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Effect of Lignite Fly Ash on the Growth and Reproduction of Earthworm *Eisenia fetida*

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Abstract: Fly ash is an amorphous ferroalumino silicate, an important solid waste around thermal power plants. It creates problems leading to environmental degradation due to improper utilization or disposal. However, fly ash is a useful ameliorant that may improve the physical, chemical and biological properties of soils and is a source of readily available plant macro and micronutrients when it is used with biosolids. Supply of nutrients from fly ash with biosolids may enhance their agricultural use. The growth and reproduction of *Eisenia fetida* was studied during vermicomposting of fly ash with cowdung and pressmud in four different proportions (T_1,T_2,T_3 & T_4) and one control *i.e.*, cow dung and pressmud alone. The growth, cocoon and hatchlings production were observed at the interval of 15 days over a period of 60 days. The maximum worm growth and reproduction was observed in bedding material alone. Next to that the T_1 was observed as the best mixture for vermiculture.

Keywords: Earthworm, Growth, Reproduction, Fly ash and Pressmud.

Introduction

Fly ash, a resultant of combustion of coal at high temperature, has been regarded as a problematic solid waste all over the world. The conventional disposal method for fly ash leads to degradation of arable land and contamination of ground water[1]. The repeated exposure of fly ash causes irritation in eyes, skin, nose and results in arsenic poisoning[2].

Coal combustion product generated each year in India is more than 100 mt per annum[3] of which 4 mt is released into the atmosphere. Coal combustion by-products were largely treated as waste materials. In fact, fly ash consists of practically all the elements present in soil except organic carbon and nitrogen. It was found that this material could be used as an additive/amendment material in agriculture applications. A careful assessment of soil and fly ash is required before its application as a soil-ameliorating agent[1]. The present outlets of fly ash disposal are using cement, concrete and grout industries[4] but such use only accounted for 38% of fly ash produced by thermal power stations[5]. The major problem faced by the coal/lignite thermal power stations all over the world is the handling and disposal of ash. In the thermal power station of the Neyveli Lignite Corporation *Ltd*., Neyveli, Tamil Nadu, India, ash pond spreads over an area of more than 45 ha and causes serious environmental hazards besides occupying fertile, cultivable land[6]. Mixtures of fly ash with organic waste have been tried by several authors[7-9].

Menon[10] studied the effect of mixed application fly ash and organic compost on soil and availability and uptake of elements by various plant species. Very little is known regarding the effects of fly ash amendment on soil biological properties[11]. Use of fly ash as a soil- amending agent has been investigated for a variety of crops[12]. Fly ash acts as a potential dust insecticide against various pests infecting rice, vegetables *etc*. And also fly ash as a carrier in pesticide formulation and the role of fly ash in soil properties have been studied by several workers[13-15].

The beneficial effect of earthworm on soil has been attributed to increase microbial populations and biologically active metabolites such as plant growth regulators[16]. Recycling of wastes through vermitechnology reduces the problems of non-utilization of agrowastes[17]. The quality and amount of food available influences the size of earthworm populations. The earthworms that are employed in organic wastes mixed with soil, to a certain extent accumulate toxic metals[18] and after vermicomposting they can be re-employed for the same purpose.

Earthworms growth, maturation, cocoon production and reproductive potential are not only influenced by environmental conditions but strongly affected by the quality and availability of food[19,20]. Growth and reproduction of different species of earthworms using different materials such as flax seeds[21], cattle manure and goat manure[22], pressmud[23], leguminous leaf litter[24] have been studied. The decomposition efficiency of *Perionyx sansibaricus* for vermicomposting was evaluated by using a variety of wastes such as agriculture waste, farmyard manure and urban solid waste. However studies are scanty on the reproductive potential of earthworms influenced by fly ash[25].

Vijayakumar and Narayanaswamy[26] applied 40 kg of fly ash/ha and reported 100% survival of earthworms. Bhattacharya and Chattopadhyay[27] found the survival of earthworms in the three different ratios (1:1, 1:3 and 3:1) of cowdung and fly ash. Ananthakrishnasamy[28] reported the survival of earthworms in three different ratios (1:1, 2:1 and 3:1) of cowdung and fly ash mixture. More recently the fly ash was vermicomposted with cowdung and the solubility of micro nutrients was analysed[29]. Apart from these, a study is available on the vermicomposting of fly ash with cowdung and reported about the variations in reproduction, zoomas and vermicast recovery[30]. So for, the studies available on the vermicomposting of fly ash are only with cowdung.

The present study was aimed to evaluate the effect of lignite fly ash on the growth and reproductive potential of *E. fetida*. During the period of 60 days the growth and reproduction were observed in fly ash (FA) with bedding material (BM – cowdung and pressmud) in various combinations where the pressmud is a solid waste produced by sugar mills.

Experimental

Collection of earthworms

Eisenia fetida was obtained from the stock culture maintained in the Department of Zoology, Annamalai University.

Collection of fly ash (FA)

Fly ash (FA) was collected from the thermal power stations I, Neyveli Lignite Corporation, Neyveli, Tamil Nadu, India.

Collection of cowdung (CD)

The urine free cowdung was obtained from the experimental dairy farm in Annamalai University. The collected cowdung was sun dried and powdered, used as the substrate for the earthworm culture.

Collection of pressmud (PM)

One month old and cured pressmud (PM) was obtained from E.I.D Parry Sugar Mill at Nellikuppam, Cuddalore district, Tamil Nadu, India.

Experimental media and inoculation of worms

The cowdung and pressmud were mixed in the ratio of 1:1 (w/w) and used as bedding material (BM). Approximately 45 days old clittellate worms *E. fetida* (15 g/kg substrate) were inoculated in five different experimental media as mentioned below. The plastic troughs were covered with nylon mesh and maintained at the room temperature 27±2°C with 60-70% of moisture, the medium without fly ash were treated as control. In each combination six replicates were maintained.

The experimental combinations were:

T_1 - FA + BM (1:9)
T_2 - FA + BM (2:8)
T_3 - FA + BM (3:7)
T_4 - FA + BM (4:6)
T_5 - BM alone

The biomass (wet weight) of worms was weighed in an electronic balance and the number of cocoons and hatchlings were counted by hand sorting method. The biomass (growth), cocoon and hatchling numbers were observed at the interval of 15 days, over a period of 60 days. The statistical significance of difference was tested using two-way ANOVA at 0.05 level.

Results and Discussion

The different levels of growth and reproduction of *Eisenia fetida* were observed in the fly ash + bedding material mixtures. Table 1-3 shows the values obtained for different parameters of growth and reproduction of *E. fetida* over the experimental period.

Growth and reproduction

The changes in worm biomass over the period of 60 days with various combinations of substrate are presented in Table 1. The percent changes in the biomass over the initial (0) day are given in brackets. Among the various experimental conditions such as T_1, T_2, T_3, T_4 and T_5, the maximum worm biomass was observed in 100% bedding material, *i.e.*, in T_5 (25.6 ± 0.65 g), the least growth was observed in T_4 (18.3 ± 0.37 g) on 60th day.

In all the experimental media the biomass have increased steadily but the biomass of *E. fetida* declined in T_3 on 15th day and in T_4 on 15th to 45th day after inoculation of worms due to mortality. After 45th day there was no mortality of worms and the biomass started to increase up to 60th day (22% increases over the initial) due to decomposition of fly ash. The results obtained from all the treatments (T_1, T_2, T_3, T_4 and T_5) clearly indicate that fly ash in combination with bedding material has positive effect on the growth of *E. fetida*. The efficiency of all the treatments supported the growth of *E. fetida* and it could be ranked in the following order T_5 (BM alone) > T_1 > T_2 > T_3 > T_4.

Table 1. Effect of fly ash – bedding material on the biomass of E. *fetida* (P<0.05)

Substrate proportions	Vermicomposting days				
	Initial (0)	15	30	45	60
T_1 FA +BM (1:9)	15 ± 0.75	16.0 ± 0.64 (+6.67)	19.2 ± 0.73 (+28.0)	21.9 ± 0.94 (+46.0)	23.1 ± 0.70 (+54.0)
T_2 FA+BM (2:8)	15 ± 0.75	15.4 ± 0.76 (+2.67)	16.1 ± 0.65 (+7.33)	18.2 ± 0.72 (+21.33)	19.8 ± 0.93 (+32.0)
T_3 FA+BM (3:7)	15 ± 0.75	14.4 ± 0.64 (-4.0)	16.3 ± 0.58 (+8.67)	18.7 ± 0.63 (+24.67)	20.1 ± 0.55 (+34.0)
T_4 FA+BM (4:6)	15 ± 0.75	13.8 ± 0.73 (-8.0)	14.8 ± 0.69 (-1.33)	13.9 ± 0.75 (-7.33)	18.3 ± 0.73 (+22.0)
T_5 BM (alone)	15 ± 0.75	16.3 ± 0.58 (+8.67)	20.7 ± 0.69 (+38.0)	23.1 ± 0.70 (+54.0)	25.6 ± 0.65 (+70.67)

ANOVA

Analysis of variance	Sum of square	Mean of square	*F*- Value
Rows	77.2744	19.3186	8.304253
Columns	146.8344	36.7086	15.77948

T- Treatments Initial (0) – worm unworked substrates Mean ±SE of six observations (+/-) – percent change of increase /decrease over the initial

The inoculated worms started to produce cocoons and they were observed on 15th day. On 60th day among all the treatments the T_5 (68.5±1.04) shows best results when compared to the other treatments, it was followed by T_1, T_2, T_3 and T_4 respectively. The variation in the cocoon production is depicted in Table 2.

Table 2. Effect of fly ash – bedding material on the cocoon production (number) of *E. fetida* (P<0.05)

Substrate proportions	Vermicomposting days					
	Initial (0)	15	30	45	60	Total
T_1 FA + BM (1:9)	-	9.2±0.77	11.1±0.72	19.01±0 .72	20.2±0.77	59.5±0.82
T_2 FA+BM (2:8)	-	8.8±0.70	10.6±0.63	18.4± 0.56	19.8±0.56	57.6±0.93
T_3 FA+BM (3:7)	-	8.4±0.51	9.2±0.77	16.2± 0.70	18.7±0.63	52.5±0.85
T_4 FA+BM (4:6)	-	8.0±0.68	8.1±0.69	14.3± 0.64	16.2±0.70	46.6±0.91
T_5 BM (alone)	-	11.1±0.71	13.0±0.71	21.8± 0.69	22.6±0.88	68.5±1.04

ANOVA

Analysis of variance	Sum of square	Mean of square	*F*- Value
Rows	178.18508	44.54627	5.461102782
Columns	9868.9032	1973.780643	241.9735471

T- Treatments Initial (0) – worm unworked substrates Mean ±SE of six observations

The hatchlings production was observed in all the treatments only on 30th day. The greatest mean number of hatchlings per cocoon was observed in the 100% bedding material (T_5) followed by T_1, T_2, T_3 and T_4 (Table 3). The highest growth (biomass) and reproduction (cocoon and hatchlings) were observed in T_5 (BM alone) when compared to other treatments. The growth (biomass) and reproduction (cocoon production and hatchlings) of *E. fetida* were tested in the fly ash with bedding material.

Table 3. Effect of fly ash –bedding material on hatchability of *E. fetida* ($P<0.05$)

Substrate proportions	Vermicomposting days					
	Initial (0)	15	30	45	60	Total
T_1 FA + BM (1:9)	-	-	18.9±0.75	23.7±0.68	34.1±0.55	76.7±0.94
T_2 FA+BM (2:8)	-	-	16.3±0.73	22.4±0.89	26.3±0.75	65.0±0.63
T_3 FA+BM (3:7)	-	-	13.2±0.74	18.0±0.71	24.7±0.94	55.9±1.03
T_4 FA+BM (4:6)	-	-	10.2±0.73	18.5±0.86	23.2±0.74	51.9±1.18
T_5 BM (alone)	-	-	20.6±0.88	35.0±0.76	42.7±0.93	98.3±0.69

ANOVA

Analysis of variance	Sum of square	Mean of square	*F*- Value
Rows	930.82133	232.7053333	4.695944401
Columns	16645.003	3329.000533	67.17852655

T- Treatments Initial (0) – worm unworked substrates Mean ± SE of six observations

Our results showed that the maximum biomass, cocoon production and hatchlings were observed in bedding material alone (*i.e.* T_5), and it was followed by T_1, T_2 and they were followed by T_3 and T_4.

The potential of earthworms as waste processors has been well documented by various authors[31-33]. Nutrition is an essential factor to determine the maximum growth of an organism. The optimal growth, maturation, cocoon production and reproductive potential of earthworms have been reported to depend on the quality and quantity of the available feed and various physicochemical parameters[32,34]. The best results regarding nutritional quality of the vermicompost and the growth and reproduction of *E. fetida* were obtained when worms were allowed to feed on cowdung exclusively[35]. On the contrary it was reported that pressmud mixed with cowdung show precocious maturation of gonads in *Lampito mauritii*[23].

Earthworms derive their nutrition from organic materials, living bacteria, fungi, diatoms, algae, protozoa, nematodes and decomposing animals. Neuhauser *et al.*[36] found that *E. fetida* gained weight, which was dependent on population density and food type. Growth and reproduction of *P. excavatus*, *E. eugeniae* in cattle dung were also reported by Reinecke *et al.*[37]. A nitrogen rich diet promotes rapid growth and increased cocoon production in *P. excavatus*[32] and a protein - rich substrate results in an increase in biomass and cocoon production in *E. fetida*[36].

Greater percentage of solid textile mill sludge (STMS) in the feed mixture significantly affected the biomass gain and cocoon production[35,38]. Kaushik and Garg[38] results showed that the total number of cocoon production was maximum (62) in 100% CD and minimum (43) in 50% STMS feed mixture. Their results showed that CD amended with 20% and 30% STMS can be suitable growth medium for *E. fetida*. From the foregoing discussion it can be concluded that *E. fetida* is able to grow well in bedding material alone. At the same time T_1 BM feed mixture seems to be the better combination than the other combinations of fly ash. This could be probably due to contribution of more quantities of nutrients to the medium by the addition of more quantity of the bedding material. Hence fly ash can be utilized for vermiculture and vermicompost production in combination with bedding material.

In our observation, the maximum worm biomass was observed in T_5 (100% bedding material) compared to other treatments and the least growth was observed in T_4. The efficiency of all the treatments to support the growth of *E. fetida* could be ranked in the following order $T_5 > T_1 > T_2 > T_3 > T_4$. The T_5 showed superior growth of earthworms because the bedding material was having higher amount of nutrients. Compared to T_3 and T_4, the combinations of T_1 and T_2 show better growth, it might be due to the higher percentage of bedding material which may provide higher nutrients for the growth of earthworms.

Conclusion

The outcome of the present investigation provides an alternative way for the utilization of fly ash apart from the conventional brick making, landfilling etc. The biomass increase and cocoon production of earthworm *Eisenia fetida* were high in 100% bedding material (cowdung and pressmud). The biomass and cocoon production were observed to have an inverse relationship with the percentage of fly ash mixed with bedding material. Our results support the findings of Ramalingam[32], Kale[17], Kaushik and Garg[38], Garg and Kaushik[39] where they have mentioned the availability of food material influences the reproduction and growth of earthworm. The reduction in the biomass of earthworm up to 60 days in T_4 and up to 30 days in T_3 indicates that the increased percentage of fly ash reduces the growth of earthworms.

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References

1. Jala S and Goyal D, *Biores Technol.,* 2006, **97**, 1136-1147.
2. Finkelman R B, Belkin H E, Zhang B S and Centeno J A, Arsenic poisoning caused by residential coal combustion. In: Proceedings of the 31st International Geological Congress, Guizhou Province, China, 2000.
3. Kumar V and Sharma P, Mission mode management of fly ash: Indian experiences. In: Verma CVJ, Lal PK, Kumar V, Lal R, Krishnamoorthy R. (Eds.), Proceedings of International Conference on fly ash Disposal and Utilization, vol. I. Central Board of Irrigation and power, New Delhi, India, 1998, 1-7.
4. ACAA (American Coal Ash Association), Coal combustion product (CCP) production and survey ACAA, Syracuse, NY. 2003.
5. Stevens G and Dunn D, *J Environ Qual.,* 2004, **33**, 343-348.
6. Senthilkumar S, Ragupathy B, Angayarkanni A and Poonkodi P, Revegetation of the Ecosystem around Neyveli mine area using Lignite fly ash. Agricultural Application of Fly ash. Proceedings of II National Seminar on use of fly ash in agriculture, Annamalai University, Annamalainagar, 2001, 143-148.
7. Sajwan K S, Ornes W H and Youngblood T V, *J Environ Sci Heal.*, 1995, **30**, 1327-1337.
8. Wong J W C, *Environ Technol.*, 1995, **16**, 741-751.
9. Arnold W, Schumann and Malcolm E, *Soil Sci Soc Am J.*, 2000, **64**, 419-426.
10. Menon M P, Elements in coal and coal combustion residues. Lewis publishers, Florida, USA, 1993, 259-285.
11. Schutter M E and Fuhrmann J J, *Soil Biol Biochem.*, 2001, **33**, 1947-1958.
12. Wallace A and Wallace G A, *Soil Sci.*, 1986, **141**, 387-389.
13. Aitken R L, Campbell D J and Bell L C, *Aust J Soil Res*, 1984, **22**, 443-453.
14. Desmukh A, Mati D B and Bharti B, *J Soil Crops*, 2000, **10**, 69-71.

15. Grewal K S, Yadaw P S, Mehta S C and Oswal M C, *Crop Res.*, 2001, **21**, 60-65.
16. Tomati U and Galli E, *Acta Zool Fennica.*, 1995, **196**, 11-14.
17. Kale R D, An evaluation of the vermitechnology process for the treatment of agro, sugar and food processing wastes. Technology Appreciation Programme on Evaluation of Biotechnological Approaches to Waste Management held on 26th October, 2000. Industrial Association-ship of IIT, Madras, 2000,15-17.
18. Leonard A O, Dolfing J, Wei-Chun M and Lexman T M, *Environ Contam Toxicol.*, 2001, **20**, 1785-1791.
19. Reinecke A J and Viljoen S A,. *Biol Fert Soils.*, 1990, **10**, 184-187.
20. Kale R D, Mallesh B C, Bano K and Bagyaraj D J, *Soil Biol Biochem.,* 1992, **24**, 1317-1320.
21. Kostecka J, *Pedobiol.*, 1999, **43**, 776-781.
22. Loh T C, Lee Y C, Liang J B and Tan D, *Biores Technol.,* 2005, **96**, 111-114.
23. Parthasarathi K and Ranganathan L S, *Euro J Soil Biol.,* 1999, **35(3)**, 107-113.
24. Manimegala G, Vermicomposting of leaf litter (Leucaena gluca (L) Benth)-cowdung mixture by *Eisenia fetida* (Savigny) and their growth and reproduction. M.Phil, Thesis, Annamalai University, Annamalainagar, 2004.
25. Suthar S, *Biores Technol.*, 2007, **98**, 1231-1237.
26. Vijayakumar N and Narayasamy P, Safety of lignite fly ash to non target organisms in the rice ecosystem. Agricultural applications of fly ash, Proceedings of II National Seminar on use of fly ash in agriculture, Annamalai University, Annamalainagar, 2001, 65-70.
27. Bhattacharya S S and Chattopadhyay G N, Vermicomposting as a tool for increasing availability of nutrients in fly ash. Agricultural applications of fly ash. Proceedings II National Seminar. On use of fly ash in Agriculture, Annamalai University, Annamalainagar, 2001, 128-132.
28. Ananthakrishnasamy S, Vermicomposting of lignite fly ash using *Lampito mauritii* (Kinberg) and *Perionyx excavatus* (Perrier). M.Phil., Thesis, Annamalai University, Annamalainagar, 2004.
29. Bhattacharya S S and Chattopadhyay G N, *Nutr Cycl Agroeco*., 2006, **17**, 223-231.
30. Gupta S K, Anamika Tewari, Richa Srivastava, R C Murthy and Saurabh Chandra, *Water Air Soil Pollut.*, 2005, **163**, 293-302.
31. Edwards C A, Breakdown of animal, vegetable and industrial organic wastes by earthworm. In: Edwards, C A., Neuhauser, E F. (Eds), Earthworms in Waste and Environmental Management. SPB, The Hague, 1988, 21-31.
32. Ramalingam R, Studies on the life cycle, growth and population dynamics of *Lampito mauritii* (Kinberg) and *Eudrilus eugeniae* (Kinberg), cultured in different organic wastes and analysis of nutrient and microbes of vermicompost. Ph.D Thesis Annamalai University, 1997.
33. Suthar S, *Biores Technol.,* 2006, **96(18)**, 2474-2477.
34. Karmegam N and Daniel T, *J Exp Zool.*, India 2000, **3(2)**, 223-226.
35. Kaushik P and Garg V K, *Biores Technol.,* 2003, **90**, 311-316.
36. Neuhauser E F, Kaplan D L, Malecki M R and Hartenstein R, *Agricultural Wastes*, 1980, **2**, 43-60.
37. Reinecke A J, Viljoen S A and Saayman R J, *Soil Biol Biochem.,* 1992, **24**, 1295-1307.
38. Kaushik P and Garg V K, *Biores Technol.,* 2004, **94**, 203-209.
39. Garg V K and Kaushik P, *Ecotox Environ Saf.*, 2006, **65**, 412-419.