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Research Article

COMPARATIVE STUDY OF MICRO - NUTRIENTS OF LIGNITE FLYASH AND COWDUNG USING *LAMPITO MAURITII* AND *PERIONYX EXCAVATUS* EARTHWORMS

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ABSTRACT

Lignite flyash is one of the major solid waste products coal based thermal power stations of India. In these waste used in cement industries, cement bricks industries, civil construction work and agricultural fields *etc*. The environmental problems generated by large-scale production of flyash in coal based power stations. This waste alternative using vermicomposting of flyash as good sources of nutrients. The availability of many nutrients is very low in flyash; available ranges of such nutrients must to be increase the effectiveness of cowdund and flyash as a soil amendment. In our experiment, we assessed the possibility of increasing total iron content (mg/Kg), total copper content (mg/Kg), total manganese (mg/Kg), total zinc (mg/Kg) and C/N ratio micronutrients in flyash through vermicomposting. Cowdung and fly ash was mixed with different trios at 1:1, 2:1, and 3:1 ratios (weight by weight). After the findings the 3:1 cow dung fly ash was the suitable combination for earthworm activity, the freshly acclamatized earthworms were inoculated in 15 gms/kg for *Lampito mauritii for* 10 -50 days. The concentration of above said micronutrient was found to increase in the earthworm-treated samples of cowdung and flyash combinations compared with the control flyash alone. The worm worked composts (vermicomposts) and worm unworked composts (control) considerable amounts of total iron, total copper, total manganese, total zinc and reduction of C/N ratio micronutrients from cowdung flyash into more soluble forms and thus resulted in increased the nutrients in the vermicompost. Among different combinations of cowdung and flyash, nutrient availability was significantly higher in the 3:1 cowdung to flyash treatment compared with the other treatments.

Keywords: Cow dung, Flyash, *Lampito mauritii* , *Perionyx excavates*, Micronutrients, and C/N ratio, Vermicompost.

INTRODUCTION

Flyash is one of the major solid waste products of coal based thermal power stations and industries. The amount of ash produced annually in India was around 90 million tons during 1995 and is likely to exceed 140 million tons in 2020. There are few uses for the tonnages produced and the disposal of flyash has become a significant problem. The common practice is to dispose these residues to the dumping sites of the power plants, which cover huge areas of otherwise agriculturally productive land. Flyash is used in civil construction, but it is not very popular because of its cost. It is also used as landfill depending on its pH, for reclaiming acid or sodic soil (Naveen kalra *et al*., 1997, Gene Stevens and David Dunn, 2004) and to alter the texture and water holding capacity of the sandy soils (Pathan *et al.*, 2003). Mittra *et al*., (2000) reported beneficial effect of flyash as soil amendment and source of nutrients for crops. The essential macronutrients like Zn, B, Mo, Si, Na and Al (Carlson *et al*., 1993). Flyash contains micronutrients and thus promotes growth of plants. In addition, it improves physical properties of soil viz., water holding capacity, aeration ect. (Vimal Kumar *et al.*, 2001). The common methods of disposal of flyashes are land filling and ash settling ponds (Suloway, 1983) and more recently in preparation of cement and bricks to some extent.

Composting is a widely acceptable alternative for converting waste into a more useful eco-friendly fertilizer to improve soil fertility (Fang *et al.,* 1998). The role of earthworms in the process of vermicomposting of waste is a physical and biochemical process. The physical process

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includes substrate aeration; mixing as well as actual grinding while the biochemical process is influenced by microbial decomposition of substrate in the intestine of earthworms various studies have shown that vermicomposting of organic waste accelerates organic matter stabilization (James Frederickson *et al.,* 1997) and gives chelating and phytohormonal elements which have a high content of microbial matter (Arancon *et al.*, 2005) and stabilized humic substances. Vermicompost is a rich source of macro and micro nutrients, vitamins, enzymes, antibiotics, plant growth hormones and immobilized microflora (Bhawalkar, 1991). Earthworms along with soil micro-organisms decompose the organic materials and produce vermicompostings of high nutrient value (Slocum, 1998). The nutrient increase in the compost products is due to the mineralization of waste in the worm gut (Ramesh and Guna Thilagaraj, 1996). The nutrients present in the wormcast are readily soluble in water for the uptake of plants (Bhawalkar and Bhawalkar, 1993). Very little works are available on the vermicomposting of flyash. Vijayakumar and Narayanasamy (2001) applied 40 Kg of flyash/ha and reported 100% survival of earthworms. Bhattacharya and Chattopadhyay (2001) found the survival of earthworms in the three different ratios of (1:1, 1:3 and 3:1) cowdung and flyash. They also reported that the maximum mineralization was found in the (1:1) ratio mixture by the earthworm *Eisenia fetida* after 50 days. Other than these no other works are available on flyash vermicomposting. Venkatesh and Eevera (2003) reported that flyash was mixed with cowdung at 1:3, 1:1, and 3:1 ratios and incubated with *Eudrilus eugeniae* for 60 days. Among different combinations of flyash and cowdung, nutrient availability was significantly higher in the 1:3 flyash to cowdung treatment compared with the other treatments. Further the mineralization of selected elements has also to be made with two indigenous species of earthworms *Lampito mauritii* and *Perionyx excavates*.

MATERILS AND METHODS

Collection and Maintenance of *Lampito mauritii* and *Perionyx excavates*

Lampito mauritii were collected from the local agricultural field. The worms were stocked in plastic containers and cowdung was used as substrate to maintain the adult earthworms. Moisture content of 70 – 80% was continuously maintained by sprinkling water. This stock culture was covered with nylon mesh and maintained at room temperature (27 ± 2^0 C) inside the laboratory. The breeding stock of *Perionyx excavates* was obtained from the local agricultural peoples. In these earthworms were maintained in a separate plastic container. Urine free cattle dung (sun dried and powered) was used as substrate to maintain the adult worms; moisture content of 70 to 80% was continuously maintained by sprinkling water on the stock culture of the plastic container. This stock culture was covered with nylon mesh and maintained at room temperature (27 ± 2^0 C) inside the laboratory.

Collection of Flyash

Flyash was collected from thermal power station I, Neyveli Corporation, Neyveli, Tamil Nadu, India.

Collection of Cowdung

The urine free cowdung was collected from the locally collected. The collected cowdung was sun dried and powdered, used as the substrate for the earthworm culture. Preparation of Different Mixture – With Cowdung and Flyash Combination of cowdung and flyash in three proportions viz., 1:1, 2:1 and 3:1 (weight by weight) were prepared and the worms were inoculated in them.

Inoculation of worms for Trial Studies

The worms were weighed and inoculated at the rate of 15gm per kg of each mixture. These set up were kept as such with required moisture for a fortnight. After 15 days the worms were observed. The population in the combinations 1:1 and 2:1 were found to have lesion on their body and shrieked in their size. Their reproductive activity was also ceased by the disappearance of clitellum. Hence 3:1 proportion of cowdung and flyash was selected for the present investigation.

Inoculation of Worms in the Experimental Combination

After finding the 3:1 cowdung flyash was the suitable combination for earthworm activity, the freshly acclamatized earthworms were inoculated in the above mentioned rate (15gms/kg). Substrate (2 kg / container) was taken 6 plastic containers (33 cm dia and 13 cm depth). The *Lampito mauritii* were inoculated in 6 containers in the suitable moisture. In another 6 plastic containers *Perionyx excavatus* were inoculated in the same way. Six containers (2 kg / containers) were maintained with the same moisture and without earthworms which were treated as control.

Collection of the Vermicast

Vermicasts from all the experimental plastic containers and compost from unworked control plastic containers were collected after 10^{th}, 20^{th}, 30^{th}, 40^{th} and 50^{th} day and air dried.

Methods

Zinc and copper content were estimated using Atomic Absorption Spectrophotometer (AAS) methods followed by Tandon (1993). The iron concentration was estimated using spectrophometer methods followed by Jackson (1973). Manganese concentration was estimated using spectrophometer methods followed by Chopra and Kanwar (1991). The ratio of the percentage of carbon to that of Carbon / Nitrogen was calculated by dividing the percentage of nitrogen estimated for the vermicast sample.

Statistical Analysis

The analysis of variance (ANOVA) was used to evaluate the statistical significance of differences in the nutrients at

0.05 levels between the worm unworked (control) substrates and worm worked composts and natural composts.

RESULTS AND DISCUSSION

Chemical analysis (selected nutrients) of worm unworked compost (control) and *Lampito mauritii* and *Perionyx excavates* worked vermicomposts (experimental obtained from cowdungg and flyash mixture substrate are given in tables 1 – 5. The differences were observed in the amount of iron present in the composts (worm-unworked and worm worked composts) and they are shown in the Table 1. A gradually increase was observed in the quantity of iron. Iron content of vermicomposts was more than the worm unworked compost. Initially (10^{th} day) the mineralization of iron was more in the compost made by *Lampito msuritii* i.e., percent changeover the worm unworked compost was +10.53, whereas the percent change in the vermicompost produced by *Perionyx excavates* was 5.11. But in all the samples collected on 20, 30, 40 and 50^{th} days the worm worked compost of *Perionyx excavatus* showed more mineralization, than the other composts. The compost made by *Perionyx excavatus* showed the best results in the iron mineralization.

Table 1. Total Iron content (Fe) (mg/kg) of worm- unworked (A) and worm–worked (B and C) Cowdung and flyash mixture (3:1) composts (N=6).

Days	Cowdung Flyash mixture (3:1)	Components				
		A	B	% Change	C	% Change
0	2004.5 ± 0.01	-	-		-	
10		2025.33 ± 4.23	2238.50 ± 1.02	+10.53	2128.83 ± 1.19	+5.11
20		2060.00 ± 1.13	2292.67 ± 1.49	+11.29	2637.00 ± 1.08	+28.01
30		2279.16 ± 1.19	2387.93 ± 1.13	+4.75	2654.00 ± 1.11	+16.45
40		2436.16 ± 1.07	2457.67 ± 1.73	+0.88	3183.17 ± 1.38	+30.66
50		2559.83 ± 1.59	2583.00 ± 1.29	+0.91	3464.00 ± 0.97	+35.32

± SE of six individual observations, Statistical significance of difference was tested at 0.05 levels, A - Worm – unworked natural composts (control), B- *Lampito mauriti* composts,C- *Perionyx excatavus* composts, % - Percent change over the control

Table 2. Total Copper content (Cu) (mg/kg) of worm- unworked (A) and worm–worked (B and C) Cowdung and flyash mixture (3:1) composts (N=6).

Days	Cowdung Flyash mixture (3:1)	Components				
		A	B	% Change	C	% Change
0	33 ± 1.36	-	-		-	
10		23.13 ± 0.29	28.50 ± 0.67	+23.22	28.50 ± 0.69	+23.22
20		25.00 ± 0.33	35.50 ± 0.91	+42.00	37.50 ± 0.90	+50.00
30		27.17 ± 0.93	44.67 ± 1.17	+64.40	45.33 ± 0.96	+66.84
40		34.17 ± 0.72	51.83 ± 1.23	+51.68	49.67 ± 0.80	+45.36
50		38.83 ± 0.98	58.50 ± 1.07	+50.67	57.00 ± 0.97	+46.79

X ± SE of six individual observations, Statistical significance of difference was tested at 0.05 levels, A - Worm – unworked natural composts (control), B- *Lampito mauriti* composts, C- *Perionyx excatavus* composts, % - Percent change over the control

Table 3. Total Manganese content (Zn) (mg/kg) of worm- unworked (A) and worm – worked (B and C) Cowdung and flyash mixture (3:1) composts (N=6).

Days	Cowdung Flyash mixture	Components				
		A	B	% Change	C	% Change

	(3:1)					
0	268.17 ± 2.29	-	-		-	
10		272.16 ± 0.55	325.83 ± 0.78	+19.72	296.00 ± 0.75	+8.76
20		282.83 ± 1.09	327.33 ± 0.81	+15.13	305.50 ± 0.70	+8.02
30		290.83 ± 1.07	322.33 ± 1.02	+14.27	318.50 ± 2.32	+3.45
40		329.50 ± 0.70	339.83 ± 0.76	+3.14	391.50 ± 2.58	+18.82
50		355.83 ± 3.16	367.83 ± 15.05	+3.37	524.00 ± 5.20	+47.26

X ± SE of six individual observations, Statistical significance of difference was tested at 0.05 levels, A - Worm – unworked natural composts (control), B- *Lampito mauriti* composts, C- *Perionyx excatavus* composts, % - Percent change over the control.

Table 4. Total zinc content (Zn) (mg/kg) of worm- unworked (A) and worm –worked (B and C) Cowdung and flyash mixture (3:1) composts (N=6).

		Components				
Days	Cowdung Flyash mixture (3:1)	A	B	% Change	C	% Change
0	143.66 ± 0.81	-	-		-	
10		145.67 ± 0.99	167.50 ± 1.22	+14.99	161.83 ± 2.78	+11.09
20		148.50 ± 0.68	174.33 ± 1.19	+17.39	176.00 ± 0.62	+18.5
30		167.17 ± 1.09	183.50 ± 0.91	+9.77	179.57 ± 1.06	+7.42
40		173.17 ± 1.23	188.00 ± 1.63	+8.56	204.00 ± 7.72	+17.80
50		201.50 ± 1.67	205.00 ± 0.61	+1.74	289.00 ± 3.77	+38.96

X ± SE of six individual observations, Statistical significance of difference was tested at 0.05 levels, A - Worm –unworked natural composts (control), B- *Lampito mauriti* composts, C- *Perionyx excatavus* composts, % - Percent change over the control

Varied quantity of copper was observed in the composts on various days (i.e., 10, 20, 30 40 and 50th days) in the environmental worm unworked and worm worked coposts (worms – *Lampito mauritii* and *Perionyx excavatus*). The variations of copper content are presented in table – 2. The rate of mineralization of copper was more in the vermicomposts, than the worm unworked compost. Same rate of mineralization of copper was observed in the vermicomposts collected on 10th day. On 20 and 30th day the mineralization was more in the compost samples produced by *Perionyx excavatus*. Later (40 and 50th day) higher mineralization was observed in the compost samples made by *Lampito mauritii*. The highest rate of copper mineralization was observed in the vermicompost made by Perionyx excavatus on 30th day (66.84 percent over the worm unworked compost). Ultimately the compost prepared by *Lampito mauritii* showed best mineralization of copper.

A step increase in the quantity of manganese was observed and it is presented in table in - 3. Initially the mineralization of manganese was more in the samples collected from worm worked compost of *Lampito mauritii* on the0th day than the other two samples collected on the same day. As the days increase the mineralization was also increased (worm unworked and worm worked composts). The maximum mineralization of manganese was observed in the 50th day vermicompost of *Perionyx excavates* i.e. 47.26 percent change over the worm unworked compost and thus the vermicompost of *Perionyx excavatus* ranked I.

Content of zinc present in the worm unworked compost and worm worked composts are produced in table – 4. The increased mineralization of zinc was observed during the early days of vermicompost produced by *Lampito mauritii* i.e 10th day the mineralization was more in *Lampito mauritii*, whereas less mineralization was observed in *Perionyx excavatus* on the same day. In all the other periods (20, 30, 40 and 50th day) the composts made by *Perionyx excavatus* showed more mineralization of zinc than the other two compost i.e. worm unworked compost *and Lampito mauritii* worked compost. The maximum mineralization occurred in the vermicompost *of Perionyx excavatus*, collected on 50th day i.e. 280 ± 3.77 mg/kg and the percent change over the worm unworked compost was 38.96. Thus the compost produced by *Perionyx excavatus* showed the best mineralization of zinc on 50th day.

Reduction in the C/N ratio was observed in all the observations. The analysed amounts are presented in Table – 5. C/N ratios were derived from the organic carbon content and the content of nitrogen. In all the samples from 10th to 50th day the vermicomposts showed better conversion (reduction) of C/N ratio than the conventional worm unworked compost. Among these two vermicomposts the best results were observed in the vermicompost made by *Lampito mauritii* from 10th day to 50th day. The maximum conservation was observed in the vermicompost of *Lampito mauritii* on 40th day since the percent change over the worm unworked compost was -68.97. Finally the least C/N ratio (10.00 ± 0.23) was found in the vermicompost of *Lampito mauritii* while the worm unworked compost (29.17 ± 0.22) and worm worked compost of *Perionyx excavatus* (10.83 ± 0.83) were showing more C/N ratio.

Table 5 . Carbon / Nitrogen ratio (C/N ratio) of worm- unworked(A) and worm worked (B and C) Cowdung and flyash mixture (3:1) composts (N=6).

	Components					
Days	Cowdung Flyash mixture (3:1)	A	B	% Change	C	% Change
0	49.5 ± 0.12	-	-		-	
10		43.17 ± 0.14	17.5 ± 0.31	-59.46	27.83 ± 0.53	-35.53
20		39.7 ± 0.83	16.5 ± 0.53	-58.44	25.33 ± 0.41	-36.11
30		38.5 ± 0.15	13.33 ± 0.23	-65.38	17.83 ± 0.31	-53.69
40		36.00 ± 0.47	11.17 ± 0.26	-68.97	13.67 ± 0.28	-63.89
50		29.17 ± 0.22	10.00 ± 0.23	-65.72	10.83 ± 0.83	-63.89

X ± SE of six individual observations, Statistical significance of difference was tested at 0.05 levels, A - Worm – unworked natural composts (control), B- *Lampito mauriti* composts, C- *Perionyx excatavus* composts, % - Percent change over the control.

Chemical analyses of selected elements present in the substrate cowdung and flyash (3:1) have been made in the conventional worm unworked compost (control) and worm worked compost (*Lampito mauritii* and *Perionyx excavatus*). The epigeic and aneciques worms are highly sensitive and selective in their medium. Vijayakumar and Narayanasamy (2001) have found that 40 kg/ha lignite flyash application was harmless for earthworm survival, where as Bhattacharya and Chattopadhya (2001) reported the survival of earthworm even in flyash without any organic amendment, they added that the best mineralization was found in the (1:1) ratio, cowdung and flyash. The quantity of Fe, Cu, Mn and Zn present in worm unworked and worm worked composts in different periods were analysed. The amount of iron present in worm worked composts was more than the worm unworked compost. On 10th day the iron content was higher in the compost made by *Lampito mauritii* i.e., percent change over the worm unworked composts was 10.53, where as the percent change in the worm worked compost produced by *Perionyx excavatus* was 5.11. But the compost of *Perionyx excavatus* on 50th day showed higher mineralization.

The worm worked compost prepared by *Perionyx excavatus* showed good results in iron mineralization. Increased content of copper was observed in the conventional and vermicompost from 0 day to 50 day. The quantity of copper was higher in the vermicompost, than the worm unworked compost. The higher rate of mineralization of copper was observed in both the worm worked composts on 10, 20 and 30th days. After that slightly decreased mineralization was observed. At the end of 50th day the highest quantity of copper was observed in the worm worked composts made by *Lampito mauritii*. Fluctuations in the content of zinc were observed in the composts. Initially on 10th & 20th day the vermicomposts showed a higher mineralization and it declined on 30th day on words in the vermicompost of *Lampito mauritii*. But it reached the pack in the compost produced by *Perionyx excavatus* on 50th day. Klein *et al*., 1975 stated that flyash is known to contain all trace elements as is rich in many chemical compounds. Fe and Cu are required for haemoglobin formation Maynard *et a*l., (1983). The higher contents of micronutrients in different flyash have been reported by Kumar *et al*., (2000). Bhattacharya and Chattopadhyay (2001) stated that vermicomposting technology would be beneficial in releasing more amounts of nutrients from flyash when treated along with organic materials. Brady (1988) stated that autotrophic bacteria obtained their energy from the oxidation of minerals contents such as Fe, Cu and ammonia. Dusserrs (1992) found that the vermicasts were richer in available manganese. Kalae *et al*., (1994) reported increase in the level of zinc in the Eudrilus eugeniae worked vermicompost producded from sugar factory waste. Ramalingam (1997) also reported both increase and decrease in the level of Fe in *the Lampito mauritii* and *Eudrilus eugeniae* worked compost. The positive effects of vermicomposting on availability micronutrients have been observed by Ghosh (1999). He has added among, different micronutrients Fe and Mn appeared to occur in higher concentrations in flyash and were followed by Zn and Cu respectively. The present observation also supports the above findings. The vermicompost was enriched with the micronutrients Fe, Zu, Mn, Cu than underlying soils Basker *et al.,* (1993): Edwards and Bohlen (1996). Vasanthi and

Kumarasamy, 1999 reported increased quantity of micronutrients (Fe, Mn and Cu) in various organic wastes when treated with earthworms and increased content of Zn in neem leaves vermicompost alone. Vijaya and Aliya (2003) observed increased mineralization of Zn and Mn in the compost made by *Perionyx excavatus* than the compost made by *Lampito mauritii*. The micronutrients such as iron were found to be increased in *Eudrilus eugeniae* worked compost Umamaheswai and vijaya lakshmi (2003). Our finding also supports the above findings since the compost made by *Perionyx excavatus* having higher mineralization.

In the present study the significantly increased Fe, Cu, Mn and Zn content in the vermicompost ($P < 0.05$) table 1, 2.3 and 4 are fall in line with observation of Bhattacharya and Chattopadhyay (2001) who have reported increased mineralization of Fe, Cu, Mn and Zn in the worm worked compost made by *Eisenia fetida* (Substrate cowdung and flyash mixture with3:1 ratio). From the beginning there was a reduction in the C/N ratio in the conventional compost and vermicompost. The reduction in the organic carbon content and increased content of nitrogen influences the C/N ratio. The higher C/N values attribute lower degree of decomposition. In our results the maximum reduction of C/N ratio was found in the vermicompost produced by *Lampito mauritii*. The carbon and nitrogen in the vermicompost added to soil, is of importance because plant content assimilate mineral nitrogen unless the ratio is in the order of 20:1 lower Edwards and Lofty (1977); Sharma and Madan (1988). Syers *et al*., (1979) reported lower level of C/N ratio in the worm cast. The recorded significant reduction in the level of C/N ratio in the vermicomposts of are in accordance with the work of Mba (1983) who found that in *Eudrilus eugeniae* worked Cassava peel C/N ratio decreased whereas total nitrogen in reased. The availability of adequate organic carbon and nitrogen in assimilable form is essential for the survival and growth of animals Lee (1985). The low C/N ratio indicates that the casts and compost would be effective sources of N through rapid N mineralization reactions Tisdale *et al*., (1993). The reduction in carbon and lowering of C/N ratio in the worm worked compost could be achieved by the respiratory activity of earthworms and microorganisms Edwards and Bohlen (1996). Vincesles-Akpa and Loquet (1997) compared the effects of composting and vermicomposting lignocellulosic maple waste and reported that the vermicompost product had alower C/N ratio. Ramalingam *et al*., (1998) also reported lower levels of C/N ratios in the vermicompost produced from different combination of organic wastes. Deka *et al*., (2003) reported that earthworms activities brings down the C/N ratio which is achieved mainly by combustion of carbon during respiration and adding nitrogen with excretion in concentration that is readily assimilable to microbes. The present findings in which the percentage of organic carbon was reduced day-by-day while the nitrogen was increased in the worm introduced substrates are fall in line with observations made by karmegam and Daniel (2000) who have reported non-significant decrease/increase of organic carbon/nitrogen after 45^{th} day and stated that it was due to the completion of composting process. Vasanthi and Kumarasamy (1999) reported that C/N ratio decreased in various organic wastes materials when treated with earthworm. Our results support the above observation.

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