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Bioremediation of Pulp and Paper Industry Secondary Sludge Spiked with Cow Dung and Effective Microorganisms Using Epigeic Earthworm *Eudrilus eugeniae* (Kinberg)

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ABSTRACT

The aim of this study was to evaluate the vermicompost formed from three different mixing ratios (T1 (1:1), T2 (2:1), T3 (3:1)) of pulp and paper industry secondary sludge with a fixed quantity of saw dust used as a carbon source and spiked with cow dung and effective microorganisms (*Rhodopseudomonas*, *Lactobacillus* and *Saccharomyces* spp.) by using it as an earthworm feed. The epigeic earthworm *Eudrilus eugeniae* (Kinberg) was used in this study to stabilise the waste under laboratory conditions. Efficacy of the resulting compost in supporting plant growth was also tested with tomato (*Lycopersicum esculentum*) F1 hybrid Ruchikar seeds. At the end of the vermicomposting process, the vermibeds showed a significant decrease in the total organic carbon (10 to 14%) content and increase in total nitrogen (51-68%), phosphorous (170-206%) and potassium (127-180%). The content of heavy metals (Pb, Cd, Ni and Cu) was lower in the final product than in initial feed mixtures. Phytotoxicity tests like germination index and vigour index showed that the resulting composts were less phytotoxic. Among the three treatments, T2 (pulp and paper sludge mixed with sawdust in a ratio of 2:1 and spiked with cow dung and effective microorganisms) showed significantly higher ($P < 0.05$) seed vigour and germination index than the other two treatments T1 (1:1 ratio) and T3 (3:1 ratio). The vigour index values were: T1 (1225), T2 (1714) and T3 (1228). The order of germination index among three treatments was T2 (77) > T3 (66.5) > T1 (60). Thus the epigeic earthworm *E. eugeniae*, along with inocula like cow dung and effective microorganisms, can easily degrade pulp and paper industry waste.

Keywords: C/N ratio, *Eudrilus eugeniae*, phytotoxicity studies, sludge treatment, vermicomposting
Abbreviations: CD, cow dung; **EM**, effective microorganisms; **GI**, germination index; **PS**, pulp and paper industry sludge; **SD**, sawdust; **VI**, vigour index

INTRODUCTION

The solid waste from pulp and paper industry especially the treatment plant sludge is an important source of organic material and nutrients (Kunzler 2001). However, the production of large quantities of this organic waste poses major environmental (contamination of ground water and soil) and disposal problems. Therefore, the disposal of this waste has become very important issue for maintaining healthy environment. In general, waste water treatment sludge constitutes the largest residual waste stream generated by the pulp and paper mills in terms of volume (Krigstin and Sain 2006). The amount and the composition of this solid waste depend on the paper grade produced, the raw materials used, the process techniques applied and the paper properties to be achieved. Although, the sludge contains high levels of organic matter and nutrients like nitrogen, potassium, phosphorus and calcium etc., these components are generally not in forms that are directly or immediately available to crops (Sharma *et al*. 2002). Mineral release will only occur when the sludge is broken down by the micro-organisms or earthworms in the soil. It has been suggested that vermicomposting by earthworms are excellent method in the management of industrial sludge (Elvira *et al.* 1998). Therefore, stabilisation (vermicomposting) of the sludge prior to application can affect all the breakdown processes prior to application, providing a humus-rich, concentrated amendment with favourable physical and structural properties.

Various investigators have established the viability of this technology as a treatment system for agro-industrial sludge (Suthar 2010), beverage industry sludge (Singh *et al.* 2010) distillery sludge (Suthar 2007; Suthar and Singh 2008), food industry sludge (Yadav and Garg 2009), sago sludge (Banu *et al.* 2008a, 2008b), sugar mill sludge (Sangwan *et al.* 2008), tannery sludge (Cardoso-Vigueros and Ramirez-Camperos 2006; Dheepa *et al.* 2006) and textile industry sludge (Garg *et al*. 2006). Though several researches were concentrated on the various industrial sludge, rich in carbon content, only a few studies are currently available that report on vermicompost from pulp and paper mill sludge with earthworms (Umamaheswari and Vijayalakshmi 2003; Garg *et al.* 2006; Nath and Deb 2008; Kaur *et al.* 2010). Nath and Deb (2008) vermicomposted the mixture of different paper mill solid wastes using locally available earthworms. Kaur *et al.* (2010) studied the efficacy of *Eisenia fetida* in bioconverting paper mill sludge mixed with cattle dung. Garg and Kaushik (2005) studied the vermistabilization of textile mill sludge spiked with poultry droppings by an epigeic earthworm *Eisenia foetida*. In the present study we used secondary sludge of pulp and paper industry because of it being a rich source of nutrients and organic matters (Kunzler 2001); however, these components are generally not in the forms that are directly or immediately available to crops. Stabilisation of the secondary sludge in the form of vermicomposting, prior to the application can affect the breakdown of complex substances with least phytotoxicity. The vermicomposting process is a result of the combined action of the earthworms and microflora

Received: 4 December, 2010. Accepted: 12 April, 2011.

Original Research Paper

Table 1 Physico-chemical characteristics of the raw materials used for vermicomposting.

Parameters	Raw materials		
	Paper mill sludge	Saw dust	Cow dung
pH	6.8	5.3	7.23
EC μmhos/cm	3.5	0.4	0.80
TOC %	22.38	54.4	26.53
TKN %	0.95	0.26	0.89
K %	0.75	0.32	0.38
P %	0.24	0.52	0.48
VS %	40.30	98	31
COD mg/g	423	1245	554
Moisture content %	55	44.50	35
Chlorides %	2.32	BDL	BDL
Sulphates %	0.57	0.003	0.002
Lead mg/kg	8.1	BDL	0.6
Cadmium mg/kg	BDL	BDL	BDL
Nickel mg/kg	13.9	BDL	BDL
Chromium mg/kg	BDL	BDL	BDL
Copper mg/kg	36	6.2	31
Iron mg/kg	33.2	3.9	220.6
Manganese mg/kg	21.53	1.3	136.8
Zinc mg/kg	15.6	BDL	193.4

BDL- Below the detection limit

living in earthworm intestines and in the growth medium (Sivakumar *et al.* 2009). Sawdust normally used as co-composting material and as a carbon source can improve the microbial activity in the composting process. Thus, the aim of recycling the nutrients in the secondary sludge of pulp and paper industry, the present investigation was started to check the effect of different ratios of pulp and paper industry sludge (secondary sludge) and sawdust as earthworm (*Eudrilus eugeniae*) food with microbial inoculants and cow dung for vermicompost preparation on nutrient status and phytotoxicity status of the resulting vermicompost.

MATERIALS AND METHODS

General

The *E. eugeniae* (Kinberg) culture was obtained from Tamil Nadu Agricultural University, Coimbatore and stock culture was maintained in our laboratory for further studies using cow dung as food.

Raw materials

Fresh, dried and urine free cow dung (CD) used in this study was collected from the local dairy farm. The secondary sludge with 40-50% moisture content sludge (PS) was collected from a Pulp and Paper industry in Kagithapuram, Karur, India. The sludge was further dried for a week to reduce the moisture levels. The saw dust (SD) was collected from the saw mill located in Kavundampalayam, Coimbatore, India. Commercially available microbial inoculum (EM) used in this investigation was obtained from Sriram Biotech, Coimbatore, India. The composition of EM has *Rhodopseudomonas*, *Lactobacillus* and *Saccharomyces* spp. The EM was prepared as prescribed by the manufacturer (4 ml/kg) and used in composting as one of the amendment to accelerate the composting process. The physico-chemical characteristics of PS, SD and CD are recorded in the **Table 1**.

Experimental design

The substrates for vermicomposting were prepared in dry weight basis by mixing a 1:1 ratio of 2.5 kg of PS and 2.5 kg of SD (T1), 3.3 kg PS and 1.7 kg of SD for 2:1 (T2) ratio and 3.75kg of PS and 1.25 kg of SD for 3:1 (T3) ratio with a fixed quantity of CD (500 g) and EM in bins made of earthen material (dimensions 60 × 29 × 20 cm). All treatments were initially subjected to pile composting for a period of 15 days to reach the thermophilic temperatures, after which they were shifted to earthen containers for vermicomposting process. The adult and uniform size of the earthworm *E. eugeniae* (635 ± 52.3 mg of single worm weight) of 75 numbers were collected from stock cultures and introduced into the vermibins. Earthworms that did not burrow into the bins by the following day were removed and new ones were introduced. The moisture content was maintained around 40-50%. To prevent the sun light penetration, the bins were closed with an iron mesh lid and they were covered by dry leaves. Once a week, the moisture loss was compensated with the addition of 400 ml distilled water. Containers were placed in humid and dark place with a temperature of about 28.5 ± 0.5°C. The vermicomposting process was carried out for 30 days time period in all the treatments and the physico-chemical parameters were analysed in all the treatments at 7 days time interval. At the end of the vermicomposting period (90 days), all parameters were reached steady state. Therefore, we present here the results obtained on the 90th day. All the samples were analysed on dry weight basis in triplicates and results were averaged.

Chemical analyses

pH and electrical conductivity (EC) were measured in 1/50 (w/v) aqueous solution after 30 minutes stirring using digital pH meter (model no.335, Elico, India) and EC meter (CM 180, Elico, India) respectively. Chemical parameters like total organic carbon (TOC), total Kjeldhal nitrogen (TKN), potassium (K), phosphorous (P), C:N ratio and heavy metals (Pd, Cr, Cd, Ni and Cu) were measured for all the treatments before the introduction of the earthworms and after each 7 day interval, up to 30 days using standard methods. Total organic carbon was determined by Walkley and Black wet oxidation with potassium dichromate as described by Jackson (1973).

Total nitrogen (N) was determined by micro Kjeldhal method (Jackson 1973). C/N ratio was calculated from the measured values of C and N. Total P was determined based on the standard vanado phosphomolybdate yellow colour method using spectrophotometer (AA 6200, Shimadzu, Japan) in the form of P_2O_5. Total K was determined by standard method using flame photometer (CL 22D, Elico, India) in the form of K_2O. Total Pb, Cd, Cr, Ni and Cu were determined by means of atomic absorption spectrophotometer (AAS) (AA 6200, Shimadzu, Japan), after digestion of the sample with concentrate HNO_3: concentrate $HClO_4$ (4: 1, v/v).

Phytotoxicity analyses: germination index (GI) and vigour index (VI)

The phytotoxicity bio-assay indeed was determined by preparing water extracts (1: 5) with the vermicompost. Fifteen seeds of tomato (*Lycopersicon esculentum*) were placed, by triplicate, in Petri dishes (7 cm diameter) lined with filter paper containing 1 ml of each extract. The control was prepared with distilled water. The percentage of germination was measured after incubating the covered Petri dishes in the dark at 28°C for 4 days (Mathur *et al.* 1993a, 1993b; Saavedra *et al.* 2006). Germinated seeds were counted in both the control (G_o- measured in the distilled water) and the sample (G) and radical growth (L) measured.

The germination index (GI) was calculated, according to the formula (Mathur *et al.* 1993a, 1993b; Saavedra *et al.* 2006):

$$\text{Germination index (GI)} = \{(G/G_o) \times 100\} \times (L/L_o)$$

where G and G_o are the number of seeds germinated in vermicompost extracts, respectively, while L and L_o are the radical /root lengths observed in the sample and control, respectively.

The vigour index (VI) of the seedlings was estimated as suggested by Abdul-Baki and Anderson (1973):

$$VI = L+SL\times G\%$$

where L is root length (cm), SL is shoot length (cm) and G% is germination percentage.

Statistical analysis

One-way ANOVA followed by Duncan's multiple-range test at the 0.05 confidence level ($P > 0.05$) was performed to evaluate the

Table 2 Physico-chemical characteristics of initial feed mixtures and vermicompost obtained from different vermicomposting bins (mean ± SD; n=3)

Treatment	pH		EC		TOC%		TKN%	
	initial	final	initial	final	initial	final	initial	final
T1	6.50 ± 0.20	7.00 ± 0.06^{a}	1.80 ± 0.01	0.89 ± 0.02^{a}	32.54 ± 2.39	13.94 ± 1.26^{c}	0.68 ± 0.04	1.50 ± 0.06^{b}
T2	6.50 ± 0.20	7.00 ± 0.00^{a}	1.80 ± 0.09	0.89 ± 0.04^{a}	31.67 ± 2.04	10.12 ± 1.06^{a}	0.62 ± 0.05	1.86 ± 0.16^{c}
T3	6.53 ± 0.06	7.07 ± 0.06^{a}	2.10 ± 0.09	0.92 ± 0.01^{b}	22.31 ± 2.51	12.30 ± 1.53^{b}	0.67 ± 0.11	1.38 ± 0.04^{a}
Treatment	**C:N**		**TP**		**TK**			
	initial	final	initial	final	initial	final		
T1	47.86 ± 5.39	9.29 ± 3.79^{c}	0.30 ± 0.02	0.81 ± 0.08^{b}	0.52 ± 0.06	1.18 ± 0.06^{a}		
T2	51.08 ± 3.58	5.44 ± 2.23^{a}	0.30 ± 0.08	0.83 ± 0.09^{b}	0.46 ± 0.06	1.29 ± 0.05^{b}		
T3	33.29 ± 1.53	8.91 ± 1.01^{b}	0.16 ± 0.06	0.49 ± 0.03^{a}	0.56 ± 0.06	1.42 ± 0.09^{c}		

Mean values followed by different letters are significantly different ($P < 0.05$, $n = 3$) according to Duncan's Multiple Range Test (DMRT)

Table 3 Heavy metal contents (mg/kg) of initial feed mixtures and vermicompost obtained from different vermicomposting bins (mean ± SD; n=3)

Treatment	Pb		Cr		Cd		Ni		Cu	
	initial	final	initial	final	initial	final	initial	final	initial	final
T1	13.2 ± 0.8	9.1 ± 1.0^{a}	BDL	BDL	0.41 ± 0.05	0.35 ± 0.03^{a}	21.5 ± 1.2	17.5 ± 2.4^{a}	32.2 ± 1.5	29.1 ± 1.5^{b}
T2	16.8 ± 2.1	11.3 ± 0.9^{b}	BDL	BDL	0.58 ± 0.02	0.41 ± 0.02^{b}	25.8 ± 2.4	20.6 ± 2.1^{b}	35.4 ± 3.9	28.6 ± 1.5^{a}
T3	16.5 ± 1.8	15.8 ± 1.4^{c}	BDL	BDL	0.62 ± 0.04	0.50 ± 0.01c	26.8 ± 2.1	22.4 ± 1.1^{c}	38.7 ± 3.9	33.4 ± 2.1^{c}

BDL- Below detectable levels
Mean values followed by different letters are significantly different ($P < 0.05$, $n = 3$) according to Duncan's Multiple Range Test (DMRT)

significance of the observed differences between the various treatments in the different parameters: physico-chemical characteristics of compost, heavy metal contents of compost and GI and VI of plants. Statistical analyses were carried out with SPSS 12.0 for Windows.

RESULTS AND DISCUSSION

Physico-chemical changes during vermicomposting process

As presented in **Table 2**, at the end of the composting process pH in all the treatments was found to be nearer to neutral pH (7) which is optimum according to Beulah and Partheeban (2001), who conducted composting with municipal solid waste. There was an increase in pH relative to their initial values (6.5) in all the treatments. However, there was no significant difference in pH values between the treatments. Apparently, pH increase is due to degradation of short-chain fatty acids and ammonification of organic N (Tognetti *et al.* 2007). The EC values also showed a decreasing tendency when compared to initial values indicating that the vermicompost is ideal for land application. Final EC values (0.89µmhos/cm for T1 and T2 and 0.92 µmhos/cm for T3) were within the recommended value as soil amendment (Rynk 1992). The EC value was significantly higher in T3 when compared to T1 and T2.

Organic C was found to be lower at the end of the experimental period, when compared to the initial level in the treatments. The organic carbon (percent lower than initial) was recorded in the order: T1 (13.9%) > T3 (12.3%) > T2 (10.1%) (**Table 2**), however, organic C values were significantly differed between the treatments. In general, vermicomposting process refers to the feeding of earthworms on organic matter and microbial degradation; therefore, both earthworm and microorganism play an important role in vermicomposting process (Suthar 2007). The type of mutualism between the earthworms and the microorganisms is very much the cause for the degradation of organic matter leading to the release of CO_2, which is nothing but mineralisation of organic C. The results obtained in this study were consistent with the findings of Suthar (2007), Kannan *et al.* (2009), Pattnaik and Reddy (2009), Veeresh *et al.* (2010), Selladurai *et al.* (2010), who worked on various industrial wastes using various earthworm species. However, the differences in the carbon losses may be attributed to the differences in the composition of the substrate used (difference in the ratio of the mixture of paper sludge and saw dust). The maximum mineralisation was observed in the treatment T1 which may be related to the suitability of the substrate for the microbial growth and activity. Total N content in the final substrate was found to be higher. Maximum N increase was noted in T2 (68% of the initial amount) followed by T1 (58%) and T3 (51%). The present study results indicated that the earthworms accelerate the microbial mediated nitrogen transformation during the process of vermicomposting. However, the nitrogen mineralisation or the increase in the N levels in the final substrate may be attributed to the addition of excretory products of the earthworms, along with the mucus, body fluids and enzymes into the substrate. The nitrogen mineralisation pattern is very much dependent on the initial nitrogen content of the substrate (paper sludge) and the activity of the earthworms in the waste composting system (Suthar 2007; Sivakumar *et al.* 2009). Lower N mineralisation occurred in the treatments which received highest proportion of paper sludge i.e., T3 (51%). The exact reason for the significant differences in the N content between the treatments is not known, however a detailed study is required.

When compared to initial values, the observed increase in the P and K in all treatments showed that the activity of earthworm *E. eugeniae* along with microorganisms promoted mineralization process and brought the nutrients to ready to use form for plant growth (**Table 2**). Maximum increase of P was recorded in T3 (206.25%) > T2 (176%) > T1 (170%). However, the levels of phosphorous were found to be high in the final vermicompost of treatment in the order as mentioned: T2 > T1 > T3. The organic matter when passes through the gut of the earthworms, the phosphorous is converted to more available forms. Similarly, further release of P is aided by the phosphate solubilising microorganisms in the vermicasts. Potassium levels were also found to show an increase in the final substrates when compared to their initial counterparts (T2 180% > T3 154% > T1 127%). However the levels of K were found to be more in T3 followed by T2 and T1. These findings were similar to that reported by Delgado *et al.* (1995) in sewage sludge vermicomposts. According to Curry and Schmidt (2007), the earthworms prime the symbiotic microflora with secreted mucus and water to increase the degradation of ingested organic matter and release of metabolites.

The final vermicomposted material showed a decrease in the heavy metal content. The reductions ranged between 18.9% and 32.7% for Pb, 14.6% and 29.3% for Cd, 16.4% and 20.2% for Ni and between 9.6% and 19.2% for Cu (**Table 3**). The highest metal reduction was observed in treatment T2. This loss or reduction of heavy metals in the final vermicasts may be attributed due to leaching and bioaccumulation of heavy metals in the earthworm body (Garg and Kaushik 2005). Thus the data reported here suggest that earthworms can effectively reduce the metal content in the substrate.

Table 4 Germination index and vigour index of vermicompost obtained from different vermicomposting bins (mean ± SD; n=3)

Treatment	Seeds germinated in (out of 15 seeds)		Germination (%)	Shoot length (SL) (cm)	Root length (RL) (cm)		Germination index (GI)	Vigour index (VI)
	Control (G_o)	Sample (G)			Control	Sample		
T1	12	9 ± 1	72 ± 5^a	10.3 ± 0.9^b	8	6.7 ± 0.1^{ab}	60 ± 4^a	1225 ± 43^a
T2	12	11 ± 1	89 ± 5^c	12.4 ± 0.3^c	8	6.9 ± 0.4^b	77 ± 2^c	1714 ± 69^b
T3	12	9 ± 1	81 ± 10^b	8.6 ± 0.3^a	8	6.6 ± 0.5^a	66.5 ± 10^b	1228 ± 152^a

Mean values followed by different letters are significantly different ($P<0.05$, n =3) according to Duncan's Multiple Range Test (DMRT)

Phytotoxicity tests

The immaturity of compost has a stronger effect on early seedling development; therefore it is necessary to evaluate the compost or vermicompost stability and maturity by these bioassays (Sanchez-Monedero *et al.* 2004). The compost is considered mature when the GI is higher than 60, compared to the control with distilled water (Zucconi and De Bertoldi 1987; Sanchez-Monedero *et al.* 2004). In the present study the GI values of T1 (60), T2 (77) and T3 (66.5) (**Table 4**) were found to be above the values indicated by Sanchez-Monedero *et al.* (2004) i.e., GI values above 60, ideal for land application. The GI values in all the treatments indicated (**Table 4**) that the end product is sufficiently mature and free from phytotoxins. Similar results were obtained by Alfano *et al.* (2009) who analysed the phytotoxicity of olive waste compost residues. Similarly, the VI also plays an important role in the determination of harmful effects on plants during the early critical stages of in their development. The VI is determined here to find out the amount toxicity posed by the compost extracts on the seedling growth and to check the ability of the seeds growth in different ratio of mixture i.e., T1, T2 and T3 (**Table 4**). Higher the VI greater the ability of the seeds to survive and grow in the vermicompost extracts indicating less phytotoxicity. The VI values were as follows: T1 (1225), T2 (1714) and T3 (1228). The order of vigour of the seedlings in the extracts was found to be as follows: T2 > T3 > T1. The present study confirms that the pulp and paper industry sludge can be recycled into value added end product without any detrimental effects on the soil and the plants, both as a fertiliser and for land application.

CONCLUSIONS

Waste materials from industries such as these treatment plant sludge should not be discarded as waste, instead can be recycled to value added end product for land reclamation or for use as a fertiliser in order to recycle the nutrients back to the soil. Vermicomposting of the pulp and paper industry secondary sludge with suitable agro industrial waste like saw dust showed positive results producing vermicompost with desirable nutritive value and less phytotoxicity.

In the present study, at the end of the vermicomposting process, the vermibeds showed (T1, T2 and T3) significant decrease in the total organic carbon (10 to 14%) content and increase in the total nitrogen (51 to 68%), phosphorous (170 to 206%) and potassium (127 to 180%), when compared to the initial level in the treatments. The phytotoxicity tests like germination index and vigour index showed that the resulting composts (T1, T2 and T3) were less in phytotoxins. The present study concluded that the treatment T2 was found to be the better of the other two treatments (T1 and T3) with respect to phytotoxicity and NPK values. The heavy metals reductions were also found to be less in the end products of T2, indicating its suitability for land application. However, the heavy metal contents in the final vermicompost of all the treatments (T1, T2 and T3) were found to be below the admissible limits (Pb-100 mg/kg, Cr-50 mg/kg, Cd-5 mg/kg, Ni-50 mg/kg and Cu-300 mg/kg) prescribed by the Pollution Control Board. Thus, it can be concluded that the secondary sludge from the pulp and paper industry has recycling value leading to sustainable waste management.

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