Bull. Environ. Contam. Toxicol. (2000) 64:294-301

DOI: 10.1007/s001289910043

Bulletin of Environmental Contamination and Toxicology

Amelioration of Fly-Ash by Selected Nitrogen Fixing Blue Green Algae

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Received: 2 July 1999/Accepted: 16 November 1999

Coal based thermal power stations have been the major source of power generation in India in the past and would continue for decades to come. Total ash generation is presently to the tune of 80 million tonnes per annum (Matani 1998) which is a major problem for disposal. The most common methods of fly ash disposal are land filling and ash settling ponds. Fly ash being light, mixes with air and water and pollutes the environment. In addition, it contaminates underground water resources with leaching of toxic metals. Thus, fly ash - a by-product of coal burning, poses a serious threat to both fauna and flora of the ecosystem. To overcome this problem, phytoremediation technology is being used to clean and revegetate fly ash landfill wasteland by suitable plantation of trees and shrubs to develop a bioaesthetic environment for local inhabitants and to arrest fly ash from rising into the atmosphere.

The main constraints faced while vegetating the fly-ash landfills are the high alkalinity (pH> 9.0) salt content and potentially harmful trace elements like As, B, Cd, Pb and Se content (Thicke 1988) which limit plant growth (Singh et al. 1994). Retardation in growth may also be assigned to the unavailability of N (since N is oxidised into gaseous constituents during the combustion) and P (although high concentration of P is present in ashes as compared with soils, but it is not in a form readily available to plants presumably due to interactions with ash Al, Fe and Ca) (Adriano et al. 1980).

The free living N_2-fixing blue-green algae (BGA) are known to make significant contribution to N-status of poor or unfertilised soil (Stewart 1976). Under ecologically adverse conditions, these organisms receive sufficient energy from various substrates to upgrade the nitrogen status of the environment. This has been mainly due to the in-built mechanism for regulation of the rate of ammonia production in blue green algae (Venkataraman 1979; Rai 1997). Despite its use as a biofertiliser for wetland rice and other crops (Venkataraman 1979; Watanbe and Roger 1984; Nanda et al. 1991; Abd-Alla et al. 1994) large scale exploitation of these organisms for upgradation of fly ash landfills with N for establishing vegetation remains largely unexplored. Therefore, it was considered important to explore the feasibility of growing N_2-fixing blue green algae on fly ash and to study its ameliorating potential.

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MATERIALS AND METHODS

Fly ash used in the study was collected from landfill of FGUTPP, Unchahar, (Raibareli, India). The fly-ash samples were air dried for 7 days and its physico-chemical properties were determined: pH and electrical conductivity (EC) by pH meter and conductivity meter, respectively using fly ash:distilled water, 1:5 (Piper 1966); total organic carbon (Walkley and Black 1934); total nitrogen (micro-Kjeldahl digestion; Nelson and Sommers 1972); available phosphorus (Olsen and Sommers 1982) and cation exchange capacity (CEC) (extracted with ammonium acetate - KCl; Allen et al. 1974).

For metal analysis, oven dried fly-ash samples (1 g each) were digested in a mixture of nitric, sulphuric and perchloric acid (6:1:2 by volume) at 100°C on aluminium digestion block. Cu, Mn, Pb, Ni, Fe, Zn, Cd, Cr, Si, Al, B and Mo contents in the diluted digests were quantified, using a Perkin Elmer 2380 Atomic Absorption Spectrophotometer and DR-3000 Spectrophotometer (HACH, USA).

For Algal culture, approximately 10 g of fly ash was placed in each Petri-plate (separately for each of the blue green algal strains) and autoclaved for 60 min at 140 kPa and 122°C and stored at 4°C for 24 h prior to inoculation. Equal amounts (10 ml) of exponentially growing cultures of seven different blue green algal strains viz., *Nostoc muscorum, N. commune, N. calcicola, Anabaena doliolum, Aulosira fertilissima, Gleocapsa magma* and *Scytonema oscillatum* were inoculated onto water (sterilised) soaked sterilised fly-ash in Petri-plates. All the cultures were kept in a culture room under standard growth conditions (115 µmoles $m^{-2} s^{-1}$ light with day fluorescent tube light at 26±2°C providing a L/D cycle of 14/10 h). Sterilized water was added regularly to avoid drying of Petri-plates.

The strain of *A. doliolum* which grew well on fly-ash plates was allowed to grow for one month under above conditions. The top mat layer of alga was harvested after every 10 days, washed thrice with double distilled water thoroughly and kept in an oven at 105°C for 48 h. Dried algal samples were wet digested in HNO_3:$HClO_4$ (3:1, v/v) mixture at 80°C. The metal content (Zn, Ni, Mn, Cu and Fe) in these digests were estimated by Perkin Elmer 2380 Atomic Absorption Spectrophotometer. The mass balance was calculated between the metal concentration before inoculation and after 30 days of algal inoculation on fly ash Petri-plates.

The fly ash was analyzed after harvesting the alga. The parameters and methodology were the same as described above.

RESULTS AND DISCUSSION

Analysis of fly ash used for bioamelioration study is presented in Table 1. Chemically, all naturally existing elements can be found in fly ash (Klein et al. 1975) and is also substantially enriched in trace elements compared with the parent

Table 1. Changes in the fly ash properties due to the growth of *A. doliolum.*

Parameter	Raw fly ash	Ameliorated Fly ash (after one month)
pH	9.6	8.3
Electrical Conductivity (m mhos cm^{-1})	8.6	2.6
Cation Exchange Capacity (meq/100g)	1.28	1.52
Total Nitrogen (%)	0.02	0.207
Available Phosphorus (%)	0.02	0.138
Organic Matter (%)	1.172	1.285
Metals ($\mu g\ g^{-1}$dw)		
Zn	82	69
Fe	415	326
Ni	204	146
Mn	12.1	9.6
Cu	24.0	22.8
Cd	42.3	40.2
Pb	40.1	34.6
Cr	23.4	23.2
B	290	146
Al	4615	4275
Si	5600	5421
Mo	33.4	27.6

coal. Among the elements enriched in ashes were Zn, Fe, Ni, Mn, Cu, Cd, Pb, Cr, B, Al, Si and Mo. Further the concentration of some of the elements like B, Al, Si, Cr and Cd were very high. Ash pH and electrical conductivity (EC) were 9.4 (highly alkaline) and 8.6 m mhos cm^{-1}, respectively. Fly ash was typically low in N (0.02% as compared to soil, 0.08%). High alkalinity of fly ash is due to the presence of high concentration of oxides of Ca and Mg, which form hydroxides in presence of water (Furr et al. 1975). Since EC is a measure (indirect) of total ions, the enrichment of fly ash with various essential and non-essential cations and anions can appreciably increase its EC. Linton et al. (1975) reported that as a result of volatilisation of elements upon combustion, followed by surface condensation and deposition, as the ambient temperature drops, these elements are preferentially enriched in a thin layer (~ 1,000 Å) at the particle surface and are readily extractable.

The comparative growth performance of all the N_2-fixing strains grown on fly ash is presented in Table 2. In the initial stage the growth of alga was reduced due to the phytotoxicity caused by fly ash. Later through step wise transfer of cultures, alga *A. doliolum* was found growing well on plates. Other strains like *N. commune* and *N. calcicola* also showed growth on the fly ash bed but their growth was quite less in comparison with *A. doliolum.* Such a difference amongst blue green algal forms has been also reported in earlier studies (Venkatraman 1979; Grant et al. 1986; Zhou et al. 1998). However, more important observation in this study was that BGA could grow in fly ash supplemented with water. This is because fly ash

contains all the essential macro and micro nutrients barring nitrogen which is not essential for heterocystous BGA growth. Blue green algae are generally alkali tolerant and can survive on low energy carbon source. Further, fly ash may have increased the growth of *A. doliolum* due to the increased availability of the elements essential for growth and their inherent tolerance for high alkalinity.

Table 2. Blue green algal species tested for growth on fly ash

BGA species	Comparative Growth
Nostoc muscorum	+
N. commune	++
N. calcicola	++
Anabaena doliolum	+++
Aulosira fertilissima	+
Gleocapsa magma	+
Syctonema oscillatum	+

+ Negligible growth
++Average growth
+++Prolific growth

Dubey and Rai (1990) have demonstrated a pH-dependent uptake and toxicity of Cr in *A. doliolum.* They reported an approximately 38% increase in growth of the cynobacterium treated with 40 µg ml^{-1} Cr at pH 9.0, however, yield decreased considerably at acidic pH. Variation in toxicity as a function of pH could possibly be due to metal speciation and mobility, as metals exist in their free ionic form at acidic pH and as complex hydroxylated forms at alkaline pH. Thus, at alkaline pH, metal ion bioavailability may be reduced (Tripathi and Chandra 1991) and consequently, the toxicity would be decreased. In the present investigation, high pH of fly ash up to 9 not only was growth stimulatory but also ameliorated the metal toxicity.

Although the fly ash contains many plant growth essential elements like Na, K, Ca, Cu, Mg, Fe, Zn, B and Mo, but some of the constituent metals e.g., zinc, copper and molybdenum, are required in trace amounts by algae for various physiological and biochemical processes (Round 1973). Cu, Zn, Mn and Fe are the essential micronutrients for normal plant metabolism as they act as cofactors of several enzymes, involved in carbohydrate and cell wall metabolism. However, the requirement of other metals like cadmium, lead, aluminium, selenium, silica, is not known in plant metabolism. In general, all metals are toxic to algae at higher concentration (Wong and Bradshaw 1981).

On studying the accumulation pattern of five important metals Cu, Mn, Zn, Fe and Ni in the algal mat it was seen that the algae showed differential accumulation of metals in order of Ni > Fe > Mn>Zn>Cu. The concentration of other metals was too low to be accurately detected. The highest metal accumulation value was of Ni

Table 3. Metal accumulation in *A. doliolum* growing on fly ash

Metals	Metal accumulation (μg g^{-1} dw)		
	10 days	20 days	30 days
Cu	14.3±3.8[a]	16.2±2.6[a]	17.5±3.6[b]
Mn	58.4±7.5[a]	58.4±6.2[a]	59.6±4.2[b]
Zn	17.2±2.6[a]	17.9±1.8[a]	18.0±2.7[a]
Ni	219.7±36.26[a]	221.2±36.8[a]	223.4±20.7[a]
Fe	168.4±20.3[a]	173.2±12.9[a]	178.5±13.8[b]

Means in rows followed by the same superscript are not significantly different at the 5% level according to Duncan's multiple range test.

(223.4 μg g^{-1} dw) followed by Fe (178.5 μg g^{-1} dw) after one month of growth and the least was of Cu (17.5 μg g^{-1} dw) (Table 3). This increased accumulation of Ni by *A. doliolum* conforms the finding of Singh et al. (1994, 1997) who also reported high concentration of Ni in both underground and aboveground parts of *Beta vulgaris* and *Vicia faba* grown on fly ash amended soil. This has been mainly due to the enhanced Ni availability in fly ash due to high fixation capacity (Trilica et al. 1985) but the availability of other metals was reduced at high soil pH.

The mass balance of the metals was determined to validate that the metal concentration was balanced between the fly ash and alga and there was no loss of metal during the experiment. The balance was perfectly matched in case of Mn, although for other metals also, the balance was nearly achieved (as tested by Least Standard Deviation at $p<0.05$).

Not only the growth of alga *A. doliolum* on fly ash Petri-plate was enhanced, but it also caused a significant improvement in the physico-chemical properties of fly ash (Table 1). Fly ash pH decreased from 9.6 to 8.3 after one month of algal growth. Similarly, electrical conductivity (EC) also decreased, the higher EC might have

Table 4. Mass balance of the metals.

Metal	Metal concentration (μg g^{-1}dw)					
	Before algal inoculation			After 30 days of algal inoculation		
	Fly ash	Algae	Total	Fly ash	Algae	Total
Cu	24.0±6.4	12.8±5.3	36.8**	22.8±4.3	17.5±3.6	40.3**
Mn	12.1±2.8	57.8±2.4	69.9*	9.6±1.7	59.6±4.2	69.2*
Zn	82.4±31.6	14.2±1.9	96.6**	69.7±12.8	18.0±2.7	87.7**
Ni	204±24.4	184±32.5	389**	146.6±14.9	223±20.7	370**
Fe	415±41.5	112±32.8	527**	326.8±29.4	178±13.8	505**

*$p<0.01$ as tested by Least Standard Deviation between Totals of a row.
**$p<0.05$ as tested by Least Standard Deviation between Totals of a row.

been responsible for reduced plant growth and yield in fly ash (Hodgson and Holliday 1966). In contrast, cation exchange capacity (CEC) increased from 1.28 meq 100 g^{-1} to 1.52 meq 100 g^{-1} for alga treated fly ash. A significant increase ($p<0.05$) in total nitrogen, available phosphorus and organic matter of fly-ash was also found. The increase in total nitrogen could be attributed to nitrogenase activity of nitrogen fixing cyanobacteria, Similar results were obtained by other workers on rice, wheat and vegetable fields (Watanabe and Roger 1984; Nanda et al. 1991: Abd-Alla et al. 1994). Increase in nitrogen in the fly ash can indirectly contribute towards the lowering of pH of fly ash. Decrease in pH may also be due to the secretion of organic acids in presence of aluminium and unavailable phosphorus as reported by Kochian (1995). The increase in available phosphorus in the ameliorated fly ash can be attributed to the lowering of pH which transforms unavailable phosphorus to available form. Further the alga reduced toxicity of fly ash by accumulating metals in their tissues with consequent detoxification responses (Kramer et al. 1996).

The results of this study indicate that the application of blue green algal inoculant *(A. doliolum)* can enhance the fertility of irrigated fly ash landfills. The positive effect of BGA inoculant, especially on N and P content of fly ash, holds a promise for use of such tolerant inoculants to enhance the nitrogen and P status of fly ash landfills. The alga is also able to reduce the metal content in the fly ash by bioaccumulation in its tissue.

Consequent upon metal remediation from waste substrate like fly ash, the problem arises what to do with the metal-enriched alga, although in some cases these might actually prove to be an economic asset. Alga containing valuable metals, like Cu and Ni, can be burned and the metals be recovered from the residue (Moffat, 1995). The residue can be buried in protected vaults, much as followed for contaminated soils now.

However, the recommendation for large-scale exploitation of blue-green algal inoculants to fly ash in field conditions cannot be made, unless a proper irrigation facility is developed in the area. Fly ash does not hold water to support algalisation which seems to be the main constraint in adopting this technology. Researches are currently underway in our laboratory to identify certain BGA strains which can grow in relatively dry conditions.

Acknowledgements: The authors are grateful to the Director, National Botanical Research Institute, for his interest in this work. The authors are also thankful to Directorate of Environment, UP for financial support (grant GAP 140725).

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