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Diversity of microflora in the gut and casts of tropical composting earthworms reared on different substrates

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Abstract: *The diversity of fungi, bacteria, yeast, actinomycetes and protozoa were analysed in the gut and casts of Eudrilus eugeniae, Lampito mauritii, Eisenia fetida and Perionyx excavatus, both qualitatively and quantitatively as influenced by different feed substrates like clay loam soil, cowdung and pressmud. While actinomycetes (Streptomyces albus, S.somaliensis, Nocardia asteroides, N.caviae and Saccharomonosporia) were not digested by any of these species of worms, protozoa (Amoeba proteus, A.terricola, Paramecium trichium, Euglena viridis, E. orientalis, Vorticella picta and Trichomonas hominis) and yeast (Candida tropicalis, C.krusei, C.albicans and Cryptococcus neoformans) were totally digested. Certain species of fungi (Saksenae vasiformis, Mucor plumbeus, Cladosporium carrionii, C. herbacium, Alternaria sp., Cunninghamella echinulata, Mycetia sterila, Syncephalostrum racemosum, Curvalaria lunata, C. geniculata and Geotrichum candidum) and bacteria (Pseudomonas aeruginosa, Bacterium antitratum, Mima polymorpha, Enterobacter aerogenes, E. cloacae, Proteus vulgaris, P. mirabilis, P.rettgeri, Escherichia coli, Staphylococus citreus, Bacillus subtilis, B.cereus, Enterococci and Micrococci) were completely digested. Certain other species were not digested fungi like Aspergillus fumigatus, A.flavus, A.ochraceous, Trichoderma koningii (except by E.eugeniae), Fusarium moniliforme (except by E. eugeniae) and Rhizopus sp., and bacteria like Klebsiella pneumoniae and Morganella morganii) and these were multiplied during the transit of the organic residues through the gut of worms. The microbial proliferation was more in the casts, due to the environment prevailing - rich in nutrient supply and large surface area available for growth and reproduction of the microbes that lead to enhanced microbial activity and humic acid contents in the casts.*

Key words: *Microflora, Earthworm gut, Wormcasts, Microbial population, Dehydrogenase, Humic acid*

Introduction

Earthworms have been scientifically studied by man right from the time of Darwin(1881) and though different aspects such as development, physiology and ecology are studied, attention has been paid to the understanding of the relationship between earthworm and microbe only in the last two decades. Soil, the major reservoir of microbes, meets the food requirement of earthworms and this has necessitated the establishment of different kinds of relationship between earthworms and microbes. They are : (1) microbes form a part of food for earthworm, (2) microbes are proliferated in the gut and vermicomposts, (3) earthworm help in the distribution of microbes and (4) together with earthworm microbes mineralise, humifies organic matter and facilitates chelation of some metal ions (Lavelle *et al.*, 1995; Parthasarathi and Ranganathan, 1999; Canellas *et al.*, 2002; Pizl and Novokova, 2003). Earthworms have the capacity to utilize soil microbes as their food (Flack and Hartenstein, 1984; Ranganathan and Parthasarathi, 1999). Growth and reproduction in earthworms require C and N and these were obtained from litter, grit and microbes (Edwards and Bohlen, 1996). Even among the microbes only few were preferentially ingested while others were rejected. Selective digestion of fungi by *Drawida calebi* (Dash *et al.,* 1984), *Lampito mauritii* and *Eudrilus ugeniae* (Parthasarathi and Ranganathan, 1998), *Lumbricus terrestris* (Wolter and Scheu, 1999), bacteria by *L. rubellus* and *Aporrectodea caliginosa* (Kristufek *et al.,* 1992), *L. mauritii* and *E. eugeniae* (Parthasarathi *et al.,* 1998), yeast by *Eisenia fetida* (Byzov *et al.,* 1995) and protozoa by *Amynthas morrisi* (Mukherjee and Julka, 1984) were reported.

Selective proliferation of microorganisms in the gut of different kinds of earthworms, due to the environment and food material available for growth of microorganisms, were reported : fungi in *P. millardi* (Ghosh *et al.,* 1989), *L. mauritii* and *E. eugeniae* (Parthasarathi and Ranganathan, 1998), *O. borincana* (Alonso *et al.,* 1999), bacteria in *A. caliginosa* (Scheu, 1987), *L. mauritii* and *E. eugeniae* (Parthasarathi *et al.,* 1998), *L. terrestris* (Wolter and Scheu, 1999) and actinomycetes in *L. terrestris*, *A. longa* and *A. caliginosa* (Parle, 1963a), *L. rubellus* and *A. caliginosa* (Kristufek *et al.,* 1993).

Higher microbial population and activity in the casts of earthworms, compared to surrounding soil, have been demonstrated by Parle (1963b), Scheu (1987), Ghosh *et al.* (1989), Edwards and Bohlen (1996) and Parthasarathi and Ranganathan (1999). Microbial biomass in the wormcasts was found to be high and their activity was essential for release of nutrients into the medium so as to be taken by the plants (James, 1991). Enhanced nutrients (N, P, K, S, Ca, Mg, Mn, Fe, Zn) in the casts of earthworm, compared to the surrounding soil, was shown to be due to mineralization taking place in the gut as well

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as in the casts (Elvira *et al.,* 1998; Parthasarathi and Ranganathan, 1999). Decomposition and humification of biodegradable organic waste materials is predominantly carried out by microorganisms in the soil but the few recent studies have shown that earthworms too have roles in humification (Edwards and Bohlen, 1996; Muscola *et al.,* 1999; Kadalli *et al.,* 2000; Manivannan *et al.,* 2004; Ranganathan and Parthasarathi, 2005). However each one of these studies was restricted to one aspect of the earthworm-microbe relationship such as selective feeding on microbes or selective digestion of microbes or proliferation of microbes in the gut and casts. In order to gain as far as possible, an insight into the holistic picture of the microbe-earthworm relationship a comprehensive study was made of the different aspects of the symbiotic relationship between the microbes found in the three different substrates such as clay loam soil, cowdung and cane sugar mill waste - pressmud and four species of earthworms differing in their mode of life - *Eudrilus eugeniae* (Eudrilidae), Lampito mauritii (Megascolicidae), *Eisenia fetida* (Lumbricidae) and *Perionyx excavatus* (Megascolicidae) and to find whether some of these microbes are commonly found in these feed and whether there is a common requirement of microbes as feed for earthworms.

Materials and Methods

Collection of organic wastes and earthworm culture: Clay loam soil (CLS, 10 cm depth) and cowdung (CD) - collected from the Agricultural Experimental Farm of Annamalai University, Annamalai Nagar and pressmud (PM) - a by-product of sugar mill, obtained from E.I.D. Parry sugar mill at Nellikuppam, Tamilnadu, were used as feed substrates for adult earthworms - *Lampito mauritii* (Kinberg) (78-80 days old), *Eudrilus eugeniae* (Kinberg) (44-48 days old), *Eisenia fetida* (Savigny) (52-55 days old) and *Perionyx excavatus* (Perrier) (45-50 days old). 20 worms of each species were maintained in separate cement containers (50 X 35 X 35 cm) containing 4 kg of feed material at 28 ± 2°C, 65% moisture, 60% RH and 12 L/12 D photoperiod. The feed was not changed during the experiment lasting for 20 days. The worms were acclimatized for 2 days in the feed substrate before the commencement of the experiment. Microbial analysis was done on vermicasts collected after 20 days of commencement of experiment.

Isolation, enumeration and identification of microflora : The population of fungi, bacteria, actinomycetes and yeasts from the substrates (CLS, CD and PM and their respective casts (C) of each species of worms) was determined by dilution plate techniques (Walksman, 1917). Each substrate of 1 gram was suspended in 1 ml sterile saline (1 g NaCl in 100 ml distilled H_2O) in a sterile test tube and was shaken thoroughly in a vortex mixer and used as inoculum for isolation and enumeration of fungi, bacteria, actinomycetes and yeast from different substrates. Using micropipette, 0.01 ml of the inoculum was inoculated into Blood agar (BA) (Anonymous, 1977), Nutrient agar (NA) (Anonymous, 1977) and MacConkey agar (MA) (Anonymous, 1977) plates and spread over each plate media by using platinum loop for bacterial growth, Sabouraud's dextrose agar (SDA) (Emmon *et al*., 1970) plates for fungal and yeast growth and actinomycetes agar (AA) (Emmon *et al.,* 1970) and SDA plates for actinomycetes growth and incubated at 30ºC and 37ºC for 18-24 hours for bacteria, 25ºC and 28ºC for 4-7 days for fungi, 30ºC and 37ºC for 10-12 days for actinomycetes and 25ºC and 37ºC for 12-14 days for yeast, respectively. The different colony forming units (CFU) developing on the media were estimated and expressed as CFU x 10^4 g^{-1} (for fungi), CFU x 10^6 g^{-1} (for bacteria) and CFU x 10^5 g^{-1} (for actinomycetes and yeast), respectively according to the method of Baron *et al.* (1994).

To identify the bacteria, actinomycetes and yeast at species level, Gram's staining and various serological and biochemical tests were carried out as described by Mahon and Manuselis (1995). In addition to these, the current toxonomic sources were used in identification procedure. To identify the fungi, light microscopic examination was carried out in the Lactophenol cotton blue stained slides of each developed colonies in the petri dish and also cultural characteristics such as colour of the fungal colony, number of days taken for the fungus to reach maximum diameter (9 cm) of the petri dish, and the texture of fungal growth were noted. The morphological and cultural features of each fungus was compared with descriptions given by Bryce (1992) and Kwon-Chung and Bennett (1992) for identification.

The gut contents (G) [(3-4 cm of gut ranging from 20-130 segments in *L. mauritii* (W), 4-5.5 cm of gut ranging from 18-185 segments in *E. eugeniae* (X), 2.5 - 3 cm of gut ranging from 17-100 segments in *E. fetida* (Y) and 3-3.5 cm of gut ranging from 18-145 segments in *P. excavatus* (Z)] of each species earthworms reared from CLS, CD and PM were dissected out using sterile scissors and the contents were transferred to 1 ml sterile saline into a sterile test tube. It was vortexed and dilution plated on NA, BA, MA, SDA and AA plates. The casts were collected after 15 days of feeding and 1g was transferred to 1ml sterile saline, shaken well and 0.01 ml taken as inoculum to spread on NA, BA, MA, SDA and AA plates. The plates were incubated and observed for fungi, bacteria, actinomycetes and yeast colonies as stated above for total counts and identification.

A wet mount was prepared from each of the substrate, gut contents and casts of each earthworm species for microscopical observation of protozoa (XCa 450). For species identification a smear was prepared and stained with Gram's stain and haematoxylin and eosin, adopted a standard manual as described by Wenyon (1926). The number of cells (protozoa) were counted using haemocytometer and by using the formula :-

$$\frac{\text{Number of cells counted} \times \text{depth factor} \times \text{dilution factor}}{\text{Area counted}}$$

The observed protozoans were expressed in number of cells/cu.mm. Data represented in the Tables (1-5) were mean of ten samples of substrates, gut contents and casts of each earthworm species.

Determination of total microbial population, microbial activity and humic acid content: The total microbial population (fungi + bacteria + actinomycetes) from the substrates [CLS, CD and PM and their respective casts (C) of each species of worms] was determined by dilution plate techniques. They total microbial colonies developing on the media were estimated by using Que-Bee colony counter and expressed as CFU x 10^6 g^{-1}. The inoculum preparation, inoculation method, using cultural media and temperature and time taken for incubation were followed as earlier mentioned procedure.

Microbial activity (in terms of estimating dehydrogenase activity) was determined from the substrates by the method of Stevenson (1959). 5g substrate were mixed with 50 mg dry $CaCO_3$ in a beaker and brought to 90% H_2O holding capacity with H_2O containing 0.5 ml 1% triphenyl tetra sodium chloride solution (TTC). The samples were incubated at 30°C for 24 hr. and then added 5ml methanol and stirred for 5 mins. The resulting slurry was washed with methanol aliquot for making enzyme extract. The density of coloured extract was determined in UV-Vis Spectrophotometer (SL 159) at 485 nm wavelength, using methanol as the reference blank. Concentrations were determined by comparison with a standard curve of triphenyl formazan (TPF) in methanol. Results were recorded in volumes of hydrogen transferred during reduction of TTC–TPF in 5g substrate according to an equation : 2, 3, 5–(TTC) + 2H→ TPF + HCl. Formation of 1 mg TPF requires 150.35 µl H. The calculated activity was expressed in µl H/5g substrates.

The humic acid content from the substrates was extracted by adopting the procedure of Schnitzer (1978). 5 g of each substrate was dissolved in 100 ml 0.5 N NaOH. The liquid was shaken well and incubated at room temperature for 24 hr. After filtration the filtrate was acidified with 6N HCl to pH 1. After 3 hrs, the coagulate was dialysed against dis. H_2O till free of chloride and finally dried in hot air oven at 40°C. The humic acid contents was expressed in mg/5g substrates.

Statistical analysis: A one way ANOVA (Analysis of variance) (XL software package, Version 5; SAS Institute Inc., 2002) was used to evaluate statistical significance of differences at 0.05% level in CFU numbers, microbial activity (dehydrogenase) and humic acid contents between substrates, gut contents and casts of different species of earthworms.

Results and Discussion

The diversity of types and number of fungi, bacteria, actinomycetes, yeast and protozoa isolated from the gut and casts of *L. mauritii, E. eugeniae, E.fetida* and *P.excavatus* as influenced by different feed substrates like CLS, CD and PM are tabulated in Tables 1-4. Of the three substrates, it was observed, that PM harbours the maximum variety and number of fungi (11 species), bacteria (13 species), actinomycetes (5 species), yeast (4 species) and protozoa (4 species), followed by CD (9 species fungi, 8 species bacteria, 2 species actinomycetes and 4 species protozoa). The least diversity of microflora was found in CLS(5 species fungi, 7 species bacteria, 2 species actinomycetes and 3 species protozoa). The following microorganisms were unique to the substrates shown against their name: *S. vasiformis* - CLS; *A. ochraceous, C. carrionii, Alternaria* sp., *C.echinulata*, *M.sterila*, *P. vulgaris, Enterococci*, *B.cereus* and *A. terricola* - CD and *T. koningii, S. racemosum, F. moniliforme, C. lunata, C. geniculata*, *G. candidum*, *M. polymorpha, S. citreus*, *Micrococci*, *N.caviae*, *Saccharomonosporia*, *V. picta, E.orientalis* and *T. hominis* - PM.

Earthworms, for their growth and reproduction, have been shown to meet their nutritional requirement by feeding on organic matter and microbes(Parthasarathi and Ranganathan, 2000a). Microorganisms constitute an important nutritional component of the earthworm diet (Edwards and Bohlen, 1996). Earthworms have been shown to be microbivorous (Flack and Hartenstein, 1984; Ranganathan and Parthasarathi, 1999). Though ingestion of microorganisms by earthworms is reported by a few authors, there is no agreed opinion as to what type of microorganism is eaten and digested by earthworms. Earthworm was shown to have minimal capacity to digest organic residues and obtain nutrition by digestion of microorganisms associated with ingested organic matter (Lavelle, *et al.,* 1995). Edwards and Fletcher (1988) concluded that fungi and protozoa were the major sources of nutrients for earthworms and that bacteria were of minor nutritional importance and soil algae were of moderate importance. On the contrary yeast are considered the most probable sources of nutrients and vitamins for earthworms (Byzov *et al.,* 1995). The earthworms were found to predate on a variety of fungi such as *Nigrospora sphaerica, Helminthosporium* sp., *Neocusmospora vasiinfecta, Chaetomium* sp., *Curvularia* sp., *Rhizopus nigrican, Mucor hiemalis, Fusarium oxysporum, Alternaria solani, Trichoderma viridi, Cunninghamella echinulate, Blastomyces* sp., *Botryotrichus* sp. and *Chaetomium glabrum* (Cooke and Luxton, 1980; Ghosh *et al.,* 1989), bacteria such as *Bacillus cereus, mycoides, Serratia marcescens, E. coli* and *Enterobacter cloacae* (Brusewitz, 1959; Pedersen and Hendriksen, 1993), yeast *Candida famata* (Byzov *et al.,* 1995) and protozoa - *Lesquereusia spiralis* (Mukherjee and Julka, 1984). Selective preference of microbes as their food by the earthworm has been studied by Ghosh *et al.* (1989), Edwards and Bohlen (1996) and Parthasarathi and Ranganathan (1998). More or less similar to these observations the present study showed that

microorganisms found in the different feed substrates before ingestion by the earthworms were not found in the casts (fungi-*S. vasiformis, M. plumbeus, C. carrionii, C.herbacium, Alternaria* sp., *C. echinulata, M. sterila, S. racemosum, C.lunata, C. geniculata and G.candidum;* bacteria - *P. aeruginosa, B.antitratum, E. aerogenes, E. cloacae, P. vulgaris, E. coli, Enterococci, B.subtilis, B. cereus, M. polymorpha, P.mirabilis P. rettgeri, S. citreus and Micrococci;* yeast - *C.tropicalis, C. krusei, C.albicans and C. neoformans and* protozoa - *A. proteus, P.trichium, E. viridis, A. terricola, E. orientalis, V. picta and T. hominis*) (Tables 1-3). The absence of these microbes in the gut of the earthworms and consequently in the casts, might be due to the digestive activity of the earthworms. The following fungi and bacteria were absent in all the four species of earthworms reared on three different substrates: *S.vasiformis, M. Plumbeus, P. aeruginosa, B. antitratum, E. aerogenes, E.cloacae* and *B. subtilis* in CLS, *C. carrionii, Alternaria* sp., *M. sterila, C.echinulate, E. aerogenes, P. vulgaris, E. coli, Enterococci, B. subtilis* and *B.cereus* in CD and *C. herbacium, S. racemosum, M. plumbeus, C. lunata, C. geniculata, G. candidum, P. aeruginosa, B. antitratum, M. polymorpha, E.aerogenes, E. cloacae, P. mirabilis, P. rettgeri, E. coli, S. citreus, Micrococci, B. subtilis* and *B. cereus* in PM (Table 4). The entire protozoan population in all the substrates and yeast population in PM were absent (Table 1-3).

The variation in the microbial populations in the earthworm gut may be because of their nutritional needs and digesting ability of the earthworms. While *E. eugeniae* is able to digest *T. koningii* and *F.moniliforme,* the others three species of earthworm are unable to digest these fungi. The population of *E.aerogenes, E. cloacae* and *E. coli* decreases in number in the gut and finally disappear in the vermicasts indicating the differential digestability of the microbes (Tables 1-4). Pedersen and Hendriksen (1993), reported qualitative and quantitative changes in the bacterial flora of ingested food materials during gut transit. Populations of *S. marcescens, E. coli, Salmonella enteritidis* and *B.cereus* var *mycoides* in *L. terrestris* (Thorpe *et*

Table - 1: Isolation and estimation of microbes in the gut and casts of earthworms reared in clay loam soil

Microbes	CLS (g^{-1})	Gut				Casts (g^{-1})			
		L.mauritii 3-4 cm^{-1}	*E.eugeniae* 4-5.5 cm^{-1}	*E.fetida* 2.5-3 cm^{-1}	*P.excavatus* 3 - 3.5 cm^{-1}	*L.mauritii*	*E.eugeniae*	*E.fetida*	*P.excavatus*
Fungi (CFU x $10^4 g^{-1}$)									
Aspergillus fumigatus	6	38	31	28	30	68	91	36	35
Aspergillus flavus	2	30	26	21	18	40	61	33	21
Saksenae vasiformis	7	-	-	-	-	-	-	-	-
Mucor plumbeus	6	-	-	-	-	-	-	-	-
Rhizopus sp.	7	18	100	29	11	100	100	29	78
Total	28	86	157	78	59	208	252	168	134
Bacteria (CFU x $10^6 g^{-1}$)									
G –ve									
Klebsiella pneumoniae	6	157	182	121	98	62	186	51	46
Pseudomonas aeruginosa	9	-	-	-	-	-	-	-	-
Bacterium antitratum	2	-	-	-	-	-	-	-	-
Enterobacter aerogenes	75	48	28	21	17	-	-	-	-
Enterobacter cloacae	26	13	9	9	6	-	-	-	-
Morganella morganii	17	112	119	89	76	290	980	186	177
G+ve									
Bacillus subtilis	5	-	-	-	-	-	-	-	-
Total	140	330	338	240	197	352	1168	237	223
Actinomycetes (CFU x $10^5 g^{-1}$)									
Streptomyces albus	3	9	15	8	6	6	10	4	5
Streptomyces somaliensis	2	8	11	7	7	5	8	6	4
Total	5	17	26	15	13	11	18	10	9
Protozoa (cells/cumm.)									
Amoeba proteus	3	-	-	-	-	-	-	-	-
Paramecium trichium	5	-	-	-	-	-	-	-	-
Euglena viridis	3	-	-	-	-	-	-	-	-
Total	11								

'-' denotes absence, G-ve = Gram negative, G+ve = Gram positive, CLS - Clay loam soil

Table - 2: Isolation and estimation of microbes in the gut and casts of earthworms reared in cowdung

Microbes	CD (g^{-1})	Gut				Casts (g^{-1})			
		L.mauritii 3-4 cm^{-1}	*E.eugeniae* 4-5.5 cm^{-1}	*E.fetida* 2.5-3 cm^{-1}	*P.excavatus* 3-3.5 cm^{-1}	*L.mauritii*	*E.eugeniae*	*E.fetida*	*P.excavatus*
Fungi (CFU x $10^4 g^{-1}$)									
Aspergillus fumigatus	10	21	33	18	17	78	63	66	55
Aspergillus flavus	11	26	41	23	22	55	68	46	44
Aspergillus ochraceous	8	36	56	31	28	30	55	33	28
Cladosporium carrionii	12	-	-	-	-	-	-	-	-
Cladosporium herbacium	11	-	-	-	-	-	-	-	-
Alternaria sp.	9	-	-	-	-	-	-	-	-
Cunninghamella echinulata	13	-	-	-	-	-	-	-	-
Rhizopus sp.	8	48	69	39	36	85	100	77	69
Mycelia sterila	6	-	-	-	-	-	-	-	-
Total	88	131	199	111	103	248	306	202	196
Bacteria (CFU x $10^6 g^{-1}$)									
G-ve									
Klebsiella pneumoniae	42	268	293	257	246	117	220	167	98
Enterobacter aerogenes	33	30	26	19	33	-	-	-	-
Morganella morganii	53	579	610	483	417	416	1016	365	319
Proteus vulgaris	26	-	-	-	-	-	-	-	-
Escherichia coli	31	29	21	18	26	-	-	-	-
G+ve									
Enterococci	13	-	-	-	-	-	-	-	-
Bacillus subtilis	16	-	-	-	-	-	-	-	-
Bacillus cereus	20	-	-	-	-	-	-	-	-
Total	234	846	950	777	622	533	1236	472	417
Actinomycetes (CFU x $10^5 g^{-1}$)									
Streptomyces albus	8	23	32	20	18	20	32	19	16
Nocardia asteroides	10	27	40	26	24	24	26	21	22
Total	18	50	72	46	42	44	58	40	38
Protozoa (cells / cumm)									
Amoeba proteus	3	-	-	-	-	-	-	-	-
Amoeba terricola	2	-	-	-	-	-	-	-	-
Euglena viridis	4	-	-	-	-	-	-	-	-
Paramecium trichium	6	-	-	-	-	-	-	-	-
Total	15								

'-' denotes absence, G-ve Gram negative, G+ve - Gram positive, CD - Cowdung

al., 1993) have been observed to be decreased during passage through gut.

Indigestion of fungi such as *Aspergillus* sp., *Fusarium* sp., *Gliocladium* sp., *Paecilomyces* sp., *Penicillium* sp., *Rhizopus* sp. and *Trichoderma* sp. in the gut of *O.borincaca* (Alonso *et al.,* 1999) and bacteria such as *K. oxytoca, E. cloacae, S. liquefaciens* and *Aeromonas hydrophilla* in the gut of *Pheretima* sp. (Toyota and Kimura, 1994) have been reported. Various reasons have been attributed to the indigestion of the microbes by the earthworms such as: (a) production of antibiotic or inhibitory substance by *Aspergillus* sp. and *Penicillium* sp. (Dash *et al.,* 1986) (b) presence of strong outer coat apparently protecting them from digestion (Aichberger, 1914) and (c) production of phytotoxic metabolite by *Fusarium* sp. (Ghosh *et al.,* 1989). In the present study, in the gut as well as in the casts of all the four species of worms, fungi such as *A. fumigatus, A. flavus, A. ochraceous, F.moniliforme* (not in *E.eugeniae*), *T. koningii* (not in *E. eugeniae*) and *Rhizopus* sp. and bacteria such as *K. pneumoniae* and *M.morganii* and actinomycetes such as *S.albus, S. somaliensis, N. asteroides, N. caviae* and *Saccharomonosporia* were found (Tables 1-4) indicating that these species were not digested, may be due to the production of antibiotic and/or inhibitory substance and/or presence of strong outer coat and/or production of phytotoxic

Table - 3: Isolation and estimation of microbes in the gut and casts of earthworms reared in pressmud

Microbes	PM (g^{-1})	Gut				Casts (g^{-1})			
		L.mauritii 3-4 cm^{-1}	*E.eugeniae* 4-5.5 cm^{-1}	*E.fetida* 2.5-3 cm^{-1}	*P.excavatus* 3-3.5 cm^{-1}	*L.mauritii*	*E.eugeniae*	*E.fetida*	*P.excavatus*
Fungi (CFU x $10^4 g^{-1}$)									
Aspergillus fumigatus	30	35	96	48	26	104	146	118	88
Aspergillus flavus	26	19	92	36	17	64	102	79	71
Trichoderma koningii	8	30	-	18	26	21	-	18	13
Cladosporium herbacium	18	-	-	-	-	-	-	-	-
Syncephalostrum racemosum	12	-	-	-	-	-	-	-	-
Fusarium moniliforme	13	11	-	13	16	34	-	15	10
Mucor plumbeus	5	-	-	-	-	-	-	-	-
Rhizopus sp.	5	100	98	69	62	78	100	69	38
Curvularia lunata	4	-	-	-	-				
Curvularia geniculata	4	-	-	-	-	-	-	-	-
Geotrichum candidum	6	-	-	-	-	-	-	-	-
Total	131	195	286	184	147	301	348	299	220
Bacteria (CFU x $10^6 g^{-1}$)									
G-ve									
Klebsiella pneumoniae	37	397	398	334	276	142	241	127	106
Pseudomonas aeruginosa	61	-	-	-	-	-	-	-	-
Bacterium antitratum	40	-	-	-	-	-	-	-	-
Mima polymorpha	13	-	-	-	-	-	-	-	-
Enterobacter aerogenes	29	23	17	9	8	-	-	-	-
Enterobacter cloacae	65	51	32	18	13	-	-	-	-
Morganella morganii	23	644	711	518	437	679	1515	518	419
Proteus mirabilis	32	-	-	-	-	-	-	-	-
Proteus rettgeri	24	-	-	-	-	-	-	-	-
Escherichia coli	16	11	9	6	7	-	-	-	-
G+ve									
Staphylococcus citreus	3	-	-	-	-	-	-	-	-
Micrococci	8	-	-	-	-	-	-	-	-
Bacillus subtilis	18	-	-	-	-	-	-	-	-
Total	369	1095	1167	888	741	821	1756	645	525
Actinomycetes (CFU x $10^5 g^{-1}$)									
Streptomyces albus	5	13	18	11	10	8	12	7	5
Streptomyces somaliensis	6	13	18	8	7	8	10	7	6
Nocardia asteroides	5	11	16	10	9	10	14	9	6
Nocardia caviae	7	21	26	19	17	15	20	14	12
Thermophilic actinomycetes like *Saccharomonosporia*	6	18	21	14	13	11	17	10	9
Total	29	76	99	62	56	52	73	47	40
Yeast (CFU x $10^5 g^{-1}$)									
Candida tropicalis	3	-	-	-	-	-	-	-	-
Candida krusei	6	-	-	-	-	-	-	-	-
Candida albicans	4	-	-	-	-	-	-	-	-
Cryptococcus neoformans	8	-	-	-	-	-	-	-	-
Total	21	-	-	-	-	-	-	-	-
Protozoa (cells / cumm)									
Amoeba proteus	8	-	-	-	-	-	-	-	-
Vorticella picta	7	-	-	-	-	-	-	-	-
Euglena orientalis	3	-	-	-	-	-	-	-	-
Trichomonas hominis	5	-	-	-	-	-	-	-	-
Total	23	-	-	-	-	-	-	-	-

'-' denotes absence, G-ve Gram negative, G+ve - Gram positive, PM – Pressmud

Table - 4: Common digestion of fungi and bacteria by four species of earthworm reared on different substrates

Microbes	Gut*					Casts				Gut*					Casts				Gut*					Cast			
	CLS (g^{-1})	*L. mauritii*	*E. eugeniae*	*E.fetida*	*P. excavatus*	*L. mauritii*	*E. eugeniae*	*E.fetida*	*P. excavatus*	CD (g^{-1})	*L. mauritii*	*E. eugeniae*	*E.fetida*	*P. excavatus*	*L. mauritii*	*E. eugeniae*	*E.fetida*	*P. excavatus*	PM(g^{-1})	*L. mauritii*	*E. eugeniae*	*E.fetida*	*P. excavatus*	*L. mauritii*	*E. eugeniae*	*E.fetida*	*P. excavatus*
Fungi																											
Aspergillus fumigatus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Aspergillus flavus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Aspergillus ochraceous	-	-	-	-	-	-	-	-	-	+	+	+	+	+	+	+	+	+	-	-	-	-	-	-	-	-	-
Saksenae vasiformis	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Trichoderma koningii	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	-	+	+	+	-	+	+
Cladosporium carrionii	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Cladosporium herbacium	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-
Alternaria sp.	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Syncephalostrum racemosum	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-
Fusarium moniliformis	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	-	+	+	+	-	+	+
Mucor plumbeus	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-
Rhizopus sp.	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Mycelia sterila	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Cunninghamella echinulata	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Curvularia lunata	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-
Curvularia geniculata	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-
Geotrichum candidum	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-
Bacteria	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-
(G – ve)																											
Klebsiella pneumoniae	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pseudomonas aeruginosa	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-
Bacterium antitratum	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-
Mima polymorpha	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-
Enterobacter aerogenes	+	+	+	+	+	-	-	-	-	+	+	+	+	+	-	-	-	-	+	+	+	+	+	-	-	-	-
Enterobacter cloacae	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+	+	+	-	-	-	-
Morganella morganii	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Proteus mirabilis	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-
Proteus rettgeri	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-
Proteus vulgaris	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Escherichia coli (G + ve)	-	-	-	-	-	-	-	-	-	+	+	+	+	+	-	-	-	-	+	+	+	+	+	-	-	-	-
Staphylococcus citreus	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-
Micrococci	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-
Enterococci	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Bacillus subtilis	+	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-
Bacillus cereus	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

'+' isolated, '-' not isolated, G + ve - Gram positive, G - ve - Gram negative, *CLS - Clay loam soil, CD - Cowdung, PM - Pressmud, * - L. mauritii* (3-4 cm^{-1}), *E. eugeniae* (4-5.5 cm^{-1}), *E. fetida* (2.5-3cm^{-1}), *P. excavatus* (3-3.5 cm^{-1}).

Table - 5: Total microbial population and its activity* and humic acid contents** in CLS, CD and PM casts ($p<0.05$)

Substrates	Fungi (CFU x 10^4 g^{-1})	Bacteria (CFU x $10^6 g^{-1}$)	Actinomycetes (CFU x 10^5 g^{-1})	Total microbial population (CFU x 10^6 g^{-1})	Microbial activity (dehydrogenase*)	Humic acid contents**
CLS (control)	28	140	5	173	3.16	0.198
CLS casts						
L. mauritii	208(+86.5)	352(+60.2)	11(+54.5)	571(+69.7)	7.75(+59.2)	0.421(+52.9)
E. eugeniae	252(+88.8)	1168(+80.0)	18(+72.2)	1438(+87.9)	9.90(+68.0)	0.507(+60.8)
E. fetida	168(+83.3)	237(+40.9)	10(+50.0)	415(+58.3)	6.58(+51.9)	0.307(+35.5)
P. excavatus	134(+79.1)	223(+37.2)	9(+44.4)	366(+52.7)	6.70(+52.8)	0.291(+31.0)
CD (control)	88	234	18	340	4.35	0.312
CD casts						
L. mauritii	248(+64.5)	533(+56.1)	44(+59.1)	825(+58.7)	16.23(+73.2)	0.708(+55.9)
E. eugeniae	306(+71.2)	1236(+81.0)	58(+68.9)	1600(+78.5)	23.49(+81.5)	0.738(+57.7)
E. fetida	202(+56.4)	472(+50.4)	40(+55.0)	714(+52.3)	12.33(+64.7)	0.512(+39.1)
P. excavatus	196(+55.1)	417(+43.8)	38(+52.6)	651(+47.7)	13.61(+68.0)	0.537(+41.9)
PM (control)	131	369	29	529	8.14	0.392
PM casts						
L. mauritii	301(+56.4)	821(+55.0)	52(+44.2)	1174(+54.9)	29.15(+72.1)	0.813(+51.8)
E. eugeniae	348(+62.3)	1756(+78.9)	73(+60.2)	2177(+75.7)	34.89(+76.0)	0.876(+55.2)
E. fetida	299(+56.1)	645(+42.7)	47(+38.3)	991(+46.6)	19.28(+57.8)	0.592(+33.8)
P. excavatus	220(+40.4)	525(+29.7)	40(+27.5)	785(+32.6)	20.11(+59.5)	0.612(+35.9)
F-value	5.656341	3.79408	0.956644	10.4634	1.735285	2.865596
P-value	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05

* µl H/5g substrates CLS – Clay loam soil; CD – Cowdung; PM - Pressmud
** mg/5g substrates
(+) Indicates percentage increase over control.

metabolites, as reported in earlier studies (Aichberger, 1914; Dash *et al*., 1984; Ghosh *et al*., 1989; Toyota and Kimura, 1994; Alonso *et al*., 1999 and Pizl and Novokova, 2003).

A number of researchers have observed increased proliferation of a variety of microorganisms in the gut of earthworms: fungi in *P. millardi* (Ghosh *et al.,* 1989)*, L. mauritii* and *E. eugeniae* (Parthasarathi and Ranganathan, 1998), *O.borincana* (Alonso *et al.,* 1999), bacteria in *A. caliginosa* (Scheu, 1987), *L.terrestris* (Wolter and Scheu 1999; Pedersen and Hendriksen, 1993), *L. mauritii* and *E. eugeniae* (Parthasarathi *et al.,* 1998) and actinomycetes in *L. terrestris, A. longa* and *A. caliginosa* (Parle, 1963a), *L. rubellus* and *A. caliginosa* (Kristufek *et al.,* 1993). Similar to these findings in the present study also it was observed that there is significant ($p<0.05$) multiplication of microorganisms in the gut of *L.mauritii, E. eugeniae, E. fetida* and *P. excavatus* reared on different substrates- fungi – 53-82% in CLS, 15-56% in CD and 11-54% in PM; bacteria – 29-59% in CLS, 62-75% in CD and 50-68% in PM and actinomycetes – 62-81% in CLS, 57-75% in CD and 48-71% in PM (Tables 1-3) (Fig. 1. a-c). Increase in microbial populations might be due to the environmental conditions prevailing and nutritional status in the gut of earthworm as reported earlier by Senapati and Dash (1984); Dash *et al.* (1986); Tiwari *et al.* (1989); Thorpe *et al.* (1993); Edwards and Bohlen (1996) and Parthasarathi and Ranganathan (1999).

A significant increase of microbial populations ($p<0.05$) in the casts of different species of earthworms reared on different substrates was observed in the present study (Tables 1-3) (Fig. 1. a-d): fungi – 79-89% in CLS, 55-71% in CD and 40-62% in PM; bacteria – 37-80% in CLS, 43-81% in CD and 30-79% in PM and actinomycetes – 44-72% in CLS, 52-69% in CD and 28-60% in PM: particularly fungi - *A. fumigatus, A. flavus, A.ochraceous, Rhizopus* sp. *T. koningii* (except in *E. eugeniae) and F. moniliforme* (except in *E. eugeniae),* bacteria - *K. pneumoniae* and *M.morganii* and actinomycetes - *S.albus, S. somaliensis, N. asteroides, N.caviae* and *saccharomonosporia* were proliferated more. Similarly increased population of fungi like *A. flavus, A. niger, A. fumigatus, A.terrestris, Fusarium* sp., *Trichoderma* sp and *Penicillium* sp. (Dash *et al.,* 1986; Ghosh *et al.,* 1989) and bacteria like *B. idozus* and *B.cereus* (Kozlovskaya and Zhdannikova, 1961) were reported in the casts. Higher microbial population in the wormcasts of different earthworm species compared with underlying soil have been observed: fungi in *O. surensis, L.mauritii* and *D. willsi* (Dash *et al.,* 1986), *P. millardi* (Ghosh *et al.,* 1989), *L.mauritii* and *E.*

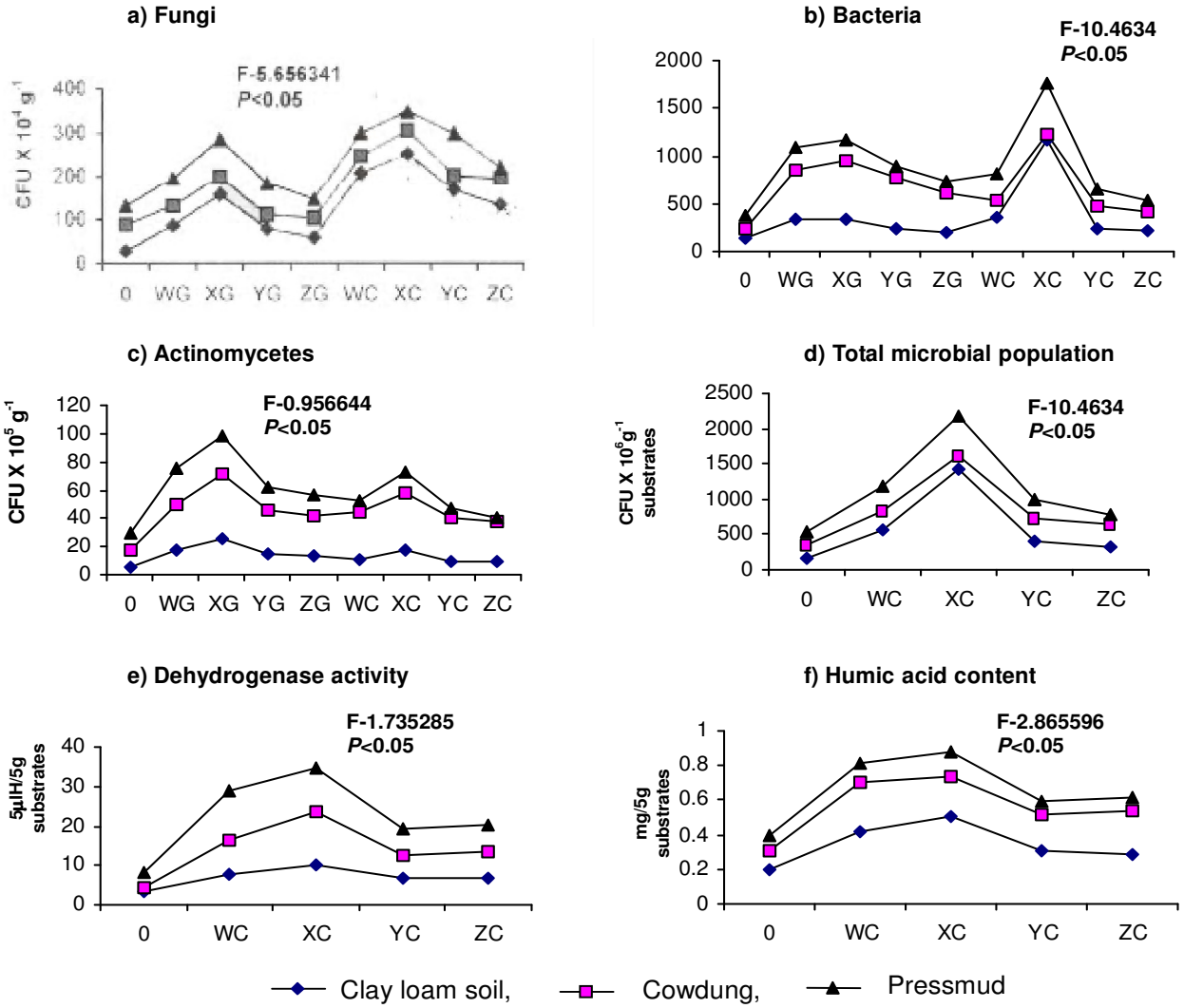

Fig. 1(a-f): Total microbial population in worm's gut and their activity and humic acid content in the different substrate vermicasts

O	-	Worm unworked initial substrates	WC	-	*L. mauritii* casts
WG	-	*L. mauritii* gut	XC	-	*E.eugeniae* casts
XG	-	*E.eugeniae* gut	YC	-	*E. fetida* casts
YG	-	*E. fetida* gut	ZC	-	*P. excavatus* casts
ZG	-	*P. excavatus* gut			

eugeniae (Parthasarathi and Ranganathan, 1998); bacteria in *A. alexundri, D. anssmensis, M. autrophyes, M. houlleti* and *N.strigosus* (Tiwari *et al.,* 1989), *L. mauritii* and *E. eugeniae* (Parthasarathi *et al.,* 1998) and actinomycetes in *L. terrestris, A. caliginosa* and *A. longa* (Parle, 1963b). Actinomycetes population, irrespective of their diversity, is not digested and they are proliferated in the gut and in the casts of all the four species (Tables 1-3). Such enhancement of microbial population in the casts was due to: (1) rich nutrient concentration, (2) multiplication of microbes while passing through the gut of worms, (3) optimal moisture and (4) large surface area of casts ideally suited for better feeding and multiplication of microbes (Parthasarathi and Ranganathan, 1999).

Wormcasts have been shown to exhibit more enzymatic and microbial activities and NPK enrichment (Tiwari *et al.,* 1989; Edwards and Bohlen, 1996; Elvira *et al.,* 1998; Parthasarathi and Ranganathan, 1999, 2000). Loquet *et al.* (1977) demonstrated an increase in cellulolytic, hemicellulolytic, amylolytic and nitrifying bacteria in wormcasts compared to the surrounding soil. Earlier studies of Parthasarathi and Ranganathan (1999, 2000) and Vinotha *et al.* (2000) have shown enhanced microbial and enzymatic activities and NPK contents in the pressmud vermicasts. In the present study, more amylolytic (*Aspergillus* sp., *Fusarium* sp., *Mucor* sp., *Rhizopus* sp. and *Bacillus* sp.), cellulolytic (*Aspergillus* sp., *Fusarium* sp., *Bacillus* sp. and *Pseudomonas* sp.), proteolytic (*Aspergillus* sp. and

Bacillus sp.), phosphate solubilizing (*Micrococci, Pseudomonas* sp., *Bacillus* sp., *Aspergillus* sp. and *Fusarium* sp.) and nitrifying (*Cladosporium sp., Alternaria sp., Aspergillus flavus, Klebsiella sp., Pseudomonas sp., Enterobacter sp. and Bacillus sp.*) microbes were found significantly in the gut and casts of all the four species of earthworms reared on CLS, CD and PM. It becomes evident from the present study that the enhanced enzymatic activities in the casts was due to these microbial activity.

The role of microbes and earthworms in decomposition of organic matter and particularly, in humification is well known (Edwards and Bohlen, 1996; Cai *et al*., 2002). Humification has been shown to be, predominantly, a microbial process (Stevenson, 1994; Filip *et al.,* 1999; Rovira *et al*., 2002) and recently earthworms, *Lampito mauritii, Eudrilus eugeniae, Eisenia fetida* and *Perionyx excavatus* have been shown to aid in humification (Manivannan *et al*., 2004). In the present study, there is a direct correlation between the microbial population and activity and humic acid content in the earthworm casts: with the increase in microbial population there is an increase of microbial activity and humic acid content (Fig. 1. d-f) (Table 5) ($p<0.05$). The actinomycetes population from all the feed substrates were found to have enhanced in the gut and cast of all the four species of earthworm indicating their role in humification since it is known that they are responsible in humus/humic acid formation (Stevenson, 1994; Edwards and Bohlen, 1996; Aswathanarayana, 1999). In addition to the established role of enhancing the nutrients in the soil by mineralisation through the enzymes secreted by the microbes and earthworms (Parthasarathi and Ranganathan, 1999; 2000; Vinotha *et al*., 2000), the increase in humic acid in the vermicasts, sequesters elements like Zn, Mn and Fe from their complex forms and chelate them (Ranganathan, unpublished observation), making them available for uptake by the plants (Parthasarathi and Ranganathan, 2002). Since the humic acid with their diverse functional groups are known to be very reactive with metal ions (Aswathanarayana, 1999). Thus the role of microbes-earthworms throws light on the flux of nutrients, particularly trace elements, between microbes → earthworms → plants.

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