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Short Communication

Vermistabilization of pressmud using *Perionyx ceylanensis* Mich.

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ABSTRACT

Sugar industry-derived pressmud was mixed with an equal amount of cow dung (1:1), and vermicomposted with *Perionyx ceylanensis* Mich. The resultant vermicompost had a pH of 7.33, electrical conductivity of 2.32 dS/m, a nitrogen, phosphorus and potassium content of 1.63%, 2.38%, and 3.13%, respectively, an organic carbon content of 29% and a C/N ratio of 17.89. The increase of NPK in vermicompost over worm-free compost was 36.94%, 28.56%, and 20.82%, respectively. The populations of bacteria, actinomycetes and fungi increased in the compost in the presence of the earthworms. In the worm guts, the microbial populations were highest in the midgut. Correlation of microbial population increase with duration of vermicomposting was statistically significant at $P > 0.05$ ($r = 0.973$, 0.99, and 0.993, respectively for bacteria, fungi, and actinomycetes). The changes in total bacterial, fungal, and actinomycetes populations positively correlated with duration of vermicomposting. The study indicates that pressmud can be effectively converted into nutrient- and microorganism-rich vermicompost with *P. ceylanensis* when mixed with cow dung in 1:1 ratio.

1. Introduction

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). During vermicomposting, temperature and moisture can act synergistically (Gunadi et al., 2003). 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 (Domínguez, 2004). The organic matter that passes through the earthworm gut results in increased levels of microbial populations, activity, and respiration, and of enzymatic activity and micro and macro nutrients (Kalam et al., 2004).

About 12 million tons of pressmud (filter cake), a major by-product of sugarcane processing, is produced annually (Parthasarathi, 2007). It consists of residue obtained from sedimentation of suspended materials such as fiber, wax, ash, soil, and other particles in cane juice. Pressmud has previously been shown to be a substrate suitable for vermicomposting (Parthasarathi and Ranganathan, 2002). The relationships between earthworms and microorganisms are keys to understand how these processes occur. However, much of the work in microbial-earthworm interactions has focused on their functional significance, and little is known about the effects of earthworms on microbial diversity. The data on the structure of microbial communities in vermiculture systems with regard to the earthworm species *Perionyx ceylanensis* is scanty. Since the vermicomposting is carried out jointly by earthworms and microorganisms, it is vital to study the total microbial population in vermiconversion systems. Hence in the present study, microbial dynamics during vermiconversion and storage of vermicompost has been studied.

Most vermicomposting experiments have used epigeic earthworm species because they possess higher potential for vermiconversion. *P. ceylanensis* was selected as suitable species for vermicomposting since it is a native species with vermicomposting potential and short life cycle (Karmegam and Daniel, 2009a, b). The present study utilizes *P. ceylanensis* in order to evaluate their vermicomposting potential under tropical conditions with reference to the organic substrate, pressmud – a sugar industry waste by-product.

2. Methods

2.1. Pressmud and earthworms

Pressmud (PM) was collected from the Co-operative Sugar Mills Ltd (Thiruvannamalai district, Tamil Nadu). *P. ceylanensis* Mich., obtained from the Department of Biology, Gandhigram Rural University, Tamil Nadu, India, was cultivated in cow dung (CD).

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2.2. Vermicomposting

The PM was subjected to preliminary vermicomposting trials in 45 × 35 × 15 cm plastic troughs containing 2 kg of PM and CD at ratios of 25:75, 50:50, and 75:25 (wt/wt), in six replicates. Control sets were also maintained without earthworms for all the combinations. The materials were maintained at 27 ± 1 °C (Karmegam and Daniel, 2009a) with a moisture content of 70 ± 5% and mixed and turned once every 15 days. Sixty *P. ceylanensis*, were placed in each trough covered with wire mesh. The amount of total organic carbon (TOC) and total nitrogen were analysed once every 10 days. Based on the outcome of the preliminary trials, PM mixed with CD in 50:50 proportions was vermicomposted for 60 days in six replicates under controlled conditions as maintained for preliminary vermicomposting trial. The physico-chemical characteristics of initial vermibed substrates, final control (worm-un-worked) and vermicompost were analysed as per standard procedures given below and the results were statistically interpreted that includes Students 't' test and ANOVA.

2.3. Physico-chemical analysis

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. Total organic carbon (TOC) was measured using the method of Walkley and Black (1934). Total Kjeldahl Nitrogen (TKN) was determined after digesting the sample with concentrated H_2SO_4 and concentrated $HClO_4$ (9:1, v/v) (Tandon, 1993). Total phosphorus (TP) was analysed using colorimetric method with molybdenum in sulphuric acid (Tandon, 1993). Total potassium (TK) and total calcium (TCa) 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-un-worked substrates was calculated [$(A - B/A) \times 100$; where A = values in the worm-worked substrate, B = values in the worm-un-worked substrate].

2.4. Microbiological analysis

One gram of each sample was transferred to test tubes containing sterilized water, mixed thoroughly with a vortex mixer for 20 min, serially diluted and 1-mL aliquots were plated in triplicate. This was used as inoculum and 1.0 mL was plated in triplicate on Nutrient agar media, Rose Bengal agar and Kenknight's media for the enumeration of bacteria, fungi and actinomycetes, respectively using pour plate method, and incubated for 24, 72 h and one week. For microbial counts of gut contents, the earthworms were surface disinfected with 25 ppm sodium hypochlorite for 10 min before dissection. The gut was divided into three portions, foregut, midgut, and hindgut based on the segment numbers. Using a sterile scalpel, forceps, and sterile knife under aseptic condition, the gut contents of each section were squeezed into sterile test tubes, and 1 g was diluted in 0.9% NaCl saline and microbial counts were determined.

2.5. Statistical analyses

Correlation of microbial population with reference to the duration of vermicomposting was analysed to know the total microbial dynamics during the process. The harvested vermicompost was stored for 45 days in gunny bags. The total microbial counts were done every five days up to 45 days using pour plate method. The correlation between microbial population and storage period was calculated using the computer software, Microcal Origin (Version 6.0).

3. Results and discussion

3.1. Vermicomposting

The results of the preliminary vermicomposting trials showed that in the absence of earthworms, the C/N ratio in the PM + CD mixtures declined only slowly whereas 31.89–44.64% reductions were observed in the presence of the worms (Fig. 1). Since PM + CD at a 50:50 ratio experienced the greatest decrease in the C/N ratio, vermicomposting was carried out with this substrate mix over a period of 60 days. The values for various parameters of the initial substrates and composted materials are listed in Table 1. As also noted by Garg and Kaushik (2005) and Sangwan et al. (2008), a decrease in pH was observed in the substrate 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 (Gunadi et al., 2003).

The physico-chemical characteristics of the compost inhabited by the worms (WOW) and those of compost without worms (WUW) are different. The pH showed a slight reduction in the WOW when compared with the WUW but the difference was not significant. The E.C., NPK, Ca, Mg, Fe, and Cu in WOW were higher than those of WUW, whereas, pH, OC, Na, Mn, S, Zn, C/P, and C/N were lower (Table 1). The NPK contents were higher in WOW (N – 1.63%; P – 2.38%; and K – 3.13%) than in WUW (N – 1.03%; P – 1.70%; and K – 2.48%). The lower C/N ratio is due to the reduction of organic carbon due to the respiratory activity of earthworms and

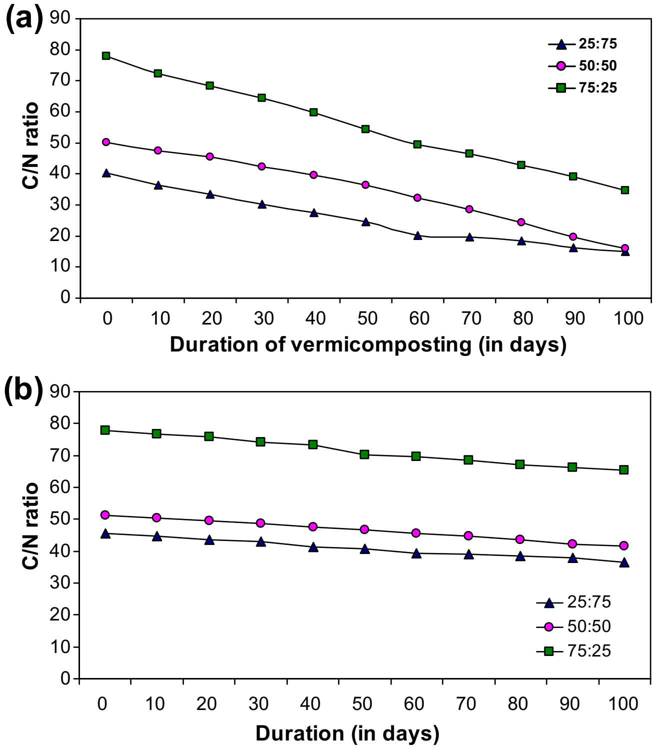

Fig. 1. Change of C/N ratio during bioconversion of PM + CD in three different combinations (a) with and (b) without (control) *P. ceylanensis* (100 days).

Table 1
Physico-chemical characteristics of PM + CD (1:1) substrate subjected to vermicomposting with *P. ceylanensis* (60 days).

Parameters	Initial	Compost[a]		Percent (%)[b] increase/ decrease of WOW over WUW
		WUW	WOW	
pH	7.45 ± 0.04	7.38 ± 0.21	7.33 ± 0.03	-0.77^{NS}
Electrical conductivity (dS/m)	1.13 ± 0.08	1.39 ± 0.08	2.32 ± 0.31	35.72^{**}
Organic carbon (%)	54.41 ± 0.23	43.77 ± 1.88	29.00 ± 1.10	-50.92^{***}
Nitrogen (%)	0.94 ± 0.03	1.03 ± 0.03	1.63 ± 0.16	36.94^{**}
Phosphorous (%)	1.50 ± 0.17	1.70 ± 0.08	2.38 ± 0.10	28.56^{*}
Potassium (%)	2.12 ± 0.07	2.48 ± 0.05	3.13 ± 0.21	20.82^{NS}
Calcium (%)	1.78 ± 0.02	1.89 ± 0.03	2.91 ± 0.06	34.92^{**}
Sodium (%)	0.30 ± 0.02	0.38 ± 0.01	0.54 ± 0.04	30.12^{*}
Magnesium (%)	0.20 ± 0.01	0.26 ± 0.03	0.33 ± 0.02	21.43^{NS}
Manganese (ppm)	10.18 ± 0.49	13.18 ± 0.68	17.17 ± 1.83	23.25^{NS}
Sulphur (%)	0.15 ± 0.02	0.20 ± 0.01	0.31 ± 0.02	33.88^{**}
Iron (ppm)	162.58 ± 2.83	171.97 ± 4.98	214.50 ± 8.57	19.83^{NS}
Copper (ppm)	8.00 ± 0.89	10.29 ± 0.37	15.67 ± 0.82	34.32^{**}
Zinc (ppm)	30.59 ± 0.82	35.25 ± 0.52	44.67 ± 2.42	21.09^{NS}
C/N	57.93 ± 1.91	34.82 ± 1.29	17.89 ± 1.72	-94.41^{***}
C/P	36.58 ± 4.14	22.59 ± 2.05	12.22 ± 0.91	-84.84^{***}

*, **, *** and NS indicates statistically significant difference at $p < 0.05$, $p < 0.01$, $p < 0.001$ and not significant by Student's 't' test.

[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.

Table 2
Range of C/N ratio of vermicomposts prepared with pressmud and other organic amendments using different species of earthworms.

Substrate combination	Species used	Days of vermicomposting	C/N ratio of vermicompost	Reference
BPS + PM (60:40)	*E. fetida*	75	17.1	Sangwan et al., 2008
BPS + PM (50:50)	*E. fetida*	75	19.9	Sangwan et al., 2008
PM	*E. eugeniae*	30*	16.0	Jayakumar et al., 2009
PM	*L. mauritii*	30*	19.4	Jayakumar et al., 2009
PM	*P. ceylanensis*	30*	18.0	Jayakumar et al., 2009
PM + CD (40:60)	*E. fetida*	75	17.3	Sangwan et al., 2010
PM + CD (50:50)	*E. fetida*	75	17.3	Sangwan et al., 2010
PM + CD (50:50)	*P. ceylanensis*	60	17.9	Present study

BPS – Biogas plant slurry.

* Vermicasts.

microorganisms, and mineralization of organic materials (Karmegam and Daniel, 2009a; Sangwan et al., 2010). Although comparisons are difficult due to different length of composting and substrate compositions, the C/N ratio achieved in the current study is similar to those observed in vermicomposting of pressmud with *Eisenia fetida, Eudrilus eugeniae, Lampito mauritii*, and *P. ceylanensis* (Table 2).

The increased level of P during vermicomposting is due to earthworm-gut derived phosphatase activity and also increased microbial activity in the cast. The presence of large number of microflora in the gut of earthworm might play an important role in increasing P and K content in the process of vermicomposting (Kaviraj and Sharma, 2003). The elevated level of Zn, Mn, and Fe in vermicompost indicates accelerated mineralization with selective feeding by earthworms on materials containing these metals. The total amounts of heavy metals increased as a consequence of the carbon losses by mineralization during vermicomposting (Domínguez, 2004). Increased levels of macro- and micro-nutrients in vermicomposts were also observed by Suthar (2007).

3.2. Total microbial populations in vermicompost and worm guts

The total microbial population in the vermicompost increased from day 0 until day 60 (Tables 3) and was significantly different ($P < 0.05$). This observation parallels that of Parthasarathi (2007) who reported increased microbial population, microbial activity and NPK content in the vermicompost at 31 °C and 60–70% moisture during vermicomposting of sugar industrial wastes. An increased number of bacteria, fungi and actinomycetes in the WOW compared to the WUW were observed (Table 3). Thus *P. ceylanensis* contributed to the increase of the microbes of the organic matter. Similar increases were also observed in other vermicomposts (Parthasarathi, 2007; Karmegam and Daniel, 2009a; Prakash et al., 2009).

The total microbial population in foregut, midgut, and hindgut of *P. ceylanensis* increased by the end of the experiment (Table 4). The bacterial, fungal, and actinomycetes populations were higher in the midgut region than in the foregut and hindgut region. The selective activity of the gut fluid of earthworms could be a significant factor for the animal's nutrition as well as for regulating the steady state of the intestinal microbial community, and modification of microbial communities in soil (Byzov et al., 2007). Idowu

Table 3
Total microbial population dynamics during vermicomposting of PM + CD (1:1) with *P. ceylanensis.*

Vermicomposting days	Total microbial population*		
	Bacteria ($CFU \times 10^6\ g^{-1}$)	Fungi ($CFU \times 10^3\ g^{-1}$)	Actinomycetes ($CFU \times 10^4\ g^{-1}$)
0	42.67 ± 1.80^{a}	53.50 ± 1.98^{a}	42.17 ± 1.86^{a}
15	50.50 ± 1.38^{a}	62.0 ± 3.27^{a}	$46.50 \pm 1.61^{a,b}$
30	60.33 ± 1.37^{a}	69.33 ± 1.89^{a}	$53.67 \pm 1.37^{a,b}$
45	87.0 ± 1.91^{b}	$75.83 \pm 1.67^{a,b}$	$62.17 \pm 1.46^{b,c}$
60	107.33 ± 3.77^{b}	89.67 ± 2.13^{b}	66.0 ± 1.63^{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 4
Total microbial population in the gut of *P. ceylanensis* cultured in PM + CD (1:1) for 60 days.

Microbial population	Total microbial population in the gut of *P. ceylanensis*[*]					
	Foregut		Midgut		Hindgut	
	Initial	Final	Initial	Final	Initial	Final
Bacteria (CFU × 10^6 g^{-1})	42.67 ± 1.80	152.33 ± 5.82	36.50 ± 3.45	209.33 ± 18.32	43.00 ± 2.16	142.83 ± 12.12
Fungi (CFU × 10^3 g^{-1})	53.50 ± 1.98	160.33 ± 10.34	80.83 ± 5.49	233.50 ± 19.71	54.17 ± 2.34	171.67 ± 12.19
Actinomycetes (CFU × 10^4 g^{-1})	42.17 ± 1.86	123.83 ± 8.84	62.33 ± 1.89	165.17 ± 3.02	35.67 ± 2.36	117.50 ± 8.60

[*] Values are mean of six replicates ± SD.

et al. (2006) reported that the aerobic bacterial counts in midgut of the earthworm, *Libyodrillus violaceous* was higher than that of foregut whereas the hindgut region recorded maximum. In the present study, midgut of *P. ceylanensis* showed higher counts than foregut and hindgut. According to Domínguez (2004), during vermicomposting earthworms fragment and homogenize the ingested material through muscular action of their foregut and also add mucus and enzymes to ingested material and thereby increase the surface area for microbial action while, microorganisms perform the biochemical degradation of waste material providing some extracellular enzymes required for organic waste decomposition within the worm's gut. The higher microbial count in midgut recorded in the present study may be due to the increased surface area of ingested materials in foregut of *P. ceylanensis*. More studies are required to confirm the exact mechanism for this increase.

3.3. Correlation of microbial population in the vermicompost

The total bacterial, fungal and actinomycetes population changes in vermicompost of PM + CD (1:1) positively correlated with that of duration of vermicomposting (Fig. 2). Through the duration of vermicomposting, vermicompost of PM + CD, the bacterial population increased from 0th day to 60th day. The correlation of bacterial population increase with the duration of vermicomposting was statistically significant ($y = 1.105x + 36.40$); ($r = 0.973$) ($p > 0.05$). Similar results were observed for fungal and actinomycetes population in vermicompost of PM + CD upon duration of vermicomposting (days) (Fig. 2).

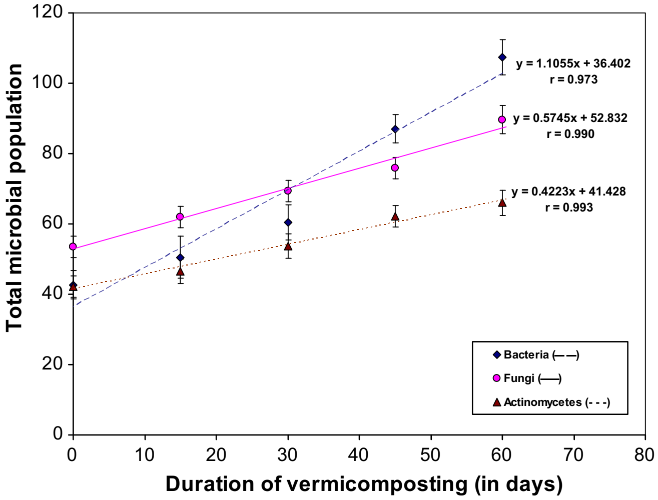

Fig. 2. Correlation of total microbial population (bacteria = CFU × 10^6 g^{-1}; fungi = CFU × 10^3 g^{-1}; actinomycetes = CFU × 10^4 g^{-1}) as a function of duration of vermicomposting PM + CD (1:1, 60 days) using *P. ceylanensis*.

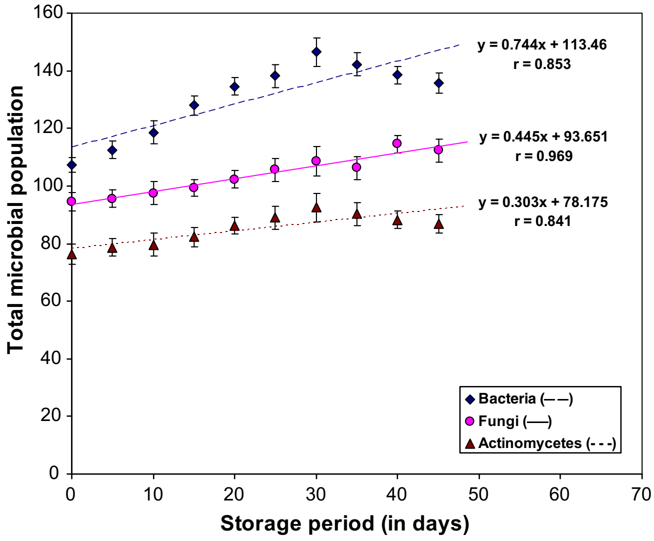

Fig. 3. Correlation of total microbial population (bacteria = CFU × 10^6 g^{-1}; fungi = CFU × 10^3 g^{-1}; actinomycetes = CFU × 10^4 g^{-1}) as a function of storage period of vermicompost (45 days) prepared with PM + CD (1:1) using *P. ceylanensis*.

The total bacterial, fungal and actinomycetes population changes in vermicompost of PM + CD (1:1) positively correlated with that of storage period. During the storage of vermicompost of PM + CD the actinomycetes population increased up to 30 days and then slightly declined. The actinomycetes colony forming units (CFU) of 76 × 10^4 g^{-1} on 0th day increased up to 92 × 10^4 g^{-1} on 30th day and decreased to 87 × 10^4 g^{-1} on 45th day of storage (Fig. 3). The correlation of actinomycetes population increase with storage period was statistically significant ($r = 0.841$) ($p > 0.05$). Similar results were observed for bacteria and fungal population in vermicompost of PM + CD upon storage (Fig. 3). Prakash et al. (2008) reported that in both vermicast and in control, the fungal population increased up to 20–30 days of incubation and afterwards showed slight decrease till 45th day. Further their study showed that the correlation between the total fungal population in vermicast with incubation period showed significant ($p < 0.05$) positive correlation. The results of the present study also fall in line.

The utilization of pressmud in the vermicomposting systems can be possible to transfer this waste into valuable end product, vermicompost. The study recommends that cow dung and pressmud in 1:1 is suitable mix for making vermicompost rich in nutrients and microorganisms which can be used as suitable organic soil amendment.

4. Conclusion

The findings of the present study reinforce the general concept that the vermicomposts and gut of earthworms tend to be much more microbiologically active than the worm-un-worked substrates. A combination of pressmud and cow dung in a 1:1 ratio was suitable to produce a valuable organic soil amendment through vermiconversion with *P. ceylanensis* within 60 days.

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