



# Assessment of heavy metal contamination and its effect on earthworms in different types of soils

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## Abstract

Earthworms encourage the mineralization of soil organic matter, the production of nutrients and the growth of plants through their feeding habits in the soil. But unsustainable development along with rapid urbanization and industrialization degrades the soils at an alarming rate which increases the level of different contaminants, i.e., heavy metals in the soil. The present study was conducted to assess the heavy metals, pH and organic carbon in agricultural, non-agricultural and industrial soils and their effects on the earthworm community structures. A total of seven earthworm species belonging to two families (Megascolecidae and Octochaetidae) were reported in this study. The earthworm species *Metaphire posthuma* was the most abundant in all collected soils, i.e., agricultural, non-agricultural and industrial soil with a relative abundance of 76.56%, 77.19% and 78.85%, respectively. The abundance and biomass of *M. posthuma* were in the order of agricultural soil > non-agricultural soil > industrial soil. It was also reported that the abundance of both anecic and endogeic species was higher in agricultural soils followed by non-agricultural and industrial soil. The findings of heavy metals indicated that industrial soil has the highest concentration of heavy metals followed by non-agricultural and agricultural soils. The contamination factor, enrichment factor, geoaccumulation index, pollution index and modified pollution index showed high, moderate and less pollution levels at industrial, non-agricultural and agricultural soil, respectively. The present study also indicated that Cd and As showed severe contamination in all studied soil types.

**Keywords** Ecological risk assessment · Geoaccumulation index · Diversity indices · Principal component analysis

## Introduction

Earthworms consist of the largest biomass of macrofauna in most soils that promotes mineralization of soil organic matter, nutrient production and plant growth (Zhang et al. 2020). They are also known as ecosystem engineers due to their soil feeding habits by which they change the structure of the soil and its physicochemical properties (Jones et al. 1994; Singh et al. 2016). When soil passed through the earthworm intestines, different enzymes degrade the organic compounds and hence increase the supply of various nutrients in the vermicast and also modify the permeability of the soil by controlling the proportion of organic matter and nutrient release (Lipiec et al. 2016; Van Groenigen et al. 2019; De Wandeler et al. 2016). Based on ecological nature, earthworms are of three types, i.e., epigeic, anecic and endogeic (Bouché 1977). Epigeic earthworms are the surface feeder and feed on the organic residues of the surface litter, anecic earthworms feed on the surface leaf litter through their permanent vertical burrows and endogeic earthworms feed on

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soil organic matter within the soil by creating nonpermanent horizontal burrows. In soil ecosystems, earthworms are also considered as keystone species due to their important role in sustaining healthy soil and provide food for other terrestrial organisms at higher tropical rates (Stroud 2019; Sizmur and Richardson 2020).

Nowadays, unsustainable development along with rapid urbanization and industrialization degrades soils at an alarming rate which increases the level of different contaminants in the soil (Kopittke et al. 2019; Kumar et al. 2019). The rise in the level of these contaminants into the soil also disturbs the earthworm communities and their structure beneath the soil. Among other various contaminants, heavy metals (HMs) are the major form of contaminants that have direct effects on earthworms (Singh et al. 2020a). However, the effects of heavy metals in the soil often depend on their concentration, i.e., earthworms can survive at a lower concentration of heavy metals due to their ability to bioaccumulate and retain heavy metals in their tissues (Shi et al. 2020). But, high concentration of heavy metals in the soil increases toxicity which causes the death of earthworm (Latif et al. 2020; Singh et al. 2020a). Heavy metals can pose persistent threats to the soil ecosystem and human health as soon as they reach the environment due to their nondegradability and bioaccumulation characteristics (Mahey et al. 2020; Zhang et al. 2020). Long-term stress on heavy metal pollution can adversely affect the life-history traits of certain species and even contribute to genetic erosion and population extinction (Audusseau et al. 2020; Rybak et al. 2020).

Keeping these things in mind, the present work was conducted to (1) evaluate the heavy metals, pH and organic carbon (OC) in agricultural, non-agricultural and industrial soils and their effects on the earthworm community structures, their abundance and biomass; (2) explore major factor in the soil which affect earthworms abundance and biomass; and (3) study the pollution level and risk assessment by enumerating contamination indices and ecological risk assessment indices. This study will provide new visions into the assessment of the ecological threats of soil pollutants from the perspective of earthworm individuals and their abundance.

## Materials and methods

### Study area

The study was conducted in the Ludhiana (Industrial hub of Punjab) situated in the northwestern part of India. Ludhiana is also known as Manchester of India (Sikka and Nayyar 2016) having different kinds of industries like bicycle, woollen, hosiery, machine tools, textile, dyeing and electroplating work, etc. These industries generate a huge amount of

industrial effluents that are directly poured into tributary Buddha Nullah through the sewers system. The untreated water of Buddha Nullah is used to irrigate crop fields (Sikka et al. 2009). Ludhiana ranked 13<sup>th</sup> in the most polluted city in the world (Majeed 2014). Ludhiana district has climatic conditions of humid subtropical as per Koppen climatic classification (Yeotikar et al. 2019). The mean rainfall is 758 mm/yr (Hadda et al. 2020).

### Earthworms sampling and identification

A total of 18 sampling sites were selected randomly which were further divided into agricultural sites, non-agricultural sites and industrial sites. The various characteristics of study sites with different vegetation/land use along with latitude and longitude are given in supplementary Table S1. The earthworm sampling was done in the monsoon and post-monsoon season. Earthworms were sampled from seven different sampling plots of size 30 cm × 30 cm in each study site by hand-sorting and digging the plot up to 30 cm. The earthworms from each sampling plots were washed with water, placed on filter paper for drying and finally weighed. The extracted earthworm individuals were killed with 70% ethanol and finally preserved in 5% formalin solution. The preserved earthworm species were dissected and studied for their morphological and anatomical features by using a stereomicroscope (Olympus). The preserved earthworm individuals were identified to species level by using earthworm morphological keys of Julka (2008). Subsequently, the identified earthworm species were categorized into epigeic, anecic and endogeic ecological category by using the method of Bouché (1977).

### Analysis of soils for pH, OC and heavy metals

Soil samples were also collected from the earthworm sampling sites. Soil samples were air-dried, powdered and sieved through 0.2 mm sieve mesh to nullify the effect of particle size during analysis. Soil samples were analyzed for pH, OC and heavy metal content. Soil pH was measured using the method of Jackson (1967). Organic carbon from soil samples was estimated by the method given by Nelson and Sommers (1996). For heavy metal estimation, 1 g of soil was digested with 15 mL of diacid mixture ( $\text{HClO}_4\text{:HNO}_3$  in 1:4 ratio) until a clear solution was obtained. The digested samples were filtered through Whatman filter paper and diluted with distilled water up to 50 ml and tested for heavy metals, i.e., Zn, Fe, Mn, Cu, Pb, Cd, Ni, As, and Co, using atomic absorption spectrophotometer (Agilent technologies Varian 20 model). The standard solutions for the above said heavy metals were procured from Agilent technologies (1000 mg/L) which were further diluted with distilled water to make the solutions of various concentrations. The



accuracy of the instruments was also checked by testing the standard solution in the instrument after every ten sample readings.

### Calculation of pollution indices

To determine the pollution level of heavy metals in soils, various pollution indices were proposed by different workers. The calculation of indices is as:

#### Contamination factor (CF)

The CF signifies the anthropogenic inputs in the soils and computed by dividing the heavy metal level in determining samples to reference environment value (Hakanson 1980). The equation used to determine CF is as:

$$CF = \frac{\text{Concentration of each heavy metal}}{\text{Background value of each heavy metal}}$$

#### Enrichment factor (EF)

EF was used to find the natural and human impacts on heavy metal concentration (Delgado et al. 2010) and computed as:

$$EF = \frac{\text{Concentration of heavy metal in samples/Fe concentration in the samples}}{\text{Background values of heavy metal/Background value of Fe}}$$

The reference values for heavy metals were adapted from Taylor and McLennan (1995), and the scores used to classify the pollution level on CF and EF values are provided in Supplementary Table S2. The values of both CF and EF were used to find out the grade of the soil, i.e., low contaminated, moderately contaminated, highly contaminated, etc.

#### Geoaccumulation index (Igeo)

Igeo index determined the pollution of heavy metals in soils which is computed by following Muller et al. (1981) as:

$$I_{geo} = \log_2 \frac{\text{Heavy metal concentration in soil samples}}{1.5 \times \text{Heavy metal concentration in background environment}}$$

The reference values of heavy metals were adapted from Taylor and McLennan (1995). The constant 1.5 implies alterations in heavy metal concentrations in the environment (Tian et al. 2017).

#### Pollution index (PI) and modified pollution index (MPI)

The PI was calculated by considering the average and maximum value of CF for each heavy metal, while MPI takes into

consideration the mean and maximum value of EF for each heavy metal. The PI and MPI were enumerated by following Nemerow (1991):

$$PI = \sqrt{\frac{(CF_{\text{average}})^2 + (CF_{\text{maximum}})^2}{2}}$$

$$MPI = \sqrt{\frac{(EF_{\text{average}})^2 + (EF_{\text{maximum}})^2}{2}}$$

The grades used to categorize the pollution level, i.e., unpolluted, slightly polluted, modest polluted, etc., are given in Supplementary Table S3.

#### Ecological risk indices (RI and MRI)

The ecological peril in the soils was resolute by using potential and modified ecological risk indices. The potential and modified risk indices were enumerated by taking into account the CF and EF values of heavy metals, respectively, and determined as:

$$RI = \sum CF \times Tr$$

$$MRI = \sum EF \times Tr$$

where Tr is the toxic response factor of apiece heavy metal PTE adapted from Heidari et al. (2019). The scores used to classify the ecological hazard are specified in Table S4.

#### Statistical analysis

One-way ANOVA followed by Tukey post hoc test was used to compare the abundance and biomass of earthworms among agricultural, non-agricultural and industrial soils.

The Similarity percentage (SIMPER) analysis was used to find out the average contribution of each earthworm in similarity/dissimilarity ranges. The diversity indices such as Simpson index of diversity, Shannon–Weiner diversity index, species richness and species evenness were also calculated. The data for soil were presented as a minimum, maximum, mean, standard error, variance, skewness, kurtosis and coefficient of variance. The principal component analysis was used to examine the order of major factors in

the soil which affects the diversity and abundance of earthworms by using Varimax rotation with Kaiser Normalization. The Pearson correlation coefficients were calculated to study the relationship between different soil variables and earthworm ecological characteristics. All the statistical analysis were done with the help of SPSS (Version 21) and Past (version 4.02) software program.

## Results and discussion

### Results

#### pH, OC and heavy metal content in soils

The descriptive statistical analysis of pH, organic carbon (OC) and heavy metals (Zn, Fe, Mn, Cu, Pb, Ni, Co, Cd and As) in agricultural, non-agricultural and industrial soils are given in Table 1. The pH was found in the range from 6.88 to 7.73 with an average value of 7.46 in agricultural soils. In non-agricultural soils, pH ranged from 7.4 to 7.73, while in industrial soil pH ranged from 7.42 to 7.92. The OC ranged from 0.04 to 1.35% in agricultural soils, 0.39 to 1.11% in non-agricultural soils and 0.42 to 1.42% in industrial soils. The mean concentration of heavy metals in agricultural soils was in the order of Fe > Mn > As > Pb > Cu > Ni > Co > Cd. Similarly, average values of heavy metals in non-agricultural and industrial soils were in the order of Fe > As > Mn > Pb > Cu > Ni > Co > Cd and Fe > As > Mn > Ni > Pb > Cu > Co > Cd, respectively. The mean value of each soil variable was significantly higher in industrial soils followed by non-agricultural and agricultural soils.

#### Assessment of pollution level of heavy metal in soils

The contamination factors (CF), enrichment factor (EF) and geoaccumulation index (Igeo) were enumerated to oversee the pollution level of heavy metals in different soils (Table 2). On the basis of grades suggested by Hakanson (1980), the CF results for agricultural soils followed the trend as As > Cd > Pb > Ni > Co > Cu > Zn > Mn. Among heavy metals, As and Cd showed high contamination, while Pb exhibited substantial contamination. In non-agricultural soils the, CF values of heavy followed the trend as As > Cd > Pb > Zn > Cu > Co > Ni > Mn. Amid heavy metals, Cd and As exhibited high contamination, whereas Pb showed significant contamination. The Zn, Cu, Co, Ni and Mn showed low contamination in the study area. In industrial soils, the CF values of different heavy metals followed the trend as: As > Cd > Ni > Pb > Cu > Co > Zn > Mn. The As, Cd, Pb and Ni showed high contamination, whereas Cu, Co, Zn and Mn exhibited considerable to modest contamination in the area. On the other hand, grades given by Sutherland (2000) were

**Table 1** Statistical analysis of pH, OC (%) and heavy metals (mg/kg) in soils collected from study area

	pH	OC	Zn	Fe	Mn	Cu	Pb	Ni	Co	Cd	As
<i>Agricultural soils</i>											
Min	6.88	0.045	12	1079.1	108.1	10.15	46.9	12	8.5	1.35	0.25
Max	7.73	1.35	68.75	1311.5	296.7	49.55	117.30	42.85	18.95	3.25	298
Mean	7.46	0.72	41.85	1277.9	242.43	31.96	84.48	27.12	14.24	2.56	118.3
S.E	0.03	0.05	1.78	7.16	6.39	1.84	3.03	1.28	0.49	0.08	15.83
<i>Non-agricultural soils</i>											
Min	7.4	0.39	70.75	1313.8	298.6	51.65	120.35	44.5	19	3.3	329.7
Max	7.73	1.11	104.05	1323	310.3	52.95	131.85	59.4	20.1	3.5	388.3
Mean	7.57	0.73	84.91	1318.7	303.93	52.36	126.62	48.73	19.34	3.38	354.03
S.E	0.07	0.14	6.81	1.72	2.59	0.21	2.03	2.71	0.20	0.04	9.88
<i>Industrial soils</i>											
Min	7.42	0.42	109.6	1323	310.5	53.2	132.3	60.15	20.1	3.6	416.3
Max	7.92	1.42	537	1418.3	483.1	278	297.1	476	27.5	5.95	631.1
Mean	7.67*	0.96*	160.5*	1345.5*	370.5*	88.12*	172.07*	205.5*	22.9*	4.41*	507.8*
S.E	0.05	0.13	41.88	9.80	14.94	21.42	17.48	49.15	0.69	0.25	26.91

\*One-way ANOVA followed by Tukey post hoc test was applied. Significantly higher values in industrial soils as compared to agricultural and non-agricultural soils

**Table 2** CF, EF and Igeo indices of heavy metals

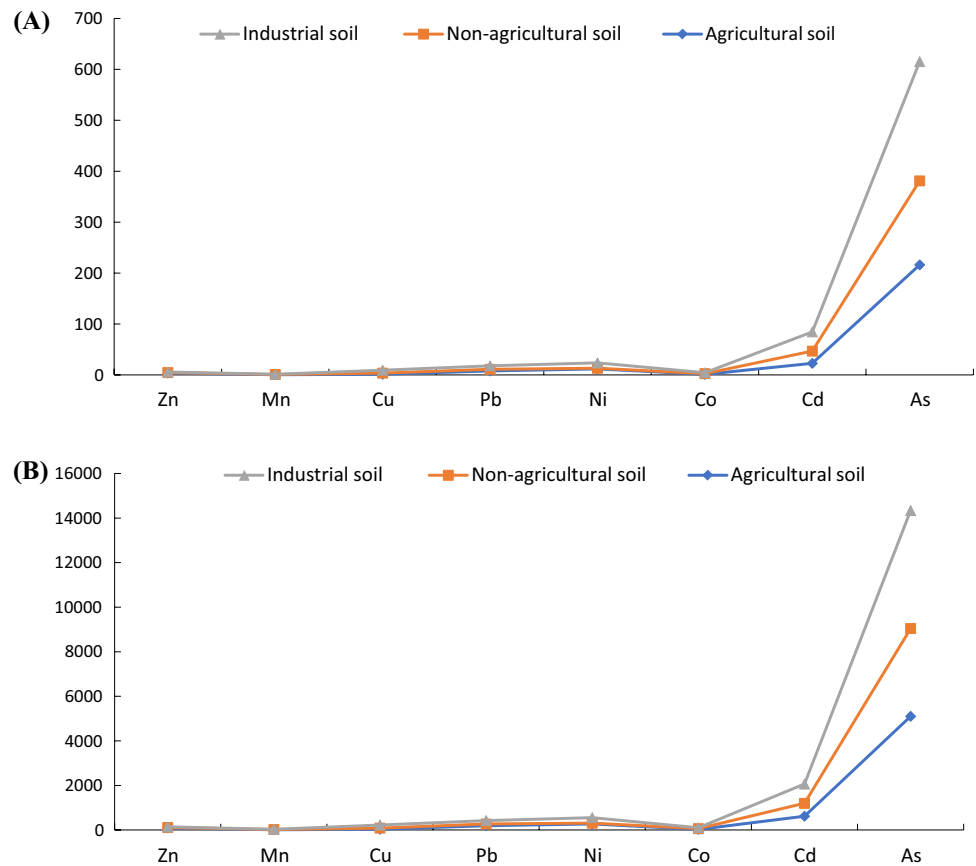
	Zn	Mn	Cu	Pb	Ni	Co	Cd	As
<b>Contamination factor (CF)</b>								
<b>Agricultural soil</b>								
Min	0.17	0.20	0.41	2.35	0.80	0.85	13.78	0.17
Max	1.76	0.89	2.62	14.86	23.80	1.90	37.24	414.97
Mean	0.66	0.47	1.40	5.00	2.10	1.41	26.54	125.36
<b>Non-agricultural soil</b>								
Min	0.60	0.35	1.19	3.49	0.60	1.60	24.49	0.50
Max	7.56	0.57	3.52	5.25	1.58	2.01	33.16	236.53
Mean	2.23	0.49	2.10	4.23	0.99	1.87	28.16	102.04
<b>Industrial soil</b>								
Min	0.65	0.43	1.05	4.17	1.77	1.86	33.67	0.43
Max	1.79	0.73	11.12	11.50	19.63	2.75	60.71	420.77
Mean	1.34	0.60	2.75	6.30	7.05	2.23	43.15	203.98
<b>Enrichment factor (EF)</b>								
<b>Agricultural soil</b>								
Min	3.97	5.08	9.80	55.23	14.55	20.16	329.62	3.95
Max	183.36	19.49	62.05	363.84	546.87	46.93	1066.11	9784.60
Mean	19.85	11.26	33.76	119.14	48.28	34.13	632.90	2907.41
<b>Non-agricultural soil</b>								
Min	14.57	10.10	38.22	81.17	14.70	43.64	604.75	1765.70
Max	37.29	18.39	81.86	126.76	58.72	48.97	863.39	6897.84
Mean	21.43	12.98	65.87	102.67	33.84	45.62	774.27	3811.64
<b>Industrial soil</b>								
Min	15.14	10.32	23.40	106.36	51.95	38.89	724.49	10.16
Max	183.36	19.49	262.20	363.84	546.87	61.51	1358.10	9784.60
Mean	46.20	14.55	78.18	172.39	211.18	53.84	1061.83	5144.42
<b>Geoaccumulation index (Igeo)</b>								
<b>Agricultural soil</b>								
Min	0.23	0.21	0.84	2.98	0.66	0.71	16.82	0.50
Max	92.21	6.53	26.94	87.20	211.35	23.90	319.00	4559.49
Mean	6.58	2.98	9.57	27.96	14.01	9.71	168.93	936.60
<b>Non-agricultural soil</b>								
Min	7.83	5.48	20.96	43.63	8.06	23.92	325.07	968.06
Max	20.21	9.83	44.10	68.71	31.37	26.16	478.42	3873.18
Mean	11.66	7.05	35.86	55.89	18.42	24.84	422.04	2089.29
<b>Industrial soil</b>								
Min	9.08	6.92	15.59	66.35	31.15	29.35	513.98	7.11
Max	28.76	10.89	175.74	171.36	322.09	40.98	904.70	6269.84
Mean	22.55	9.21	41.34	103.09	125.67	35.26	689.78	3132.99

used to categorize the pollution level based on EF. The EF results of agricultural soils indicated that Cd, As, Ni and Pb exhibited extreme enrichment in the area, Co and Cu showed high enrichment, while Zn and Mn showed considerable enrichment of studied heavy metals. In non-agricultural soils, Cd, As, Co, Pb and Cu showed extreme enrichment, while in industrial soils Cd, As, Co, Ni, Pb, Cu and Zn indicated extreme enrichment. The Igeo results in agricultural soil indicated that Cd, As, Co, Ni, Pb, Cu and Zn showed extreme pollution, while Mn exhibited high pollution in the

area. In non-agricultural and industrial soil, all studied heavy metals showed extreme pollution in the studied area. Pollution index (PI) and modified pollution index (MPI) also showed high, moderate and less pollution levels at industrial, non-agricultural soil and agricultural soil, respectively (Fig. 1a, b).

The ecological risks were enumerated by potential ecological risk (RI) and modified potential ecological risk (MRI) for different heavy metals in soils (Table 3). The potential ecological risk (Er) value of Cd in agricultural

**Fig. 1** Pollution index (a) and modified pollution index (b) of different heavy metals in soils



soils was recorded above 320, signifying the very risk of this metal in the agricultural soils of the study area. In non-agricultural soils, Cd exhibited very high risk, As showed high risk, while Zn, Mn, Cu, Pb, Ni and Co exhibited low risk. In industrial soils, Cd and As showed very high risk, Ni reflected modest, while Zn, Mn, Cu, Pb and Co showed low risk in the study area. The modified potential ecological risk (mEr) of Cd, As and Pb in agricultural soils indicated very high risk, while Cu, Ni and Co showed high risk. In non-agricultural soils, Cd, As, Pb and Cu showed very high risk, Zn and Mn exhibited low risk, but Ni and Co showed high risk in the studied area. In industrial soils, Cd, As, Ni and Pb showed very high risk, Zn and Mn displayed low risk, while Cu and Co showed high risk in the area. Based on RI and MRI values, the trend followed by different soils was industrial soil > non-agricultural soil > agricultural soil.

### Earthworm community structure

A total of seven earthworm species belonging to two families, i.e., Megascolecidae and Octochaetidae, were reported in this study (Table 4). The earthworm species *Amyntas morrissi*, *Lampito mauritii*, *Metaphire posthuma* and *Polypheretima elongata* belong to the

family Megascolecidae, while *Eutyphoeus incommodus*, *Eutyphoeus waltoni* and *Octochaetona beatrix* belong to the family Octochaetidae. All seven earthworm species were reported in non-agricultural fields soil followed by four and three earthworm species in the agricultural and industrial soil, respectively. The earthworm species *O. beatrix*, *E. incommodus* and *E. waltoni* were only reported from non-agricultural soil. *M. posthuma* was the most abundant species in agricultural, non-agricultural and industrial soil with a relative abundance of 76.56%, 77.19% and 78.85%, respectively. SIMPER analysis was used to assess the contribution of each earthworm species among agricultural, non-agricultural and industrial soils which showed that maximum contribution among earthworm individuals was due to *Metaphire posthuma* and *Lampito mauritii* (Table 5). The abundance and biomass of *M. posthuma* was significantly different ( $p < 0.05$ ) and were in the order of agricultural soil (8.17 individuals/m<sup>2</sup> and 9.94 g/m<sup>2</sup>) > non-agricultural soil (5.79 individuals/m<sup>2</sup> and 6.62 g/m<sup>2</sup>) > industrial soil (4.1 individuals/m<sup>2</sup> and 3.23 g/m<sup>2</sup>). The abundance and biomass of *L. mauritii* in industrial soil were significantly ( $p < 0.05$ ) less as compared to agricultural and non-agricultural soil, while no significant ( $p > 0.05$ ) difference was reported in abundance and biomass of *L. mauritii* among agricultural and non-agricultural soil. Similarly, both abundance and



**Table 3** Ecological risk assessment of heavy metals in soils collected from study area

	Zn	Mn	Cu	Pb	Ni	Co	Cd	As	Ecological risk (RI)
Potential ecological risk of each metal (Er)									
<b>Agricultural soils</b>									
Min	0.17	0.20	2.03	11.73	3	4.25	413.27	0.33	473.37
Max	7.56	0.89	13.12	74.28	119	10.05	1117.35	829.93	1710.56
Mean	0.83	0.47	7.06	24.81	10.21	7.15	793.62	243.32	1087.47
<b>Non-agricultural soils</b>									
Min	0.63	0.42	8.19	17.46	3.15	9.28	780.61	151.33	1020.13
Max	1.54	0.72	17.61	26.24	11.45	9.68	1071.43	578.27	1706.23
Mean	0.89	0.54	13.68	21.28	6.93	9.49	964.29	317.12	1334.21
<b>Industrial soils</b>									
Min	0.65	0.44	5.23	21.46	11.13	10.05	1056.12	0.87	1139.69
Max	1.79	0.73	55.60	57.50	98.16	13.75	1821.43	841.53	2801.77
Mean	1.46	0.60	13.35	33.43	40.28	11.48	1345.41	412.46	1858.46
Modified potential ecological risk of each metal (mEr)									Modified ecological risk (MRI)
<b>Agricultural soils</b>									
Min	3.97	5.08	48.99	276.15	72.73	100.80	9888.66	7.91	11,227.39
Max	183.36	19.49	310.27	1819.18	2734.34	234.65	31,983.36	19,569.2	46,238.15
Mean	19.85	11.26	168.78	595.70	241.41	170.63	18,987.09	5814.82	26,009.53
<b>Non-agricultural soils</b>									
Min	14.57	10.10	191.12	405.85	73.51	218.18	18,142.44	3531.40	23,709.2
Max	37.29	18.39	409.28	633.80	293.58	244.86	25,901.81	13,795.69	40,705.47
Mean	21.43	12.98	329.33	513.33	169.21	228.11	23,228.05	7623.28	32,125.73
<b>Industrial soils</b>									
Min	15.14	10.32	116.99	531.80	259.75	236.97	24,902.58	20.32	26,873.13
Max	44.06	16.35	1311.01	1286.20	2301.25	307.57	40,742.92	18,823.97	62,671.87
Mean	34.83	14.31	316.93	790.05	957.16	272.83	31,978.06	9720.75	44,084.91

**Table 4** Distribution of earthworm species and their relative abundance in different study sites

Family	Species	Ecological category	Agricultural	Non-agricultural	Industrial	Relative abundance (%)		
						Agricultural	Non-agricultural	Industrial
<i>Megascolecidae</i>	<i>Metaphire posthuma</i>	Endogeic	+	+	+	76.56	77.19	78.85
	<i>Lampito mauritii</i>	Anecic	+	+	+	15.63	17.19	15.38
	<i>Amyntas morrisi</i>	Anecic	+	+	+	6.25	0.70	5.77
	<i>Polypheretima elongata</i>	Anecic	+	+	–	1.56	2.46	–
<i>Octochaetidae</i>	<i>Octochaetona beatrix</i>	Endogeic	–	+	–	–	1.40	–
	<i>Eutyphoeus incommodus</i>	Anecic	–	+	–	–	0.35	–
	<i>Eutyphoeus waltoni</i>	Endogeic	–	+	–	–	0.70	–

biomass of *A. morrisi* were significantly ( $p < 0.05$ ) higher as compared to non-agricultural and industrial soil. The maximum abundance (individuals/m<sup>2</sup>) and biomass (g/m<sup>2</sup>) were also reported in the order of agricultural > non-agricultural > industrial soil.

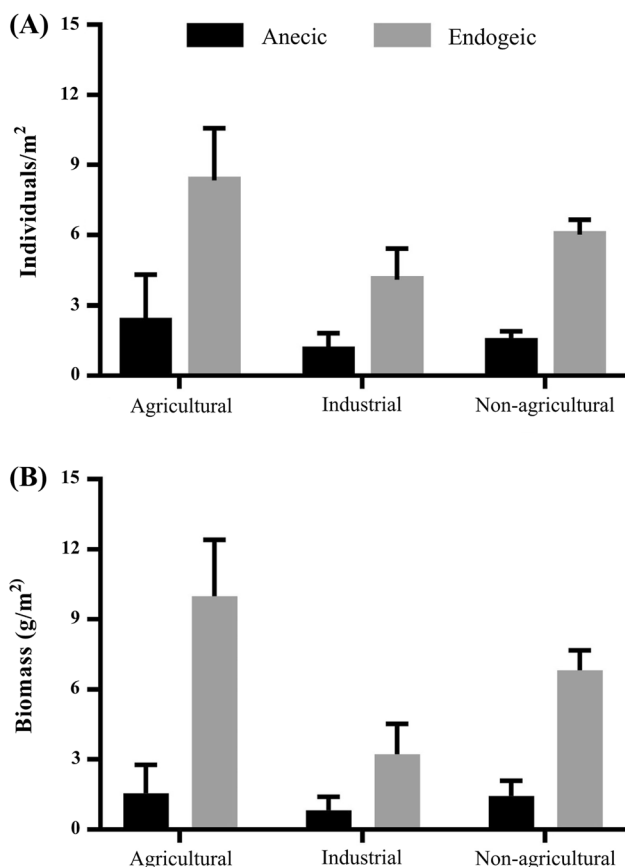
Based on the ecological category, four earthworm species belong to the anecic ecological category, while three species belong to the endogeic ecological category (Table 4). No epigeic earthworm species were reported in this study. The abundance (individuals/m<sup>2</sup>) and biomass (g/m<sup>2</sup>) of both anecic and endogeic earthworm species among agricultural,

**Table 5** Earthworm abundance and biomass among different study sites

Species	Contribution* (%)	Individuals/m <sup>2</sup>			Biomass (g/m <sup>2</sup> )		
		Agricultural	Non-agricultural	Industrial	Agricultural	Non-agricultural	Industrial
<i>Metaphire posthuma</i>	<b>71.22</b>	8.17 ± 2.29a	5.79 ± 0.61b	4.1 ± 1.33c	9.94 ± 2.36a	6.62 ± 0.71b	3.23 ± 1.31c
<i>Lampito mauritii</i>	<b>19.66</b>	1.67 ± 1.31a	1.29 ± 0.43a	0.8 ± 0.47b	1.3 ± 0.97a	1.2 ± 0.41a	0.66 ± 0.4b
<i>Amyntas morrisi</i>	3.26	0.67 ± 0.67a	0.05 ± 0.05b	0.3 ± 0.3b	0.25 ± 0.25a	0.07 ± 0.07b	0.17 ± 0.17ab
<i>Polypheretima elongata</i>	0.58	0.17 ± 0.17	0.18 ± 0.08	0	0.05 ± 0.05	0.14 ± 0.06	0
<i>Octochaetona beatrix</i>	2.74	0	0.11 ± 0.11	0	0	0.14 ± 0.14	0
<i>Eutyphoeus incommodus</i>	2.09	0	0.03 ± 0.03	0	0	0.04 ± 0.04	0
<i>Eutyphoeus waltoni</i>	0.45	0	0.05 ± 0.05	0	0	0.07 ± 0.07	0
Total		10.67 ± 3.72a	7.5 ± 0.65b	5.2 ± 1.91b	11.54 ± 3.01	8.27 ± 0.75	4.05 ± 1.74

\* SIMPER analysis was used to assess the contribution of each earthworm species in diversity in agricultural, non-agricultural and industrial soils. The earthworm communities contributed with more than 10% to the total percentage of dissimilarity were highlighted

One-way ANOVA followed by Tukey post hoc test was applied for individuals/m<sup>2</sup> and Biomass among agricultural, non-agricultural and industrial sites. The mean followed by different letters was significantly different at 5% significance level



**Fig. 2** Abundance (Individuals/m<sup>2</sup>) and biomass (g/m<sup>2</sup>) of earthworms on the basis of ecological category among agricultural, industrial and non-agricultural soils

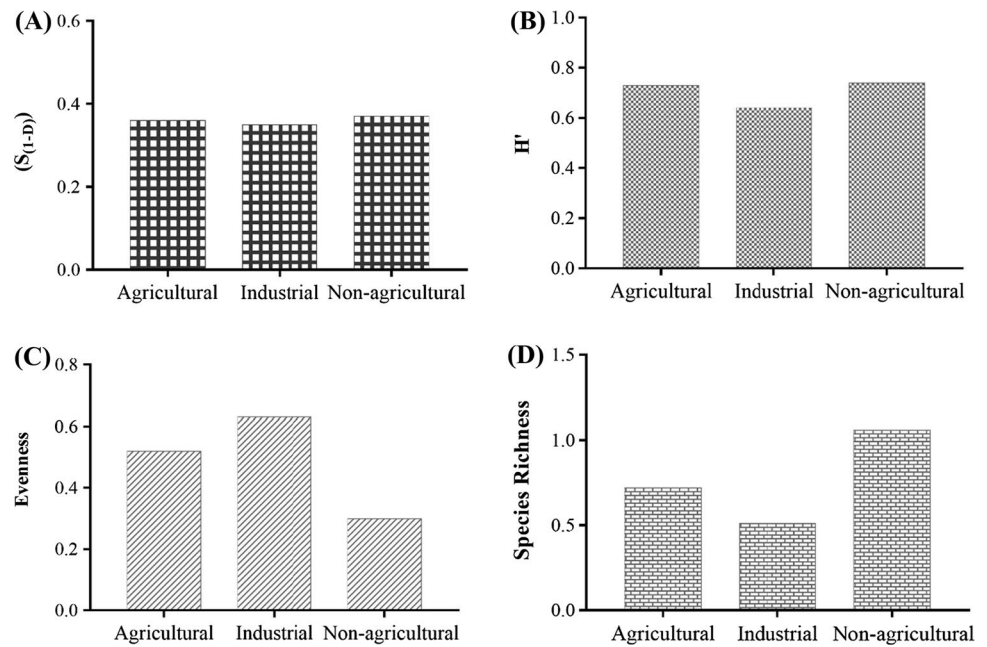
non-agricultural and industrial soils are given in Fig. 2. It was reported that the abundance of both anecic and endogeic species was higher in agricultural soils (2.34 and 8.35 individuals/m<sup>2</sup>, respectively) followed by non-agricultural (1.47

and 6.02 individuals/m<sup>2</sup>, respectively) and industrial soil (1.1 and 4.1 individuals/m<sup>2</sup>, respectively). Similarly, the biomass of both anecic and endogeic species was higher in agricultural soils (1.55 and 9.99 g/m<sup>2</sup>, respectively) followed by non-agricultural (1.44 and 6.83 g/m<sup>2</sup>, respectively) and industrial soils (0.83 and 3.22 g/m<sup>2</sup>, respectively). The Simpson index of diversity, Shannon–Weiner diversity index and species richness were found to be highest in non-agricultural soil (0.37; 0.74 and 1.06, respectively) which was due to the high number of earthworm species and their individuals/m<sup>2</sup> (Fig. 3a–d). On the other hand, Simpson index of diversity, Shannon–Weiner diversity index and species richness among the agricultural soil (0.36, 0.73 and 0.72, respectively) and industrial soil (0.35, 0.64, and 0.51, respectively) have intermediate and lowest diversity indices, respectively. The high evenness (0.63) was reported in industrial soil due to less number of species along with their even distribution.

The ranges of all the soil variables for earthworm species *Metaphire posthuma*, *Lampito mauritii*, *Amyntas morrisi* and *Octochaetona beatrix* are given in Table 6. The ranges of *Eutyphoeus incommodus*, *E. waltoni* and *Polypheretima elongata* were not given due to their presence at a single site only. The pH of soil was slightly acidic to moderate alkaline (6.8–7.9) for *M. posthuma* while neutral to moderate alkaline for *Lampito mauritii* (7.2–7.92), *Amyntas morrisi* (7.2–7.72) and *Octochaetona beatrix* (7.29–7.73). The range of OC (0.05–1.43%) for *M. posthuma* was also observed high as compared to other species which might be due to its wide range of distribution at all study sites. Similarly, the wide ranges of heavy metals (Zn: 12–537 mg/kg; Fe: 1079.1–1418.3 mg/kg; Mn: 108.1–483.1 mg/kg; Cu: 10.9–88.05 mg/kg; Pb: 46.9–297.1 mg/kg; Ni: 12–476 mg/kg; Co: 8.5–27.5 mg/kg; Cd: 1.35–5.95 mg/kg and 0.25–631.1 mg/kg) were also reported for *M. posthuma* among all the land use pattern.



**Fig. 3** Simpson index of diversity ( $S_{1-D}$ ), Shannon–Wiener diversity index ( $H'$ ), Species evenness and species richness among the agricultural, industrial and non-agricultural soils



**Table 6** Range of different soil variables for different earthworm species

Soil variables	<i>Metaphire posthuma</i>	<i>Lampito mauritii</i>	<i>Amyntas morrisi</i>	<i>Octochaetona beatrix</i>
pH	6.88–7.92	7.2–7.92	7.2–7.72	7.29–7.73
OC (%)	0.05–1.43	0.27–1.26	0.51–1.11	0.30–0.86
Zn (mg/kg)	12–537	12–126.8	34.4–80.55	33.6–537
Fe (mg/kg)	1079.15–1418.3	1079.5–1344.3	1273.4–1326	1274.5–1302.4
Mn (mg/kg)	108.1–483.1	180.7–369.3	180.7–267	188.1–242.2
Cu (mg/kg)	10.9–88.05	10.9–61.6	10.9–52.4	16.4–57.1
Pb (mg/kg)	46.9–297.1	48.7–156.2	62.1–136.6	60.7–124.4
Ni (mg/kg)	12–476	17.7–476	18.9–39.7	12–38.6
Co (mg/kg)	8.5–27.5	8.95–24.6	8.9–16.7	8.5–15.9
Cd (mg/kg)	1.35–5.95	1.35–5.15	1.7–2.65	1.4–2.75
As (mg/kg)	0.25–631.1	0.55–572.8	199.4–587.2	0.25–451.2

The ranges of *Eutyphoeus incommodus*, *E. waltoni* and *Polypheretima elongata* were not given due to their presence at single site only

### Impact of soil properties on earthworm abundance

The principal component analysis was used to find the impact of soil variables on earthworm abundance. The principal component analysis was applied to 12 different soil variables which resulted in four major principal components, i.e., PC1, PC2, PC3 and PC4, with a total variance of 32.22%, 15.46%, 13.31% and 11.99%, respectively. The different soil factors, their loading values, respective eigenvalues and total variance (%) for each component are given in Table 7. The method of Liu et al. (2003) was

used to classify the factor loadings as strong, moderate and weak having absolute loading values > 0.75, 0.75–0.50 and 0.50–0.30, respectively. The PC1 contributes 32.22% of the total variance with strong positive loading of OC and moderate negative loading of pH, which results in positive loading for earthworm abundance. The PC2 contributes 15.46% of the total variance with strong negative loading of Zn and Cu while moderate negative loading of Mn which also results in positive loading of earthworm abundance. The PC3 contributes 13.31% of variance due to positive loading of Fe and Cd, while PC4 causes 11.99% of variance due to the positive loading of Pb and Ni. Thus, both PC3 and

**Table 7** Loading values for different soil variables

Variables	PC1	PC2	PC3	PC4
pH	<b>-0.626</b>	-0.064	-0.154	0.144
OC	<b>0.846</b>	-0.11	-0.058	0.148
Zn	0.014	<b>-0.853</b>	0.038	0.132
Fe	0.191	0.126	<b>0.861</b>	-0.055
Mn	-0.206	<b>-0.505</b>	-0.161	0.158
Cu	0.389	<b>-0.846</b>	-0.074	-0.42
Pb	-0.012	-0.132	-0.054	<b>0.816</b>
Cd	0.066	-0.208	<b>0.697</b>	0.202
Ni	0.192	0.525	0.093	<b>0.604</b>
As	0.113	<b>-0.716</b>	-0.061	-0.152
Co	0.202	<b>-0.839</b>	0.136	0.058
Individuals	0.853	0.968	-0.815	-0.762
Eigenvalue	3.204	2.077	1.592	1.352
Variance (%)	32.22	15.46	13.31	11.99
Cumulative Variance (%)	32.22	47.68	60.99	72.98

Extraction method: principal component analysis; rotation method: Varimax with Kaiser normalization

PC4 cause negative loading for earthworm abundance. The loading plot for the loading values of principal component analysis is given in Fig. 4. The loading plot clearly showed that the loading values of industrial sites were much different as compared to agricultural and non-agricultural sites.

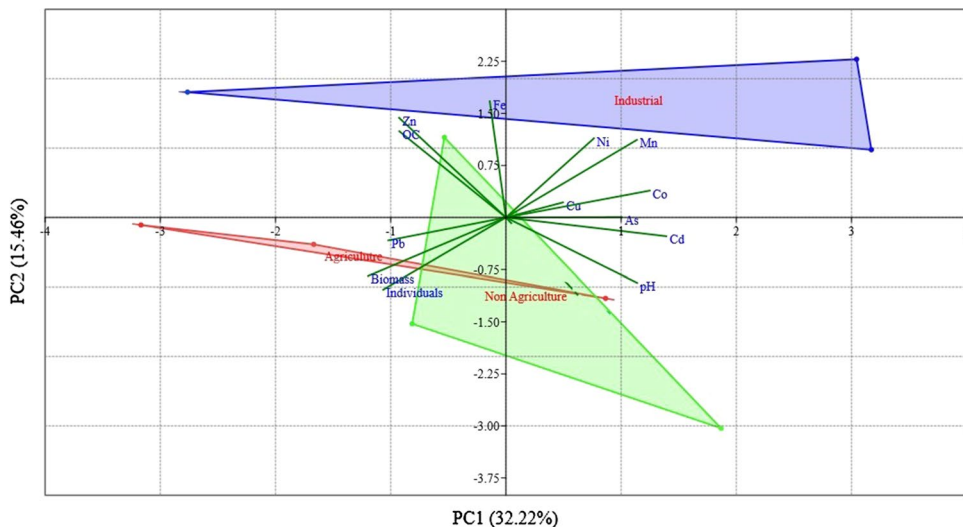
The correlation coefficient of different physicochemical variables with earthworm abundance is given in Table 8. The correlation coefficient reveals that there was a significant positive correlation of earthworm abundance and biomass with OC, while a significant negative correlation was observed with Zn, Mn, Cu, Pb, Cd, Ni and Co.

**Discussion**

**Heavy metal contamination among agricultural, non-agricultural and industrial soils**

The mean concentration of heavy metals in agricultural soils was in the order of Fe > Mn > As > Pb > Cu > Ni > Co > Cd. Similarly, the heavy metals contents in non-agricultural and industrial soils were in the order of Fe > As > Mn > Pb > Cu > Ni > Co > Cd, and Fe > As > Mn > Ni > Pb > Cu > Co > Cd, respectively. After a comparison of heavy metals of agricultural soils with Indian background soils, it was inferred that 97.5%, 82.5%, 47.5%, 100% and 42.5% sampling sites

**Fig. 4** Loading plot for different soil variables collected from three different soil types



**Table 8** Correlation coefficients of earthworm abundance and biomass against different soil variables

Soil Variables	Abundance	Biomass
pH	−0.049	−0.096
OC	0.170*	0.219*
Zn	−0.198*	−0.275**
Fe	0.111	0.018
Mn	−0.118	−0.229**
Cu	−0.212*	−0.220*
Cn	−0.195*	−0.225*
Pb	−0.197*	−0.164
Cd	−0.241*	−0.373**
Ni	−0.153	−0.236*
As	0.071	−0.002
Co	−0.309**	−0.392**

\*Significant at 0.05; \*\* Significant at 0.01

surpassed their contents for Zn, Mn, Ni, Pb and Co, respectively (Gowd et al. 2010). Arsenic concentration exceeded 75% in contrast to Poland soil guidelines, while Cd concentration was found low in comparison with their limits (Wcisło 2012). Similarly, the comparison of heavy metal in non-agricultural soils with Indian background values and Poland soil limits indicated that 100% of sampling sites surpassed their values for Zn, Mn, Pb, Ni, Co and As (Gowd et al. 2010; Wcisło 2012). In industrial soils, heavy metals exceeded 100% for Zn, Mn, Pb, Ni, Co and As, while 80% for Cu, and 60% for Cd in contrast to Indian background values and Poland soil limits (Gowd et al. 2010; Wcisło 2012).

The contamination factor (CF), enrichment factor (EF) and geoaccumulation index (Igeo) were enumerated to oversee the pollution level of heavy metals in different soils types. The EF results of agricultural soils indicated that Cd, As, Ni and Pb exhibited extreme enrichment in the area, Co and Cu showed high enrichment, and Zn and Mn showed considerable enrichment of studied heavy metals. In non-agricultural soils Cd, As, Co, Pb and Cu showed extreme, Zn and Ni showed high, and Mn exhibited considerable enrichment, respectively. In industrial soils, Cd, As, Co, Ni, Pb, Cu and Zn indicated extreme enrichment, while substantial enrichment showed by Mn. On the other hand, Muller (1981) suggested grades to classify the pollution level based on Igeo results. The Igeo results in agricultural soil indicated that Cd, As, Co, Ni, Pb, Cu and Zn showed extreme pollution, while Mn exhibited high pollution in the area. In non-agricultural and industrial soil, all studied heavy metals showed extreme pollution in the studied area. The grades suggested by Nemerow (1991) for PI and MPI and based upon that in agricultural soils Zn, Pb, Ni, Cd and As exhibited severe pollution, Cu and Co modest, and Mn showed slight pollution. In non-agricultural soils Cd, Pb and As showed severe

pollution, Cu exhibited heavily, Ni and Co modest, and Zn and Mn showed slight pollution. In industrial soils, PI values of Cd, As, Ni, Pb and Cu showed severe pollution, Co and Zn showed moderate, and Mn exhibited slight pollution in the studied area. The MPI values for all studied heavy metals in agricultural, non-agricultural and industrial soils were found above 10, signifying severe pollution of these heavy metals in the area.

The potential ecological risk (Er) value of Cd in agricultural soils was recorded above 320, signifying a very high risk of this metal in the agricultural soils of the study area. Er value of As showed high risk, whereas Zn, Mn, Cu, Pb, Ni and Co showed low risk in the area. In non-agricultural soils, Cd exhibited very high risk, As showed high risk, and Zn, Mn, Cu, Pb, Ni and Co exhibited low risk. In industrial soils, Cd and As showed very high risk, Ni reflected modest, and Zn, Mn, Cu, Pb and Co showed low risk in the study area. Based on grades used by Kumar et al. (2018) for the classification of ecological risk and based on these grades it was inferred that Mn, Zn and Co showed low ecological peril for all samples. Cu showed low to modest ecological risk in the area. The modified potential ecological risk (mEr) of Cd, As and Pb in agricultural soils indicated very high risk, Cu, Ni and Co showed high risk, and Zn and Mn exhibited low risk. In non-agricultural soils, Cd, As, Pb and Cu showed very high risk, Zn and Mn exhibited low risk, and Ni and Co showed high risk in the area. In industrial soils, Cd, As, Ni and Pb showed very high risk, Zn and Mn displayed low risk, and Cu and Co showed high risk in the area. The results of ecological risk indicated inferred that Cd is the vital pollutant in the area. Our results on ecological risk assessment are in corroboration with Pandit et al. (2020) and Kumar et al. (2018). They also reported in their studies that Cd is the main contaminant responsible for polluting the soils. Based on RI and MRI values, the trend followed by different soils was as follows: industrial soil > non-agricultural soil > agricultural soil.

### Effect of soil variables on Earthworm abundance

The principal component analysis was applied to 12 different soil variables which resulted in four major factors, i.e., PC1, PC2, PC3 and PC4, which caused 72.98% variance in the data. The PC1 contributes 32.22% of the total variance with strong positive loading of OC and moderate negative loading of pH causing positive loading for earthworm abundance which showed a negative and positive effect of pH and OC on earthworm distribution. This pH at a particular site is an important factor for earthworm distribution as earthworms can survive only in moderate salt concentration with neutral pH (Sanchez et al. 1997). McCallum et al. (2016) reported that earthworms are very sensitive to pH and their abundance decreases as the pH in the soil shifts to an acidic

range or basic range. But most of the studies reported a high abundance of earthworms near neutral pH, i.e., 7 (De Wandeler et al. 2016). In this study, the agricultural soil had pH near to neutral and also had a higher abundance and biomass of earthworm individuals, while both non-agricultural and industrial soil had moderate alkaline pH and also had less abundance and biomass of earthworm individuals. However, on the other hand, OC acts as a kind of food for the earthworm and various studies also reported that soil having high OC content usually has higher earthworm abundance (Singh et al. 2020b; Zhang et al. 2020). In the present study, a significant positive correlation was also reported among earthworm biomass and abundance with OC. Thus, PC1 represents the edaphic factor for the earthworm abundance. The PC2, PC3 and PC4 contributed 40.76% of total variance which was due to negative loading of Zn, Mn, Cu, As and Co while positive loading of Fe, Cd, Pb and Ni. These heavy metals have a very toxic effect on the earthworm. The Zn, Pb, and Cd in the soil directly affect earthworm biomass and species richness (Wang et al. 2018). But the effects of the heavy metal in the soil are also dependent on their concentration, i.e., earthworms can survive in less concentration of heavy metals (Shi et al. 2020). The survival of earthworms in less heavy metals concentration might be due to their capability to bioaccumulate and storage of heavy metals in their yellow tissues (Suthar 2009; Audusseau et al. 2020). But as the concentration of heavy metals in the soil increases, more bioaccumulation of heavy metals is done by the earthworm which causes toxicity and hence mortality in the earthworm (Latifi et al. 2020). Hence, the PC2, PC3 and PC4 represent heavy metals effect on earthworm abundance.

## Conclusion

The abundance and biomass of earthworms were much higher in agricultural soils but the diversity of earthworm species was much more in non-agricultural soils. The industrial soil was reported with lowest abundance and diversity of earthworm species. The present study also concluded that industrial soil has the highest concentration of heavy metals in contrast with non-agricultural and agricultural soils. After comparison of industrial soils with Indian background values and Poland soil limits, it was revealed that 100% of samples surpassed their values for Zn, Mn, Pb, Ni, Co and As, while 80% for Cu and 60% for Cd. 97.5%, 82.5%, 47.5%, 100% and 42.5% sampling sites surpassed their contents for

Zn, Mn, Ni, Pb and Co in comparison with their limits in agricultural soils. Similarly, in non-agricultural soils 100% of sampling sites surpassed their values for Zn, Mn, Pb, Ni, Co and As in contrast to their limits. The results of CF, EF, Igeo, PI, MPI, RI and MRI showed that industrial areas were much polluted with heavy metals as compared to agricultural and non-agricultural soils which might be the reason for less abundance and diversity of earthworm species in industrial soils.

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