are believed to determine the bioavailability of metals for organisms living in soil. Acidity of dredged sediment substrate was responsible for the absence of earthworms, since an older landfill with a higher pH harbored earthworms.

In the present study sewage water contain toxic substances and heavy metals more as compared to the canal water which adversely affected the earthworm biodiversity and abundance. Such type of effect of sewage water, sewage solidified sludge and other medium on earthworms are on record (Dayananda *et al*., 2008 and Fordham and Wilber 1992) [34, 35].

In the present study the earthworms prefer to live in loamy soil and have higher abundance than the sandy soil. Similarly Hendrix *et al*. (1992) [36] worked on the distribution and abundance of EWs in relation to landscape dynamics. There results showed that the sandy clay loam supported higher earthworms abundance and biomass than with higher sandy content.

Table 1: Diversity and relative abundance (%) of earthworms in cauliflower (*Brassica oleracea*) at sampling site 1 (Chack 204 RB).

Order	Family	Genus	Species	No of earthworms (%ge)
Haplotaxida	Megascoledidae	Pheretima	*P. hawayana*	85 (22.19)
			P. posthuma	74 (19.32)
			P.morrisi	46 (12.27)
			P. heterochaeta	50 (13.31)
			P. minima	58 (15.40)
			L. willeyi	67 (17.49)
	Lumbricidae	Aporrectodea	*A. longa*	3 (0.78)
Total	2	2	8	383 (100)

Table 2: Relative abundance (%) of earthworms in cauliflower (*Brassica oleracae*) at different depth and sampling sites.

Depth (cm)	Site 1	Site 2	Total
0-10	65 (12.54)	158 (30.50)	223 (43.05)
11-20	50 (9.65)	135 (26.06)	185 (35.72)
21-30	20 (3.86)	90 (17.37)	110 (21.23)
Total	135 (26.07)	383 (73.93)	518 (100)

Table 3: Monthly variation in average (range) relative abundance (%) of earthworms in cauliflower crop at different depths.

Depth (cm)	November	December	January	Total
0-10	147 (28.37	55 (10.61)	21 (4.05)	223 (43.05)
11-20	120 (23.166	50 (9.65)	15 (2.89)	185 (35.71)
21-30	78 (15.05)	24 (4.63)	8 (1.54)	110 (21.23)
Total	345 (66.60)	129 (24.90%)	44 (8.49)	518 (100)

Table 4: Physico-chemical characteristics of sewage water and canal water.

Analytes	Sewage water	Canal water	pValue
Temperature	23.00±1.53	23.00±1.53	0.999
pH	5.8333 ± 0.0882	7.6667±0.0667	0.0043*
DO (mg/l)	3.7967± 0.0504	6.433±0.233	0.0112*
TDS (mg/l)	34.13±2.97	60.67±5.21	0.0439*
TSS (mg/l)	183.33±8.82	60.00±5.77	0.0115*
Turbidity (NTU)	191.67±6.01	105.67±7.22	0.0028*
Co (mg/kg)	2.4167±0.0833	1.0833±0.0833	0.0572
.Cd (mg/kg)	1.500 ±0.144	0.250±0.00	0.5000
Pb (mg/kg)	2.8767±0.0722	2.367±0.186	0.0076*
Zn (mg/kg)	71.92 ±1.80	30.32±2.2	0.0192*
Cr (mg/kg)	0.583±0.167	0.250±0.00	0.4226
Mg (mg/kg)	226.00± 5.86	244.33±5.21	0.0533
Ca (mg/kg)	940.67±7.88	737.5±86.6	0.1941

*(p<0.05)

Table 5: Physico-chemical characteristics of sewage water irrigated soil and canal water irrigated soil (Mean± SEM).

Analyte (mg/kg)	Sewage water irrigated Soil	Canal water irrigated soil	P-Value
Co	2.5800±0.0693	1.608±0.227	0.8670
Cd	0.3533±0.0501	0.210±0.0253	0.1036
Pb	3.1456±0.0068	0.248±0.481	0.0014**
Zn	50.25±1.70	20.58±3.34	0.0001**
Cr	4.248±0.155	3.915±0.465	0.0366*
Mg	234.4±21.4	827.5±48.1	0.0001**
Ca	1193.20±84.1	577.83±37.7	0.0003**
Soil texture	Sand, silt	Clay	

*(p<0.05), ** (p<0.001)

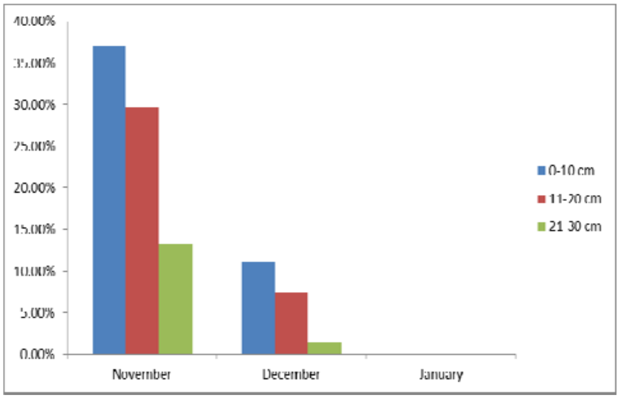

Fig 1: Relative abundance % of earthworms at sampling Site 1

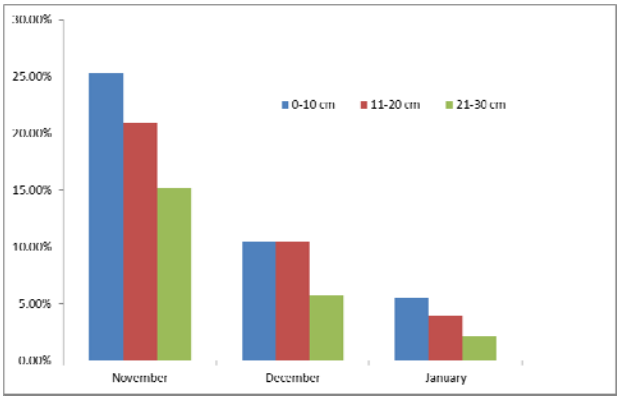

Fig 2: Relative abundance % of earthworms at sampling Site 2.

Conclusion

It is conceivable from the present data that earthworm population proves to be better and beneficial for non-polluted soil. Environmental factor, such as temperature is very important for earthworm management. The abundance of earthworms was low in cold months of winter. The harsh condition of winter seems to be limiting factor for abundance of earthworm. Acidic pH and high concentration of heavy metals badly affect the diversity and abundance of earthworms.

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