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For

Subject- BOTANY

Entitled ***'Green technology for cleanup of soil contaminated by coal fly ash'***

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CERTIFICATE

I, Prof. Anita Mukherjee, Ph.D., declare that the work presented in this report is original and carried throughout independently by me during the complete tenure of major research project of UGC, New Delhi.

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Green technology for cleanup of soil contaminated by coal fly ash

Section I: To evaluate the genotoxicity of Fly ash contaminated soil by applying the integrated physico-chemical-biological approach using different cells or organisms from more than one trophic level

Fly ash collected from Kolaghat thermal power plant and Titagarh thermal power plant was subjected to subsequent characterization, bioassay for toxicity and phytoremediation studies.

Characterization of fly ash (FA) and fly ash leachate (FL)

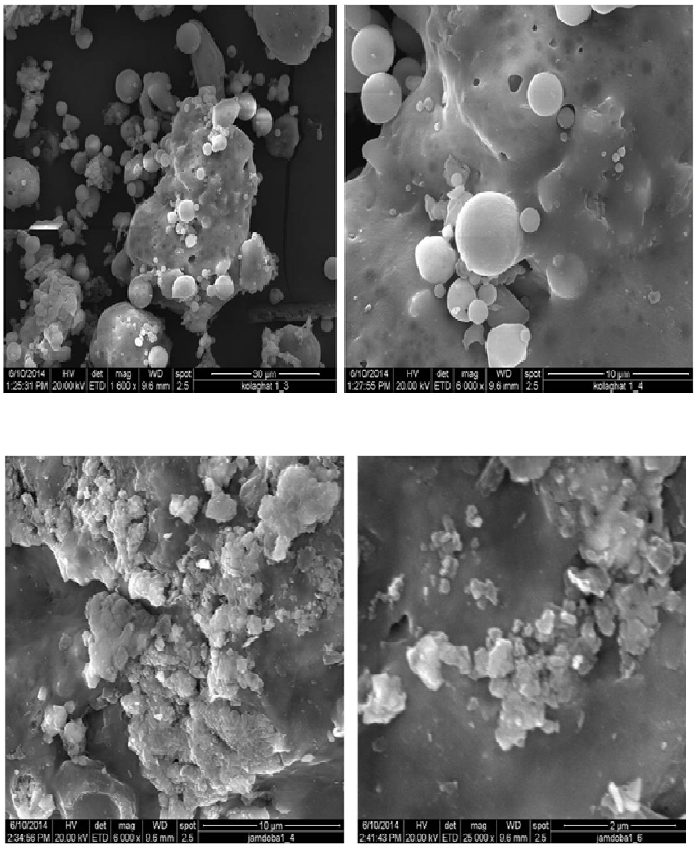
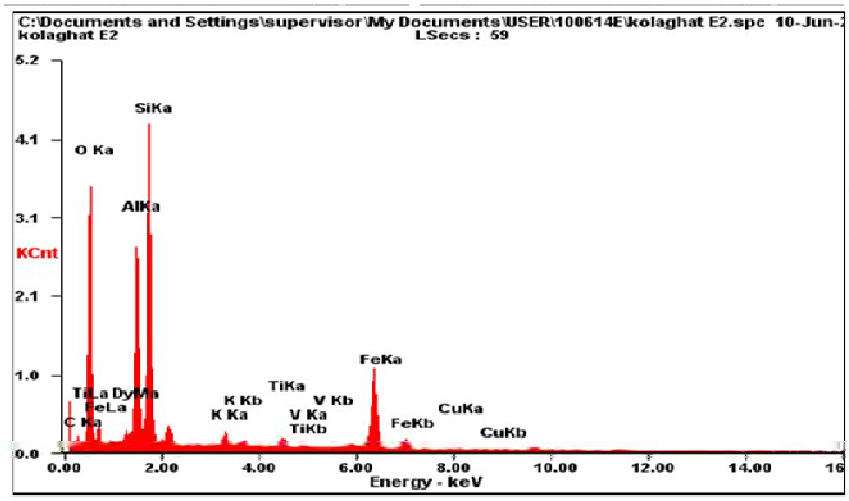
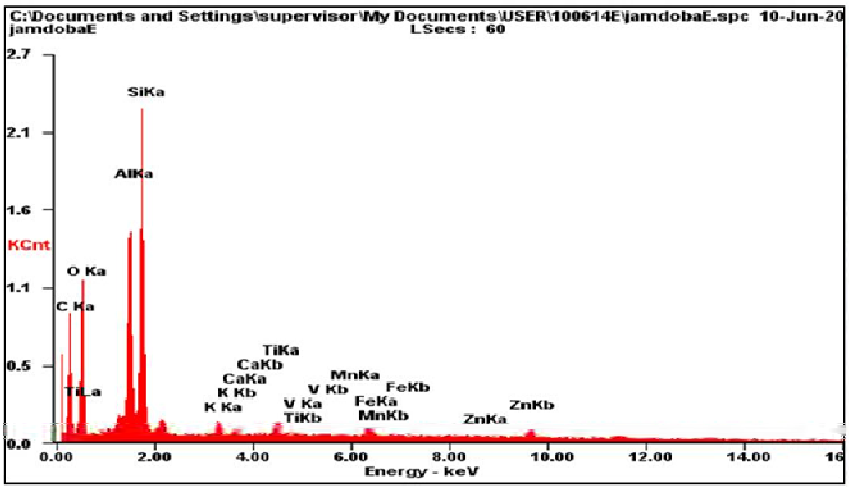


Figure 1: Scanning electron microscopic image of fly ash (A) dumpsites of Kolaghat thermal power plant (B) dumpsites of Titagarh thermal power plant



Element	Wt %	At %
C K	06.94	12.97
O K	36.11	50.68
DyM	02.71	00.37
AlK	13.69	11.40
SiK	20.17	16.13
K K	01.16	00.67
TiK	01.19	00.56
V K	00.12	00.05
FeK	17.50	07.03
CuK	00.41	00.15



Element	Wt %	At %
C K	44.57	58.49
O K	27.29	26.89
AlK	09.76	05.70
SiK	12.64	07.09
K K	00.92	00.37
CaK	00.51	00.20
TiK	01.26	00.41
V K	00.21	00.07
MnK	00.38	00.11
FeK	01.86	00.52
ZnK	00.60	00.15

Figure 2: EDX analysis of fly ash sample. (A) Kolaghat fly ash; (B) Titagarh fly ash

Table 1: Atomic absorption spectroscopic (AAS) analysis of fly ash and fly ash leachate prepared from fly ash collected from Kolaghat Thermal Power Plant and Titagarh Thermal Power Plant. Values are replicate of 3 samples ±SD. BDL- Below detection limit

Heavy metal	Kolaghat fly ash (mg/kg±SD)	Kolaghat fly ash leachate (mg/L±SD)	Titagarh fly ash (mg/kg±SD)	Titagarh fly ash leachate (mg/L±SD)
Nickel	50.00±1.12	12.00±0.78	49.00±0.04	<0.092
Cadmium	1.50±0.50	0.40±0.25	BDL	<0.147
Copper	5680.00±1.12	8.50±0.62	86.00±0.05	<0.198
Lead	48.20±1.12	3.10±1.13	36.00±0.02	<0.088
Arsenic	8.50±0.90	2.50±0.99	BDL	BDL
Cobalt	23.00±3.45	0.90±0.12	15.00±0.05	0.213±0.09

Inference:

SEM images revealed particle sizes ranging from 46.6±3.23 nm-492.6±2.91 µm and 59.4±0.23 nm-741.2±2.32 µm respectively for Kolaghat fly ash and Titagarh fly ash. Surface morphology study of Kolaghat fly ash revealed mostly spherical shape with smooth outer surface whereas in Titagarh fly ash mostly irregular shaped particles were observed. In both fly ash, EDX analysis showed presence of higher amount of aluminium, iron, silica, carbon, titanium.

Atomic absorption study (AAS) of Kolaghat fly ash and fly ash leachate showed presence of significant amount of nickel, lead, arsenic and cobalt. In Titagarh fly ash nickel, copper, lead and

cobalt were found in significant amount. In fly the leachate only cobalt was found to be present in significant amounts.

B. Bioassay for toxicity assessment of fly ash and fly ash leachate:

Onion bulbs were treated with fly ash (control and 100 % FA) and fly ash leachate (control, 1:16 FL, 1:8 FL and 1:4 FL) to evaluate fly ash induced toxicity. To assess toxicity, cell viability, DNA damage and oxidative stress was evaluated.

Cell Viability

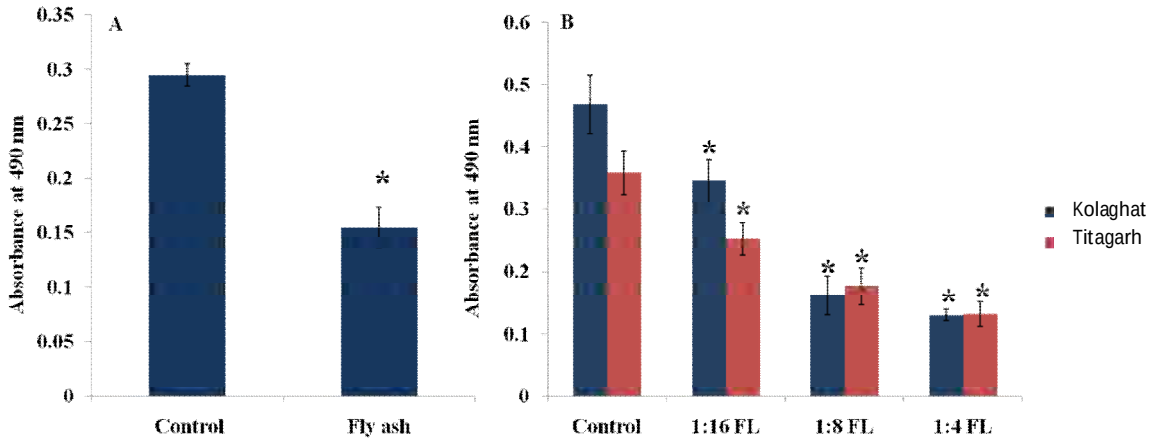


Figure 3: TTC test in root cells of *Allium cepa* exposed to fly ash (A) and Fly ash leachate (B). Values are replicate of 3 samples ±SEM. * statistically significant with respect to control ($p \leq 0.05$).

Genotoxicity evaluation:

Alkaline comet assay

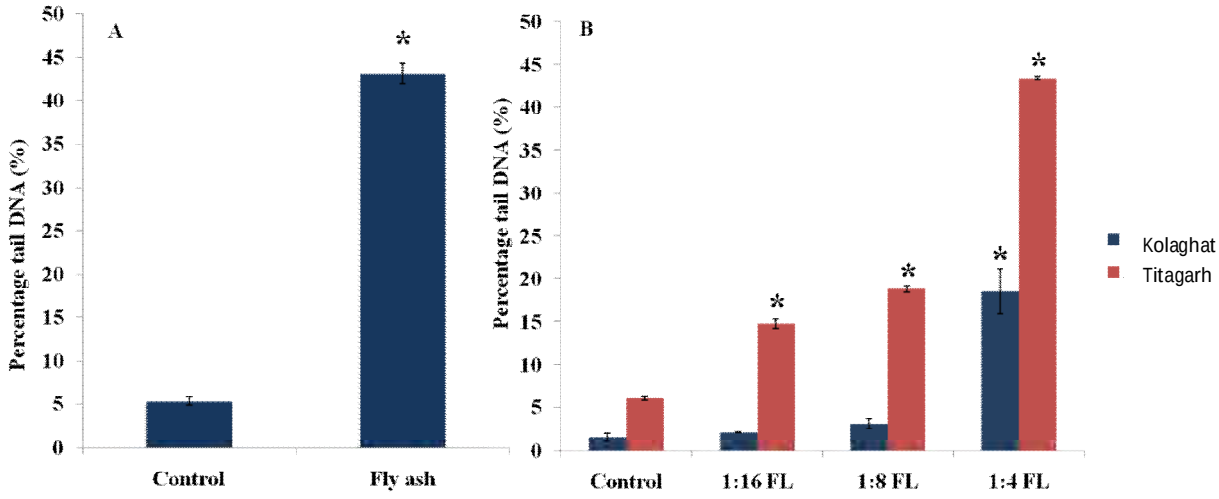


Figure 4: Alkaline comet assay in root cells of *Allium cepa* exposed to fly ash (A) and Fly ash leachate (B). Values are replicate of 3 samples ±SEM. * statistically significant with respect to control ($p \leq 0.05$).

Oxidative stress:

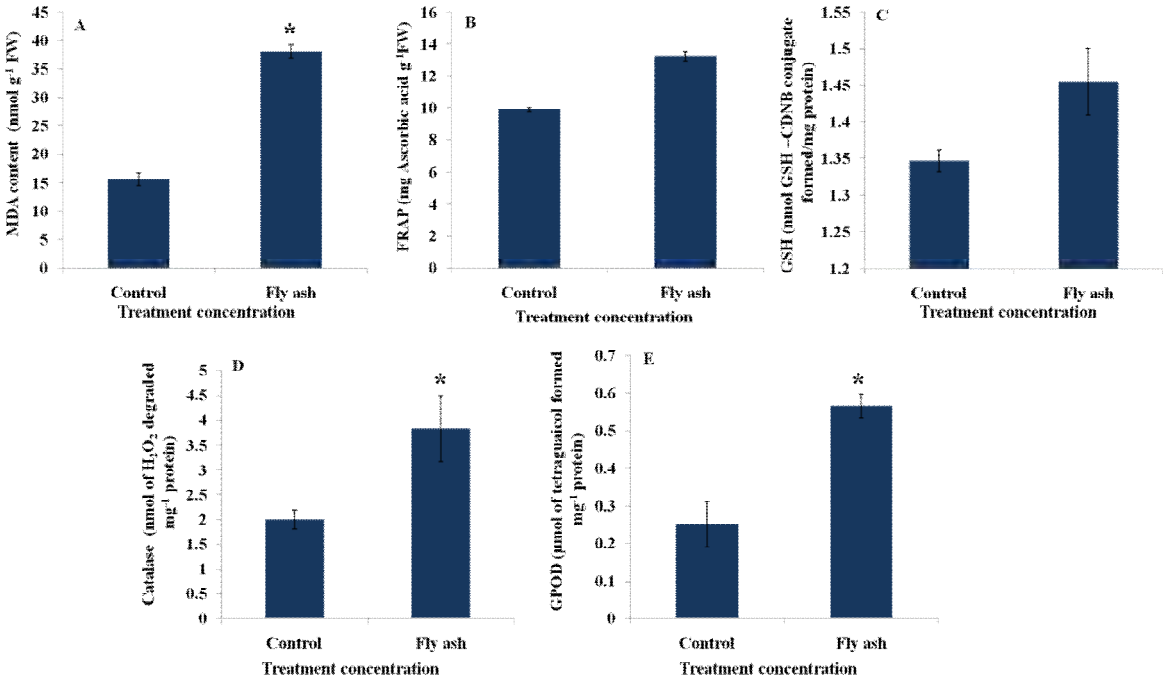


Figure 5: Oxidative stress biomarker in root cells of *Allium cepa* exposed to fly ash. (A) Lipid peroxidation; (B) Total non enzymatic antioxidant value (FRAP value) (B) GSH content, (C) Catalase activity, (D) Guaiacol peroxidase activity. Values are replicate of 3 samples ±SEM. * statistically significant with respect to control ($p \leq 0.05$).

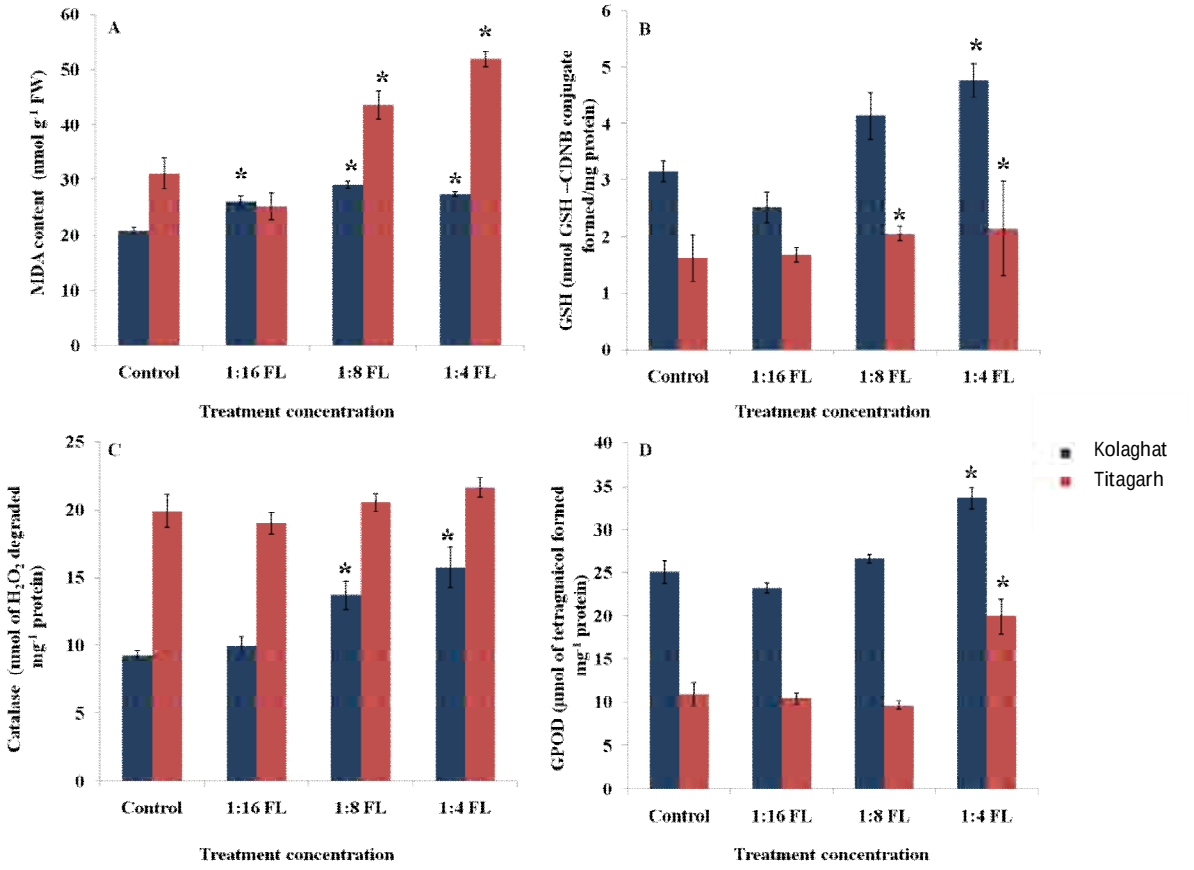


Figure 6: Oxidative stress biomarker in root cells of *Allium cepa* exposed to Fly ash leachate. (A) Lipid peroxidation; (B) GSH content, (C) Catalase activity, (D) Guaiacol peroxidase activity. Values are replicate of 3 samples ±SEM. * statistically significant with respect to control ($p \leq 0.05$).

Inference:

TTC test revealed a reduction in the number of viable cells. Decrease in cell viability of fly ash and fly ash leachate was statistically significant and concentration dependent.

Alkaline comet assay results indicated that there was an increase in DNA damage in onion roots treated with fly ash and fly ash leachate. The DNA damage was more pronounced in fly ash leachate sample collected from Titagarh.

The results of *Allium* test show a decrease in mitotic index with increase in chromosome aberrations and number of bi nucleate cell. Similar trend was noted in root tips exposed to fly ash leachate. The values were statistically significant. In onion roots treated with fly ash and leachate, a significant increase in lipid peroxidation was noted at the highest concentration.

In fly ash exposed Allium root tissues, no significant increase was noted in reduced glutathione (GSH) content and in total non enzymatic antioxidant activity. A significant increase in GSH content was observed in fly ash leachate treated sets when compared to control. Catalase activity increased significantly (1.5 times) in fly ash treated onion root tissue. On the other hand, increase in catalase activity in fly ash leachate sets (Kolaghat fly ash) was significant at the concentrations i.e.1:8 and 1:4 fly ash: water (w/v). In the case of fly ash leachate prepared from Titagarh ash, no change in catalase activity was observed. Guaiacol peroxidase (GPOD) activity was significantly induced by fly ash and fly ash leachate compared to the control plants.

Section II: To identify the plant species best adapted for the uptake, sequestration, or degradation of FA and evaluate the phytoremediating potential of the species

The present study pivots around the phytoremediating potentials of *Saccharum spontaneum* and *Saccharum munja*- two strikingly tolerant members of the Poaceae family. The performance of these grasses was analyzed in two different stressed conditions- One being the soil contaminated with industrial wastes while the other being coal Fly Ash (FA).

Saccharum spontaneum and *Saccharum munja* have proved their success in growing in all types of unfavorable and disturbed areas. They accelerate the ecological processes in an adverse environment, lending to long term sustainable eco-restoration. They can well tolerate stress conditions due to nature of their vigorous and extensive fibrous root system which could have a phytostabilizing effect as well. To handle this problem judiciously a study was undertaken where these two grasses owing to their aggressive growth and capability to produce substantial biomass were grown on coal fly ash and contaminated soil. They were maintained for a prolonged period and were evaluated periodically for genotoxicity by single cell gel electrophoresis. Analysis of metal content in FA and contaminated soil and in roots and shoots of these two grasses were carried out before commencement and after termination of treatment period. In addition, *Allium* bulbs were planted on that very pot where *S. spontaneum and S.munja* are growing and *Allium* test was conducted to assess the genotoxicity of fly ash and contaminated soil. Experiments were undertaken to find out whether plantation of *S. spontaneum* and *S. munja* are eco friendly choices to remediate polluted growth surfaces or not.

Coal fly ash (FA) was collected from the dumpsites near Kolaghat and Titagarh Thermal Power station (West Bengal, India) in the month of January (2013). Plastic drums were used for the said purpose and was stored in the laboratory at room temperature. The soil was collected from the contaminated sites near Industrial areas. Authenticated specimen of *Saccharum spontaneum* and *Saccharum munja* were collected from Bolpur, West Bengal, India and grown in the Experimental Garden of department of Botany, University of Calcutta for the purpose of the purpose of acclimatization and multiplication.

Estimation of heavy metals was carried out by AAS in contaminated soil and fly ash immediately after sample collection. Then after 12 months, plant sample (roots and shoot of *S. spontaneum* and *S. munja*) as well as soil and FA sample were studied for AAS.

S. spontaneum and *S. munja* were grown on contaminated soil and FA in earthen pots (size-diameter; 20cm, height; 17 cm) for a period of 12 months. Comet assay was performed in shoot and root cells of the two plants on day 1 of planting to detect DNA damage. After 12 months comet assay was again performed in shoot and root cells of the plants to detect the changes in DNA throughout the treatment period. Before the commencement of treatment for *Saccharum, Allium cepa* was grown on FA and soil sample for 5 days and thereafter the roots were processed

for comet assay. At the end of the 12th month, *Allium* bulbs were once again planted on those very pots for 5 days where *S. spontaneum* and *S.munja* were planted, to study the soil clearing potential of these grasses.

Allium cepa bulbs were grown on contaminated soil as well as fly ash (for 5 days) immediately after their respective collections i.e before the commencement of treatment period. *Allium* was again planted on the same sample after the termination of treatment period as well.

Comet assay-The nuclei of both the roots as well as young leaves of *S. spontaneum* and *S. munja* and root nuclei of *Allium* were processed for comet assay.

In addition phytoremediation of fly ash was performed utilizing the plant *Vetiver*, planted in control soil and fly ash for 18 months. Effect of fly ash on the pigment, genetic material and antioxidative enzymes were evaluated from leaf tissue. Metal uptake potential of plant resulting into lowering of the metal concentration was also evaluated.

Results:

Table 2 Estimation of heavy metals in shoot and root tissues of *Saccharum spontaneum* (SS) and *Saccharum munja* (SM) plants (N=3)grown on fly ash

Metals	Plants	Amount (µg/ g of shoot tissue)		Amount (µg/ g of root tissue)	
		Day 1	12 Months	Day 1	12 Months
Zinc	SS	18.00 ± 1.40	22.00 ± 1.95	124.18 ± 3.64	201.48 ± 2.78*
	SM	20.00 ± 1.05	24.02 ± 2.05	131.11 ± 2.45	221.68 ± 2.84*
Lead	SS	17.00 ± 1.32	19.25 ± 1.05	11.25 ± 1.03	20.75 ± 1.08*
	SM	18.00 ± 0.98	21.08 ± 1.98	20.25 ± 1.78	25.75 ± 1.37*
Copper	SS	0.40 ± 0.01	0.48 ± 0.02	41.68 ± 2.40	49.18 ± 3.47*
	SM	0.45 ± 0.01	0.57 ± 0.02	48.29 ± 1.02	52.78 ± 1.12
Nickel	SS	2.00 ± 0.90	2.00 ± 0.7	10.65 ± 0.11	13.00 ± 0.13
	SM	2.03 ± 1.00	2.50 ± 0.7	8.01 ± 1.03	10.65 ± 1.13
Cadmium	SS	0.10 ± 0.01	0.10 ± 0.03	0.73 ± 0.22	1.51 ± 0.24*
	SM	0.14 ± 0.02	0.14 ± 0.02	0.85 ± 0.07	1.48 ± 0.47
Arsenic	SS	0.10 ± 0.01	0.10 ± 0.03	0.27 ± 0.14	0.45 ± 0.15
	SM	0.18 ± 0.03	0.20 ± 0.03	0.26 ± 0.15	0.38 ± 0.15

*Values are Mean of three samples ± SD; *- Statistically significant at p≤0.05 when compared to day1*

Bio accumulation and translocation factors (BAF and TF respectively) are very useful parameters to study the soil to plant transfer pattern of heavy metal accumulation (Rezvani and Zaefarian 2011). BAF and TF values greater than unity are indicative of the fact that the plant is a potential heavy metal accumulator and is effective in translocation of the metals from root to harvestable parts (Tu and Ma et al, 2002). Thus BAF and TF values determine the role of the

plants in remediation of heavy metals from FA. For most of the metals studied here, higher ranges of BAF values were observed in roots of both the grasses. Among the metals Zn, Cu and Cd had the highest BAF values for roots in *S. spontaneum* (Zn-6.97, Cu-3.01, Cd-1.65). Among the two *Saccharum* species *S. munja* was efficient in extracting additional number of metals from FA (Zn-7.67, Pb-1.10, Cu-3.24, and Cd-1.62) (Table 4). The BAF values in the shoots of the plants are less than unity and of higher values for Pb and Zn. This is in coherence with TF values which indicates that relative translocation of these metals did not occur to a significant amount in the aerial parts (Table3). Different trends on BAF and TF values are available in literature that varied depending on the metals (present in FA) analyzed and the plants selected (Ahmad and Ahmad 2014, Jambhulkar and Juwarkar 2009) favoring the best one for phytoextraction of the metals. Based on the results of BAF and TF we can say that *S. spontaneum* and *S. munja* are suitable for extracting Zn, Cu, Cd and Pb from FA and could be used as sustainable alternative in the management of FA dumpsites.

Table 3 Phytoextraction of heavy metals by *Saccharum spontaneum* and *Saccharum munja* Plants (N=3) grown on fly ash

	Saccharum spontaneum			*Saccharum munja*		
Heavy metals	TF	BAF_{Shoot}	BAF_{Root}	TF	BAF_{Shoot}	BAF_{Root}
Zinc	0.11	0.76	6.97	0.11	0.83	7.67
Lead	0.93	0.82	0.88	0.82	0.90	1.10
Copper	0.01	0.02	3.01	0.01	0.03	3.24
Nickel	0.15	0.14	0.92	0.23	0.17	0.75
Cadmium	0.07	0.10	1.65	0.09	0.73	1.62
Arsenic	0.02	0.19	0.88	0.03	0.39	0.74

The presence of heavy metals is often reported to have genotoxic effects in plants. Investigations made by various authors and data from literature validated the fact that heavy metals like Zn, Pb, Cu have acute toxic effects in plants (Herawati et al., 2000; Ivanova et al., 2008). Rank and Nielsen (1998) established that the genotoxicity of heavy metals in plants can be correlated to industrial loads and their concentrations. Therefore, comet assay was used to study the genotoxic potential of the FA. The % tail DNA was the parameter selected (Kumaravel and Jha 2006). DNA damage in *Saccharum spontaneum* and *S munja* shoots in plants grown on FA or garden

soil do not show any statistically significant values over the period of experiment. Table 4 presents results of ANOVA test followed by Duncan's New Multiple Range test (DNMRT). The values of the % tail DNA in FA samples for both the plants increased significantly in the root cells at the end of the study period (Table 4). This can be correlated to the elevated retentions of heavy metals in the shoots and roots of the plants.

Table 4 Detection of DNA damage in shoot and root cells of *Saccharum spontaneum* (SS) and *Saccharum munja* (SM) plants (N=3) grown on garden soil (GS) and fly ash

Metals	Plants	Shoot		Root	
		Day 1	12 Months	Day 1	12 Months
		Tail DNA (%)	Tail DNA (%)	Tail DNA (%)	Tail DNA (%)
GS	SS	15.93±1.56a	16.15±1.96a	7.46±0.08m	9.40±0.37m
	SM	7.34 ± 1.06b	9.05 ± 0.98b	8.26±0.05m	11.44±0.18n
FA	SS	16.73±1.28a	17.95±4.24a	7.46±0.51m	10.51±0.56n
	SM	10.28±2.28b	12.15±0.84b	7.95 ± 0.22m	11.82±0.08n

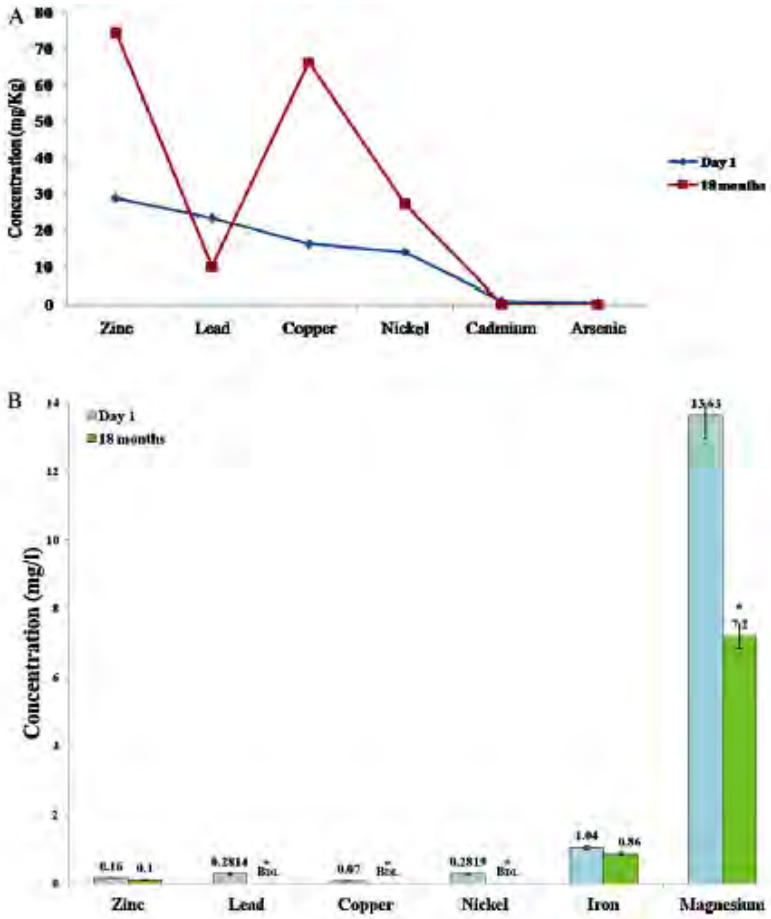
Values are expressed as Mean of three replicates ± SD. One way ANOVA test followed by Duncan's New Multiple Range Test. Values in a vertical column followed by the same letter (a,a) or (m,m) are not statistically different at the 5% level whereas values with different letters (a, b) or (m, n,) are significant at the 5% level.

The overall performance of *Saccharum* plants has proved its potential in growing on coal fly ash. The plants can extract Zn, Cu, Cd and Pb from FA and stabilize them in the underground part. At the end of the study period a considerably less amount of the metals were detected in the shoots. Since these plants are perennial and a natural colonizer unlike the introduced plants they can be promoted as an excellent candidate for remediation and restoration of FA dumpsites.

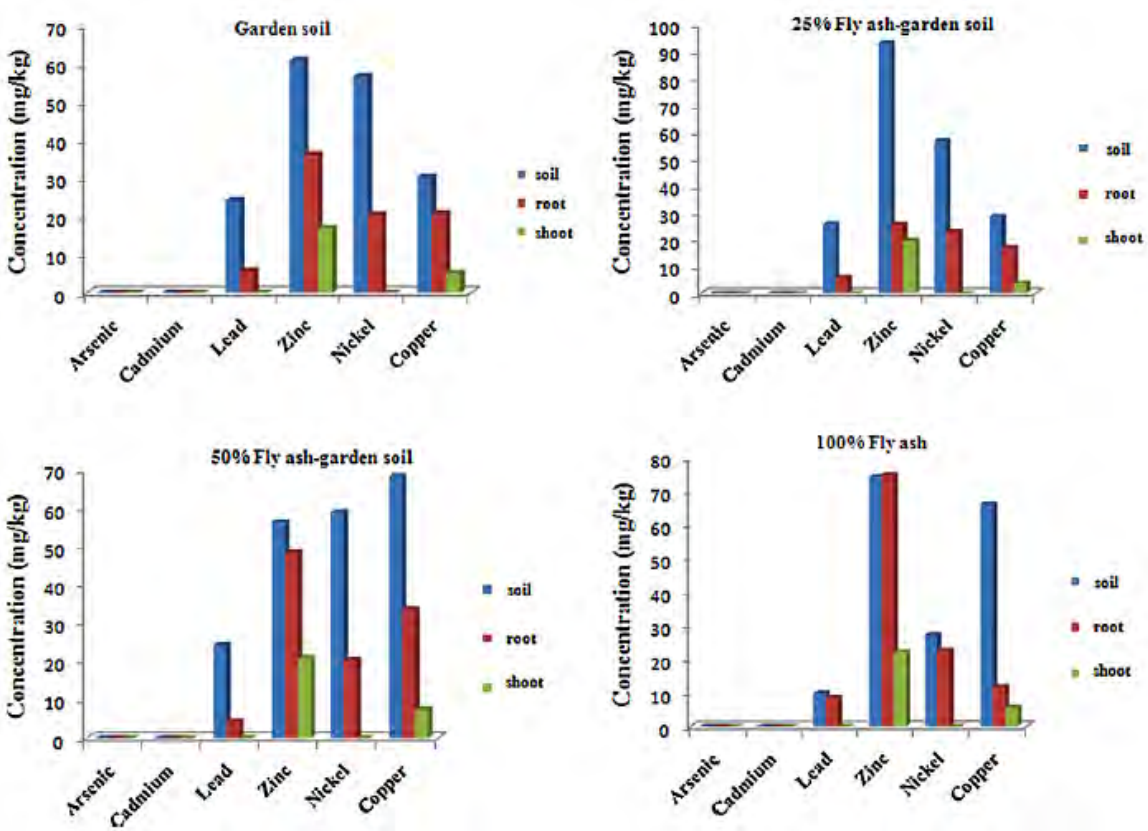
Re-vegetation of FA dump site would require the plant to be tolerant to extreme environmental conditions, heavy metals, acidic/alkaline pH, and severe drought/rain. Vetiver system is known

to be tolerant to extreme conditions and at the same time capable of controlling soil erosion (Truong and Baker, 1998a,b; Truong, 1999; Roongtanakiat and Chairoj, 2001; Roongtanakiat et al., 2003; Yang et al., 2003; Roongtanakiat, 2009). The present study thus explores the possibility of using Vetiver system for phytoremediate of FA, in a controlled laboratory based study. The study has been divided into the following parts for easier understanding and interpretation of results viz.: (a) Characterization of FA using scanning electron microscopy and elemental analysis. (b) Uptake of heavy metals by vetiver grass system. (c) Effect of heavy metal uptake in reducing toxic potential of the FA.

RESULTS:



(A) Heavy metal estimation in fly ash on day 1 and at the end of 18 months. (B) Heavy metal estimation in fly ash leachate on day 1 and at the end of 18 months; *$P \leq 0.05$.



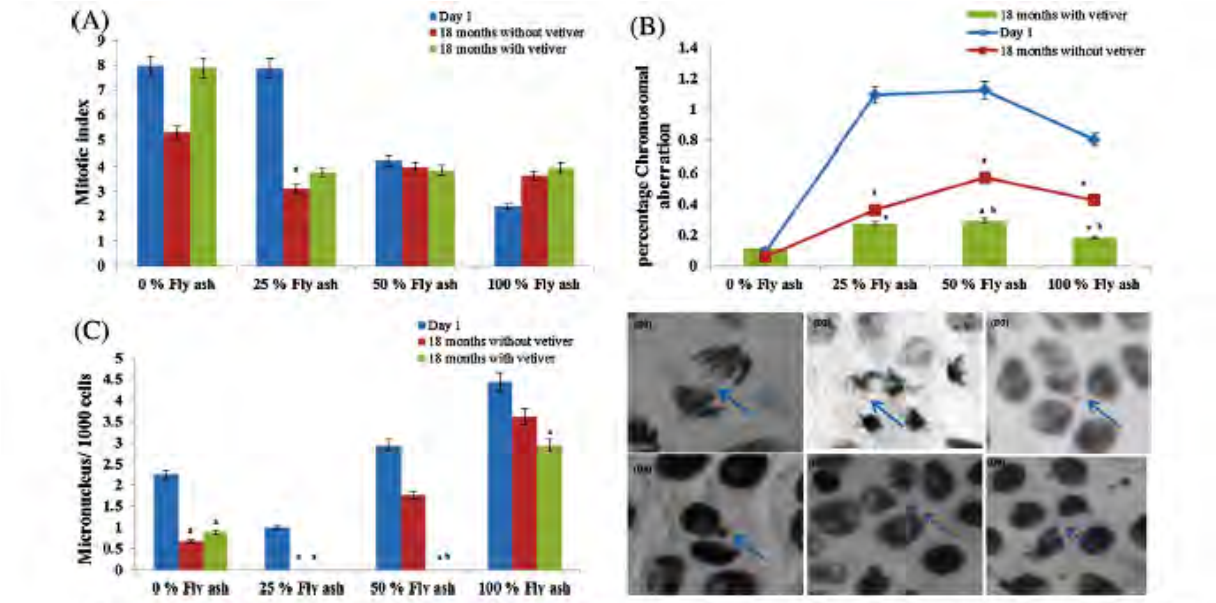
Comparative distribution of heavy metals in fly ash–soil amendments, and root and shoot of vetiver.

Root quality of vetiver under the influence of fly ash and its amendments.

Table 5

Relative translocation and bioaccumulation of heavy metals by vetiver at various amendments of fly ash-garden soil (0, 25, 50, and 100%); BDL: below detectable range.

	Arsenic	Cadmium	Lead	Zinc	Nickel	Copper
Garden soil						
TF	BDL	BDL	BDL	0.467089	BDL	0.25012
$BF_{(shoot)}$	BDL	BDL	BDL	0.277987	BDL	
		0.170836	$BF_{(root)}$	BDL	BDL	
		0.235173		0.359972		
			0.595148		0.683015	
25% Fly ash-garden soil amendment						
TF	BDL	BDL	BDL	0.766914	BDL	
		0.217723	$BF_{(shoot)}$	BDL	BDL	
BDL		0.210227	BDL	0.130039		
$BF_{(root)}$	BDL	BDL	0.222093	0.274121	0.400106	
		0.597266				
50% Fly ash-garden soil amendment						
TF	BDL	BDL	BDL	0.431753	BDL	
		0.219751	$BF_{(shoot)}$	BDL	BDL	
BDL		0.370948	BDL	0.108112		
$BF_{(root)}$	BDL	BDL	0.176689	0.859167	0.346082	
		0.491975				
100% Fly ash-garden soil amendment						
TF	BDL	BDL	BDL	0.296286	BDL	
		0.462901	$BF_{(shoot)}$	BDL	BDL	
BDL		0.298391	BDL	0.08278		
$BF_{(root)}$	BDL	BDL	0.857	1.007105	0.833517	0.17883



Comparative toxicity of FA amendments at day 1 and 18 months (with and without vetiver grass); (A) results of mitotic index; (B) results of % Chromosomal aberration; (C) results of micronucleus assay; (D) representative figures of typical aberrations scored in *Allium* test, (D1)/(D6) anaphase bridge, (D2) fragment in anaphase, (D3)/(D5) micronucleus formation, (D4) nuclear bud; [a]indicates statistically significant changes in values compared to day 1 of that specific FA amendment; [b]indicates statistically significant changes in toxicity of FA amendments with vetiver compared to FA amendments without vetiver at 18 months, $P \leq 0.05$.

Root length, Mitotic index (MI), number of micronuclei/1000 cells, % binucleate cells, and % chromosomal aberrations revealing the genotoxic potential of fly ash-soil amendments in *Allium cepa* roots as analyzed by *Allium* test and *Allium* anaphase – telophase chromosome aberration assay.

Fly ash-soil amendments	Mean root length ± SD		Mitotic index	Micronuclei/1000 cells		%Binucleate cells		%Chromosomal aberration	
	Day 1	18 Months	(18 months)	Day 1	18 Months	Day 1	18 Months	Day 1	18 Months
Garden soil	7.986 ± 0.33	5.275 ± 0.167	9.43 ± 1.23	2.25 ± 0.23	0.89 ± 0.46	–	0.09 ± 0.02	0.09 ± 0.02	0.11 ± 0.03
25% Fly ash	7.864 ± 0.23	3.098 ± 0.424[a,b]	8.06 ± 0.83	0.99 ± 0.17	–	–	0.26 ± 0.09[b]	1.09 ± 0.25[a]	0.27[b] ± 0.12
50% Fly ash	4.221 ± 0.10[a]	3.950 ± 0.334[a,b]	9.00 ± 1.58	2.93 ± 0.12	–	–	0.64 ± 0.06[a,b]	1.12 ± 0.16[a]	0.29[b] ± 0.08
100% Fly ash	2.396 ± 0.11[a]	3.625 ± 1.611[a]	6.97 ± 0.58[a]	4.44 ± 0.36[a]	2.94 ± 0.86[a,b]	0.1 ± 0.04[a]	0.58 ± 0.12[a,b]	0.80 ± 0.07[a]	0.18[b] ± 0.02

Root length represented here is an average of reading from 10 roots per concentration/time point and is represented as Mean root length ± standard deviation.
[a] Indicates statistically significant increase in values compared to control of that specific day.
[b] Indicates decrease in value with respect to the value on day 1 for that particular amendment.

Evaluation of genotoxicity of fly ash and amendments using comet assay over a period 18 months at regular interval.

Concentration of fly ash–soil amendments (w/w)		%Tail DNA±SD						
		Day 1	Day30	Day 45	Day 60	Days 75	Days 90	18 Months
0%	Planting of *Allium cepa* in pots	8.29 ± 4.53	9.58 ± 4.91	7.05 ± 0.98	9.16 ± 0.1	6.97 ± 0.88	5.97 ± 0.52	6.00 ± 1.08
25%	containing *Vetiver zizanoides*	40.49 ± 2.43[a]	19.37 ± 2.79[a,b]	16.26 ± 1.01[a,b]	9.15 ± 4.51[b]	12.68 ± 0.71[b]	11.12 ± 1.95[b]	11.12 ± 1.95[b]
50%	growing on different fly ash-	62.07 ± 4.20[a]	21.04 ± 5.77[a,b]	15.17 ± 1.18[a,b]	15.1 ± 3.65[a,b]	14.18 ± 1.53[a,b]	13.26 ± 5.1[b]	13.26 ± 5.1[b]
100%	garden soil amendments	54.75 ± 5.11[a]	45.85 ± 3.15[a]	27.48 ± 1.92[a,b]	24.04 ± 1.92[a,b]	30.88 ± 5.26[a]	28.72 ± 0.9[a,b]	28.72 ± 0.9[a,b]

Comet assay parameter (% tail DNA) represented here is an average of reading from 75 nuclei (25 × 3) scored per concentration/time point and is represented as % tail DNA ± standard deviation.
[a] Indicates statistically significant increase in values compared to control of that specific day.
[b] Indicates decrease in value with respect to the value on day 1 for that particular amendment.

Papers published in Journals

1. **Arpita De**, Anita Mukherjee. Study on the genotoxic effects of coal fly ash leachate and consequent soil amendments in *Allium cepa* L. (2013). Journal of the Botanical Society of Bengal- ISSN 0971, Vol 67, No. 2, 91-101.

2. Manosij Ghosh, Jit Paul, Aditi Jana, **Arpita De**, Anita Mukherjee. Use of the grass, *Vetiveria zizanioides* (L.) Nash for detoxification and phytoremediation of soils contaminated with fly ash from thermal power plants (2015). Ecological Engineering (Elsevier) 74, 258-265.IF-2.74.

3. **Arpita De**, Anita Mukherjee. *Saccharum spontaneum* and *Saccharum munja* can phytoremediate coal fly ash. Journal of Environmental Sciences (Springer), 2015, (Communicated).

Abstracts published in Proceedings

1. **Proceedings of the 16th All India Congress of Cytology and Genetics and National Symposium on "Gene Environment and Health"**(16th AICCG, October 22-24,2013)Organized by –Department of Botany,University of Kerala, Kariyavattom, Thiruvanantapuram, Kerala. **Abstract No.P7,pg-41. Amelioration of Soil contaminated with Coal Flyash"**
2. **Proceedings of the International Symposium on "Trends in Plant Science Research"** (February 15-16, 2014), Organized by – Centenary Celebration Committee, Department of Botany, University of Calcutta, Kolkata. **Abstract No.P58,pg-29-"*Saccharum spontaneum* L. –an eco friendly choice for remediation of coal flyash"** .

growth and production (Bilski *et al.*, 1995; Siuta 2001; Bilski *et al.*, 2011). Coal fly ash, used as soil amendment, or on a soil covered fly ash landfills, can be very effective as a provider of certain essential nutrients to plants, such as B, Mg, Mo, S, and Zn (ElMogazi *et al.*, 1988; Gibczynska *et al.*, 2006). Fly ash also has been reported to improve the nutritional status of soils by providing plants with micronutrients. However, there is a great concern, that plants either cultivated or voluntarily growing on soil with high content of FA may absorb toxic amounts of heavy metals (Bilski *et al.*, 2011).

Genotoxicity of soil contaminated with FA is relatively unexplored. Higher plants particularly *Vicia faba* and *Allium cepa* possess many advantages that make them ideal for use by scientists for screening and monitoring of genotoxic agents according to the standard protocol for the plant assay established by the International Program on Chemical Safety (IPCS) and the World Health Organization (IPCS 1998). *Allium* test is one of the best-established test systems used in order to determine the toxicity in the laboratories. Moreover, this system is well correlated with the data obtained from eukaryotic and prokaryotic systems (Matsumoto *et al.*, 2006).

The objective of the present study is to study the effects of FA leachate and FA mixed with soil on *Allium cepa*. We have evaluated the cytotoxicity and genotoxicity considering the endpoints mitotic index, chromosomal aberrations, nuclear abnormalities as well as root growth inhibition and viability.

MATERIALS AND METHODS

Collection of coal fly ash and preparation of leachate and soil amendments with FA

Coal fly ash (FA) was collected from the dumpsites near Kolaghat Thermal Power Station (West Bengal, India) in the month of January 2013. Plastic drums were used for the said purpose and was stored in the laboratory at room temperature. FA leachate was prepared according to the European pre-standard leaching test prEN 12457-1 (European Committee for Standardization, 1998) with some modifications (Chakraborty and Mukherjee, 2009). Leachate was prepared by mixing 25 g of FA in 100 ml of double distilled water and placed for 3 days on shaker in dark. The leachate thus prepared (from 1 part of FA dissolved in 4 parts of water) was further diluted with distilled water to give the following concentrations- 25%(1:3), 50%(1:1) and 100% (i.e. no water added). The pH was found to be 7.4.

Garden soil sample was mixed with FA in varying proportion to obtain the different per cent of FA in mixtures (0,25,50 and 100%).

Plant material

Small sized onion bulbs (*A. cepa*, 2n=16) of purple variety were purchased from the local markets and planted on thoroughly moistened sand for the purpose of root initiation. The roots were allowed to attain a size of 2-3 cm. The bulbs were then gently taken out of sand and thoroughly washed with water.

Chemicals required

45% acetic acid, 1:3 aceto ethanol solution, 70% alcohol, 2% aceto orcein stain, ethyl methane sulfonate (EMS), double distilled water, triphenyl tetrazolium chloride (TTC), 95% ethanol, Evans blue dye and dimethyl formamide (DMF) were of analytical grade and purchased locally.

Estimation of metals

Metal estimation of FA leachate was done according to the standard method of the American Public Health Association, 1998 (Chakraborty and Mukherjee, 2009). The metals analysed by AAS in leachate are Na, K, Ca, Ni, Mg, Fe, Mn, Zn, Cd, Cu, Pb, As, F, SO_4, Co, P, Cr and Si and expressed as mg/L and those analysed in FA sample are Zn, Pb, Cu, Ni, Cd, and As and expressed as µg/g of FA.

Experimental design

Treatment protocol with FA leachate- Treatment was carried out by suspending the roots in the leachate solution of varying concentrations (0, 25, 50 and 100%,) for 6 h. After termination of treatment period, it was allowed to recover in absence of test reagent for 20 h (1 cell cycle). Double distilled water and 2 mM EMS solution were used as negative and positive control sets respectively.

Protocol for soil amendment with FA - Onion bulbs were allowed to grow in soil mixed with various proportions of FA (0, 25, 50 and 100% FA). After 5 days, the roots were processed for the following experiments-

1. Root length inhibition assay- This assay shows inhibition of root length in onion exposed to various concentrations of FA leachate as well as soil mixed with various proportions of FA.

2. Cell viability or cytotoxicity test - Cell viability assay includes Evans blue and TTC assay.

3. Genotoxicity test- Genotoxicity test includes- mitotic index , chromosomal aberrations and nuclear abnormality assays.

Fixation, staining and slide preparation

Fixation

The first 3 mm of the root was gently cut with a razor blade and fixed in 1:3 aceto ethanol solution for 24 h. Thereafter the roots were preserved in 70% ethanol for future use. The roots were then washed with distilled water for 10 min and then hydrolysed in 45% acetic acid.

Staining

The excised root tips were dipped in stain solution (9 parts of 2% acetorcein and 1 part of 1 (N) HCl mixture) and subjected to mild heat. It was then allowed to stay for 45 min.

Slide preparation

The M region (the first 1mm calyptra) and the F_1 region (2mm) were squashed separately. Preparation was carried out following the squash technique (Sharma and Sharma, 1980).

Assessment of cytotoxic and genotoxic effects of FA by performing Allium assay
Root length inhibition assay

FA leachate

The onion bulbs were allowed to grow in sand until the roots attained a length of approximately 1.5-2 cm. They were exposed to various concentrations of FA leachate (0, 25, 50 & 100%) for 6 h and after termination of treatment period they were allowed to undergo a recovery period of 20 h in single distilled water in absence of test reagent. 5 longest roots were measured (in cm) from each bulb before exposure to treatment solution (Chakraborty *et al*., 2009). After termination of recovery period the lengths of those very roots were again measured and the differences were noted.

Soil mixed with FA

Onion bulbs were allowed to germinate directly in soil as described earlier. After termination of treatment period the roots were thoroughly washed under tap water. 5 longest roots were measured (in cm) from each bulb and noted.

Cell viability assay

Evans blue dye test

A dilute solution (0.025%w/v) of Evans blue dye stains dead or damaged cells while the living or viable cells repel the dye and stay unstained.

2-3 roots (2.0-3 cm in length) from each onion bulb of different concentration of leachate solution were dipped in Evans blue dye for 30 min. The roots were then thoroughly washed with water to get rid of the excess stain. They were then dipped in DMF overnight to allow the stain to get extracted out into the DMF solution. The absorbance of the coloured DMF solution is recorded at 600 nm.

TTC test

This study is aimed at showing the 2,3,5-triphenyl tetrazolium chloride (TTC) reduction for assessment of cell survival following exposure of onion bulbs to FA leachate / FA in soil. 2-3 roots (2.0-3.0 cm in length) from each onion bulb of different concentration of leachate solution were dipped in 0.5% TTC solution for 10-15 minutes in dark at 37°C. They were then dipped in 95% ethanol overnight at –20°C to allow the stain to get extracted out into ethanol. The absorbance of the coloured ethanol solution is recorded at 490 nm.

Genotoxicity test

The slides thus prepared were scored for mitotic index, chromosomal aberrations and nuclear abnormalities (Gupta *et al*., 2012).

Mitotic index (MI)

MI was found by observing 1000 cells and counting all stages of mitotic cells (Rank and Nielsen, 1997). Formulae used for MI calculation-
MI(%)=Number of cells in mitosis/Total number of dividing cells × 100

Chromosomal aberrations (CA)

Chromosomal abnormalities reflect an anomaly either in the chromosome structure or number generated spontaneously or induced by some chemical agents. To evaluate chromosome abnormalities by *Allium* assay, several types of CA are considered in different phases of the cell division (prophase, metaphase, anaphase and telophase).

Nuclear abnormalities (NA)

Nuclear abnormalities (NA) are alterations in the morphology of interphasic nuclei caused by the actions of the test chemicals. The NAs that were found to have been induced by FA in this experiment are binucleated and micronucleated cells and were scored from the F_1 region.

Statistical analysis

Statistical analysis has been performed using Sigma Stats.3 software (SPSS Inc., Chicago, Illinois, USA). The experiments were repeated once to establish the reproducibility of the results. Pooled data were statistically analysed for analysis of variance (ANOVA). The level of significance was established at $p \leq 0.05$.

RESULTS

Estimation of metals

The metals analysed by AAS in FA leachate are shown in Table 1a. The metals present in the leachate shows the amount of K to be highest followed by Ca, Na, SO_4, Mg, P and Si. Fluoride was below the level of detection.The metals present in the garden soil and FA are presented in the Table 1b. The amount of Zn was found to be highest followed by Pb in fly ash sample.

Root length inhibition assay

There was a reduction in the percentage of increase in root length with increasing concentration of FA leachate. The average percentage of root length increase (following treatment of 6 h and recovery period of 20 h) for control set (0% FA leachate), 25% FA leachate, 50% FA leachate and 100% FA leachate are 90.63, 88.04, 61.06 and 44.14% respectively (Table 1c). There is a dose dependent inhibition of root length with maximum inhibition at 100% FA leachate concentration and minimum at 0% concentration (double distilled water).

Table 1a: Metal estimation of fly ash leachate by atomic absorption spectrophotometer (Mean of 3 samples ± Standard Deviation).

Metal	Amount in mg/l
Sodium	1250.0 ± 6.00
Potassium	1680.0 ± 9.00
Calcium	1478.0 ± 10.00
Nickel	12.0 ± 2.00
Magnesium	650.0 ± 3.00
Iron	2.31 ± 0.31
Manganese	10.0 ± 1.00
Zinc	0.26 ± 0.06
Cadmium	0.40 ± 0.08
Copper	8.50 ± 1.50
Lead	3.10 ± 0.41
Arsenic	2.50 ± 0.35
Fluoride	BDL*
Sulphate	1250.0 ± 9.00
Cobalt	0.90 ± 0.07
Phosphorus	218.0 ± 18.00
Chromium	1.50 ± 0.19
Silicon	120.0 ± 0.80

*- Below Detection Level

Table 1b: Metal estimation of fly ash sample by atomic absorption spectrophotometer (Mean of 3 samples ± Standard Deviation).

Metal	Amount (µg/g of fly ash)
Zinc	28.90 ± 3.04
Lead	23.39 ± 2.32
Copper	16.29 ± 2.13
Nickel	14.06 ± 0.93
Cadmium	0.91 ± 0.24
Arsenic	0.51 ± 0.14

FA amendments in soil caused a decrease in root length at doses like 25 and 100 % FA. Bulbs grown exclusively on 50% FA showed growth of roots comparable to garden soil. Dose dependent root length inhibition did not occur in case of onions exposed to various proportions of FA in combination with garden soil (Table 1d). Bulbs grown exclusively on garden soil showed maximum growth of roots while those grown on 100% FA showed minimum growth of roots.

Table 1c: Root Length Inhibition assay, Evans blue dye test and TTC test in *Allium cepa* treated with varying concentrations of fly ash leachate solution

Concentrations of Fly ash leachate (%)	Root Length Inhibition Average Root Length (cm)	Evans blue dye test Absorbance at 600 nm	TTC test - Absorbance at 490 nm
0[a]	9.06	0.07	0.94
25	8.80	0.14 *	0.66 *
50	6.10	0.16 *	0.51 *
100	4.41 *	0.27 *	0.33 *

[a] Distilled water used as vehicle control.
* Statistically significant at $p<0.05$.

Table 1d: Root Length Inhibition assay, Evans blue dye test and TTC test in *Allium cepa* treated with varying concentrations of fly ash mixed with garden soil

Concentrations of Fly ash leachate (%)	Root Length Inhibition Average Root Length (cm)	Evans blue dye test Absorbance at 600 nm	TTC test - Absorbance at 490 nm
0[a]	7.98	0.14	0.06
25	4.22 *	0.15	0.11 *
50	7.86	0.17	0.05
100	2.39 *	0.19	0.002 *

[a] Garden soil
* Statistically significant at $p<0.05$.

Cell viability test
Evans blue dye test

Evans blue or T-1824 is an azo dye that has been used as a viability assay on the basis of its penetration into non-viable cells. The staining with Evans blue was used as a marker of cell death. The roots of onion treated with FA leachate showed higher capacity to fix the dye in a dose dependent fashion in comparison to the same in control root. In case of soil amendment experiment, the uptake of the dye was maximum in roots grown in 100% FA and minimum in roots grown in garden soil (control).

TTC test

TTC is a redox indicator commonly used in biochemical experiments especially to indicate cellular respiration. The compound is enzymatically reduced to red TPF (1,3,5-triphenyl formazan) in living tissues due to the activities of various dehydrogenases (Mikula *et al.* 2006). The intensity of formazan staining was found to be maximum in case of onion roots from negative control set up (in absence of test reagent) followed by 25% and 50% FA leachate solution and minimum in the case of 100% FA leachate solution (Table 1c). In case of soil amendment with FA, the intensity of formazan staining was maximum in onions grown on 25% FA and minimum for 100% FA.

Genotoxicity test
Mitotic index (MI)

Mitotic index (MI) is used as parameter to assess the cytotoxicity of FA as leachate and as amendment with soil. The results from testing of FA leachate and EMS are shown in Table 2. It is seen that there is a dose dependant decrease in the MI. It has been observed that the mean of MI for 25% FA leachate treatment is less than that of 50% FA leachate treatment. In case of onions exposed to various proportions of FA in combination with garden soil there was a dose dependent reduction in mitotic index (Table 2).

Chromosomal aberrations

In the present study, chromosomal abnormalities studied from the root tip region show the induction of clastogenic aberrations like chromosome sticky bridges and breaks in late anaphase and telophase stages of

Table 2: Chromosomal aberrations (CA) induced in *Allium* root meristem cells by exposure to various concentrations of FA leachate solution.

Concentrations of FA leachate solution (%)	Mitotic index (Mean ± SD)	Chromosomal aberrations per 5000 cells			%CA (Mean ± SD)
		Bridge	Vagrant	Fragment	
0[a]	8.12 ± 0.56	1	0	0	0.01 ± 0.04
25	6.75 ± 0.59*	5	0	0	0.09 ± 0.06
50	6.87 ± 0.85*	4	2	0	0.11 ± 0.07
100	4.97 ± 0.35*	6	2	0	0.15 ± 0.05*
2mM EMS	4.65 ± 0.27*	12	1	0	0.27 ± 0.11*

[a] Distilled water used as vehicle control.
* Statistically significant at $p<0.05$.

mitosis. In general, the frequency of CA was higher in FA leachate than in the FA- soil amendments (Table 3). There is a dose–related response for chromosomal aberrations as well but not so in the case of MN assay.

Nuclear abnormalities

Nuclear abnormalities including micronucleated and binucleated cells were studied from the F_1 region of the root and did not show significant differences between the treatment sets (Table 4&5).

DISCUSSION

Ecological studies on effects of FA contaminating terrestrial and aquatic habitats have been well documented (Twardowska, 1990). On the other hand fly ash is known to have many benefits as an input material for agricultural applications. Literature review on FA does not warrant much concern on the heavy metals, radioactivity etc. Further confirmatory studies in this field are needed for the judicious use of this useful material which is otherwise being wasted or underutilized. In the present study we have evaluated the effect of FA and FA leachate on common plant test system keeping into account to what extent it could be considered safe for amendment of soil.

Inhibition of root length elongation is an important parameter of cell death. A decrease in the viability of root cells was observed after exposure to FA and FA leachate. This is the most visible symptom of damage caused by heavy metals present in FA. TTC test and Evans blue test are reliable tools that enable precise characterization of experimental results on cell viability of plant material. Evans blue or T-1824 is an azo dye that has been used as a viability assay on the basis of its penetration into non viable cells. The staining with Evans blue was used as a marker of cell death. The uptake of dye thus determines loss of membrane integrity which signifies membrane deterioration (Gaff and Okongo, 1971). TTC is a redox indicator commonly

Table 3: Chromosomal aberrations (CA) induced in *Allium* root meristem cells by exposure to various concentrations of FA in combination with garden soil.

Concentrations of FA mixed with garden soil (%)	Mitotic index (Mean ± SD)	Chromosomal aberrations per 5000 cells			Mean of %CA (Mean ± SD)
		Bridge	Vagrant	Fragment	
0[a]	9.33 ± 1.05	2	0	0	0.03 ± 0.05
25	7.85 ± 1.21	7	0	0	0.14 ± 0.08
50	6.97 ± 1.20*	7	0	0	0.13 ± 0.05
100	5.17 ± 0.55*	9	3	0	0.25 ± 0.11*

[a] Garden soil.
* Statistically significant at pd" 0.05.

Table 4 : Nuclear abnormalities (NA) induced in *Allium* root meristem cells by exposure to various concentrations of FA leachate solution.

Concentrations of FA leachate Solution (%)	Nuclear abnormalities in F_1 region			
	MN studied from 5000 cells	Mean (±SD)	BN studied from 5000 cells	Mean (±SD)
0[a]	2	0.38 ± 0.53	1	0.01±0.04
25	12	2.34 ± 0.51*	1	0.01±0.04
50	7	1.35 ± 0.86	1	0.01 ±0.04
100	9	1.71 ± 1.04*	2	0.03 ±0.05
2mM EMS	13	2.53 ± 0.52*	2	0.04 ± 0.02

[a] Distilled water used as vehicle control.
* Statistically significant at $p<0.05$
BN- Binucleate cells, MN- Micronucleate cells

Table 5: Nuclear abnormalities (NA) induced in *Allium* root meristem cells by exposure to various concentrations of FA sample in combination with garden soil

Concentrations of FA mixed with garden soil (%)	Nuclear abnormalities in F_1 region			
	MN studied from 5000 cells	Mean (±SD)	BN studied from 5000 cells	Mean (± SD)
0[a]	3	0.58 ± 0.86	0	0.0 ± 0.0
25	11	2.17 ± 1.29	0	0.0 ± 0.0
50	6	1.19 ± 1.09	3	0.06 ±0.09
100	9	1.78 ± 1.76	2	0.04 ± 0.05

[a] Garden soil.
* Statistically significant at pd" 0.05
BN- Binucleate cells, MN- Micronucleate cells

used in biochemical experiments especially to indicate cellular respiration. The compound is enzymatically reduced to red TPF (1,3,5-triphenyl formazan) in living tissues due to the activities of various dehydrogenases (Mikula *et al.*, 2006). Our results show that both FA and leachate were capable of inducing such damages.

The increase or decrease in MI determines the cytotoxicity level of an agent (Fernandes *et al.*, 2007). Both the reduction and increase in the MI are important indicators in monitoring the environmental pollution, especially for assessments of contaminants that present genotoxic and cytotoxicity potentials (Akinboro and Bakare, 2007). MI thus studied is found to have decreased in dose dependent manner; therefore fly ash as well as fly ash leachate might have a mito-depressive effect which is able to induce cytotoxicity in *Allium cepa* roots.

The primary objective of using chromosomes as monitoring system is to determine whether or not a particular chemical is a clastogen. If the chemical is a clastogen then this would permit exchanges with subsequent cytological or genetic damage. CAs such as chromosome bridges and breaks are indicators of clastogen action (Leme and Morales, 2009). These clastogen manifestations originated from DNA strand breaks followed by mis-repair or no repair (Achary *et al.*, 2013). Chromosomal abnormalities studied from the root tip region show the induction of clastogen aberrations like chromosome sticky bridges and breaks

in late anaphase and telophase stages of mitosis. Klasterska *et al.* (1976) suggested that chromosome stickiness arises from improper folding of chromosome fibre into single chromatids and chromosomes. As a result there is an intermingling of fibres and the chromosomes become attached to each other by means of subchromatid bridges (Tripathi and Kumar, 2010).

Hence fly ash is capable of inducing some common signs of toxic influence on the chromosomes of onion. Generally, the anomalous alterations observed in the *Allium* assay are micronucleated and binucleated cells (Narayananswamy *et al.*, 2010; Vieira *et al.*, 2011), lobulated nuclei, nuclei carrying nuclear buds and mini cells (Leme *et al.*, 2008; Migid *et al.*, 2007; Carita *et al.*, 2008). Nuclear abnormalities including micronucleated and binucleated cells were studied from the F_1 region of the root. The greater synchrony of interphasic cells in the F_1 region facilitates the scoring of MNC from this region. It was known that micronucleus bearing cells usually do not divide in the F_1 cells and tend to degenerate in the F_2 generation (Arora, 1961). Acentric fragments or entire chromosomes both can contribute to the formation of MN which is not incorporated into the main nucleus during the cell cycle. Therefore, any substance able to promote MN is said to be clastogenic. Binucleated cells on the other hand, are consequences of disturbed cell cycle control.

Based on the results of the present study we can conclude that FA leachate and FA-as amendment can induce cytotoxicity, genotoxicity and can cause root growth inhibition. FA leachate is a more potent inducer of cell damages than the FA. Fly ash used as amendment in soil should be used carefully and further studies should be carried to find the optimum combination with low genotoxicity to the plants grown.

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Study on the genotoxic effects of coal fly ash leachate and consequent soil amendments in *Allium cepa* L.

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Coal fly ash (FA) is a byproduct of coal combustion at electricity generation plants. Despite having significant contribution to environmental pollution, scientists have proved the efficacy of using this hazardous waste to amend soil used in agricultural purposes. Owing to its basic mineral contents, FA can promote growth of crops on nutrient deficient soils.

The present study was undertaken to evaluate the genotoxicity of FA leachate and soil amended with FA. Various concentrations of FA leachate used are 25 (1:3), 50 (1:1) and 100% and the proportion in which FA was mixed with soil are as follows- 0 (garden soil), 25, 50 and 100%. The results show that there was a fall in mitotic index along with root length inhibition in a dose dependent manner with a maximum effect at 100% concentration of FA leachate and FA in combination with garden soil as well. The damage inducing capacities of FA have been confirmed by cell viability assays. FA leachate is more potent in provoking cytotoxic and genotoxic effects in *Allium cepa*. Studies should further be undertaken to look for the best combination of FA and soil that caters to be a promising option for augmenting the nutrient status of agricultural soil.

Key words: Coal fly ash, soil amendment, genotoxicity, mitotic index, cytotoxicity

INTRODUCTION

Fly ash (FA) is the finest of coal ash particles formed from mineral matter in coal. In India production of coal FA (both FA and bottom ash) is likely to touch 140 million tons per annum by 2020 (Gupta *et al*., 2007). Apart from Indian subcontinent, coal is also the major source of energy in many European countries including Turkey. By 2010 around 64 million tons of lignite/ hard coal was burnt in Turkish thermal power plants and it is expected that the amount will increase by 30% by 2020. This problematic solid waste has unanimously been regarded as a menace that can jeopardize terrestrial as well as aquatic ecosystems on Earth if not disposed with meticulous care.

Fly ash consists of oxides of Si, Al, Fe and Ca and also consists of 0.5 to 3.5% Na, P, K and S and the remainder is composed of trace elements. The trace elements in FA are mobilized when introduced into terrestrial, aquatic and atmospheric environments (Baba *et al*., 2004). Practically all the elements in soil except organic carbon and nitrogen are present in fly ash. Thus it has been found that this material could be used as an additive / amendment material in agriculture applications and thus can serve as an alternative for disposal of FA in landfills. In view of the above, the use of fly ash as an amendment to agricultural soils has been investigated to explore its effects on crop

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Tissue specific overexpression of endogenous *ferritin* gene in rice

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P-57

Abstract:

In mature rice seeds, external aleurone layer and embryo accumulate maximum amount of iron, which are removed during milling leaving behind very low iron content in the edible seed. Ferritin, a large protein molecule can accumulate ca. 4500 ferric ions in its central cavity as bioavailable form. Therefore, we focused to develop high iron rice by endosperm specific overexpression of endogenous *ferritin* gene. Firstly, the endosperm specific activities of two seed specific promoters (*OsglutelinA2* and *Osglobulin1*) have been studied through *gus* expression analysis. *OsglutelinA2* promoter exhibited stronger gus expression in transgenic seeds over *Osglobulin1*. After confirmation of the activities, two promoters have been subcloned separately into the upstream region of *Osfer2* recombinant binary vector. The two recombinant overexpression constructs have been used in genetic transformation of Pusa Sugandh II. The resulting transgenic milled seeds of Pusa sugandh II showed up to 7.8-fold of *ferritin* overexpression and 2.09-fold of iron increment. The zinc and magnesium content of the seeds were also enhanced without affecting other metals concentration. Moreover, the transgenic plants possess no significant morphological differences with non-transgenic Pusa Sugandh II.

Saccharum spontaneum L. - an eco friendly choice for remediation of coal fly ash

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P-58

Abstract:

Coal fly ash is generated as a byproduct of coal combustion in coal fired thermal power plants. The practices adopted for the disposal of this global waste are mainly slurry, open lands or ash ponds adjacent to the plants. Wind and leaching are often the potential causes of off-site contamination from the dumpsites. The components of fly ash vary considerably depending upon the source and specific makeup of the coal bed. The heavy metal contaminants in fly ash pose a serious threat to the environment as they have high bioaccumulation rate in living systems. To handle this problem judiciously a study was undertaken where *Saccharum spontaneum* was grown on coal fly ash for a period of 12 months. Comparative study of metal analysis in fly ash carried out before commencement and after termination of the treatment period (1 year) shows that *Saccharum spontaneum* can sequester a considerable amount of heavy metals without incurring significant DNA damages in itself. Thus the results of comet assay (to detect DNA damages) and metal estimation by Atomic Absorption Spectroscopy (AAS) establish the fact that plantation of *Saccharum spontaneum* is an eco friendly choice to remediate coal fly ash.

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PROGRAM BOOK

P5 : Studies on the Cytotoxic Effect of Copper Oxychloride on the Seeds of *Pisum sativum* and *Vigna radiata*

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Food shortage is a serious global problem in the current century thus the agricultural sector has to increase the rate of production for grains and pulses. Although different diseases may damage the crop production, many types of fungicide have been formulated for management of fungus in the crop fields as well as home gardens during cultivation. They act quickly and not only cure fungal disorders but also affect the cell division at the chromosomal level. The *Vigna radiata* and *Pisum sativum* are important source of proteinaceous pulse crop belonging to Fabaceae having chromosome number 2n= 22 and 2n= 14 respectively. The seeds of *V. radiata* and *P. sativum* were treated with copper oxychloride, a blue label chemical fungicide. It was observed that copper has significantly increased the seed germination. The mitotic inhibition in both plants increased with increasing concentration of copper oxychloride. The root /shoot ratio have interesting result with increasing percentage of copper first act as plant growth stimulator and then act as an essential micronutrient up to concentration of 1% but higher concentration caused the decrease value of root/shoot ratio, thereby causing decrease plant height due to accumulation and inactivation of proteins resulting increased in total seed protein content. The rate of pollen germination and flowering decreased with increasing percentage of copper oxy chloride in both testing plants. The *Vigna radiata* and *Pisum sativum* recorded very low percentage of abnormality index in lower concentration of copper oxychloride therefore it may act as a safe and ideal fungicide for protection of *P. sativum* and *V. radiata.*

P6 : Impact of Aluminium (Nanoparticle) Stress is More Pronounced in *Vicia Faba* L. in Comparison to *Allium Cepa* L.

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Aluminium (Al) is the 3rd most abundant metallic element in the soil. At lower pH (~5.8) plants represent several signals of Al toxicity. Aluminium exposure leads to root growth inhibition that causes a decrease in the levels of plant production. This inhibition is primarily attributed to cytotoxicity, genotoxicity and ROS generation in the root cells. In the present study, the most remarkable symptoms of Al nanoparticle toxicity in two model plant systems- *Allium cepa* L. and *Vicia faba* L. are addressed. A comparative account of a battery of harmonized experimental data is presented here which documents the fact that *Vicia faba* L. is more susceptible to stress induced by Al nanoparticles when compared to *Allium cepa* L. The antioxidative stress enzymes (CAT, GPX) and lipid peroxides (MDA content) showed variations in dose dependent manner. Results of comet assay performed to assess for DNA strand breaks and Evans blue uptake by root meristem as a cell death parameter serving as an indicator of cytotoxicity leads to the conclusion that Al –induced stress is mediated through genotoxic pathways. The effects are more pronounced in *Vicia faba* L. than that in *Allium cepa* L.

P.7
Amelioration of Soil by Coal Fly Ash

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Coal fly ash is a byproduct of coal combustion at electricity utility plants. Though it has got a significant contribution to environmental pollution, scientists have proved the efficacy of using this hazardous waste to amend soil used in agricultural purposes. Fly ash (FA) when amended to soil can cater to be a promising option for augmenting the nutrient status of the soil which manifests into higher crop yield. Owing to its basic mineral contents, FA can even promote growth of crops on nutrient deficient soils. Based on relevant literature, the present study was under taken to evaluate the genotoxicity of soil amended with FA. Various proportions in which FA were mixed with soil are as follows- 0%, 25%, 50% and 100% fly ash. Thereafter Allium test was carried out to test their genotoxicity. The results show that there was a fall in mitotic index and root length inhibition in a dose dependent manner with a maximum effect at 100% concentration of FA. The damage inducing capacities of FA have been confirmed by cell viability assays like TTC and Evans Blue assays. Hence it is found that 25% concentration of FA serves to be the best amendment that can be used for agricultural soil amelioration.

P.8
Testing the Efficacy of *Cerbera odallam* Extracts as a Potential Anti-Tumour Agent

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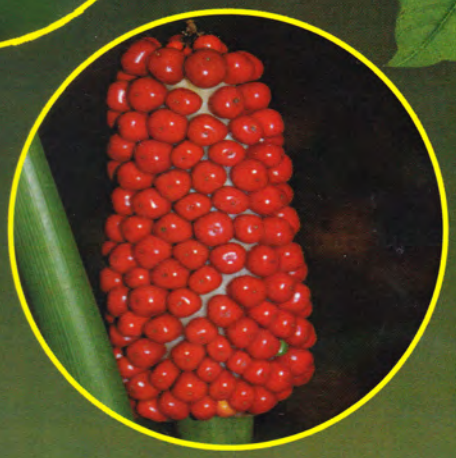
Cardiac glycosides have been reported to be anti-proliferative in nature owing to its ability to bind and inhibit Na+ Ka+ATPase. The present study was designed to analyse if the reported toxic properties of Cerberin, a cardiac glycoside from *Cerbera odallam* could be exploited to develop it into an effective anticancer drug. For this, dichloromethane extract of *Cerbera odallam* was fractionated on silica column and the fraction was subjected to qualitative phytochemical analysis. On the basis these analyses coupled with the results of HPTLC we concluded that fraction five is relatively pure with a minor band and constituted the cereberin rich fraction. This fraction was used for testing the antitumor activity in vitro, for which A431 and PBMCs were selected as the tumor cell and normal cell model. These were cultured in parallel, and treated with various concentrations of the serially diluted fraction 5. IC50 and safety index were calculated from regression curve plotted using

ABSTRACTS



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