

## Earthworm Communities in the Waste Deposit Sites (Cowdung Heaps and Municipal Solid Wastes) of West Tripura, India

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### ABSTRACT

Community characteristics of earthworms were studied in two waste deposit sites - the cow dung heaps and municipal solid wastes - in West Tripura, India. A total of eleven earthworm species belonging to four families and ten genera were collected. Sample based rarefaction curve, revealed almost completeness of inventory in the studied sites with eleven species in cow dung deposit sites and seven species in municipal solid wastes. Cow dung heaps had higher ( $p < 0.05$ ) earthworm density, biomass, diversity index, evenness, richness index and significantly lower index of dominance than the municipal solid wastes. Rank abundance curve showed dominance of *Perionyx excavatus* in cow dung and *Lampito mauritii* in the municipal solid wastes. Occurrence of five earthworm species namely, *Lampito mauritii*, *Metaphire houlleti*, *Perionyx excavatus*, *Dichogaster bolau* and *Metaphire posthuma* in both cowdung and municipal solid wastes with wide range of tolerance to environmental parameters (euracious) indicates their usefulness in degradation of organic wastes. According to principal components analysis, soil organic matter, moisture, pH, bulk density, water holding capacity, nitrogen, phosphorus, potassium were the most potent regulators of earthworm population density and biomass with highest positive loading scores.

Key Words: Organic Wastes; Sample Based Rarefaction Curve; Rank Abundance Curve; Diversity; Dominance.

### INTRODUCTION

Charles Darwin (1881) in his book "The Formation of Vegetable Mould Through the Action of Worms" mentioned the role of earthworms in the disappearance of surface plant litter. Since then a century has been passed to evaluate the role of earthworms in the breakdown of organic wastes, plant litter and their redistribution and incorporation in the soils (Edwards and Lofty 1977, Kale et al. 1982, Lee 1985). Organic matter is pulverized and subjected to digestive enzymes in their alimentary canal and excreted as plant nutrient rich earthworm casts. It has been confirmed that the worm cast contains a higher amount of nitrate nitrogen and plant available nutrients (compared to parent soils) required for crop production (Edwards and Lofty 1977, Asawalam and Hauser 2001, Chaudhuri et al. 2009). The

burrowing activity of earthworms enhances aeration and porosity of the soil, thereby improving its water holding capacity (Chaudhuri et al. 2009). Dramatic rise in human population and increased agricultural activities have generated a large amount of organic wastes, the disposal of which is one of the major hazards for keeping a clean environment. In recent days vermicomposting technology has been developed to recycle organic wastes through the agency of earthworms (Chaudhuri 2017). Most of the vermicultivists use one of four species, *Eisenia fetida* (Savigny), *Eisenia andrei* (Bouche), *Perionyx excavatus* (Perrier) or *Eudrilus eugeniae* (Kinberg). In vermicomposting a few other species with restricted geographical distribution such as *Perionyx ceylanensis* (Michaelsen), *Perionyx sansibaricus* (Michaelsen, 1891), *Lampito mauritii* (Kinberg), *Dendrobaena veneta* (Rosa) have been reported to have

potential for vermicomposting or vermistabilization of organic wastes (Dominguez and Edwards 2011, James and Guimaraes 2011). Earthworm species suitable for waste management can only be selected on the basis of their biological and ecological characteristics. A wide range of ecological tolerance and high fecundity are the basis of selection of earthworm species for vermiculture (Chaudhuri 2017).

In most of the developing countries municipal solid waste (MSW) disposal has become a great problem particularly in areas with population pressure (Choudhury and Choudhury 2014). According to Shekdar (2009), metropolitan cities of India alone generate about 14 million Mg (tons) of MSW yearly. In fact urban population in India has increased from 26% in 2001 to 32% in 2011 (Census of India 2011) which results in the rapid increase of waste generation.

According to the report of the Central Pollution Control Board (2013), about 360 Mg of municipal solid wastes are generated every day in Tripura, but only a fraction of it (40 Mg day<sup>-1</sup>) undergoes degradation in chemical or microbial treatment plant. In the majority of Indian cities and towns, municipal solid waste management is unscientific and involves dumping in low lying areas (Lal and Rajanikant 2010). Cow (*Bos indicus*) is major cattle generating 9-11 kg dung day<sup>-1</sup> (Werner 1987, Brown 2003) and cow dung is used in various practices though it is usually stored as an open dump in the rural areas of India.

Although earthworms are predominant waste degrading fauna in terrestrial ecosystem and vermicomposting species in general, inhabit waste deposit sites, only a few studies have been carried out to explore earthworm resource in waste deposit sites (Paliwal and Julka 2007, James and Guimaraes 2011). According to Paliwal and Julka (2007), in addition to vermicomposting species viz. *Perionyx excavatus*, *Eisenia fetida* and *Eudrilus eugeniae*, other epigeic earthworm species, such as *Perionyx sansibaricus*, *Perionyx bainii*, *Amyntas corticis*, *Bimastos parvus* and *Dichogaster bolau* could also be used in vermicomposting because of their affinities for the high organic matter. Kaushal et al. (1995, 1999) have used *Drawida nepalensis* and *Metaphire houlleti* in waste degradation. Paul and Selvi (2007) produced vermicompost from biogas slurry of municipal solid wastes mixed with cow dung using *Perionyx ceylanensis*. Kale (2011), reported *Dichogaster curgensis* (Michaelsen 1921) and *Dichogaster bolau* (Michaelsen, 1891) as potential vermicomposting species under tropical condition. Based on their survey, James

and Guimaraes (2011) reported *Dichogaster annae* (Horst 1893), *Amyntas gracilis* (Kinberg 1867) and *Metaphire schmardae* (Horst 1883) as probable species in waste management.

The present study aims to characterise the earthworms community in the two kinds of wastes, namely municipal wastes and cowdung heaps, to identify earthworm species with higher relative abundance and preference to higher organic matter content in soils, and to select species for their potential for vermicomposting on the basis of their ecological characteristics.

## MATERIALS AND METHODS

### Study Sites

The studies were conducted for 2 consecutive years (April 2015 to July 2017) in the two waste deposit sites viz. municipal solid waste deposit sites (MSW) and cow dung heaps (CD) of West Tripura (22°51' - 24°32' N and 90°21' E). The average elevation of West Tripura is 15 m above sea level. Tripura is encircled by Bangladesh except in the north east where it meets neighboring state Assam and Mizoram.

Both CD and MSW dumps were 4-5 months old. A total of at least 50 dumps each for MSW and CD were surveyed for two consecutive years. Size of the individual dump varied from 50 to 65 m<sup>2</sup>. MSW was an open dumping ground (with direct exposure to sunlight) under Agartala Municipal Council, with a few small grasses and bushes and being used for disposal of municipal solid wastes comprising of both degradable and non-degradable wastes. In Agartala city, municipal wastes generally comprise of house hold wastes, commercial, construction debris, electronic wastes, biomedical wastes etc. (Choudhury and Choudhury 2014) which are generally utilized in land filling.. Replicate plots for MSW were selected in Nagicherra, Debendra Chandra Nagar, Kalitala of West Tripura, which was 10-20 km apart from the Agartala city.

Cow dung deposit sites, 7-20 km away from the Agartala city, were in the village areas of Mohanpur, Khayerpur, Jirania of West Tripura. Cow dung heaps had a little canopy cover of banana, bamboo, betel nut along with different kinds of herbs and grasses. Cowdung is utilized in organic farming. Anthropogenic disturbances in MSW due to the frequent dumping of wastes by trucks and human (road pickers) activities were comparatively much higher than that in the CD.

### Earthworm Sampling

Earthworms in general, during activity period (as revealed by casting activity) are concentrated within 0-40 cm soil depth. Earthworms depending chiefly or particularly on soils rich in organic matter occupy 0-15 cm depth. Earthworms were sampled by conventional digging of 600 quadrats (25cm x 25cm x 15 cm each) and hand sorting (Chaudhuri et al. 2008) at soil waste juncture. Sampling points were 10 m apart from each other. Collected earthworms were counted, rinsed and weighted on an electronic balance under field conditions. Earthworm species were identified with the help of the keys adopted by Gates (1972) and Julka (1988). Earthworm species difficult to identify were sent to Dr. Paliwal (Zoological Survey of India, Solan) for confirmation. Following synecological parameters were estimated for each sampling point: earthworm biomass (fresh weight,  $\text{g m}^{-2}$ ), density (individuals  $\text{m}^{-2}$ ), diversity and community indices, including relative abundance, frequency, index of general diversity (Shannon and Weaver 1963), index of dominance (Simpson 1949), evenness (Dash and Dash 2009), Menhinick's species richness index (Menhinick 1964). To determine the completeness of inventory Sample-based Rarefaction Curve (SRC) with 95% confidence interval was employed (Gotelli and Colwell 2001). Contrasting patterns of species richness amongst the assemblages (MSW and CD) were viewed through the application of Individual-based Rarefaction Curves (IRC). 'X' axis of the curve either represents the number of individuals, or the samples as the standardization by a number of species is necessary to evaluate patterns of richness at a comparable level of sampling effort when data sets are likely to differ systematically in the mean number of individuals per sample (Gotelli and Colwell 2001).

### Soil Analysis

Soil samples ( $n=150$ ), collected at 0-15 cm depth from each quadrat, were air dried, ground with mortar and pestle and sieved (1-2 mm mesh sieve). Composite soil samples comprising of 4 sub samples were analyzed to determine soil moisture (Gravimetric wet weight method), water holding capacity (Upadhyay and Sharma 2001), bulk density (Upadhyay and Sharma 2001), pH (1:2.5 dilution methods) and soil organic matter content (Walkley and Black method 1934). Soil temperature was recorded at a depth of 0-15 cm using a digital soil thermometer. Soil samples for estimation of

nitrogen (N), phosphorus (P), potassium (K) and heavy metals were sent to the D.R.D.O, Assam, India.

### Data Analysis

Variations among soil physico-chemical properties and some biological parameters like earthworm density and biomass in CD and MSW sites were compared using Student's t test at the 5% level of significance. Differences among the species indices data viz. Shannon diversity index, dominance index, Menhinick species richness index and evenness were tested non-parametrically by using Kolmogorov - Smirnov goodness of fit test (Kolmogorov 1933, Smirnov 1939a, 1939b). Similarities of earthworm species composition in the two waste deposit sites (CD, MSW) were identified using single link Bray-Curtis cluster analysis (McAleece et al. 1997) and Sorenson similarity index (Sorenson 1948). Combination of both the habitat data was subjected to Pearson correlation coefficient for determining the relationships among earthworm density, biomass and different soil abiotic factors. For correlation studies biological samples and soil samples ( $N=150$  each) from the same sampling plot were considered. In order to determine the most effective factors that influence the earthworm distribution, Principal Component Analysis (PCA) was adopted where the soil temperature ( $^{\circ}\text{C}$ ), pH, organic matter (g%), moisture (%), bulk density ( $\text{g cm}^{-3}$ ), water holding capacity (%), nitrogen (%), phosphorus (%), potassium (%) were considered as physicochemical variables and earthworm density ( $\text{ind. m}^{-2}$ ), biomass ( $\text{g m}^{-2}$ ) as biological variables (Arenki et al. 2010). Eigen values one and above was considered as a standard for the extraction of principal components that explained the total variability (Shrestha and Kazama 2007). Effects of variables in each principal component were clarified through factor loading values of PCA. Factors loading are classified as strong, moderate and weak corresponding to absolute loading values  $>0.75$ ,  $0.75-0.50$  and  $0.50-0.30$  (Liu et al. 2003).

## RESULTS

### Site Characteristics

Details of abiotic factors as well as nutrient contents of the studied habitats (MSW, CD) are shown in Table 1. Among the soil physicochemical properties, pH, moisture content, water holding capacity and organic

Table: 1 Physicochemical properties and biosynecological parameters of earthworm species under cowdung heaps and municipal solid waste deposit sites. (Mean  $\pm$  S.E.)

Parameters *	Habitats		p	t
	MSW	CD		
Temperature ( $^{\circ}$ C)	28.7 $\pm$ 0.09	28.12 $\pm$ 0.15	>0.05	-3.24
pH	7.3 $\pm$ 0.1	7.02 $\pm$ 0.013	<0.05	-5.53
Moisture (%)	24.21 $\pm$ 0.06	38.31 $\pm$ 0.073	<0.05	15.53
Bulk density (g cm <sup>-3</sup> )	0.30 $\pm$ 0.15	0.37 $\pm$ 0.001	>0.05	0.4
Water holding capacity (%)	30.6 $\pm$ 0.48	53.1 $\pm$ 1.7	<0.05	-24.39
Organic matter (g %)	3.76 $\pm$ 0.12	8.32 $\pm$ 0.12	<0.05	-11.38
Nitrogen (%)	0.17 $\pm$ 0.02	0.23 $\pm$ 0.23	>0.05	2.04
Phosphorus (%)	2.27 $\pm$ 0.19	2.14 $\pm$ 0.18	>0.05	2.04
Potassium (%)	2.34 $\pm$ 0.22	2.48 $\pm$ 0.07	>0.05	2.04
Density (ind. m <sup>-2</sup> )	86.1 $\pm$ 2.42	156.31 $\pm$ 20.5	<0.05	-7.47
Biomass (g m <sup>-2</sup> )	52.6 $\pm$ 1.7	80.4 $\pm$ 6.1	<0.05	-6.26
Diversity indices**	MSW	CD	p	D
Shannon's diversity index	0.42 $\pm$ 0.07	1.16 $\pm$ 0.02	<0.005	1
Simpson's index of dominance	0.79 $\pm$ 0.03	0.42 $\pm$ 0.03	<0.05	1
Evenness	0.03 $\pm$ 0.07	0.43 $\pm$ 0.08	<0.05	0.66
Menhinick richness index	0.24 $\pm$ 0.05	0.38 $\pm$ 0.01	<0.05	0.33
Similarity index				
Sorensen's index	77.77%			
Bray Curtis index	78%			

(\* Student's t test, \*\* Kolmogorov-Smirnov goodness of fit).

matter contents greatly differed between MSW and CD. Analysis of habitat soil revealed the presence of a few heavy metals, such as lead (2.4 mg kg<sup>-1</sup>), copper (22.8 mg kg<sup>-1</sup>) and cadmium (0.3 mg kg<sup>-1</sup>) in the soils of MSW, whereas heavy metals were absent in the CD.

### Population Characteristics

A total of 4344 earthworms were extracted from the soil samples, comprising of 11 species belonging 4 families and 10 genera, with five species under the family Megascolecidae [*Metaphire houlleti* (Perrier), *Lampito mauritii* Kinberg, *Metaphire posthuma* (Vailant), *Perionyx excavatus* Perrier, *Kanchuria* sp.], family Octochaetidae with four species [*Lenogaster chittagongensis* (Stephenson), *Dichogaster bolaii* (Michaelsen), *Eutyphoeus gammiei* (Beddard), *Octochaetona beatrix* (Beddard)], family Glossoscolecidae and Moniligastridae with one species each, *Pontoscolex corethrurus* (Muller) and *Drawida assamensis* Gates respectively. Distributions of earthworms among the studied sites are presented in Table 2. Present study revealed the occur-

rence of seven native (*L. chittagongensis*, *E. gammiei*, *O. beatrix*, *L. mauritii*, *P. excavatus*, *D. assamensis*, *Kanchuria* sp.) and four exotic earthworm species (*D. bolaii*, *M. posthuma*, *M. houlleti*, *P. corethrurus*) in the studied sites. Three ecological categories of earthworms namely epigeic (*P. excavatus*, *L. chittagongensis*, *D. bolaii*), anecic (*L. mauritii*, *M. houlleti*), endogeic (*E. gammiei*, *M. posthuma*, *Kanchuria* species, *D. assamensis*, *P. corethrurus*, *O. beatrix*) were collected from MSW and CD. Seven earthworm species (*L. mauritii*, *M. posthuma*, *P. excavatus*, *M. houlleti*, *D. bolaii*, *P. corethrurus*, *O. beatrix*) were recorded in MSW while CD had 11 species (*M. houlleti*, *L. mauritii*, *M. posthuma*, *P. excavatus*, *Kanchuria* sp., *L. chittagongensis*, *D. bolaii*, *E. gammiei*, *O. beatrix*, *P. corethrurus*, *D. assamensis*). Except for four species (*Kanchuria* sp., *L. chittagongensis*, *D. assamensis* and *E. gammiei*) which were restricted to CD; other species (*L. mauritii*, *M. posthuma*, *M. houlleti*, *P. excavatus*, *D. bolaii*, *O. beatrix*, *P. corethrurus*) were common to both the studied sites. A sample-based rarefaction curve (Figure 1A) showed an initial exponential phase of a

Table 2. Earthworm population characteristics and their association with different soil abiotic factors under cowdung heaps and municipal solid waste deposit sites. (Mean  $\pm$  S.E.)

Earthworm Species	Habitat	Density <sub>2</sub> (ind.m <sup>-2</sup> )	Population characteristics				Soil abiotic factors		
			Biomass <sub>2</sub> (g m <sup>-2</sup> )	Relative (%)	Frequency (%)	Temperature (°C)	pH	Moisture (%)	Organic matter (%)
<i>L. mauritii</i> (Anecic)	MSW	82.3 $\pm$ 2.5	46.2 $\pm$ 1.7	87.2	81.2	28.4 $\pm$ 0.89 (20-34)	7.3 $\pm$ 0.3 (5.4 - 8.0)	25.7 $\pm$ 3.22 (15.6 - 39.2)	3.8 $\pm$ 1.03 (0.7 - 5.2)
	CD	76.7 $\pm$ 11.5	38.2 $\pm$ 3.21	12.8	17.9	27.9 $\pm$ 0.78 (20.3 - 31)	7.62 $\pm$ 2.04 (6.6 - 8.4)	32.12 $\pm$ 8.17 (19.0 - 41.7)	9.23 $\pm$ 0.4 (4.7 - 15.7)
<i>M. posthuma</i> (Endogeic)	MSW	39.5 $\pm$ 9.2	52.9 $\pm$ 8.1	5.8	7.2	29.6 $\pm$ 0.86 (25.0 - 31.2)	7.4 $\pm$ 1.1 (6 - 8)	23.91 $\pm$ 2.4 (18.8 - 31.0)	3.9 $\pm$ 0.98 (1.2 - 6.2)
	CD	54.2 $\pm$ 7.3	58.8 $\pm$ 10.1	6.9	10.8	28.1 $\pm$ 0.22 (20 - 32)	7.1 $\pm$ 0.7 (6 - 8)	38.7 $\pm$ 4.1 (34.5 - 46.3)	7.11 $\pm$ 1.7 (3.7 - 11.8)
<i>M. houlletii</i> (Anecic)	MSW	16.00	59.52	0.12	0.61	32.05	7.26	17.42	1.5
	CD	40.08 $\pm$ 22.3	30.17 $\pm$ 6.03	1.7	3.29	28.8 $\pm$ 0.9 (25.3 - 29.3)	7.6 1.06 (6.2 - 8)	33.8 $\pm$ 8.8 (28.6 - 41.5)	8.03 $\pm$ 1.2 (4.7 - 14.7)
<i>Kanchuria</i> sp. (Endogeic)	MSW	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	CD	16.00	3.2	0.07	0.38	28.65	7.25	36.88	4.44
<i>P. excavatus</i> (Epigeic)	MSW	30.51 $\pm$ 7.37	12.22 $\pm$ 4.54	1.69	4.0	29.87 $\pm$ 0.07 (28.0 - 34.2)	7.02 $\pm$ 0.19 (6.2 - 8.1)	27.32 $\pm$ 1.24 (11.0 - 36.3)	4.45 $\pm$ 0.9 (1.1 - 8.2)
	CD	126 $\pm$ 12.3	58.9 $\pm$ 7.2	61.8	39.4	28.22 $\pm$ 0.15 (25 - 32)	7.02 $\pm$ 0.04 (6.0-8.3)	40.2 $\pm$ 0.59 (19.2-46.1)	8.43 $\pm$ 1.01 (5.7 - 15.7)
<i>D. bolau</i> (Epigeic)	MSW	59.8 $\pm$ 31.12	1.76 $\pm$ 0.72	3.25	4.3	28.85 $\pm$ 0.42 (26 - 2)	7.1 $\pm$ 0.04 (6.5 - 8.0)	26.86 $\pm$ 2.82 (19.2 - 41.1)	3.55 $\pm$ 0.81 (0.9 - 9.8)
	CD	67.6 $\pm$ 10.3	3.1 $\pm$ 0.45	9.1	10.2	28.8 $\pm$ 0.41 (20.0 - 31.4)	7.19 $\pm$ 0.09 (6.2 - 8.1)	48.41 $\pm$ 3.13 (29 - 52.3)	10.35 $\pm$ 1.49 (7.6 - 15.8)
<i>O. beatrix</i> (Endogeic)	MSW	16.00	12.56	0.12	0.61	31.2	7.43	22.8	1.3
	CD	16.00	12.32	0.07	0.38	28.06	7.19	46.25	6.83
<i>E. gammiei</i> (Endogeic)	MSW	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	CD	19.5 $\pm$ 3.1	94.3 $\pm$ 14.39	0.33	1.36	28.55 $\pm$ 0.41 (27 - 32)	6.93 $\pm$ 0.07 (6.4-7.2)	29.20 $\pm$ 0.61 (20.3-37)	7.15 $\pm$ 2.67 (4.0-9.7)
<i>L. chittagongensis</i> (Epigeic)	MSW	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	CD	24.00	1.92	0.11	0.38	29.55	7.77	45.34	7.74
<i>D. assamensis</i> (Endogeic)	MSW	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	CD	24.00	9.28	0.11	0.38	27.3	6.71	46.84	6.28
<i>P. corethrurus</i> (Endogeic)	MSW	16.0	9.2	0.12	0.61	29.7 $\pm$ 0.3 (29.4 - 30)	7.03 $\pm$ 0.06 (7 - 7.1)	24.68 $\pm$ 6.02 (18.7-30.7)	3.07 $\pm$ 1.0 (5.06 - 7.08)
	CD	37.3 $\pm$ 6.4	15.1 $\pm$ 3.8	5.9	10.5	26.14 $\pm$ 0.7 (19.7 - 30.6)	6.5 $\pm$ 0.05 (5.0 - 8.5)	32.2 $\pm$ 1.31 (15.2 - 36.0)	3.6 $\pm$ 0.4 (1.6 - 4.5)

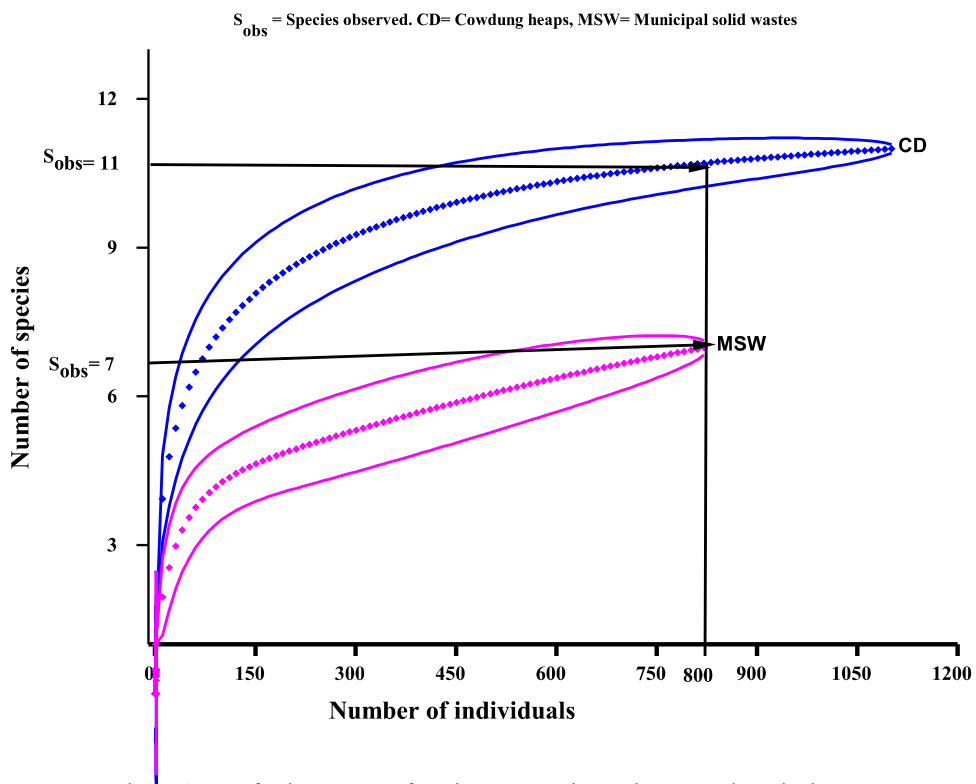
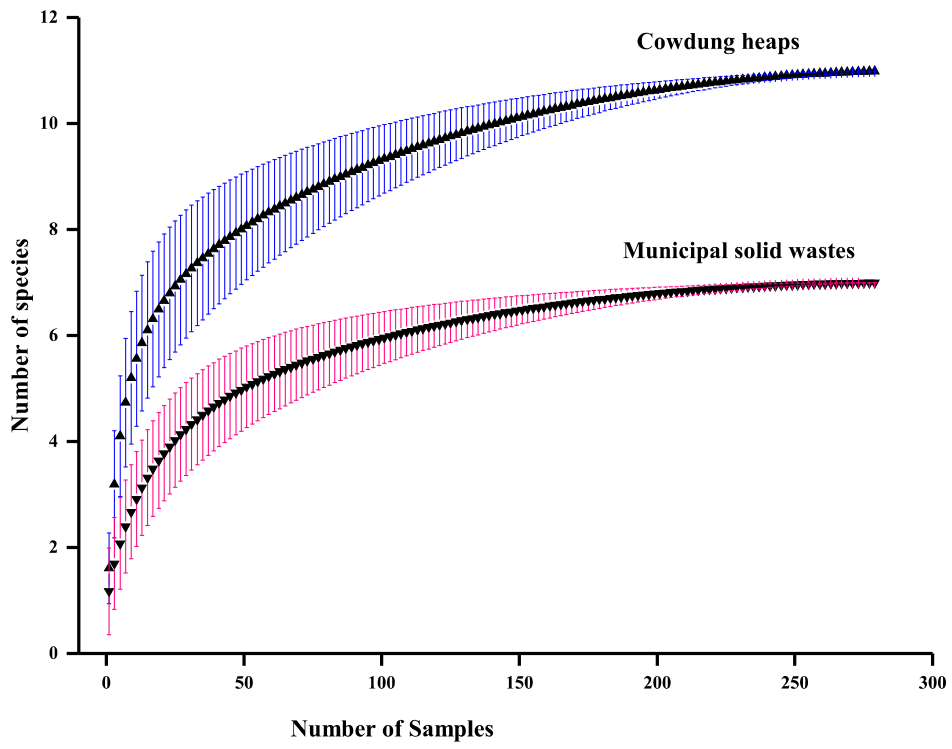


Figure 1. Rarefaction curves of earthworm species under waste deposit sites - (A) Sample based, (B) Individual based

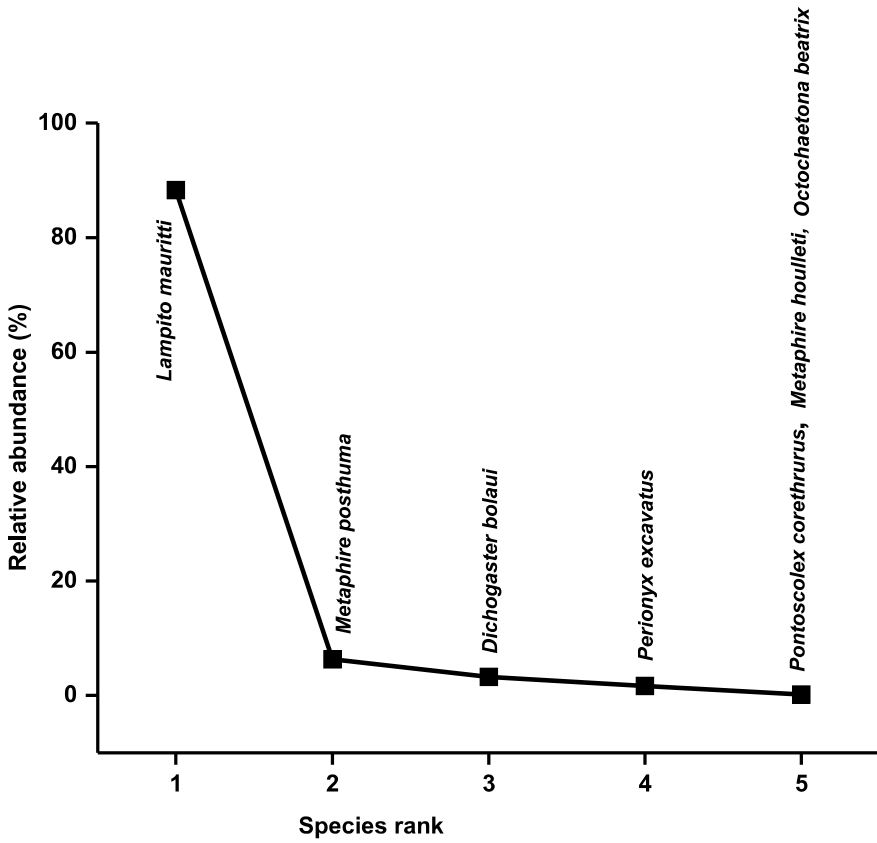
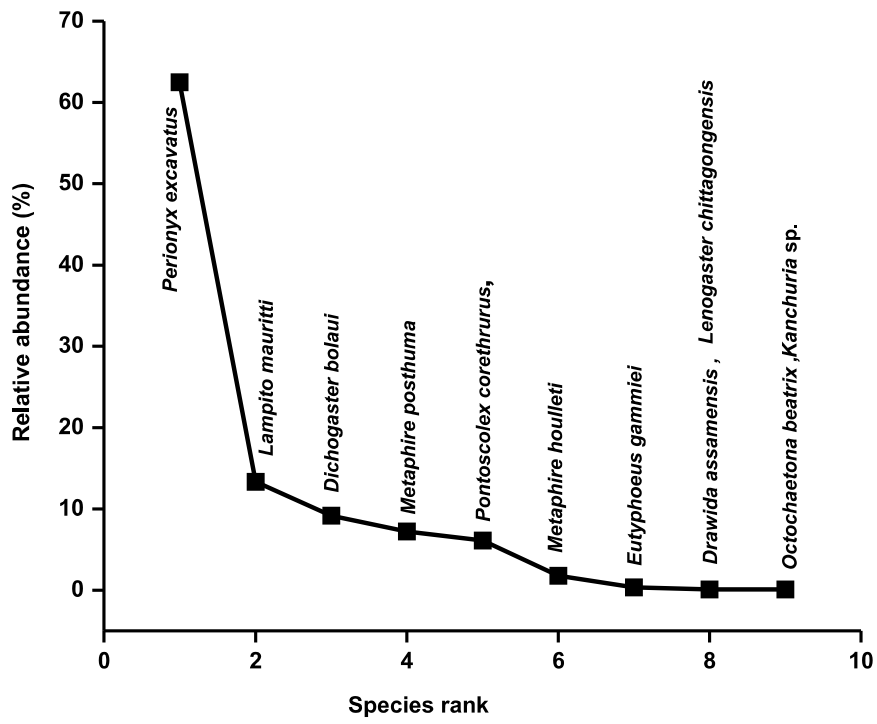


Figure 2: Rank abundance curves showing the abundance pattern of earthworm species under (A) Cowdung heaps and (B) Municipal solid wastes

sharp rise and then gradually attained asymptotic level for both CD and MSW at 11 and 7 species richness respectively. Differences in species richness of the assemblages (MSW and CD) were visualized through the inspection of 95% confidence interval of individual based rarefaction curve that indicated that the CD had species richness of 11 species which was much higher than the MSW with 7 species from a common assemblage of 800 individuals with non overlapping 95% confidence interval (Figure 1B). A dominant species was generally recognized by its 10% or above relative abundance in the community (Engelmann 1973). Thus anecic species *L. mauritii* was dominant in both MSW (relative abundance 87.2%, frequency 81.2%, density 82.3 ind. m<sup>-2</sup>, biomass 46.2 g m<sup>-2</sup>) and CD (relative abundance 12.8%, frequency 17.9%, density 76.7 ind. m<sup>-2</sup>, biomass 38.2 g m<sup>-2</sup>) (Figure 2B and Table 2). Epigeic species, *P. excavatus* was dominant only in CD with relative abundance 61.8%, frequency 39.4%, density 126 ind. m<sup>-2</sup> and biomass 58.9 g m<sup>-2</sup> (Figure 2A and Table 2). While the smallest earthworm species, *D. bolau* had low biomass (3.1 g m<sup>-2</sup>) with high density (67.6 ind. m<sup>-2</sup>), the largest earthworm species, *E. gammiei* had highest biomass (94.3 g m<sup>-2</sup>) and low density (19.5 ind. m<sup>-2</sup>) in the CD. Other earthworm species such as *M. posthuma* had high biomass values (above 50 g m<sup>-2</sup>) in the waste deposit sites (Table 2). Geophagous earthworm species viz. *P. corethrurus*, *D. assamensis*, *O. beatrix* and *Kanchuria* sp. had very low biomass values in the waste deposit sites (Table 2).

### Interhabitat Variation in Community Characteristics

The CD had 11 earthworm species which included three epigeic (*P. excavatus*, *D. bolau*, *L. chittagongensis*), six endogeic (*M. posthuma*, *Kanchuria* sp., *O. beatrix*, *E. gammiei*, *D. assamensis*, *P. corethrurus*) and two anecic species (*L. mauritii*, *M. houletti*). The MSW had two epigeic (*P. excavatus*, *D. bolau*), three endogeic (*M. posthuma*, *O. beatrix*, *P. corethrurus*) and two anecic species (*L. mauritii* and *M. houletti*). CD had high biomass value of 27 g m<sup>-2</sup> for epigeic species, 45 g m<sup>-2</sup> for endogeic species, while in MSW biomass value of 47.5 g m<sup>-2</sup> was contributed by anecic species. The highest relative abundance of epigeic was 72% and endogeic 14% in CD and anecic 88% in MSW. Thus in spite of highest number (6) of endogeic species in the waste deposit sites their relative abundance was very low.

Overall earthworm density and biomass were significantly higher ( $P < 0.05$ ) in the CD than in the

MSW (Table 1). Shannon diversity index, evenness and species richness index were significantly higher ( $P < 0.05$ ) and index of dominance was significantly lower ( $P < 0.05$ ) in the CD than those in the MSW (Table 1). In spite of interhabitat variations, similarity index between CD and MSW was 77.7% (Sorenson index) and Bray-Curtis index 78%.

### Earthworm and Soil Properties Relationship

Edaphic parameters and biosynecological parameters of earthworm species in the waste deposit sites (WDS) in general (mean values of MSW and CD) are shown in Table 1. Distribution of earthworm species on CD and MSW with reference to their ecological parameters namely temperature, moisture, pH and soil organic matter is given in Table 2. Environmental variables such as, temperature, moisture, pH etc. and their relationship with density and biomass of earthworms are given in Table 3. Earthworm species in the WDS in general, experienced a mean soil temperature of 28.41°C, moisture 31.26%, bulk density 0.36 g cm<sup>-3</sup>, water holding capacity 41.85%, organic matter 5.9%, nitrogen 0.16%, phosphorus 2.45% and potassium 2.56%. There were no significant differences ( $p < 0.05$ ) in the contents of soil N, P, and K between MSW and CD. Heavy metals (lead, Cadmium, Copper) were present in the soils of MSW and absent in CD. Among the soil parameters, moisture, water holding capacity and organic matter content were significantly higher ( $P < 0.05$ ) in the CD than the MSW

Table 3. Pearson's correlation coefficient of environmental variables with earthworm density and biomass.

Environmental variables	Density	Biomass
Temperature(°C)	r = -0.69, p <0.05	r = -0.51, p <0.05
pH	r = 0.74, p <0.05	r = 0.75, p <0.05
Moisture (%)	r = 0.79, p <0.05	r = 0.79, p <0.05
Water holding capacity (%)	r = 0.75, p <0.05	r = 0.63, p <0.05
Bulk density (g cm <sup>-3</sup> )	r = 0.55, p <0.05	r = 0.82, p <0.05
Organic matter (g %)	r = 0.86, p <0.05	r = 0.88, p <0.05
Nitrogen (%)	r = 0.69, p <0.05	r = 0.51, p <0.05
Phosphorus (%)	r = 0.71, p <0.05	r = 0.67, p <0.05
Potassium (%)	r = 0.64, p <0.05	r = 0.66, p <0.05

(Table 1). Earthworm species such as, *L. mauritii*, *M. posthuma*, *M. houletti*, *P. excavatus*, *D. bolau*, *O.*

*beatrice*, *P. corethrurus* exhibited a wide range of tolerance while *Kanchuria* sp., *L. chittagongensis*, *D. assamensis* and *E. gammiei* had a narrow range of tolerance to edaphic factors (Table 2).

PCA was executed for evaluating most effective factors that control the earthworm distribution (Figure 3). This was based on eigen values and factors loading values of the principal components (PCs) (Table 4a, 4b). Nine physicochemical parameters (temperature, pH, moisture, bulk density, water holding capacity, organic

matter, nitrogen, potassium and phosphorus) and two biological parameters (earthworm density and biomass) were subjected to PCA that resulted in two PCs (PC1 and PC2).

Table 4b. Factor loading values of soil physicochemical and earthworm community parameters for the Principal Components.

Table 4a. Eigen- analysis of the correlation matrix for the Principal Components.

PC	Eigenvalue	Variance (%)	Cumulative variance (%)
1	7.33817	66.711	66.71
2	1.24674	11.334	78.04
3	0.678679	6.1698	84.21
4	0.618639	5.624	89.84
5	0.346288	3.1481	92.99

	PC 1	PC 2
Density	0.9206	0.14209
Biomass	0.91143	0.072649
Temperature	-0.66761	0.50259
pH	0.84182	-0.06704
Moisture	0.87804	0.17182
Water holding capacity	0.70257	0.65732
Bulk density	0.78083	-0.28326
Organic matter	0.91398	0.25649
Nitrogen	0.72719	0.23178
Phosphorus	0.81208	-0.28831
Potassium	0.77955	-0.46875

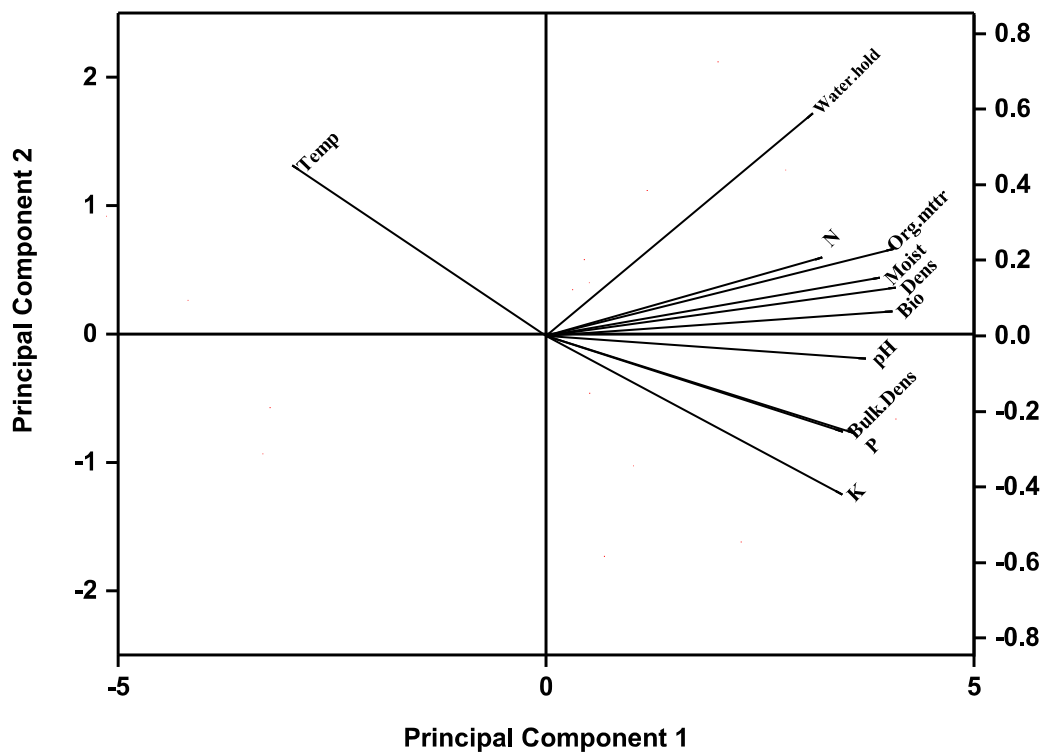


Figure 3. Biplot of Principal Component analysis, showing general multivariate relationship between earthworm density, biomass and soil physicochemical parameters.

The different PCs and their respective eigen values and proportion of variance along with cumulative variance are given in Table 4a. First axis of PCA having eigen value (7.33) accounted for 66% of total variance and had strong positive loading score of earthworm density and biomass for moisture, pH, bulk density, water holding capacity, organic matter, nitrogen, potassium, and phosphorus, whereas temperature accounted for a moderate negative loading score and the variance was 11% (Table 4b).

## DISCUSSION

A total of 11 earthworm species are found in the waste deposit sites (cow dung and municipal wastes) of West Tripura. All these species occur in the CD. While only seven species are found in the MSW. Four species namely *Kanchuria* sp., *E. gammiei*, *L. chittagongensis* and *D. assamensis* are restricted to CD. The differences in the species composition in the earthworm community of CD and MSW indicate the importance of habitat heterogeneity ( $\beta$ -diversity) in the diversity of earthworms (Fragoso and Lavelle 1987). Among the earthworm species of the waste deposit sites, most dominant species are Megascolecid earthworms, *L. mauritii* and *P. excavatus*. Earlier Chaudhuri et al. (2012) reported the occurrence of *L. mauritii*, *P. excavatus*, *M. posthuma*, *M. houlleti*, *D. bolau*, *O. beatrix*, *P. corethrurus*, *E. gammiei* in the cowdung deposit sites. Occurrence of *M. posthuma*, *P. excavatus*, *D. bolau* and *L. mauritii* with high relative abundance were also recorded by Goswami and Mondal (2015) and Rai (2017) in the sewage canal side and compost pit respectively. Earthworm species with rare occurrence viz. *O. beatrix*, *L. chittagongensis*, and *Kanchuria* sp., in the waste deposit sites were also recorded in the soils of rubber plantation (Chaudhuri et al. 2008) and tea plantation (Jamatia and Chaudhuri 2017) of Tripura.

Earthworm species in the waste deposit sites of West Tripura are represented by a minimum of 6 and maximum of 11 species which is well within the reported range of 3-17 species in the earthworm communities of the tropical and temperate ecosystem (Fragoso et al. 1999). According to Edwards and Bohlen (1996) earthworm communities are species poor in a given ecosystem. Thus in order to get true species richness in a community sufficient number of samples are to be taken. Sample based rarefaction curve seems to attain asymptote at 275 samples for both the studied habitats

(MSW, CD). This suggests that inventory was almost complete in the representative sites and species population collected from these sites is very likely to resemble almost entire earthworm fauna (Coddington et al. 1996, Valckx et al. 2011). The principal use of rarefaction curve is to evaluate the pattern of richness at a comparable level of sampling effort where data sets are in the total number of individuals. The steeper curve indicates diverse community (Colwell et al. 2012). The steeper rarefaction curve observed for CD supports the occurrence of a higher number (11 species) of earthworm species that differ significantly from MSW (7 species) as shown by non overlapping 95% confidence intervals for equal number ( $n=800$ ) of individuals (Magurran 2004). Land use patterns can exert a strong influence on the overall abundance, diversity, and community composition of soil macro fauna (Barrios et al. 2005). Sample based and individual based rarefaction curve for both the habitats clearly reveal that MSW is a less diverse habitat than CD in respect of earthworm species richness probably due to absence of canopy cover, soil physical and chemical properties and presence of heavy metal contents that perhaps made the habitat less suitable for sensitive earthworm species and favored some metal accumulating earthworms, such as, *L. mauritii* and *M. posthuma* (Koul et al. 2015). Dominance of the earthworm species differed in MSW and CD. While in MSW, *L. mauritii* was the only dominant earthworm species with high density (82.3 ind.  $m^{-2}$ ), biomass (46.2  $g\ m^{-2}$ ) and relative density (87.2%). In CD dominance was shared by two species namely, *P. excavatus* (density 126 ind.  $m^{-2}$ , biomass 58.9  $g\ m^{-2}$ , relative abundance 61.8%) and *L. mauritii* (density 76.7 ind.  $m^{-2}$ , biomass 38.2  $g\ m^{-2}$ , relative abundance 12.8%). Due to shared dominance in CD and single dominance in MSW, dominance index was significantly higher ( $p < 0.05$ ) in MSW than CD. Comparatively more anthropogenic disturbances and exposure to sunlight with less vegetation than the CD was probably the main cause of less diversity and more dominance in the MSW than in the CD. Shannon diversity index (1.16), species richness index (0.38) and evenness (0.43) in the CD are significantly higher ( $p < 0.05$ ) than those in MSW. Shannon diversity index (1.16) in CD is close to the Shannon diversity indices 1.19 and 1.33 in the agricultural fields and home gardens (Sharma and Bharadwaj 2014), but much lesser than that of mixed forest (Chaudhuri and Nath 2011) and mixed fruit plantation (Dey and Chaudhuri 2014). In the rank abundance curve, steeper slope in MSW signifies higher dominance and

shallow slope in CD indicate higher diversity and lower dominance than the former (Smith and Wilson 1996, Dey and Chaudhuri 2014). The relative abundance value 61.8% of *P. excavatus* in CD and 87.2% of *L. mauritii* in MSW confirms these facts. Dominance of *L. mauritii* in MSW and *P. excavatus* in CD indicates their survival superiority in these wastes and strong competitive edge over other earthworm species in waste deposit sites. *P. excavatus* is an epigeic species and *L. mauritii* an anecic species. Both are natural colonizer in CD as indicated by their co-dominance with much higher density (*P. excavatus* 126 ind. m<sup>-2</sup>, *L. mauritii* 76.7 ind. m<sup>-2</sup>). Because of their species association with affinities for dung, Ismail (2005) utilized both the species together in vermicomposting technology.

Density (156.31 ind. m<sup>-2</sup>) and biomass (80.4 g m<sup>-2</sup>) values of earthworm species in CD are significantly higher ( $p < 0.05$ ) than those in MSW (density 86.1 ind. m<sup>-2</sup> and biomass 52.6 g m<sup>-2</sup>). According to Shakir and Dindal (1997) earthworm population is negatively correlated with species diversity. Chaudhuri and Nath (2011) reported significantly higher earthworm diversity with low population densities in the mixed forest compared to the monoculture rubber plantations of Tripura. High population density with low earthworm diversity in pineapple and tea plantation respectively were recorded by Dey and Chaudhuri (2014) and Jamatia and Chaudhuri (2017). In contrast, our studies revealed that CD with high earthworm population densities and MSW with low earthworm population density had high and low earthworm diversity respectively. This deviation may be due to fact that presence of hazardous heavy metals in the organic wastes of MSW affected the gametogenesis, thereby the reproductive potential of earthworms (Reinecke and Reinecke 1997, Kale 1998). Electron microscopic studies of Reinecke and Reinecke (1997), revealed damage of spermatozoa of *Eisenia fetida* fed with cowdung rich heavy metals. Besides these, both MSW and CD were artificially made and fragile ecosystems with a concentration of organic wastes above the soils which form a food base for both epigeic and anecic earthworm species. Soils with neutral pH, favorable moisture (39.7%), organic matter content (8.3%) are some of other important soil physicochemical factors that favor high density and biomass of earthworms in CD in spite of its higher earthworm diversity than MSW. In a really diverse community, Shannon diversity index generally ranges from 1.5 to 3.5 (Stilling 2006). Thus lower Shannon diversity indices ranging from 0.42 (MSW) to 1.16 (CD) in the studied

sites was probably due to anthropogenic interference, unstable environmental conditions due to frequent removal of wastes and occurrence of heavy metals in the MSW. It also supports the view of Odum (1973), Dash and Dash (2009) that unstable communities and communities under severe stress exhibit low diversity. Earthworms with more food availability, gain more weight (biomass) than those with little supplementary food (Muldowney et al. 2003). Parthasarathi and Ranganathan (2000), Parthasarathi (2010) pointed out better microbial activity with increase soil moisture and favorable temperature results in significant increase in biomass in earthworms. Our present study also recorded strong correlation of earthworm population density, biomass with pH, moisture, and organic matter content (Table 3). A strong positive correlation between earthworm densities and soil organic matter contents in the present study supports Hendrix et al. (1992) who also reported a strong positive correlation between earthworm population densities and soil organic matter content across ten sites including conventional and non tillage agro ecosystems, grassy meadows and mixed deciduous forests in the South eastern USA. Joshi et al. (2010) also reported a significant positive correlation between earthworm population densities and soil organic matter. Significant positive correlation between earthworm species richness and soil moisture and organic matter from Kashmir valley was also reported by Najjar and Khan (2011). Thus there is a little doubt that the soil moisture and organic matter are important factors influencing earthworm abundance in the waste deposit sites in Tripura.

Texture, nutrient status and moisture condition of soils determine the ecological categories of the earthworms in an ecosystem (Chaudhuri et al. 2008). Ecological categories in waste deposit sites comprise three species of epigeic (*P. excavatus*, *D. bolau*, *L. chittagongensis*), six species of endogeic (*M. posthuma*, *Kanchuria* sp., *O. beatrix*, *E. gammiei*, *D. assamensis*, *P. corethrurus*) and two species of anecic (*M. houletti*, *L. mauritii*) earthworms. Frago et al. (1999) advocated that earthworm communities of tropical agroecosystem are mostly composed of endogeic species of earthworms. The dominance of endogeic species is due to oxidative burning of soil organic carbon under tropical conditions. Later Chaudhuri et al. (2008), Dey and Chaudhuri (2014), Jamatia and Chaudhuri (2017) reported dominance of endogeic earthworm species with higher biomass values in the plantation crops such as rubber, pineapple and tea respectively. In contrast, dominance of

epigeic and anecic earthworm species with highest biomass and lowest relative abundance of endogeic species in waste deposit sites under the tropical condition is due to high organic matter contents in the habitat soils. High density and biomass value of *L. mauritii* in MSW was due to its heavy metal accumulation capacities in habitat (Koul et al. 2015).

Earthworms are thin-skinned invertebrate with little protection against the changes in physical and chemical conditions of the soils (Edwards and Bohlen 1996). Soil physicochemical characteristics and climatic condition of an area determine earthworm diversity (Hackenberger and Hackenberger 2014). In the PCA two principal components together explains 78% of the total variance. PC1 explains 66.71% of the total variance exhibiting strong positive loading score for soil moisture, pH, organic matter content, bulk density, water holding capacity, nitrogen, phosphorus and potassium against the earthworm population (density, biomass) of the studied sites. In PC2 temperature accounts for moderate negative loading score indicating soil temperature not as much as an important regulatory factor for earthworm distribution in the waste deposit sites. This is because high and low soil moisture diminishes the temperature response of soil respiration (Welsch and Hornberger 2004). Significant positive correlation of the earthworm population density with soil moisture, organic matter content, phosphorus and potassium shown by Lanthanzara (2011) and Kalu et al. (2015) are in favor of our study. High phosphorus and potassium content of the soil supports high earthworm densities (Edwards 2004, Lanthanzara 2011). This supports our findings of strong positive loading score of PC1 for phosphorus, potassium and earthworm densities and biomass. Higher positive loading score for nitrogen content of the soil along with earthworm density and biomass in the PC1 suggests a strong positive correlation among the variables that supports Haokip and Singh (2017).

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