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Vermicomposting of Sugarcane Trash and Leaf Litter in Combination with Pressmud Using the Earthworm, *Perionyx ceylanensis*

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ABSTRACT

In the present study, initial vermicomposting trials were conducted for 100 days using leaf litters of *Polyalthia longifolia* (LPL) and sugarcane trash (SCT), each in combination with pressmud (PM) (i.e., 0:100, 25:75, 50:50, 75:25 and 100:0) by inoculating an epigeic earthworm, *Perionyx ceylanensis*. Based on C/N ratio, worm biomass and numbers recorded in vermicomposting trials, organic substrates + PM in 50:50 (1:1) proportion was selected for 60 days vermiconversion studies. Nutrient and microbiological changes during vermicomposting was studied with a special focus on the enzymes in the gut and vermicasts of *P. ceylanensis*. The NPK contents in worm worked substrates were higher than worm unworked LPL + PM and SCT + PM substrates. The C/N ratio of vermicompost showed decrease over compost (worm-unworked) and percent decrease observed was significant ($P < 0.001$). The total microbial population (bacteria, fungi and actinomycetes) present at the end of experiment (60^{th} day) was significantly higher ($P < 0.05$) than the microbial population present at the start of the experiment in both the substrates. Amylase, cellulase, invertase, phosphatase and protease in fresh vermicasts of *P. ceylanensis* showed higher activities when reared in LPL + PM substrate. The activities of the enzymes cellulase, invertase and protease in vermicasts of *P. ceylanensis* were significantly higher in LPL + PM. The activity of these enzymes while ageing showed an increase up to 15-20 days and then declined. In the vermicasts recovered from LPL + PM and SCT + PM (1:1), cellulase showed highest activity at the end of 30 days. The present study reveals that the clitellate *P. ceylanensis* exhibit higher activity of amylase, cellulase, invertase, phosphatase and protease.

Keywords: earthworms, microbial activity, sugarcane waste, vermicasts, waste recycling
Abbreviations: CFU, colony forming units; **LPL**, leaf litter of *Polyalthia longifolia*; **NPK**, nitrogen, phosphorus and potassium; **PM**, pressmud; **SCT**, sugarcane trash; **TOC**, total organic carbon; **WOW**, worm-worked; **WUW**, worm un-worked

INTRODUCTION

The organic matter degraded by earthworm activity is called vermicompost and it can be used as top soil or as organic manure in fields to prevent organic carbon deficiency. The process of compost preparation using earthworms is well-known as vermicomposting technology. Though more than 400 species are listed under Indian earthworms, only about 20 species are employed for waste disposal through vermicomposting technology (Karmegam and Daniel 2011). Ample knowledge on vermicomposting of various organic wastes by exotic species of earthworms like *Eudrilus eugeniae*, *Eisenia fetida*, *Lumbricus rubellus*, *Perionyx excavatus*, *Lampito mauritii*, etc., is available through published works (Edwards and Arancon 2004; Munnoli *et al.* 2010). However, the utilization of the earthworm, *Perionyx ceylanensis* Mich. for vermicomposting technology has been recently reported (Karmegam and Daniel 2009a; Prakash and Karmegam 2010a; John Paul *et al.* 2011). *P. ceylanensis* was selected as suitable species for vermicomposting since it is an epigeic native species with vermicomposting potential and short life cycle (Karmegam and Daniel 2009a, 2009b). The nutrient level of vermicompost depends on the nature of the organic material used as food source for earthworms (Garg *et al.* 2006; Suthar and Singh 2008; Jayakumar *et al.* 2009; Daniel *et al.* 2010). Knowledge about the food requirement and digestive capability of the earthworms is essential to understand the decomposition process during vermicomposting.

The enzymes and the enzyme activity in the gut of earthworms and in casts are extremely important since they are responsible for the degradation of organic materials and to make the nutrients in available form for plants. In vermicomposting, earthworms transform organic residues into more humified materials (Ranganathan and Parthasarathi 2005). Bound extra-cellular enzymes are constituents of humus. Extra-cellular enzymes bound to soil humus can persist in hostile soil environments ensuring metabolic activity during periods of limited soil microbial activity due to scarce energy supplies or environmental stress (Benitez *et al.* 1999). All biochemical reactions important in nutrient cycling or recycling in the soil are catalysed by enzymes which are proteins with catalytic properties owing to their power of specific activation (Tabatabai 1982). Soil enzymes are involved in metabolic processes and energy transfer associated with plant and microbial growth. They are thus potential indicators of soil biological activity and fertility (Mulongoy and Bedoret 1989). It is essential to know the enzyme activities in gut and casts to completely understand the process of vermicomposting and the beneficial role of vermicompost in soil, so that various kinds of wastes with different chemical composition can be subjected to vermicomposting process to produce valuable vermicompost.

The studies on vermistabilization of different organic materials including pressmud (PM), leaf litters of trees and agricultural residues alone or in combination with cow dung

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are available (Gajalakshmi *et al.* 2005; Suthar 2007; Karmegam and Daniel 2009a; Pramanik 2010; Prakash and Karmegam 2010b; Yadav and Garg 2011a, 2011b). A recent study by Kumar *et al.* (2010a) demonstrated that the compost produced by pre-decomposing of PM with other by-products of sugar processing industries (sugarcane trash and bagasse), with efficient microbes (composting) followed by vermicomposting point towards the feasibility of an integrated system of vermicomposting to produce nutrient rich vermicompost. Now-a-days, the availability of cowdung is becoming scarce due to the reduction in agricultural practices and the use of cowdung for the production of biogas, farm yard manure and compost (John Paul 2005). Since the PM is nutritionally rich and more or less equivalent in texture to that of cowdung, in the present study, a trial has been carried out to utilize PM (as a substitute for cowdung) in combination with *Polyalthia longifolia* leaf litter (locally abundant organic material) and sugarcane trash. So that the leaf litter residues along with PM could be utilized for the production of valuable vermicompost. Hence the present study has been carried out with the following objectives: (i) to assess the potential of the earthworm species, *P. ceylanensis* in vermicomposting of two different organic materials, leaf litters of *P. longifolia* and sugarcane trash (leaves) mixed with PM; (ii) to analyse the activity of different enzymes in the casts and gut of *P. ceylanensis* cultured in of *P. longifolia* and sugarcane trash mixed with PM; (iii) to find out the enzyme activities with reference to different developmental stages of the earthworm, *P. ceylanensis*.

MATERIALS AND METHODS

Collection of materials and earthworms

The raw materials, leaf litters of *P. longifolia* L. (LPL) was collected locally. The filter mud or PM was collected from The Cheyyar Co-operative Sugar Mills Ltd., located in Thenthandalam, Anakkayur, Thiruvannamalai District, Tamil Nadu. The sugarcane trash (SCT, sugarcane leaves left in the field after harvesting) was collected from a private farm near Vandavasi, Thiruvannamalai District, chopped and used for the study. The earthworm, *P. ceylanensis* Mich. for the study, originally collected from culture bank of the Department of Biology, Gandhigram Rural University, Tamil Nadu, India was mass multiplied in cow dung and used for the study.

Vermistabilization of organic materials

The cultures of *P. ceylanensis* were acclimatized to the laboratory conditions using the standard culture medium, cow dung. After acclimatizing the worms to the laboratory conditions in cow dung, the worms were mass multiplied in the laboratory in plastic troughs of 45 × 30 × 15 cm size. Since the worms initially procured were 100 in numbers, mass multiplication using cowdung was done to increase the worm numbers required for the study. The PM, LPL and SCT were subjected to pre-decomposition for 15 days under shade by sprinkling water and turning. The combinations of pre-decomposed organic materials were mixed with PM in different proportions as indicated in **Table 1** and the vermibeds were prepared accordingly.

The vermibed materials were maintained with a moisture content 70 ± 5% by sprinkling water and the vermibed substrates were subjected to mixing and turning once in 15 days. All the experiments were carried out at an ambient temperature (26 ± 3°C) (Karmegam and Daniel 2009a).

Table 1 Combination of vermibed substrates used for preliminary vermicomposting trial (100 days).

Press mud (%)	Organic substrates (LPL/SCT in %)
0	100
25	75
50	50
75	25
100	0

Optimization of vermicomposting days

The clitellate-adult epigeic earthworms, *P. ceylanensis*, numbering 60 were inoculated in each trough and no earthworms were introduced in control sets of all the combinations. The culture troughs were placed indoor in the laboratory and covered with wire mesh. The amount of total organic carbon (TOC) and total nitrogen were analysed once in 10 days from 0^{th} day to 100 days (Karmegam and Daniel 2009a). C:N ratio was considered as a criterion for assessing the process of vermicomposting.

Vermicomposting and characterization of vermicompost

Based on the results of vermistablization and optimization studies, vermicomposting of organic substrates, LPL and SCT mixed with PM in 50:50 proportion was carried out for 60 days using *P. ceylanensis* in six replicates under controlled conditions. The physico-chemical characteristics of initial vermibed substrates, final control (worm-unworked) and vermicompost were analysed as per standard procedures given below.

Physico-chemical analysis of vermibed substrates

The vermibed substrates, i.e., worm-worked (substrates introduced with worms) and the worm-unworked substrates (control set) were analysed for various physico-chemical parameters using standard procedures. Determination of pH was done by a digital pH meter, electrical conductivity by a conductivity meter (Elico) using 1:10 (w/v) compost-water (double distilled) suspension. The moisture content was determined after drying at 105°C for 24 h. TOC was measured and using the method (Walkley and Black 1934). Total Kjeldhal Nitrogen (N) was determined after digesting the sample with concentrated H_2SO_4 and concentrated $HClO_4$ (9:1, v/v) (Tandon 1993). Total phosphorus (P) was analysed using colorimetric method with molybdenum in sulphuric acid (Tandon 1993). Total potassium (K) and total calcium (Ca) were determined after digesting the samples in concentrated HNO_3: $HClO_4$ (4:1, v/v), by flame photometer (Tandon 1993). Total Fe and Zn were determined by atomic absorption spectrophotometer after digestion of the sample by the dry ashing method (Tandon 1993). The percent increase/decrease of various physico-chemical (nutrient) parameters over the worm-unworked substrates was calculated [(A-B/A) × 100; where A= values in the worm-worked substrate, B= values in the worm-unworked substrate].

Microbiological analysis

At periodic intervals of 15 days from the start of the experiment, the total colony forming units (CFU) of bacteria, fungi and actinomycetes in the vermibed substrates were counted up to 60 days. The methodology adopted was based on the 'Standard Dilution Plate Technique' where three random sub-samples were collected. One gram of each sample was taken in a sterile conical flask containing nine ml of distilled water and shaken in a vortex mixer for 30 min. From this stock, various dilutions were prepared from 10^{-1} to 10^{-7} with sterile distilled water. One ml of the diluted sample was poured into Petri dishes containing respective media for bacteria, fungi and actinomycetes as indicated in **Table 2** (Kannan 1996). Three replicates were maintained for each observation. The Petri dishes with 30-300 colonies were selected for enumeration total microbial population. The bacterial, fungal and actinomycetes population was expressed as CFU per gram of the sample.

Enzymatic analysis of vermicasts and gut of *P. ceylanensis*

A separate set of experiments was conducted in this phase of work as described below in triplicates. The vermibed materials in 1:1 ratio were prepared in plastic troughs in six replicates and worms were introduced. In one set of experiment, worms were sacrificed for enzyme analysis and in another set, the vermicasts were taken and subjected to enzymatic analysis. The vermicasts were carefully collected in glass containers and transferred to the laboratory for enzymatic analysis. The enzymes, amylase (EC 3.2.1.1), cel-

Table 2 Media used for enumeration of Colony Forming Units (CFU) of bacteria, fungi and actinomycetes in different vermibed substrates.

Micro-organism	Media used	Dilution of the sample	Incubation in day(s)
Bacteria	Nutrient Agar Media	10^{-6}	1
Fungi	Martin's Rose Bengal Agar Media	10^{-3}	3
Actinomycetes	Kenknight's Media	10^{-4}	7

Table 3 Total number of *P. ceylanensis* recovered after 100 days of vermicomposting of different organic substrates (initial worm numbers: 60/trough; values are mean ± S.E.).

Vermibed substrate combinations (LPL/SCT : PM)	Worms recovered after 100 days (no./trough)	
	LPL + PM	SCT + PM
100 : 0	407.6 ± 21.6 Aa	387.5 ± 18.2 Aa
75 : 25	523.5 ± 23.3 Ab	489.7 ± 24.4 Ab
50 : 50	680.4 ± 20.4 Ac	597.5 ± 17.9 Ac
25 : 75	687.5 ± 21.8 Ac	606.4 ± 28.6 Bc
0 : 100	691.8 ± 23.3 Ac	615.5 ± 26.5 Bc

The superscript letters indicate statistical significance of difference in mean values between the columns (A, B and C) and between the rows (a, b and c) respectively at $P < 0.05$ (ANOVA); The mean values followed by same letters indicate the difference is not significant at $P < 0.05$.

Table 4 Total biomass of *P. ceylanensis* recovered after 100 days of vermicomposting of different organic substrates (initial worm numbers: 60/trough; values are mean ± S.E.).

Vermibed substrate combinations (LPL/SCT: PM)	Total biomass of worms after 100 days (g/trough)	
	LPL + PM	SCT + PM
100 : 0	214.9 ± 12.3 Aa	209.0 ± 11.9 Aa
75 : 25	253.1 ± 12.7 Ab	244.5 ± 12.7 Ab
50 : 50	290.0 ± 13.3 Ac	283.1 ± 14.2 Ac
25 : 75	298.2 ± 14.9 Ac	291.3 ± 11.3 Ac
0 : 100	302.0 ± 15.6 Ac	294.5 ± 13.3 Ac

The superscript letters indicate statistical significance of difference in mean values between the columns (A, B and C) and between the rows (a, b and c) respectively at $P < 0.05$ (ANOVA); The values followed by same letters indicate the difference is not significant at $P < 0.05$.

lulase (EC 3.2.1.4.), protease (EC 3.4.0), phosphatase (EC 3.1.3.1, 2) and invertase (EC 3.2.1.20) in the vermicasts were analysed. The activity of amylase, cellulase and invertase was done according to method described by Galstyan (1965). Protease and phosphatase activities were analysed adopting the methods of Sarath *et al.* (1989) and Jannossy (1963), respectively. The worm casts were incubated to study the effect of ageing on enzyme activities. Another set of experiment was designed to collect three categories of worms: (i) juveniles, (ii) pre-clitellates and (iii) clitellate adults to assess the presence of gut enzymes in different stages of *P. ceylanensis*. For the enzymatic analysis of worm guts, gut content cleared tissue was used.

Statistical analysis

The total number and biomass of *P. ceylanensis* recovered after 100 days of initial vermicomposting was compared between different vermibed combinations by ANOVA at the significant level of $P < 0.05$ using Microcal Origin (Version 3.1). The percentage increase / decrease of physico-chemical characteristics of WUW and WOW composts was compared using Student *t*-test at $P < 0.05$, $P < 0.01$ and $P < 0.001$. The initial microbial CFU, the final microbial CFU and the microbial CFU at intervals (WOW and WUW) in the vermibed substrates were subjected to one-way ANOVA using the Computer Software, Microcal Origin (Version 3.1) at $P < 0.05$. Similarly, the enzyme activity between vermicasts obtained from LPL+PM and SCT + PM, and between different stages of the earthworm, *P. ceylanensis* (juvenile, pre-clitellate and clitellate) were tested using ANOVA at $P < 0.05$.

RESULTS AND DISCUSSION

Vermicomposting trials and worm growth (number and biomass)

The results of the initial vermicomposting trials, conducted for 100 days using three different combinations of each organic substrate, LPL, SCT and PM (i.e., 0:100, 25:75, 50:50, 75:25 and 100:0) by inoculating *P. ceylanensis*, showed (based on C:N ratio) that the organic substrates mixed with cowdung in 50:50 (1:1) proportion was suitable for this earthworm for efficient vermiconversion. The substrates without worms (0 worms) showed very slow decline in C/N ratio. The C/N ratios at the end of the experiment i.e. on the 100th day for *P. ceylanensis* are shown in **Fig. 1**. The values of C/N ratio in 25:75 of organic substrates + PM were found to be closer to those of 50:50 combinations of all the three substrates, and the difference was not significant ($P < 0.05$).

The highest worm biomass and worm numbers were recorded in 1:1 combinations of vermibed substrates (**Tables 3, 4**). In LPL + PM (0:100), a maximum of 692 worms/trough were recovered after 100 days which showed no significant difference with that of 25:75 and 50:50 combinations at $P < 0.05$ but the number of *P. ceylanensis* were significantly different from 100:0 and 75:25. A clear trend of increase of worm number with reference to the increase of PM proportion was observed for both the substrates (**Table 3**).

In 100:0 LPL + PM, 387 worms/trough were recovered after 100 days of vermicomposting which was lower than all the other vermibed combinations and the difference was statistically significant ($P < 0.01$). In the same proportion i.e., 100:0 of SCT and PM, 408 worms were recovered which showed increasing trend up to 0:100 (**Table 3**). In SCT + PM vermibed substrate, slightly lower worm counts

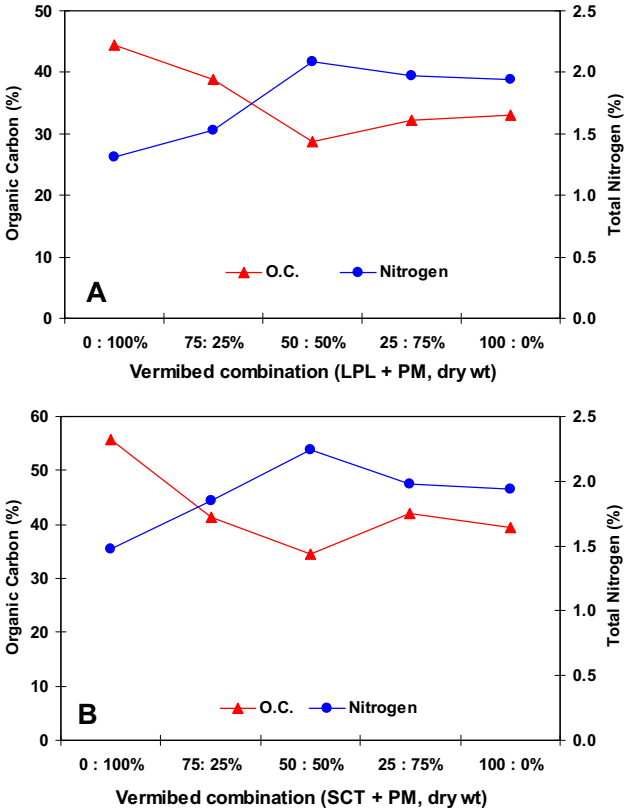

Fig. 1 Changes in organic carbon and total nitrogen content during preliminary vermicomposting trial using different combinations of LPL (**A**) and SCT (**B**) with pressmud (PM).

were recorded. A total of 688 worms/trough were recovered in 25:75 combination of LPL + PM substrate whereas the same proportion SCT + PM showed significantly lower worm counts (606 worms/trough). The data clearly showed that the vermibed substrates influence the worm recovery which was directly related to the proportion of PM, i.e., the higher the PM proportion, the higher the number of worms.

In LPL + PM (0:100), a maximum of biomass of 302 g/trough were recovered after 100 days which showed no significant difference with that of 25:75 and 50:50 combinations at $P < 0.05$ but the biomass values of *P. ceylanensis* were significantly different from 100:0 and 75:25. A clear trend of increase of worm biomass with reference to the increase of PM proportion was observed for both the substrates (**Table 4**). In 100:0 LPL + PM, 214 g of worms/trough were recovered after 100 days of vermicomposting which was lower than all the other vermibed combinations and the difference was statistically significant ($P < 0.01$). In the same proportion i.e., 100:0 of SCT and PM, 209 g of worms were recovered which showed increasing trend up to 0:100 (**Table 4**).

In SCT + PM vermibed substrate, slightly lower worm biomass was recorded in all the combinations when compared with LPL + PM substrate. A total worm biomass of 298 g/trough were recovered in 25:75 combination of LPL + PM substrate whereas the same proportion of SCT + PM showed lower worm biomass of 291 g/trough where the values did not show significant different at $P < 0.05$. The data clearly showed that the vermibed substrates influence the worm biomass recovery which was directly related to the proportion of PM, i.e., higher the PM proportion, higher the worm biomass (**Table 4**).

Vermicomposts are produced from organic wastes through interactions between earthworms and microorganisms, and can be utilized as plant growth media or soil amendments (Edwards and Arancon 2004). Although, microbes are responsible for biochemical degradation of organic matter, earthworms are the important drivers of the process, conditioning the substrate and altering the biological activity (Lavelle and Spain 2001; Domínguez 2004). The microbial respiration may lead to rapid carbon loss through CO_2 production and also, digestion of carbohydrates, lignin, cellulose and other polysaccharides from the substrates by inoculated earthworms may cause carbon reduction during the decomposition of organic waste. Some part may be converted to worm biomass through the assimilation process, which consequently reduces the carbon budget of vermicomposted wastes (Suthar 2009). The addition of N in the form of mucous, excretory substances which were not initially present in feed substrates has been reported (Sangwan *et al.* 2008; Karmegam and Daniel 2009a). The higher percent increase of EC and NPK in vermicompost produced by *P. ceylanensis* than in the compost in this study may be attributed to the mineralization process caused by earthworm action along with microorganisms on organic materials. The recovery of high worm numbers and biomass in the present study is in association with the proportion of PM. The data clearly shows that higher proportion of PM increases the worm growth. The similar trend of growth of *P. ceylanensis* has been found during vermicom-

Table 5 Nutrient status of LPL + PM (1 : 1) substrate subjected to vermicomposting with *P. ceylanensis* (60 days).

Parameters	Compost [a]			Percent (%) [b] increase/decrease of WOW over WUW
	Initial value of substrate	WUW	WOW	
pH	7.3 ± 0.1	7.4 ± 0.1	7.6 ± 0.09	2.6 [NS]
Electrical conductivity (dS m^{-1})	1.3 ± 0.1	1.6 ± 0.1	2.5 ± 0.3	36.0 **
Organic carbon (%)	53.4 ± 3.2	46.2 ± 4.4	33.1 ± 3.2	-39.6 **
Nitrogen (%)	0.8 ± 0.1	1.2 ± 0.2	1.8 ± 0.2	33.3 *
Phosphorus (%)	0.7 ± 0.1	0.9 ± 0.1	1.4 ± 0.3	35.7 *
Potassium (%)	0.4 ± 0.1	0.6 ± 0.1	1.3 ± 0.5	53.8 ***
Calcium (%)	1.1 ± 0.1	1.2 ± 0.2	1.7 ± 0.1	29.4 *
Sodium (%)	0.3 ± 0.1	0.3 ± 0.1	0.4 ± 0.1	25.0 *
Magnesium (%)	0.6 ± 0.02	0.6 ± 0.02	0.7 ± 0.1	14.3 [NS]
Manganese (ppm)	18.3 ± 0.7	19.2 ± 0.6	27.7 ± 1.3	30.7 *
Sulphur (%)	0.1 ± 0.01	0.1 ± 0.01	0.2 ± 0.01	50.0 **
Iron (ppm)	82.7 ± 1.1	96.8 ± 2.0	129.2 ± 2.0	25.1 *
Copper (ppm)	8.7 ± 0.3	9.1 ± 0.6	14.1 ± 1.3	35.5 **
Zinc (ppm)	34.6 ± 1.4	37.2 ± 0.4	49.3 ± 3.0	24.5 *
C/N	66.8 ± 2.10	38.5 ± 3.0	18.4 ± 3.3	-109.4 ***

[a] Values are mean of six replicates ± SD.
[b] Values without sign and values with negative sign are percent increase and percent decrease respectively; WUW= worm un-worked; WOW= worm-worked.
*, **, *** and NS indicates statistically significant difference at $P < 0.05$, $P < 0.01$, $P < 0.001$ and not significant by Student's *t*-test.

Table 6 Nutrient status of SCT + PM (1 : 1) substrate subjected to vermicomposting with *P. ceylanensis* (60 days).

Parameters	Compost [a]			Percent (%) [b] increase/decrease of WOW over WUW
	Initial value of substrate	WUW	WOW	
pH	7.2 ± 0.1	7.3 ± 0.1	7.4 ± 0.1	1.4 [NS]
Electrical conductivity (dS m^{-1})	1.2 ± 0.2	1.4 ± 0.3	2.1 ± 0.3	33.3 *
Organic carbon (%)	49.8 ± 3.0	43.2 ± 5.3	33.1 ± 3.2	-30.5 *
Nitrogen (%)	0.83 ± 0.1	0.91 ± 0.1	1.4 ± 0.2	35.0 *
Phosphorus (%)	1.2 ± 0.2	1.4 ± 0.1	1.8 ± 0.3	22.2 *
Potassium (%)	0.5 ± 0.1	0.6 ± 0.1	1.2 ± 0.3	50.0 **
Calcium (%)	0.9 ± 0.1	1.0 ± 0.2	1.5 ± 0.4	33.3 *
Sodium (%)	0.4 ± 0.1	0.5 ± 0.1	0.7 ± 0.3	28.6 *
Magnesium (%)	0.3 ± 0.04	0.3 ± 0.02	0.5 ± 0.6	40.0 **
Manganese (ppm)	22.2 ± 0.6	24.1 ± 0.6	31.7 ± 1.1	24.0 *
Sulphur (%)	0.1 ± 0.01	0.1 ± 0.01	0.2 ± 0.01	50.0 **
Iron (ppm)	75.7 ± 1.3	83.3 ± 2.2	107 ± 2.1	22.1 *
Copper (ppm)	8.7 ± 0.5	10.1 ± 0.9	14.0 ± 1.1	27.9 *
Zinc (ppm)	32.6 ± 1.6	36.3 ± 3.6	46.5 ± 3.0	21.9 *
C/N	60.0 ± 3.1	47.5 ± 3.0	23.6 ± 2.1	-100.9 ***

[a] Values are mean of six replicates ± SD.
[b] Values without sign and values with negative sign are percent increase and percent decrease respectively; WUW= worm un-worked; WOW= worm-worked.
*, **, *** and NS indicates statistically significant difference at $P < 0.05$, $P < 0.01$, $P < 0.001$ and not significant by Student's *t*-test.

Table 7 Total microbial population dynamics during vermicomposting of LPL + PM (1 : 1) with *P. ceylanensis*.

Vermicomposting days	Total microbial population*		
	Bacteria (CFU× 10^6 g^{-1})	Fungi (CFU×10^3 g^{-1})	Actinomycetes (CFU×10^4 g^{-1})
0	42.3 ± 0.8 a	53.0 ± 1.9 a	32.2 ± 3.1 a
15	55.7 ± 4.5 a	76.7 ± 3.1 b	49.2 ± 3.1 ab
30	76.3 ± 3.0 b	94.1 ± 3.6 cd	58.7 ± 2.8 b
45	96.0 ± 3.3 c	106.7 ± 2.2 d	63.8 ± 1.5 bc
60	130.0 ± 3.3 d	129.2 ± 2.6 d	79.7 ± 2.7 c

* Values are mean of six replicates ± SD; Values with same letter in columns are not significantly different at $P < 0.05$ by ANOVA.

Table 8 Total microbial population dynamics during vermicomposting of SCT + PM (1 : 1) with *P. ceylanensis*.

Vermicomposting days	Total microbial population*		
	Bacteria (CFU × 10^6 g^{-1})	Fungi (CFU × 10^3 g^{-1})	Actinomycetes (CFU × 10^4 g^{-1})
0	32.7 ± 1.6 a	35.5 ± 2.0 a	43.2 ± 1.9 a
15	41.5 ± 1.4 a	43.0 ± 3.3 a	49.1 ± 1.6 ab
30	63.3 ± 1.4 b	61.3 ± 1.9 b	56.7 ± 1.4 ab
45	74.2 ± 2.0 bc	65.9 ± 1.7 b	68.2 ± 1.5 bc
60	87.3 ± 3.8 c	78.7 ± 2.1 b	70.0 ± 1.6 c

* Values are mean of six replicates ± SD; Values with same letter in columns are not significantly different at $P < 0.05$ by ANOVA.

posting of organic substrates in combination with cowdung, where cowdung served as instant resource for nutrients and microorganisms to activate the earthworms (Karmegam and Daniel 2009a; John Paul *et al.* 2011). The recovery of a high number of earthworms in the treatment inoculated with *P. ceylanensis* showed the short life cycle of this earthworm species which is an essential characteristic of vermicomposting species (Karmegam and Daniel 2009b).

Vermicomposting of LPC and SCT with PM (1:1) using *P. ceylanensis*

1. Physico-chemical characteristics of vermibed substrates (1:1)

Based on the results of initial vermicomposting trials given above for optimization of vermicomposting of the substrates, LPL and SCT mixed with PM in 50:50 proportion (1:1, wt/wt) was selected for vermicomposting. The initial values of pH and E.C. (dS/m) in LPL+PM mixture were 7.3 and 1.3 respectively; for SCT + PM the values were 7.2 and 1.2 (**Table 5**). The initial organic carbon values of LPL + PM was higher (53.4%) than SCT + CD (49.8%). The C/N values at the start of the experiment recorded for LPL + PM and SCT + PM showed the same trend. The C/N ratio observed for LPL + PM was 66.8, which was higher than SCT + PM (60.0). The iron, copper and zinc contents in vermibed substrates recorded at the start of the experiment for LPL+PM substrate were 82.7, 8.7 and 34.6 ppm, respectively; for SCT + PM, the values were 75.7, 8.7 and 32.6 ppm respectively (**Tables 5, 6**).

The NPK contents in worm worked substrates of LPL + PM were higher (N-1.8; P-1.4; and K-1.3%) than worm unworked substrates (N-1.2; P-0.9; and K-0.6%). The C/N ratio of worm worked LPL + PM showed decrease (18.4) over worm unworked substrates (38.5) and the percent decrease observed was 109.4 which was highly significant at 0.01% level by Student's *t*-test (**Table 5**). The physico-chemical characteristics of SCT + PM (1:1) substrates subjected to vermicomposting with *P. ceylanensis* for 60 days showed changes in nutrient contents (**Table 6**). The physico-chemical characteristics such as E.C., NPK, Ca, Mg, Fe and Cu in SCT + PM (1:1) vermicompost showed increase over worm unworked and initial substrates. Whereas, OC and C/N in the vermicompost of SCT + PM (1:1) showed decrease over worm unworked and initial substrates. The NPK contents in worm worked substrates were higher (N-1.4, P-1.8, and K-1.2%) than worm unworked substrates (N-0.91; P-1.4; and K-0.6%). The C/N ratio of worm worked SCT + PM showed decrease (23.6) over worm unworked substrates (47.5) and percent decrease observed was 100.9 which was significant at $p<0.001$ level according to Student's *t*-test. The percentage increase of 33.3 for E.C., 35.0 for N, 22.2 for P and 50.0 for K were recorded (**Table 6**).

As noted by Garg and Kaushik (2005) and Sangwan *et al.* (2008), a decrease in pH was observed in all the three substrates during vermicomposting. The decrease may be due to mineralization of nitrogen and phosphorus into nitrites/nitrates and orthophosphates and bioconversion of organic material into organic acids (Garg *et al*. 2006). The lower C/N ratio is due to the reduction of organic carbon due to the respiratory activity of earthworms and microorganisms, and mineralization of organic materials (Karmegam and Daniel 2009a; Sangwan *et al.* 2010). The increased level of P during vermicomposting is due to earthworm-gut derived phosphatase activity and also increased microbial activity in the cast. Le Bayon and Binet (2006) concluded that the impact produced by earthworm on P biogeochemical transformations in the soil depends on the close relationship between the properties of the organic P source and the specific burrowing behaviour and food preferences of worms. The elevated level of Zn, Mn and Fe in vermicompost indicates accelerated mineralization with selective feeding by earthworms on materials containing these metals. Increased levels of macro- and micro-nutrients in vermicomposts were also observed by Suthar (2007).

Total microbial population in the vermicompost

The results on the total microbial population in the vermicompost analysed from 0^{th} day till the termination of the study, i.e., 60^{th} day showed increasing trend towards the end of vermicomposting process of both the substrates. These results along with statistical significance of difference are summarized in **Tables 7** and **8**. The total microbial population (bacteria, fungi and actinomycetes) present at the end of experiment (60^{th} day) was significantly different ($P < 0.05$) from the microbial population present at the start of the experiment in both the substrates. The total bacterial population of 42, 56, 76, 96 and 130 CFU × 10^6 g^{-1} were recorded during vermicomposting of LPL + PM (1:1) with *P. ceylanensis* on 0, 15, 30, 45 and 60 days respectively (**Table 7**). Similar trend of increase of fungal and actinomycetes were observed during vermicomposting of LPL + PM (1:1) (**Table 7**). On the 60^{th} day, total fungal and actinomycetes recorded in LPL + PM vermicompost were 129 CFU × 10^3 g^{-1} and 80 CFU × 10^4 g^{-1}, respectively. The total fungal population of 53, 78, 94, 106 and 129 CFU × 10^3 g^{-1} were recorded during vermicomposting of LPL + PM (1:1) with *P. ceylanensis* on 0, 15, 30, 45 and 60 days respectively. A similar trend was noticed in SCT + PM (1:1) substrate also (**Table 8**).

Similar increases in microbial population were reported in other vermicomposting systems also. Prakash and Karmegam (2010a), while studying the vermicomposting of pressmud using *P. ceylanensis*, reported that the bacterial, fungal and actinomycetes population showed increase towards the progression of vermicomposting period. The present observation is well supported by Parthasarathi (2007) who reported enhanced microbial population, microbial activity and NPK content in the vermicompost at 31°C and 60-70% moisture during vermicomposting of sugar industrial wastes. Stability of vermicompost depends considerably on their age, their microbial population and activity and the organic matter content. The existence of symbiotic relationship between earthworms and microorganisms and also the presence of increased number of microorganisms in the vermicompost has been reported by certain workers (Parthasarathi 2007; Karmegam and Daniel 2009a; Prakash *et al.* 2009). The findings of the present study thus confirm the concept that the earthworm gut might be a specialized

Table 9 Enzyme activities in the vermicasts (fresh) of the earthworm, *P. ceylanensis*.

Enzymes studied	Fresh vermicasts recovered from	
	LPL + PM (1 : 1)	SCT + PM (1 : 1)
Amylase	4.71 a	4.12 a
Cellulase	7.12 a	5.33 b
Invertase	8.63 a	4.44 b
Phosphatase	5.40 a	4.32 a
Protease	7.34 a	5.76 b

Activities of amylase, cellulase and invertase are expressed as mg of glucose/g of oven dry samples for 24 hr of incubation; Protease activity: mg of glutamic acid/g of oven dry substrates for 24 hr incubation; Phosphatase activity: mg/phenol/g of oven dry substrates for 24 hr of incubation; Mean values followed by same letters are not significantly different from each other at 5% level (ANOVA, $P < 0.05$)

microhabitat of enhanced microbial activities in soils. Some of the intestinal mucus secreted during passage through the earthworm gut is ingested with the casts, where it continues to stimulate microbial activity and growth. The increase of fungal population in vermicompost is because, the vermicompost is usually rich in ammonia and partially digested organic matter and thus provides a good substrate for growth of microorganisms. This kind of significant increase ($P < 0.05$) of microbial population in casts has been reported by Jayakumar *et al.* (2009) and Prakash *et al.* (2009) during vermicomposting of pressmud and paper mill sludge. This clearly indicated that the organic substrates used in the present study could initiate the proliferation of the microorganisms and the earthworm species used in the present study also acted as a medium for the rapid microbial colonization whereby increases the microbial activity.

Enzyme activities in vermicasts and gut of *P. ceylanensis*

The activities of amylase, cellulase, invertase, phosphatase and protease in fresh vermicasts of *P. ceylanensis* showed higher activities when reared in LPL + PM substrate. In LPL + PM (1:1), 4.71, 7.12 and 8.63 mg glucose/g of oven dry samples incubated for 24 h was recorded whereas, significant lower activities were observed of these enzymes in SCT + PM (1:1). The activities of the enzymes cellulase, invertase and protease in vermicasts of *P. ceylanensis* were significantly higher in LPL + PM (**Table 9**).

The activity of these enzymes while ageing showed an increase up to 15-20 days and then declined (**Figs. 2, 3**). The activity of amylase, 4.71, 5.2, 5.5, 6.5, 7.3, 6.4 and 5.2 mg glucose/g of oven dry samples incubated for 24 h were recorded on 0, 5, 10, 15, 20, 25 and 30 days of ageing of vermicasts recovered from LPL + PM respectively. In the vermicasts recovered from both the substrates, cellulase showed highest activity at the end of 30 days. The activity of cellulase, 7.12, 7.5, 8.3, 9.2, 9.5, 9.1 and 8.7 2 mg glucose/g of oven dry samples incubated for 24 h for the vermicasts recovered from LPL + PM and 5.33, 6.3, 7.0, 9.0, 9.1, 8.2 and 7.1 mg glucose/g of oven dry samples incubated for 24 h for the vermicasts recovered from SCT + PM were recorded (**Figs. 2, 3**).

As observed from both the substrates, higher activity was shown by clitellate stage of *P. ceylanensis*. The activity of amylase, 1.20, 3.63 and 7.42 mg glucose/g of oven dry samples incubated for 24 h were recorded for juvenile, preclitellate and clitellate *P. ceylanensis* collected from LPL + PM substrate respectively. Similar trend was observed for cellulase, invertase, phosphatase and protease enzymes also (**Table 10**). In all the stages, invertase showed maximum activity when compared with other enzymes studied. The activity of invertase, 3.26, 6.58 and 11.56 mg glucose/g of oven dry samples incubated for 24 h were recorded for juvenile, preclitellate and clitellate *P. ceylanensis* collected from LPL + PM substrate respectively. Invariably, activity of all the enzymes in preclitellate worms were significantly higher than juveniles ($P < 0.05$), whereas, the activity of enzymes in clitellate worms showed significantly higher

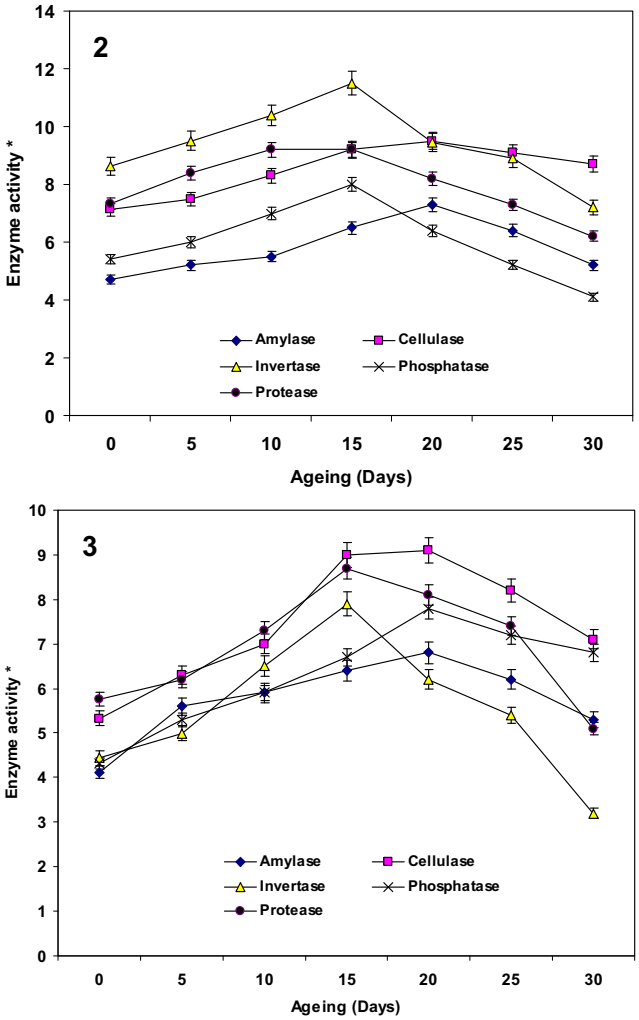

Fig. 2 Enzyme activities during ageing of vermicasts collected from LPL+PM (1:1). *Activities of amylase, cellulase and invertase are expressed as mg of glucose/g of oven dry samples for 24 h of incubation; Protease activity: mg of glutamic acid/g of oven dry substrates for 24 h incubation; Phosphatase activity: mg/phenol/g of oven dry substrates for 24 h of incubation; Error bars indicate ± SD of six replicates.

Fig. 3 Enzyme activities during ageing of vermicasts collected from SCT + PM (1:1). *Activities of amylase, cellulase and invertase are expressed as mg of glucose/g of oven dry samples for 24 h of incubation; Protease activity: mg of glutamic acid/g of oven dry substrates for 24 h incubation; Phosphatase activity: mg/phenol/g of oven dry substrates for 24 h of incubation; Error bars indicate ± SD of six replicates.

Table 10 Enzyme activities in the gut of different stages of *P. ceylanensis* collected from LPL + PM (1 : 1).

Enzymes	Stages of *P. ceylanensis*		
	Juvenile	Preclitellate	Clitellate
Amylase	1.20 a	3.63 b	7.42 c
Cellulase	2.30 a	5.46 b	9.13 c
Invertase	3.26 a	6.58 b	11.56 c
Phosphatase	1.42 a	3.90 b	8.03 b
Protease	3.10 a	6.43 b	12.57 c

Activities of amylase, cellulase and invertase are expressed as mg of glucose/g of oven dry samples for 24 h of incubation; Protease activity: mg of glutamic acid/g of oven dry substrates for 24 h incubation; Phosphatase activity: mg/phenol/g of oven dry substrates for 24 h of incubation; Mean values followed by same letters are not significantly different from each other at 5% level (ANOVA, $P < 0.05$)

activity than juveniles and preclitellates. The clitellate *P. ceylanensis* collected from SCT + PM showed 6.82, 8.90, 10.83, 8.60 and 11.89 mg/g activity of amylase, cellulase, invertase, phosphatase and protease respectively (**Table 11**).

Lattaud *et al.* (1997) observed that the study of the specific glucoside activities conducted in *Polypheretima elongata* revealed major amylase and maltase activities which

Table 11 Enzyme activities in the gut of different stages of *P. ceylanensis* collected from SCT + PM (1 : 1).

Enzymes	Stages of *P. ceylanensis*		
	Juvenile	Preclitellate	Clitellate
Amylase	0.93 a	2.30 b	6.82 c
Cellulase	2.10 a	5.61 b	8.90 c
Invertase	2.68 a	6.12 b	10.83 c
Phosphatase	1.56 a	4.33 b	8.60 b
Protease	2.96 a	5.80 b	11.89 c

Activities of amylase, cellulase and invertase are expressed as mg of glucose/g of oven dry samples for 24 h of incubation; Protease activity: mg of glutamic acid/g of oven dry substrates for 24 h incubation; Phosphatase activity: mg/phenol/g of oven dry substrates for 24 h of incubation; Mean values followed by same letters are not significantly different from each other at 5% level (ANOVA, $P < 0.05$)

showed that this earthworm is able to degrade starch, a root substrate, up to glucose. Marshall *et al.* (1981) attribute the increase in available P in the earthworm faeces to physical breakdown of the plant material and trituration of the mineral fraction for which the presence in casts of an increased proportion of fine particles provides support. Dehydrogenase is an intracellular enzyme linked to the respiratory electron transport system. Dehydrogenase activity plays an essential role in the initial stages of oxidation of soil organic matter. It is more dependent upon the metabolic state of the microbial population than the activity of free enzymes available in the soil (Tiwari *et al.* 1989). Lee (1985) reported increased enzyme activities and microbial populations in worm casts as compared with the underlying soil. Microorganisms and soil enzymes are closely associated with soil fertility; information on biological characteristics of worm casts can define the importance of worm casting activity in soil fertility. Application of vermicompost to the soil has multitude of advantages such as conservation of energy, increasing the fertilizer use efficiency, improvement of soil properties and enhancement of soil microbial activity.

Ranganathan and Vinotha (1998) reported that posterior gut of reproductively active, clitellate stage of the compost worm, *E. eugeniae* when reared in PM exhibits enhanced amylase, protease, acid and alkaline phosphatase and cellulase activity compared to the activity of these enzymes observed in immature, pre-clitellate stage worms reared in the same media. In a similar work, Lakshmi Prabha *et al.* (2007) reported that the gut of mature *E. eugeniae* and *E. fetida* contain higher activity of amylase, cellobiase, endoglucanase, acid phosphatase and nuclease enzymes. The expression of alkaline phosphatase in developing embryo and mature stages of the earthworm, *Eisenia andrei* was investigated by Park *et al.* (1996). It has been reported that cellulase, amylase, invertase, protease and phosphatase activities in PM and vermicasts of fresh, 15- and 30-day-old casts of *L. mauritii* and *E. eugeniae* decreased considerably with reference to ageing (Parthasarathi and Ranganathan 2000).

Digestive enzymes of *Perionyx millardi* have been assayed by Mishra (1993) and her result shows the presence of protease, cellulase, amylase, invertase and urease. The earthworm showed maximum activity for protease and amylase, minimum activity for invertase, cellulase and urease in comparison to other tropical earthworms. Further, the activities of microorganisms in earthworm gut and vermireactors are related to enzyme activities in vermicompost (Kumar *et al.* 2010b). Benítez *et al.* (2005) reported that β-glucosidase, phosphatase and urease activities of the organic extracts either increased or remained the same after a nine month period of vermicomposting.

The results obtained by Aira *et al.* (2005) showed that ageing favoured the release of microbial retained N, mainly as dissolved organic nitrogen, and which was associated with the high protease activity observed. In addition, found an age-dependent decrease in both microbial biomass and activity, which were stimulated by the addition of glucose. Inoculation of fungi in to the substrates, water hyacinth and cow manure during predecomposition for vermicomposting significantly increased cellulase, protease and acid and alkaline phosphatase activities (Pramanik 2010). In the present study reduction in the cellulase was observed in aged 15 and 30 days old casts due to lowered organic matter and reduced microbial population and activity (Parthasarathi *et al.* 1997; Parthasarathi and Ranganathan 1998, 2000). The lost of moisture in aged casts is also another factor for the reduced enzyme activity. The reasons for more enzyme activities in the casts of *P. excavatus* are: (i) greater consumption rate, (ii) enhanced gut microbial population (Parthasarathi and Ranganathan 1998 and 2000) and (iii) enhanced microbial population in the casts (Parthasarathi *et al.* 1997; Pramanik 2010) and (iv) more moisture (Jayakumar *et al.* 2009). The triggering of microbial growth in association with the decomposition process of SCT and LPL mixed with PM by *P. ceylanensis* action might be the reason for enhanced enzyme activities. The elevated enzyme activity in the gut of clitellate *P. ceylanensis* is because of the worm size. Normally the clitellate is the adult stage of earthworm which is larger in size, shows higher activity in both organic matter consumption and reproduction.

CONCLUSION

The vermicomposting potential of *P. ceylanensis* over two different organic substrates in proper combination with PM could result in the production of nutrient-rich vermicompost. The organic substrates mixed with PM in 50:50 (1:1) proportion was suitable for this earthworm for efficient vermiconversion. The study clearly indicates that the organic materials, LPL and SCT in combination with PM (1:1) could help the earthworms to increase their population and vermiconversion efficiency. The data regarding the enzymatic analysis of vermicasts and worm gut obtained in the present study reveals that the action of earthworms enhances the enzymatic activity and hence the vermicompost can be applied to the soil as fertilizer, definitely it would help to improve the soil biological activity. Since the clitellate stage of *P. ceylanensis* showed higher enzymatic activity, clitellate stage of this earthworm species can be used as an inoculum.

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REFERENCES

Aira M, Monroy F, Domínguez J (2005) Ageing effects on nitrogen dynamics and enzyme activities in casts of *Aporrectodea caliginosa* (Lumbricidae). *Pedobiologia* **49**, 467-473

Benitez E, Nogales R, Elvira C, Masciandaro G, Ceccanti B (1999) Enzyme activities as indicators of the stabilization of sewage sludges composting with *Eisenia fetida*. *Bioresource Technology* **67**, 297-303

Benitez E, Saniz G, Nogales R (2005) Hydrolytic enzyme activities of extracted humus substances during the vermicomposting of a lignocellulosic olive waste. *Bioresource Technology* **96**, 785-790

Daniel T, Sivasankari B, Malathy M (2010) Microbial and nutrient enhancement of *Gliricidia sepium* and *Leucaena leucocephala* leaf materials using *Eisenia fetida*. In: Karmegam N (Ed) Vermitechnology II. *Dynamic Soil, Dynamic Plant* **4 (Special Issue 1)**, 152-154

Domínguez J (2004) State-of-the art and new perspectives on vermicomposting research. In: Edwards CA (Ed) *Earthworm Ecology* (2nd Edn), CRC Press, Boca Raton, pp 401-424

Edwards CA, Arancon NQ (2004) The use of earthworms in the breakdown of organic wastes to vermicomposts and animal feed proteins. In: Edwards CA (Ed) *Earthworm Ecology* (2nd Edn), CRC Press, Boca Raton, pp 345-380

Gajalakshmi S, Ramasamy EV, Abbasi SA (2005) Composting–vermicomposting of leaf litter ensuing from the trees of mango (*Mangifera indica*). *Bioresource Technology* **96**, 1057-1061

Galstyan ASH (1965) Method of determining activity of hydrolytic enzymes of soil. *Pochvovedenie* **2**, 68-74

Garg VK, Kaushik P (2005) Vermistabilization of textile mill sludge spiked with poultry droppings by an epigeic earthworm *Eisenia foetida*. *Bioresource Technology* **96**, 1189-1193

Garg VK, Kaushik P, Dilbaghi N (2006) Vermiconversion of waste water

sludge from textile spiked with anaerobically digested biogas plant slurry employing *Eisenia foetida*. *Ecotoxicology and Environmental Safety* **65**, 412-419

Janossy G (1963) Changes in the phosphatase activity of soil microorganisms in relation to phosphorus supply. *Agrokemia es Talajtan* **12**, 285-292

Jayakumar M, Karthikeyan V, Karmegam N (2009) Comparative studies on physico-chemical, microbiological and enzymatic activities of vermicasts of the earthworms, *Eudrilus eugeniae*, *Lampito mauritii* and *Perionyx ceylanensis* cultured in pressmud. *International Journal of Applied Agricultural Research* **4 (1)**, 75-85

John Paul JA (2005) Municipal solid waste generation, characterization, microbial activity, vermicomposting and management in Dindigul Town. PhD thesis, The Gandhigram Rural Institute-Deemed University, Gandhigram, Tamil Nadu, India, 220 pp

John Paul JA, Karmegam N, Daniel T (2011) Municipal solid waste (MSW) vermicomposting with an epigeic earthworm, *Perionyx ceylanensis* Mich. *Bioresource Technology* **102**, 6769-6773

Kannan N (1996) *Laboratory Manual in General Microbiology*, Palani Paramount Publication, Palani, India, 315 pp

Karmegam N, Daniel T (2009a) Investigating efficiency of *Lampito mauritii* (Kinberg) and *Perionyx ceylanensis* Michaelsen for vermicomposting of different types of organic substrates. *The Environmentalist* **29**, 287-300

Karmegam N, Daniel T (2009b) Growth, reproductive biology and life cycle of the vermicomposting earthworm, *Perionyx ceylanensis* Mich. (Oligochaeta: Megascolecidae). *Bioresource Technology* **100**, 4790-4796

Karmegam N, Daniel T (2011) Biodiversity of earthworms in India: Utility, threats and conservation. In: Daniel T (Ed) *Proceedings of National Conference on Biodiversity: Richness, Uses, Threats and Conservation*, 19-21 January 2011, The Gandhigram Rural Institute-Deemed University, Gandhigram, India, pp 41-45

Kumar R, Verma D, Singh BL, Kumar U, Shweta (2010a) Composting of sugar-cane waste by-products through treatment with microorganisms and subsequent vermicomposting. *Bioresource Technology* **101**, 6707-6711

Kumar R, Singh BL, Kumar U, Verma D, Shweta (2010b) *Drawida willsi* Michalsen activates cellulolysis in pressmud vermireactor. *Bioresource Technology* **101**, 9086-9091

Lakshmi Prabha M, Jayaraaj IA, Jeyaraaj R, Rao S (2007) Comparative studies on the digestive enzymes in the gut of earthworms, *Eudrilus eugeniae* and *Eisenia fetida*. *Indian Journal of Biotechnology* **6**, 567-569

Lattaud C, Zhang BG, Locati S, Rouland C, Lavelle P (1997) Activities of the digestive enzymes in the gut and in tissue culture of a tropical geophagous earthworm, *Polypheretima elongata* (Megascolecidae). *Soil Biology and Biochemistry* **29**, 335-339

Lavelle P, Spain AV (2001) *Soil Ecology*, Kluwer Academic Publishers, London, pp 12-90

Le Bayon RC, Binet F (2006) Earthworm changes the distribution and availability of phosphorous in organic substrates. *Soil Biology and Biochemistry* **38**, 235-246

Lee KE (1985) *Earthworms - Their Ecology and Relationships with Soils and Land Use*, Academic Press, New York, 411 pp

Marshall GP, Syers JK, Gregg PEH (1981) Plant availability of phosphorus in dead herbage ingested by surface casting earthworms. *Soil Biology and Biochemistry* **13**, 163-167

Mishra SL (1993) Digestive enzymes of the tropical earthworm *Perionyx millardi*. *Journal of Ecobiology* **5 (2)**, 77-79

Mulongoy K, Bedoret A (1989) Properties of wormcasts and surface soils under various plant covers in the humid tropics. *Soil Biology and Biochemistry* **21**, 197-203

Munnoli PM, Teixeira da Silva JA, Bhosle S (2010) Dynamics of the soil-earthworm-plant relationship. In: Karmegam N (Ed) *Vermitechnology II. Dynamic Soil, Dynamic Plant* **4 (Special Issue 1)**, pp 1-21

Park SC, Parka HW, Smith TJ (1996) Expression of alkaline phosphatases during embryonic development and immature stages of the earthworm, *Eisenia andrei*. *Soil Biology and Biochemistry* **28**, 579-582

Parthasarathi K (2007) Influence of moisture on the activity of *Perionyx excavatus* (Perrier) and microbial–nutrient dynamics of pressmud vermicompost. *Iranian Journal of Environmental Health Science and Engineering* **4 (3)**, 147-156

Ranganathan LS, Parthasarathi K (2005) Humification of cane sugar mill wastes by *Eudrilus eugeniae* (Kinberg). *Journal of Annamalai University* **41**, 1-8

Parthasarathi K, Ranganathan LS (1998) Pressmud vermicasts are 'hot spots' of fungi and bacteria. *Ecology, Environment and Conservation* **4**, 81-86

Parthasarathi K, Ranganathan LS (2000) Aging effect on enzyme activities in pressmud vermicasts of *Lampito mauritii* (Kinberg) and *Eudrilus eugeniae* (Kinberg). *Biology and Fertility of Soils* **30**, 347-350

Parthasarathi K, Ranganathan LS, Anandi V (1997) Predation of fungi by *Lampito mauritii* (Kinberg) reared on different substrates. *Indian Journal Microbiology* **37**, 41-42

Prakash M, Jayakumar M, Karmegam N (2009) Vermistabilization of paper mill sludge using the earthworm *Perionyx ceylanensis*: Influence on physico-chemical and microbiological status. *Indian Journal of Applied Microbiology* **10**, 20-25

Prakash M, Karmegam N (2010a) Vermistabilization of pressmud using *Perionyx ceylanensis* Mich. *Bioresource Technology* **101**, 8464-8468

Prakash M, Karmegam N (2010b) Dynamics of nutrients and microflora during vermicomposting of mango leaf litter (*Mangifera indica*) using *Perionyx ceylanensis*. *International Journal of Global Environmental Issues* **10**, 339-353

Pramanik P (2010) Changes in enzymatic activities and microbial properties in vermicompost of water hyacinth as affected by pre-composting and fungal inoculation: A comparative study of ergosterol and chitin for estimating fungal biomass. *Waste Management* **30**, 1472-1476

Ranganathan LS, Vinotha SP (1998) Influence of pressmud on the enzymatic variations in the different reproductive stages of *Eudrilus eugeniae* (Kinberg). *Current Science* **74**, 634-635

Sangwan P, Kaushik CP, Garg VK (2008) Feasibility of utilization of horse dung spiked filtercake in vermicomposters using exotic earthworm *Eisenia fetida*. *Bioresource Technology* **99**, 2442-2448

Sangwan P, Kaushik CP, Garg VK (2010) Vermicomposting of sugar industry waste (press mud) mixed with cow dung employing an epigeic earthworm *Eisenia fetida*. *Waste Management Research* **28**, 71-75

Sarath G, Dela Motte RS, Wagner FW (1989) *Proteolytic Enzymes: A Practical Approach*, IRL Press, Oxford, England, pp 25-55

Suthar S (2007) Vermicomposting potential of *Perionyx sansibaricus* (Perrier) in different waste materials. *Bioresource Technology* **98**, 1231-1237

Suthar S (2009) Vermicomposting of vegetable-market solid waste using *Eisenia fetida*: Impact of bulking material on earthworm growth and decomposition rate. *Ecological Engineering* **35**, 914-920

Suthar S, Singh S (2008) Vermicomposting of domestic waste by using two epigeic earthworms (*Perionyx excavatus* and *Perionyx sansibaricus*). *International Journal of Environmental Science and Technology* **5 (1)**, 99-106

Tabatabai MA (1982) Soil enzymes in methods of analysis, Part-2. *Agronomy* **9**, 903-947

Tandon HZ (1993) *Methods of Analysis of Soils, Plant, Water and Fertilizers*, Fertilizer Development and Consultation Organization, New Delhi, 148 pp

Tiwari SC, Tiwari BK, Mishra RR (1989) Microbial population, enzyme activities and nitrogen-phosphorus-potassium enrichment in earthworm casts and in the surrounding soil of a pineapple plantation. *Biology and Fertility of Soils* **8**, 178-182

Walkley A, Black IA (1934) An examination of the Degtjareff method for determining soil organic matter and proposed modification of the chromic acid titration method. *Soil Science* **34**, 29-38

Yadav A, Garg VK (2011a) Recycling of organic wastes by employing *Eisenia fetida*. *Bioresource Technology* **102**, 2874-2880

Yadav A, Garg VK (2011b) Vermicomposting – An effective tool for the management of invasive weed *Parthenium hysterophorus*. *Bioresource Technology* **102**, 5891-5895