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Vermicomposting of Lignite Flyash Reduces its Genotoxicity and Phytotoxicity and Improves its Agronomic Values for Application as Soil Amendments in Farmlands: A Study of Vermicomposted Flyash on Onion Crops (*Allium cepa*)

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ABSTRACT

The aim of this study was to evaluate the toxic potential of flyash by *Allium cepa* and also to understand the effect of vermicomposting on the reduction of toxicity, if any. Morphological studies of *A. cepa* roots indicated coiled and wavy roots following exposure to flyash, but no root abnormality was reported in vermicompost (VC). Under genotoxic studies, mitotic index (MI) was inhibited. The MI in cow dung (CD) was 9.52 which was reduced to 7.30 in FA, whereas MI increased to 11.43 in the CD + FA mixture and 15.76 in vermicomposted FA. The wet weight of roots and chlorophyll (Chl) contents were reduced in FA, but not so in VC. The wet weight of *A. cepa* roots in CD was 28.1 g on the 60th day which was reduced to 23.9 g in FA, while VC showed an increase to 36.4 g on the 60th day. The content of both Chl *a* and *b* decreased in FA and increased more in VC than in CD. Simultaneously Chl *a* and *b* content increased more in the CD + FA mixture than in FA but decreased in VC. It can be concluded that FA was toxic but vermicomposting of FA might be beneficial for bioremediation and thus could be used in agriculture.

Keywords: bio-accumulate, earthworms, nutritive soil amendment, toxic materials

INTRODUCTION

Flyash, a solid waste generated from coal-fired thermal power plants, contains both macro and micronutrients and can be used as a valuable soil amendment in farmlands. Flyash is alkaline in nature and also contain small quantities of Fe, Ca, Mg, Na, K, Ti and P oxides (Aitken *et al*. 1984). It contains several toxic heavy metals such as Cr, Pb, Hg, Ni, V As and B (Elseewi *et al*. 1980; Dalmau *et al*. 1990). Moreover coal continues to be a major source of power production in India and therefore flyash disposal is a major environment problem (Pandey *et al.* 2009).

Repeated exposure to FA can cause irritation in eyes, skin, nose, throat and respiratory tract and result in arsenic poisoning to man. Inhalation of FA by rats shows an increase in the metal concentration (Cd, Cr, Cu, Mn and Pb) in the liver which leads necrotic changes in hepatocytes (Mani *et al*. 2007).

When FA particles are inhaled and deposited in the lung, they increase enzymes (elastase and proteases), which stimulate fibrosis and induce silica toxicity (Borm 1997). FA constituents have the potential to induce oxidative stress in fish (Alia *et al.* 2004).

Plant bioassays provide meaningful parameters to assess the toxicity of complex mixture like flyash wastes without even knowing its chemical composition. Previously many workers have successfully employed plant bioassay *viz.*, *Allium cepa*, *Vicia faba* and *Tradescantia paludosa* as sensitive and rapid bio-tools for genotoxicity for screening the environmental contaminants in soil, surface and ground water, landfill leachates, waste water/sludge, etc. (Odeigah *et al.* 1997; Cotelle *et al*. 1999; Steinkellner *et al*. 1999; Jain *et al*. 2004).

Allium test generally provides useful estimate of the total toxic effect resulting from the treatment of root tip cells by mixture of wastes (De marini 1991; Fiskesjo 1993). When flyash is treated by earthworms (vermicomposted) it becomes a valuable biofertilizer. Karmegam and Daniel (2008) have reported that the application of vermicompost made from flyash increases the yield of hyacinth bean.

Vermicomposting by *Eisenia fetida* can be an important tool to reduce the toxicity of any hazardous organic wastes e.g. municipal sludge as evidenced by the results of genotoxicity and phytotoxicity by *A. cepa* (Srivastava *et al.* 2005). Saxena *et al*. (1998) studied flyash vermicomposting. Sinha *et al.* (2002) studied the action of three earthworms species *Eisenia fetida*, *Perionyx excavatus* and *Eudrilus eugeniae* on biodegradation of some community wastes in India and Australia. Dharani (2009) reported that vermicomposting reduces metal toxicity in flyash. It is reported that incorporation of untreated flyash in soil delays germination of crops (wheat, chickpea, mustard, lentil, rice and maize) (Kalra *et al.* 1997). They have observed reduced growth of crops in the earlier stages, which subsequently may lead to reduced yield under unfavourable environments.

The present study has been aimed to evaluate the genotoxicity, phytotoxicity and mitotic index of *A. cepa* in lignite flyash untreated and vermicompost.

MATERIALS AND METHODS

Collections of flyash (FA), cowdung (CD), earthworms and onion

FA was collected from the Thermal Power Station I, Neyveli Lignite Corporation, Neyveli, Tamil Nadu, India. The urine-free CD was obtained from the experimental dairy farm of Annamalai University. The collected CD was sun dried and powdered and used as the substrate for the experiment. *Eisenia fetida* earthworms were obtained from the stock culture maintained in the Department of

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Zoology, Annamalai University. Healthy onion bulbs of *Allium cepa* (2n = 16) were purchased from the local market at Annamalainagar.

Vermicomposting

FA (20%) and CD (80%) w/w were mixed (1 kg) and kept in plastic container, pre composted for 15 days as suggested by Nair *et al*. (2006). Then 30 g of healthy and clitellate earthworms (*E. fetida*) were inoculated in the mixture for vermicomposting. After 60 days, the vermicast was collected and used as the medium (T_4) for the culture of *A. cepa*.

Preparation of media

One kg of CD was used as first medium (T_1), 1 kg of 100% FA was used as second experimental medium (T_2), FA (20%) with CD (80%) (total one kg) was used as third medium (T_3). In the other experiment, 1 kg of vermicast obtained from FA + CD (T_4) was used as fourth medium.

Culture of *A. cepa*

One kg of each mixture as described in the preparation of media was placed in separate plastic containers. The substrates were mixed thoroughly and adequate water was also added. Then 20 onion bulbs (*A. cepa*) were planted in each experimental media and allowed to grow for 60 days. Sufficient experimental pots were maintained in each medium.

Genotoxicity studies

Cultured onion bulbs in all the experimental media were not disturbed for 2 days to get 2-3 cm long roots.

Fixation, slide preparation and scoring

After 2 days, the onion bulbs were uprooted, the root tips were exposed and fixed in freshly prepared fixative I (acetic acid + absolute alcohol + chloroform 2: 3: 2) for 4 min and then transferred to fixative II [absolute alcohol + conc. HCl (1: 1)] for 3 min. After that, the roots were washed with distilled water for 4 times and fixed in 70% alcohol. Thereafter, root tips were squashed and stained in acetocarmine to study the frequency of cell division.

Phytotoxicity studies and weight of roots

On 15th day, onion bulbs from 3 pots were uprooted and used for the experiments. Likewise on 30th, 45th and 60th days, onion bulbs from 3 pots (each time) were uprooted and used for the study. Each time, after uprooting, the roots were removed from the base of bulbs and washed with tap water. The water particles were removed by keeping the roots on the filter paper and they were weighed with an electronic balance.

The root elongation was observed in all the above media. Wet weight of *A. cepa* roots was observed once in 15 days up to 60 days. Up to 60 days at 15 days interval, the chlorophyll in the leaves of *A. cepa* was estimated (Arnon 1949).

Statistical analysis

Triplicates were made for each media. For the mitotic index, mean ± SE was calculated. The statistical significance of difference was tested with two-way ANOVA at the 0.05% level using SPSS® computer software for Windows (version 9.05).

RESULTS AND DISCUSSION

To evaluate the effect of FA in the plant *A. cepa*, mitotic index (on the second day), wet weight of root and Chl *a* and *b* were studied for a period of 60 days, on 15th, 30th, 45th and 60th day in all proportions (T_1, T_2, T_3 and T_4).

Table 1 summarizes the effect of FA on the mitotic index of the root meristem cells of *A. cepa*. In T_2 the mitotic index is very less (7.30 ± 0.29) whereas the other experiments show more mitotic index (T_1, T_3, and T_4).

The wet weight of *A. cepa* roots exposed to different media over a period of 60 days is presented in the **Table 2**. Among the different media tested, T_4 *i.e.*, application of vermicompost, recorded the highest value in all the days. The Chl content was significantly affected by the application of FA over a period of 60 days (**Table 3**). The chlorophyll content progressively increased in all the other experiments (T_1, T_3, and T_4). Likewise the Chl *b* content was also more in T_4. This was followed by the other experiments *viz.*, T_3, T_1 and T_2 respectively. In the study, the application of FA *i.e.*, T_2 experiment shows poorer results than the other experiments.

The lower concentration of flyash enhanced the rate of cell division whereas its higher concentrations inhibit the rate of division (Roychoudhry and Giri 1989). The inhibition of mitotic index can also be attributed to be the effect of environmental chemicals on DNA/protein synthesis of the biological systems (Chauhan *et al*. 1998; Jain *et al*. 2004). Various heavy metals are known to induce chromosome breaks, fragments and micronucleus formation in plants and mammalian test systems (Knasmuller *et al*. 1998).

It was reported that the FA contains heavy metals such as Cr, Cu, Pb, Zn (Su and Wong 2002; Gupta *et al*. 2005). In *A. cepa*, whenever there is root growth inhibition, there is always reduction in the number of dividing cells (Bakare and Wale-Adeyemo 2004; Babatunde and Bakare 2006). In

Table 1 Effect of flyash on the mitotic index of the root meristem cells of *Allium cepa* for 2 days.

Substrate proportions			Mitotic index	
T_1 (CD)			9.52 ± 0.23	
T_2 (FA)			7.30 ± 0.29	
T_3 (CD + FA)			11.43 ± 0.30	
T_4 (VC)			15.76 ± 0.31	
Source of variation	**Sum of square**	**df**	**Mean of square**	**F value**
Between groups	144.58501	1	144.585013	19.8411032
Within groups	43.722875	6	7.28714583	

Values are mean of three observations ± S.E.; T-Treatment; CD – cowdung; FA – flyash; VC – vermicompost

Table 2 Wet weight of *Allium cepa* roots exposed to various media (g).

Substrate proportions	Days			
	15	**30**	**45**	**60**
T_1 (CD)	15.3 ± 0.53	19.8 ± 0.52	23.4 ± 0.48	28.1 ± 0.51
T_2 (FA)	12.1 ± 0.64	16.3 ± 0.46	20.6 ± 0.52	23.9 ± 0.54
T_3 (CD + FA)	18.4 ± 0.48	21.5 ± 0.56	26.7 ± 0.59	31.3 ± 0.53
T_4 (VC)	20.6 ± 0.60	24.8 ± 0.58	31.4 ± 0.48	36.4 ± 0.69
Source of variation	**Sum of square**	**df**	**Mean of square**	**F value**
Rows	219.1325	3	73.0441667	84.9076526
Columns	403.7825	3	134.594167	156.454311

Values are mean of three observations ± S.E.; T-Treatment; CD – cowdung; FA – flyash; VC – vermicompost

Table 3 Chlorophyll content of *Allium cepa* in different days exposed to various media (mg/g).

Substrate proportions	Chlorophyll *a/b*	Days			
		15	**30**	**45**	**60**
T_1 (CD)	*a*	0.023 ± 0.0054	0.028 ± 0.0050	0.032 ± 0.0053	0.025 ± 0.0055
	b	0.036 ± 0.0053	0.030 ± 0.0051	0.049 ± 0.0066	0.023 ± 0.0054
T_2 (FA)	*a*	0.015 ± 0.0036	0.028 ± 0.0050	0.034 ± 0.0062	0.025 ± 0.0055
	b	0.018 ± 0.0044	0.036 ± 0.0053	0.039 ± 0.0051	0.024 ± 0.0054
T_3 (CD + FA)	*a*	0.023 ± 0.0054	0.037 ± 0.0068	0.041 ± 0.0058	0.034 ± 0.0060
	b	0.035 ± 0.0065	0.049 ± 0.0067	0.064 ± 0.0065	0.040 ± 0.0069
T_4 (VC)	*a*	0.032 ± 0.0057	0.045 ± 0.0062	0.050 ± 0.0064	0.042 ± 0.0056
	b	0.049 ± 0.0067	0.061 ± 0.0055	0.077 ± 0.0063	0.055 ± 0.0061
Source of variation		**Sum of square**	**df**	**Mean of square**	**F value**
Rows		0.025729	7	0.00367557	0.434104
Columns		0.0132543	3	0.00441808	0.317033

Values are mean of three observations ± S.E.; T-Treatment; CD – cowdung; FA – flyash; VC – vermicompost

the present study the reduced MI of *A. cepa* in T_2 may be due to the presence of heavy metal in FA. The observed MI in T_3 is higher than that of T_1 and T_2. The results confirm the report of the previous work that the higher seed germination rate and increased root length of lettuce are observed in FA (5%) added soil (Lau and Wong 2001). But at the same time the MI in the T_4 is more than that of T_2 and T_3. The earthworms are capable of bioaccumulating heavy metals in their body tissues, especially chloragocytes and the intestinal microflora have the capacity to detoxify most of the pesticides (Jain *et al.* 2004; Gupta *et al.* 2005; Suthar 2008). Apart from these, it shows considerable reduction in heavy metals concentration in vermicomposted FA and also accumulation of heavy metals in earthworm tissue (Gupta *et al.* 2005; Dharani *et al.* 2010). Earthworms helped to transform considerable amount of insoluble P from flyash in to more soluble forms thus resulted in the increased bioavailability of the nutrients (Bhattacharya and Chattopadhyay 2002). Vermicomposting can be an important tool to reduce the toxicity in flyash as evidenced by our results of MI. It is recommended that the vermicomposting might be beneficial for bioremediation before land filling (Srivastava *et al.* 2005).

Some of the possible reasons for the decrease in chlorophyll content may be the formation of enzyme chlorophyllase, which is responsible for chlorophyll degradation (Srivastava *et al.* 2005). Under saline conditions, compost supply led to an enhancement in total chlorophyll, Chl *a* and Chl *b* contents which could be related to a proportional increase in plant nitrogen content, as a consequence of the exogenous nitrogen supply to the soil (Dordos and Sioulas 2008). Consistent with previous findings (Santos *et al.* 2001; Netondo *et al*. 2004), we found a decrease in the contents of photosynthetic pigments more in T_2 and T_3 than in T_4. This could be attributed either to decrease of synthesis and/or an increase of degradation of chlorophyll and to chlorophyllase activity stimulation (Santos 2004).

The decrease in plant weight of FA treatment (T_2) may be due to toxicants (heavy metals) present in the FA. The rate of growth of *A. cepa* in differently polluted waters could provide evidence for the inhibition of root growth, decreased percentage of cell division and increased number of aberrant cells (Smak-Kincl *et al.* 1996). Being rich in macro and micronutrients, the flyash vermicompost has been found to be an ideal organic manure enhancing biomass production of a number of crops (Pashanai *et al.* 1996; Hidlago 1999). The importance of vermicompost (made from various organic wastes including flyash waste) in agriculture, horticulture, and soil conservation has been studied by many workers (Edwards 1995; Kaviraj Sharma 2003; Sinha *et al.* 2009). Inoculation of earthworms attributed to increased microbiological activity in the vermicomposted samples and also increases the nitrogen-fixing bacteria (Bhattacharya and Chattopadhyay 2004). In addition to increased N availability C, P, K, Ca and Mg availability in earthworms' vermicompost is also greater than in the starting feed material (Orozco *et al.* 1996). Earthworm cast amendment has been shown to increase plant dry weight (Edwards 1995) and plant N uptake (Tomati *et al.* 1994). Hence it is strongly suggested that flyash must be treated by vermitechnology (vermicomposted) before using for agricultural purposes.

CONCLUSION

Burned lignite flyash waste from coal power plants are invariably used as soil amendment in farmlands for agriculture as it is rich in nitrogen (N) and other plant nutrients. It is also disposed inlandfills. Flyash also contain complex mixtures of toxic chemicals including heavy metals of unknown specificity and is potentially genotoxic to plants. If used in farmlands or disposed in landfills, without any treatment, it may cause serious health and environmental hazards.

Earthworms can play important role in the treatment and safe management of flyash waste converting it into a valuable resource for farms and *Eisenia fetida* can be used very successfully. Onion crops (*Allium cepa*) can be used as an important tool in the screening of genoxicity of flyash on crop plants. Vermicomposting of flyash by earthworms also results into significant reduction in its toxic nature. Earthworms have the capacity to detoxify and disinfect hazardous materials. Present findings are also supported by Sinha *et al.* (2010) where they found that vermicomposting caused significant reduction in total concentration of metals like Zn, Fe, Mn, Cu, Cd and Pb from biodegradable hazardous wastes.

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