

## Microbial Load and Diversity in Vermicompost Process under Varied Environmental Conditions

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### Abstract

Composting earthworms, indigenous *Perionyx excavatus* and exotic *Eisenia foetida* and *Eudrilus eugeniae* were reared in two substrates, water hyacinth-cowdung mixture (Ex-I) (1 : 1) and banana residues-cowdung mixture (Ex-II) (1 : 1). The vermicomposting of mixtures were carried out for 45 days. The total bacterial and total fungal population increased throughout the experiments and especially in casts of two exotic species. Two types of fungi, *Drechslera* sp. and *Aspergillus niger* were found in the casts formed by three species.

**Key words :** Vermicomposting, Cowdung, Casts, Bacteria, Fungi.

With increasing human population and its demands there is increase in production of various types of wastes, ranging from agricultural, domestic, municipal and industrial wastes. All these are causing various types of pollution problems, which cause health hazards and put tremendous stress on our economic resources. Thus there is urgent need to maintain environmental and agricultural sustainability without reducing productivity. At this point of view, earthworms provide various levels of solutions (1). Earthworms breakdown the dead plant and animal materials in soil and forest litter, and in the maintenance of soil structure, aeration and fertility (2). Earthworms are potentially important vectors of microbial propagules since they live in the upper part of the soil profile and transport a large amount of soil (200—400 t/ha per year) through their bodies (3). Waste materials harbor microorganisms and during the process of decomposition there occurs qualitative and quantitative changes in the organic wastes (4, 5). The gut of the earthworm contains beneficial symbiotic microorganisms (6). Increased number of microorganisms in casts of earthworm has been reported by many authors (3). During the process of vermicomposting earthworms maintain aerobic conditions in the organic wastes through proper mixing and the biochemical process is enhanced by microbial decomposition of the substrate in the intestines and the earthworms convert a portion of the organic

present in the wastes into worm biomass and excrete undigested or partially digested matter as worm cast. Further, earthworms also enhance soil microbial activity by improving the environment for microbes. In order to evaluate the efficacy of the casts formed by the three different species of worms viz. indigenous *Perionyx excavatus* and exotic *Eisenia foetida* and *Eudrilus eugeniae* when they were fed with different media like, water hyacinth-cowdung mixture and banana residues-cowdung mixture, a quantitative analysis of bacteria and fungi and identification of fungi were made.

### Methods

Substrates water hyacinth (5 kg) and banana residues (5 kg) were mixed with cowdung (5 kg) separately in (1 : 1)<sup>1</sup> and used as feeding substrates for earthworms, *Eisenia foetida*, *Eudrilus eugeniae* and *Perionyx excavatus*. The three earthworm species were collected from the vermigarden of Paribesh Unnayan Parishad, Kolkata ; 1.3 kg of the substrates were weighed corresponding to each earthworm species after primary decomposition, moistened and allowed in six bamboo bucket. Forty earthworms of *Eisenia foetida* and *Eudrilus eugeniae* and 30 *Perionyx excavatus* were introduced into separate sets of buckets. Control was also maintained without earthworm addition in each of two experi-

**Table 1.** Changes of bacterial population in CFU / g dry compost during composting in Ex-I.

Experiment type	Dilution factor	No. of colonies per plate	SPC/ml = Colonies counted/dilution factor	Moisture content (%)	CFU/g dry compost
Ex-I/0	10 <sup>-2</sup>	5	5×10 <sup>2</sup>	50	10×10 <sup>2</sup>
Ex-I/C (15)	10 <sup>-2</sup>	6	6×10 <sup>2</sup>	40	10×10 <sup>2</sup>
Ex-I/P (15)	10 <sup>-2</sup>	11	11×10 <sup>2</sup>	40	18.3×10 <sup>2</sup>
Ex-I/Eu (15)	10 <sup>-2</sup>	10	10×10 <sup>2</sup>	40	16.7×10 <sup>2</sup>
Ex-I/E (15)	10 <sup>-2</sup>	15	15×10 <sup>2</sup>	40	25×10 <sup>2</sup>
Ex-I/C (30)	10 <sup>-2</sup>	12	12×10 <sup>2</sup>	30	17.1×10 <sup>2</sup>
Ex-I/P (30)	10 <sup>-2</sup>	25	25×10 <sup>2</sup>	30	35.7×10 <sup>2</sup>
Ex-I/Eu (30)	10 <sup>-2</sup>	32	32×10 <sup>2</sup>	30	45.7×10 <sup>2</sup>
Ex-I/E (30)	10 <sup>-2</sup>	35	35×10 <sup>2</sup>	30	50×10 <sup>2</sup>
Ex-I/C (45)	10 <sup>-2</sup>	17	17×10 <sup>2</sup>	20	21.3×10 <sup>2</sup>
Ex-I/P (45)	10 <sup>-2</sup>	34	34×10 <sup>2</sup>	20	42.5×10 <sup>2</sup>
Ex-I/Eu (45)	10 <sup>-2</sup>	56	56×10 <sup>2</sup>	20	70×10 <sup>2</sup>
Ex-I/E (45)	10 <sup>-2</sup>	55	55×10 <sup>2</sup>	20	68.8×10 <sup>2</sup>

ments. Water sprinkling was carried out on alternate days to maintain the optimal moisture (40—50%) (7) for the growth of worms. The experimental buckets

**Table 2.** Changes of bacterial population in CFU/g dry compost during composting in Ex-II.

Experiment type	Dilution factor	No. of colonies per plate	SPC/ml = Colonies counted/dilution factor	Moisture content (%)	CFU/g dry compost
Ex-II/0	10 <sup>-2</sup>	6	6×10 <sup>2</sup>	50	12×10 <sup>2</sup>
Ex-II/C (15)	10 <sup>-2</sup>	8	8×10 <sup>2</sup>	40	13.3×10 <sup>2</sup>
Ex-II/P (15)	10 <sup>-2</sup>	9	9×10 <sup>2</sup>	40	15×10 <sup>2</sup>
Ex-II/Eu (15)	10 <sup>-2</sup>	12	12×10 <sup>2</sup>	40	20×10 <sup>2</sup>
Ex-II/E (15)	10 <sup>-2</sup>	14	14×10 <sup>2</sup>	40	23.3×10 <sup>2</sup>
Ex-II/C (30)	10 <sup>-2</sup>	11	11×10 <sup>2</sup>	30	15.7×10 <sup>2</sup>
Ex-II/P (30)	10 <sup>-2</sup>	23	23×10 <sup>2</sup>	30	32.9×10 <sup>2</sup>
Ex-II/Eu (30)	10 <sup>-2</sup>	36	36×10 <sup>2</sup>	30	51.4×10 <sup>2</sup>
Ex-II/E (30)	10 <sup>-2</sup>	30	30×10 <sup>2</sup>	30	42.9×10 <sup>2</sup>
Ex-II/C (45)	10 <sup>-2</sup>	15	15×10 <sup>2</sup>	20	18.8×10 <sup>2</sup>
Ex-II/P (45)	10 <sup>-2</sup>	35	35×10 <sup>2</sup>	20	43.8×10 <sup>2</sup>
Ex-II/Eu (45)	10 <sup>-2</sup>	55	55×10 <sup>2</sup>	20	68.8×10 <sup>2</sup>
Ex-II/E (45)	10 <sup>-2</sup>	52	52×10 <sup>2</sup>	20	65×10 <sup>2</sup>

**Table 3.** Changes of fungal population in CFU / g dry compost during composting in Ex-I.

Experiment type	Dilution factor	No. of colonies per plate	SPC/ml = Colonies counted/dilution factor	Moisture content (%)	CFU/g dry compost
Ex-I/0	10 <sup>-3</sup>	2	2×10 <sup>3</sup>	50	4×10 <sup>3</sup>
Ex-I/C (15)	10 <sup>-3</sup>	2	2×10 <sup>3</sup>	40	3.33×10 <sup>3</sup>
Ex-I/P (15)	10 <sup>-3</sup>	3	3×10 <sup>3</sup>	40	5×10 <sup>3</sup>
Ex-I/Eu (15)	10 <sup>-3</sup>	3	3×10 <sup>3</sup>	40	5×10 <sup>3</sup>
Ex-I/E (15)	10 <sup>-3</sup>	9	9×10 <sup>3</sup>	40	15×10 <sup>3</sup>
Ex-I/C (30)	10 <sup>-3</sup>	4	4×10 <sup>3</sup>	30	5.71×10 <sup>3</sup>
Ex-I/P (30)	10 <sup>-3</sup>	6	6×10 <sup>3</sup>	30	8.57×10 <sup>3</sup>
Ex-I/Eu (30)	10 <sup>-3</sup>	9	9×10 <sup>3</sup>	30	12.9×10 <sup>3</sup>
Ex-I/E (30)	10 <sup>-3</sup>	10	10×10 <sup>3</sup>	30	14.3×10 <sup>3</sup>
Ex-I/C (45)	10 <sup>-3</sup>	6	6×10 <sup>3</sup>	20	7.5×10 <sup>3</sup>
Ex-I/P (45)	10 <sup>-3</sup>	7	7×10 <sup>3</sup>	20	8.75×10 <sup>3</sup>
Ex-I/Eu (45)	10 <sup>-3</sup>	11	11×10 <sup>3</sup>	20	13.8×10 <sup>3</sup>
Ex-I/E (45)	10 <sup>-3</sup>	12	12×10 <sup>3</sup>	20	15×10 <sup>3</sup>

were kept in such way as to avoid direct sunlight (to prevent evaporation) and rain (to prevent excess moisture) and to protect from predation (1, 2, 7). For the analysis of total bacterial load, two to three drops were taken on the plate from 10<sup>-2</sup> dilution and then nutrient agar medium was poured on the each plate. Plates were given for incubation at 37 C for 24 h in a bacterial incubation chamber (8,9).

For fungal analysis, two to three drops were taken from 10<sup>-3</sup> dilution and then Czapekdox agar medium was poured on each plate and given for incubation at 28 C for 7 days in a fungal incubation chamber. The bacterial and fungal colonies developing on the plate were counted after incubation and expressed as colony forming units (CFU) × 10<sup>-2</sup> / g and (CFU) × 10<sup>-3</sup> / g respectively. Gram staining was done after every sampling and bacterial culture and identification of fungi was done after pure culture (3, 8, 9).

The presence of gram positive or gram negative bacteria were determined microscopically on the basis of their violet or reddish-pink color. The fungal

**Table 4.** Changes of fungal population in CFU/g dry compost during composting in Ex-II.

Experiment type	Dilution factor	No. of colonies per plate	SPC/ml = Colonies counted/dilution factor	Moisture content (%)	CFU/g dry compost
Ex-II/0	10 <sup>-3</sup>	2	2×10 <sup>3</sup>	50	4×10 <sup>3</sup>
Ex-II/C (15)	10 <sup>-3</sup>	2	2×10 <sup>3</sup>	40	3.33×10 <sup>3</sup>
Ex-II/P (15)	10 <sup>-3</sup>	4	4×10 <sup>3</sup>	40	6.66×10 <sup>3</sup>
Ex-II/Eu (15)	10 <sup>-3</sup>	3	3×10 <sup>3</sup>	40	5×10 <sup>3</sup>
Ex-II/E (15)	10 <sup>-3</sup>	5	5×10 <sup>3</sup>	40	8.33×10 <sup>3</sup>
Ex-II/C (30)	10 <sup>-3</sup>	5	5×10 <sup>3</sup>	30	7.14×10 <sup>3</sup>
Ex-II/P (30)	10 <sup>-3</sup>	7	7×10 <sup>3</sup>	30	10×10 <sup>3</sup>
Ex-II/Eu (30)	10 <sup>-3</sup>	8	8×10 <sup>3</sup>	30	11.4×10 <sup>3</sup>
Ex-II/E (30)	10 <sup>-3</sup>	7	7×10 <sup>3</sup>	30	10×10 <sup>3</sup>
Ex-II/C (45)	10 <sup>-3</sup>	6	6×10 <sup>3</sup>	20	7.5×10 <sup>3</sup>
Ex-II/P (45)	10 <sup>-3</sup>	8	8×10 <sup>3</sup>	20	10×10 <sup>3</sup>
Ex-II/Eu (45)	10 <sup>-3</sup>	11	11×10 <sup>3</sup>	20	13.8×10 <sup>3</sup>
Ex-II/E (45)	10 <sup>-3</sup>	10	10×10 <sup>3</sup>	20	12.5×10 <sup>3</sup>

species were identified microscopically on the basis of their morphological structure.

### Results and Discussion

The results of the estimation of bacterial load in the casts of the three species of earthworms when reared in water hyacinth-cowdung mixture (Ex-I) and banana residues-cowdung mixture (Ex-II) are presented in Tables 1 and 2 respectively. The results show that the casts of *Eudrilus eugeniae* had 1.02 times more CFU than the casts of *Eisenia foetida*, 1.6 times more CFU than the casts of *Perionyx excavatus* when three species are reared in Ex-I and the casts of *Eudrilus eugeniae* also showed 1.06 times more CFU than the casts of *Eisenia foetida*, 1.6 times more CFU than the casts of *Perionyx excavatus* when three species are reared in Ex-II.

On account of fungal count, the casts of the three species of earthworms when reared in Ex-I and Ex-II are presented in Tables 3 and 4 respectively which showed that the casts of *Eisenia foetida* showed 1.09 times more CFU than the casts of *Eudrilus eugeniae*, 1.71 times more CFU than the

casts of *Perionyx excavatus* in Ex-I and the casts of *Eudrilus eugeniae* showed 1.1 times more CFU than the casts of *Eisenia foetida*, 1.4 times more CFU than the casts of *Perionyx excavatus* in Ex-II.

The result of the determination of gram positive or gram negative bacteria revealed that the bacteria showing violet color after every gram staining during the course of investigation, so the bacteria are gram positive in both experiments. The fungi, *Drechslera* sp. and *Aspergillus niger* are found through this investigation. Increased number of microorganisms in casts of earthworm has been reported by many authors (3). Vermicompost produced by *Eudrilus eugeniae* had higher population of bacteria ( $5.7 \times 10^7$ ) and fungi ( $22.7 \times 10^4$ ) (9).

The gram bacteria belonged to *Bacillus* sp., the fungi were *Mucor* sp., *Aspergillus* sp., *A. flavus*, *A. niger*, *Fusarium* sp., *Penicillium* sp. and *Trichoderma* sp. (9). The *Aspergillus niger*, *A. flavus*, *A. versicolor*, *A. terreus* and *Mucor* sp. were the important and dominated species in the casts of *Eudrilus eugeniae* and *Rhizopus stolonifer* was the dominant species of the casts of *Lampito mauritii* (3).

It is revealed that *Eudrilus eugeniae* and *Eisenia foetida* are best for using them in vermicomposting and are better suited for any substrate and the experimental substrate water hyacinth is better than other substrates due to presence of large number of microorganisms.

### References

1. Bhatnagar R. K. and R. K. Palta. 1996. Earthworm vermiculture and vermicomposting. Kalyani Publ., Ludhiana, India.
2. Sultan A. Ismail 1997. Vermiculture, the biology of earthworms. Orient Longman, New Delhi, India.
3. Umamaheswari S., V. Balamurugan, and G. S. Vijayalakshmi. 2003. Fungal predation by *Lampito mauritii* (Kinberg) and *Eudrilus eugeniae* (Kinberg) cultured in different organic wastes, Asian J. Microbiol. Biotech. Env. Sci. 5 : 63—65.
4. Anandan M. 2000. An integrated approach of biofertilizer for sustainable agriculture. Intens. Agric., pp. 9—11.
5. Jat R. and D. Kumar. 2004. Need and opportunities of organic farming in India. Intens. Agric., pp.

- 28—30.
6. Kale R. D. 1998. Earthworm—Cinderella of organic farming. Prism Books Pvt. Ltd., Bangalore, India.
  7. Santra S. C. 2005. Environmental science, New Central Book Agen. Pvt. Ltd., Kolkata 700009, India.
  8. Nirmalnath P. J., A. P. Biradar and B. S. Nandahalli. 2000. Vermicompost microflora as influenced by different crop residues. *Karnataka J. Agri. Sci.* 13 : 207—208.
  9. Prabhakumari P., S. K. Nair, A. Naseema, K. S. Meenakumari and C. K. Peethambaran. 1997. Microflora associated with earthworms and vermicompost. *J. Trop. Agric.* 35 : 68—70.