

Vetiver can grow on Fly ash without DNA damage

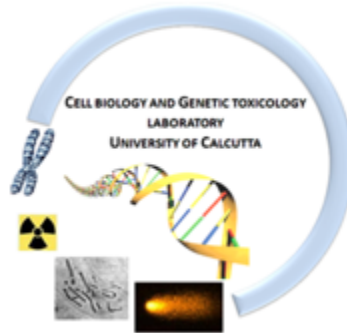
Prof. Anita Mukherjee,

Cell Biology and Genetic Toxicology Laboratory

Faculty: CAS, Department of Botany & Head: Department of Genetics

University of Calcutta. Kolkata (Calcutta), India.

Email: Prof.amukherjee.cu@gmail.com



University of Calcutta

কলিকাতা বিশ্ববিদ্যালয়



DEPARTMENT OF BOTANY



University Of Calcutta

Fly ash...

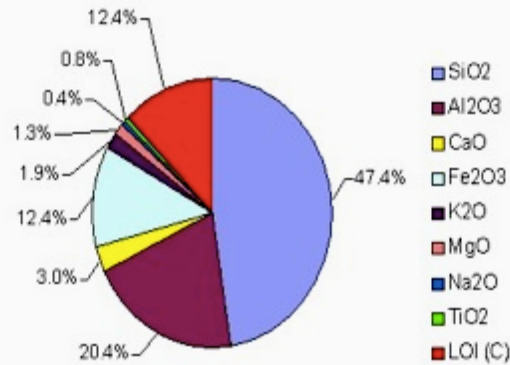
Pulverized fuel ash (PFA) or fly ash is generated from the combustion of coal in electricity generating power stations. It is collected from the exhaust gas stream of the furnace either in electrostatic precipitators (ESP's) or sometimes in a filter (bag house). It has a much finer particle size, which means that it does not fall to the bottom of the furnace but rather exits with the exhaust gases - hence the name fly ash. It is mainly derived from the inorganic component of the coal (clay, shales etc) but also contains residual carbon from un-burnt fuel.



Physical Properties

- Fly ash consists of fine, powdery particles that are predominantly spherical in shape, either solid or hollow, and mostly glassy (amorphous) in nature.
- The specific gravity of fly ash usually ranges from 2.1 to 3.0, while its specific surface area (measured by the Blaine air permeability method) may range from 170 to 1000 m²/kg.
- The color of fly ash can vary from tan to gray to black, depending on the amount of unburned carbon in the ash. The lighter the color, the lower the carbon content.

Chemical composition of Fly ash

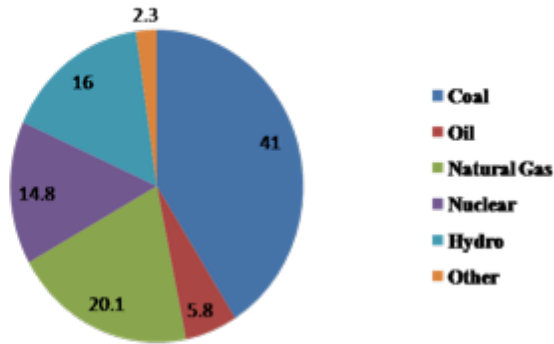


	ClassF*	ClassF*	ClassC*	Class C*
	low-Fe	high-Fe	high-Ca	low-Ca
SiO ₂	46-57	42-54	25-42	46-59
Al ₂ O ₃	18-29	16.5-24	15-21	14-22
Fe ₂ O ₃	6-16	16-24	5-10	5-13
CaO	1.8-5.5	1.3-3.8	17-32	8-16
MgO	0.7-2.1	0.3-1.2	4-12.5	3.2-4.9
K ₂ O	1.9-2.8	2.1-2.7	0.3-1.6	0.6-1.1
Na ₂ O	0.2-1.1	0.2-0.9	0.8-6.0	1.3-4.2
SO ₃	0.4-2.9	0.5-1.8	0.4-5.0	0.4-2.5
LOI	0.6-4.8	1.2-5.0	0.1-1.0	0.1-2.3
TiO ₂	1-2	1-1.5	<1	<1

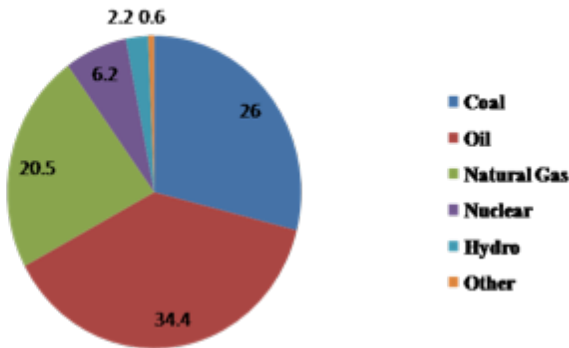


Fly ash scenario: world

Total World Electricity Generation by Fuel (2006)

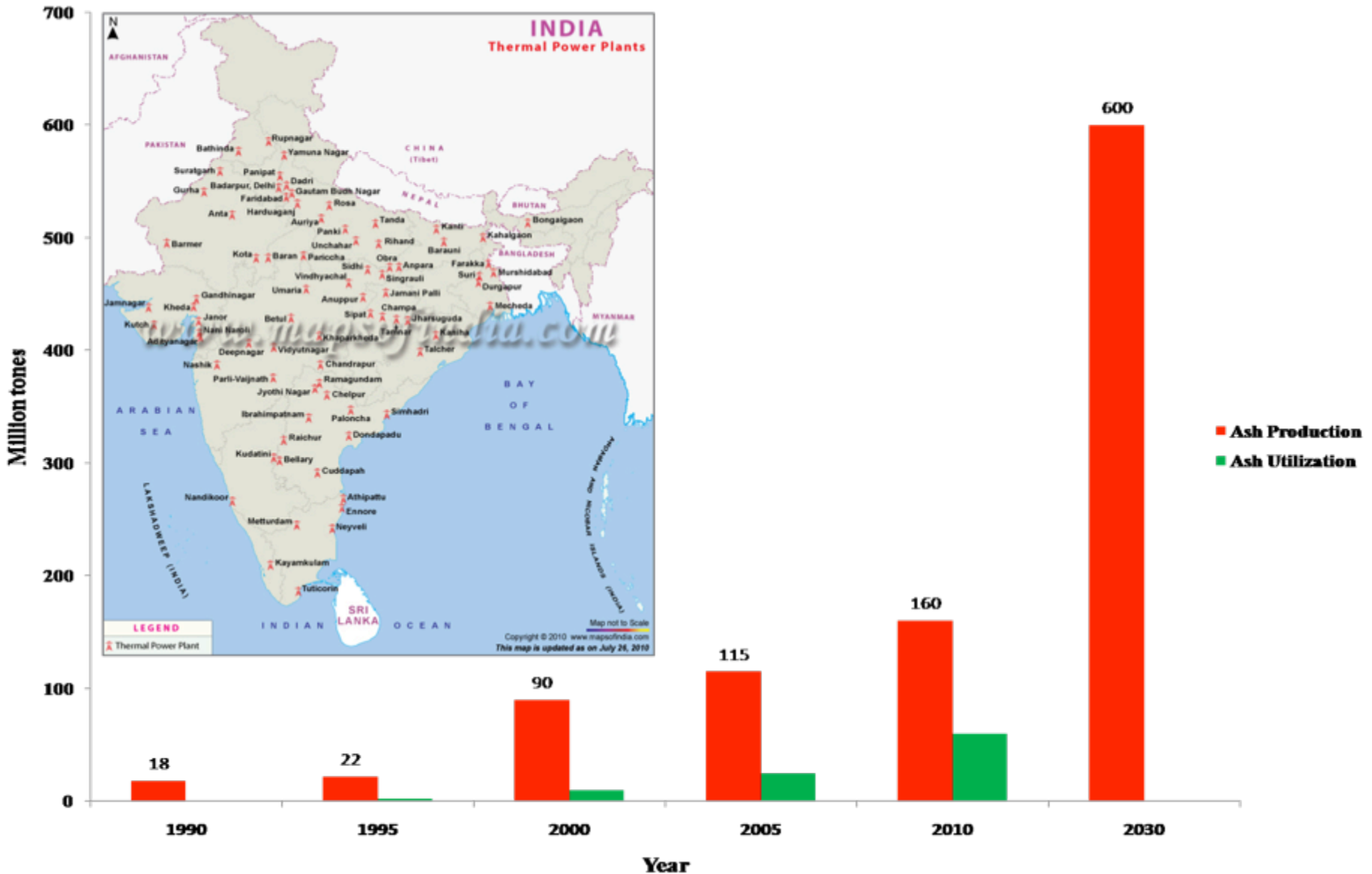


Total World Primary Energy Supply by Fuel (2006)

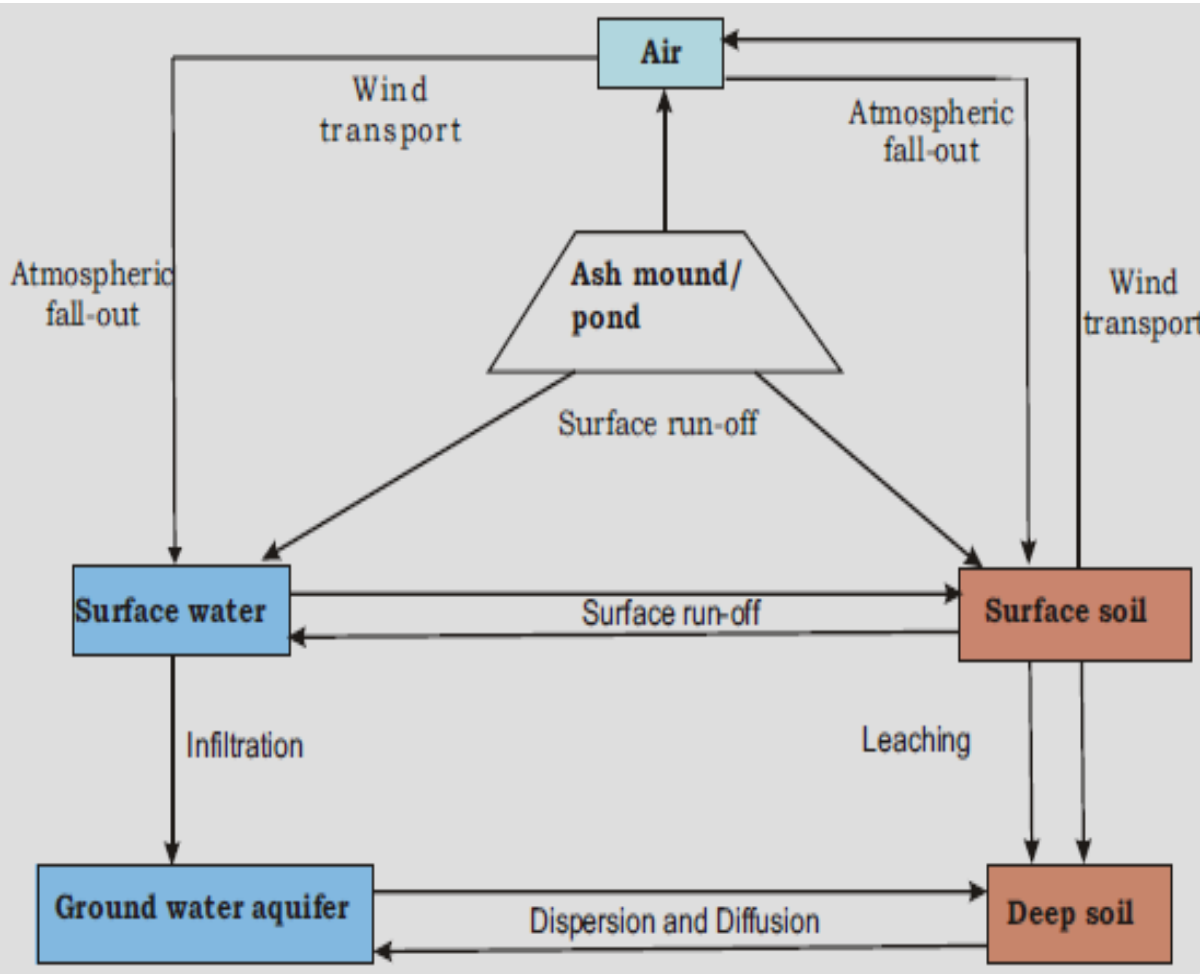


SL NO	Country	Annual ash production, MT	Ash utilization %
1	India	112	38
2	China	100	45
3	USA	75	65
4	Germany	40	85
5	UK	15	50
6	Australia	10	85
7	Canada	6	75
8	France	3	85
9	Denmark	2	100
10	Italy	2	100
11	Netherlands	2	100

Fly ash scenario: India



How dumping of fly ash leads to pollution of air, land and water





Concern: Effect on human health

- Haemolysis
- Fibrosis
- Effects on immune system and inflammatory cells
- Pneumoconiosis; an occupational lung disease lung disease caused by the inhalation of dust, often in mines.
- Chronic obstructive pulmonary disorders (COPD)
- Genetic risk

Type ash ^a	Exposure	Species	Effect(s)	Ref
CFA, not specified	8 h/day, 180 days 0.6 and 4.25 mg/m ³	Rat	Mild response, No fibrosis	Raabe <i>et al.</i> , 1982
CFA (FBC and PCC)	4 weeks, 5 days/week, 7 h/day 36–38 mg/m ³	Rat (F344) male/female	Chronic cell influx Sil > PCC > FBC	Bice <i>et al.</i> , 1987
CFA (2 types) and COM	Intratracheal (5, 15, 45 mg) quartz positive control	Rat (SD)	Fibrosis at 15 mg Mild inflammation all doses of CFA. COM less toxic.	Schreider <i>et al.</i> , 1985
CFA (FBC)	Intratracheal (30 mg)	Hamster (SG)	Transient (30 days) recruitment of PMN and AM	Lantz and Hinton, 1986
CFA (PFA)	Intratracheal (2, 10, 50 mg) TiO ₂ and quartz as controls	Rat (Wistar) male	Dose-related influx of PMN, NOEL of 2 mg/kg	Arts, 1993
CFA	Intratracheal (10 mg), other dusts incl. quartz as controls	Rat (Wistar) female	Ranking: quartz > CFA > mica	Bajpai <i>et al.</i> , 1992 ^b
CFA (PCC?)	Intratracheal (12.5 mg), 60 days; quartz controls	Rat	No fibrosis; dust laden AM and cells dusts including quartz	Kaw <i>et al.</i> , 1988

^aFBC: fluidized bed combustion; PCC: pulverized coal combustion; COM: coal oil mixture fly ash; PFA; pulverized coal fuel ash; NOEL: No observed effect level; AM: alveolar macrophages; PMN: polymorphonuclear neutrophils.

^bFibrosis was not studied as an endpoint.

Is there a way out ???????

Utilization ...

Area of Utilisation	Quantity (in Million Tons)
Land Development	7.73
Cement manufacturing	7.20
Ready Mix Concrete and asbestos cement products	0.40
Roads embankments	1.34
Ash Dyke Raising	3.51
Bricks and other building products	2.04
Mine Filling	1.13
Export	0.90
Others	3.36
Total	27.61

Phytoremediation as an alternative

Jatropha curcas



Prosopis juliflora



Vetiveria zizanioides



Phytoremediation using Vetiver

Vetiver



Kingdom:	Plantae
(unranked):	Angiosperms
(unranked):	Monocots
(unranked):	Commelinids
Order:	Poales
Family:	Poaceae
Subfamily:	Panicoideae
Genus:	<i>Chrysopogon</i>
Species:	<i>C. zizanioides</i>

Species	Distribution
1. <i>V. elongata</i> (R.Br.) Stapf ex C.E.	New Guinea, Australia
2. <i>V. festucoides</i> (Presl.)	Ohwi Japan
3. <i>V. filipes</i> C.E.Hubbard	New Guinea, Australia (Queensland)
4. <i>V. fulvibarbis</i> Stapf	Central and East Africa
5. <i>V. intermedia</i> S.T. Blake	Australia (Queensland)
6. <i>V. lawsonii</i> (Hook.f) Blatt. Et McCann.....	India
7. <i>V. nemoralis</i> (Balansa) A. Camus	Southeast Asia
8. <i>V. nigritana</i> Stapf	Central and East Africa
9. <i>V. pauciflora</i> S.T. Blake.....	Australia (Queensland)
10. <i>V. rigida</i> B.K. Simon.....	Australia (Queensland)
11. <i>V. zizanioides</i> Nash	India, Central and Southeast Asia

Morphological Characteristics

- Vetiver grass does not produce above or underground runners. The plant has a strong and massive root system, which is vertical in nature descending 2-3 meters in the first year, ultimately reaching some five meters under tropical conditions. The depth of root structure provides the plant with great tolerance to drought, permits excellent infiltration of soil moisture and penetrates through compacted soil layers (hard pans).
- Above ground, the shoots can grow to two meters and when planted close together it forms a solid vegetative barrier that retards water flow and filters and traps sediment in runoff water. Growth occurs from the crown, which rises relative to soil build-up. It is also highly resistant to pests, diseases, fire and heavy grazing pressure.

Physiological Characteristics

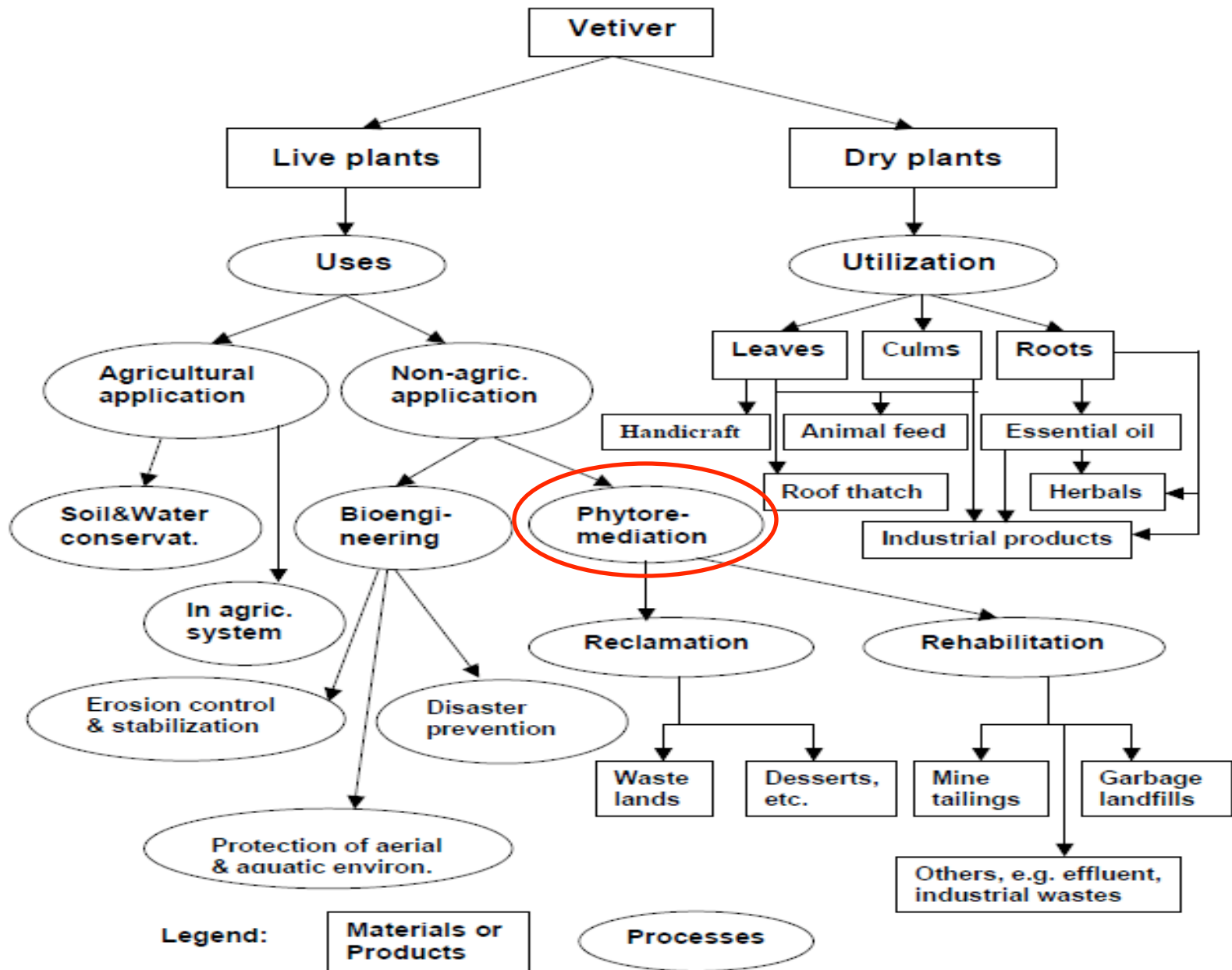
- Tolerance to extreme climatic variations such as prolonged drought, flood, submergence and temperature levels ranging from -20°C to 55°C.
- Vetiver has been found to thrive under rainfall ranging from 300 mm to 6000 mm per annum.
- Ability to regrow rapidly after being affected by drought, frost, fire, saline and other adverse conditions when the adverse effects are removed.
- Adaptability to a wide range of soil types (pH 3.0 to 10.5) (Truong and Baker 1998).
- Highly tolerant to growing media that are high in acidity, alkalinity, salinity, sodicity and magnesium (Truong 1994; Truong *et al.* 2003).
- Highly tolerant to Al, Mn, As, Cd, Cr, Ni, Pb, Hg, Se and Zn in the soil (Truong and Baker 1998).

Data depicting the tolerance level of vetiver

Heavy Metals	Threshold levels in soil (mgKg ⁻¹)(a)		Threshold levels in plant (mgKg ⁻¹)	
	Vetiver	Other plants (b)	<i>Vetiver</i>	Other plants (c)
Arsenic	100-250	2.0	21-72	1-10
Cadmium	20-60	1.5	45-48	5-20
Copper	50-100	Not available	13-15	15
Chromium	200-600	Not available	5-18	0.02-0.20
Lead	>1 500	Not available	>78	Not available
Mercury	>6	Not available	>0.12	Not available
Nickel	100	7-10	347	10-30
Selenium	>74	2-14	>11	Not available
Zinc	>750	Not available	880	Not available

Effects of the Vetiver System on soil loss and runoff in agricultural lands

Countries	Soil loss (t/ha)			Runoff (% of rainfall)		
	Control	Conventional	VS	Control	Conventional	VS
Thailand	3.9	7.3	2.5	1.2	1.4	0.8
Venezuela	95.0	88.7	20.2	64.1	50.0	21.9
Venezuela (15%)*	16.8	12.0	1.1	88	76	72
Venezuela (26%)*	35.5	16.1	4.9			
Vietnam	27.1	5.7	0.8			
Bangladesh		42	6-11			
India		25	2			
		14.4	3.9		23.3	15.5



Environ Monit Assess (2009) 153:351–357
DOI 10.1007/s10661-008-0361-z

Evaluation of genotoxicity of coal fly ash in *Allium cepa* root cells by combining comet assay with the *Allium* test

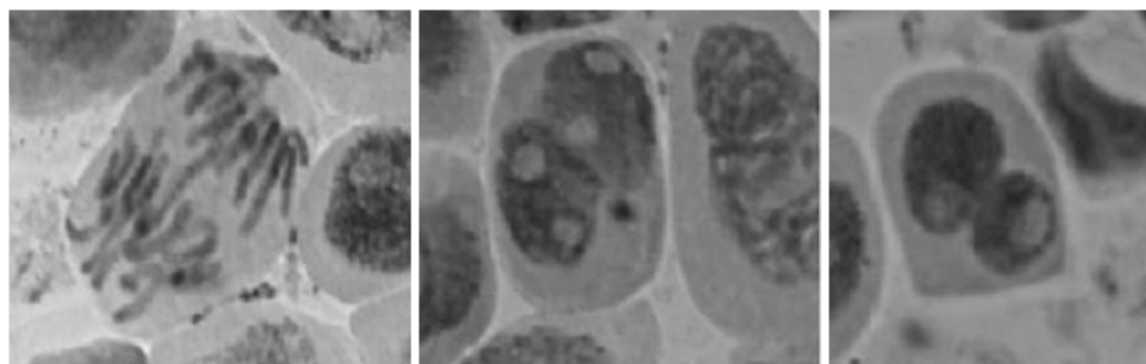
**Rajarshi Chakraborty • Ashit Kumar Mukherjee •
Anita Mukherjee**

Estimation of heavy metals in FA

Metal	Amount ($\mu\text{g/g}$ of fly ash)
Zn	28.90 ± 3.04
Pb	23.39 ± 2.32
Cu	16.29 ± 2.13
Ni	14.06 ± 0.93
Cd	0.91 ± 0.24
As	0.51 ± 0.14

Effects of treatments with different concentrations of fly ash for 5 days in the root meristems of *A. cepa*

Con ^a (%)	Root Length (cm) Mean±SD	Mitotic Index Mean±SD	Prophase (%) ^b Mean±SD	MP-AP-TP (%) ^c Mean±SD	BN Cell ^d / 1,000 Mean±SD	MN ^e /1,000 Mean±SD	CHR ^f Breaks and Bridges/1000 Mean±SD
0 ^g	3.91±0.14	11.46±1.43	9.12±0.78	2.17±1.02	0.57±0.81	0.00±0.00	0.00±0.00
5	3.84±0.98	13.81±3.00	12.71±3.11	1.78±0.51	2.52±1.79	0.00±0.00	0.84±0.80
10	4.25±1.56	12.03±3.49	9.00±2.33	3.04±1.25	7.26±3.68*	0.00±0.00	1.16±0.86
25	4.22±0.90	15.62±2.63	12.98±3.03	2.48±1.27	8.21±4.49*	1.92±1.45	0.48±0.67
50	3.17±1.34	14.59±0.60	12.43±0.17	2.16±0.42	3.80±1.43	0.84±0.73	0.00±0.00
75	3.41±0.71	14.72±1.31	14.20±1.91	0.83±0.25	3.66±2.86	0.42±0.40	0.00±0.00
100	1.38±0.99*	4.34±0.21*	3.82±0.39*	0.52±0.60*	6.55±1.27*	0.95±0.90	0.00±0.00
EMS(2 mM) ^h	–	12.33±0.58	10.00±1.00	2.33±0.58	0.42±0.70	5.33±0.58	2.33±0.58



Detection of DNA damage in nuclei of *A. cepa* root meristems following 5 days exposure to fly ash using the Comet assay

Amount of fly ash in mixture (%)	Tail DNA (%) Mean \pm SD	Tail length (μm) Mean \pm SD	Olive tail moment (arbitrary unit) Mean \pm SD
0 ^a	18.83 \pm 1.26	44.68 \pm 6.75	6.39 \pm 0.63
5	17.09 \pm 3.56	43.57 \pm 3.61	5.40 \pm 0.92
10	28.51 \pm 5.02	51.58 \pm 3.04	10.54 \pm 1.42
25	37.76 \pm 9.28*	53.28 \pm 4.11	11.13 \pm 1.54
50	31.69 \pm 1.39*	56.75 \pm 10.78	9.58 \pm 0.845
75	85.65 \pm 4.02*	101.82 \pm 7.93*	42.05 \pm 4.85*
100	88.07 \pm 6.08*	109.87 \pm 4.23*	49.23 \pm 6.97*
EMS (2 mM) ^b	43.80 \pm 1.53	88.54 \pm 5.58	20.14 \pm 0.92





Contents lists available at ScienceDirect

Ecotoxicology and Environmental Safety

journal homepage: www.elsevier.com/locate/ecoenv



Mutagenicity and genotoxicity of coal fly ash water leachate

Rajarshi Chakraborty, Anita Mukherjee *

Department of Botany, Centre of Advanced Study in Cell and Chromosome Research, University of Calcutta, 35 Ballygunge Circular Road, Kolkata 700 019, India

Analyses of metals present in the fly ash leachate

Metals	Amount, mean \pm SD (mg/l)
Na	30.90 \pm 2.92
Si	28.43 \pm 1.60
K	16.35 \pm 2.31
Ca	15.95 \pm 1.84
Mg	13.63 \pm 1.21
Fe	1.04 \pm 0.15
Mn	0.23 \pm 0.03
Zn	0.16 \pm 0.02
Cd	< 0.02
Ni	< 0.2819
Cu	< 0.0691
Pb	< 0.2814
Al	< 0.02
As	< 0.014
F	< 0.03
SO ₄ ^a	32.67 \pm 5.29

Detection of DNA damage in *Nicotiana* leaf cells following 24 h exposure to fly ash leachate assessed by the comet assay

Leachate concentration [fly ash (g):water (ml)]	Tail DNA, mean \pm SD (%)	Tail length, mean \pm SD (μ m)	Olive tail moment, mean \pm SD (arbitrary unit)
Distilled water ^a	6.63 \pm 0.55	13.95 \pm 3.35	1.21 \pm 0.54
1:16	7.40 \pm 1.03	19.32 \pm 1.61	2.17 \pm 0.26
1:8	7.51 \pm 1.75	14.49 \pm 4.26	1.63 \pm 0.98
3:16	15.64 \pm 1.87*	31.66 \pm 3.35*	4.46 \pm 0.33*
1:4	16.19 \pm 1.30*	28.98 \pm 1.61*	3.97 \pm 0.40*
EMS ^b	26.94 \pm 1.48*	33.81 \pm 5.81*	6.05 \pm 0.40*

Detection of DNA damage in human whole blood cells (BC) and lymphocytes (L) following 3 h exposure to fly ash leachate assessed by the comet assay

Leachate concentration [fly ash (g): water (ml)]	Tail DNA, mean \pm SD (%)		Tail length, mean \pm SD (μ m)		Olive tail moment, mean \pm SD (arbitrary unit)	
	BC	L	BC	L	BC	L
Distilled water ^a	15.24 \pm 2.85	8.33 \pm 1.38	24.15 \pm 1.61	28.98 \pm 3.22	3.30 \pm 0.57	1.56 \pm 0.34
1:16	15.17 \pm 2.72	9.06 \pm 0.88	28.98 \pm 5.58	31.13 \pm 3.35	3.50 \pm 0.64	1.50 \pm 0.19
1:8	16.59 \pm 1.46	8.52 \pm 1.42	30.59 \pm 1.61	29.52 \pm 4.92	4.10 \pm 0.30	1.27 \pm 0.34
3:16	67.55 \pm 5.92*	16.88 \pm 0.79*	47.76 \pm 3.72*	46.15 \pm 4.05*	12.38 \pm 2.51*	3.75 \pm 0.59*
1:4	71.74 \pm 4.94*	20.00 \pm 2.11*	48.84 \pm 1.86*	48.84 \pm 1.86*	14.92 \pm 0.80*	4.45 \pm 0.57*
EMS ^b	74.64 \pm 3.81*	19.10 \pm 2.30*	50.98 \pm 3.35*	54.73 \pm 5.80*	15.48 \pm 3.50*	4.30 \pm 0.44*

TECHNICAL NOTE: VETIVER CAN GROW ON COAL FLY ASH WITHOUT DNA DAMAGE

Rajarshi Chakraborty and Anita Mukherjee

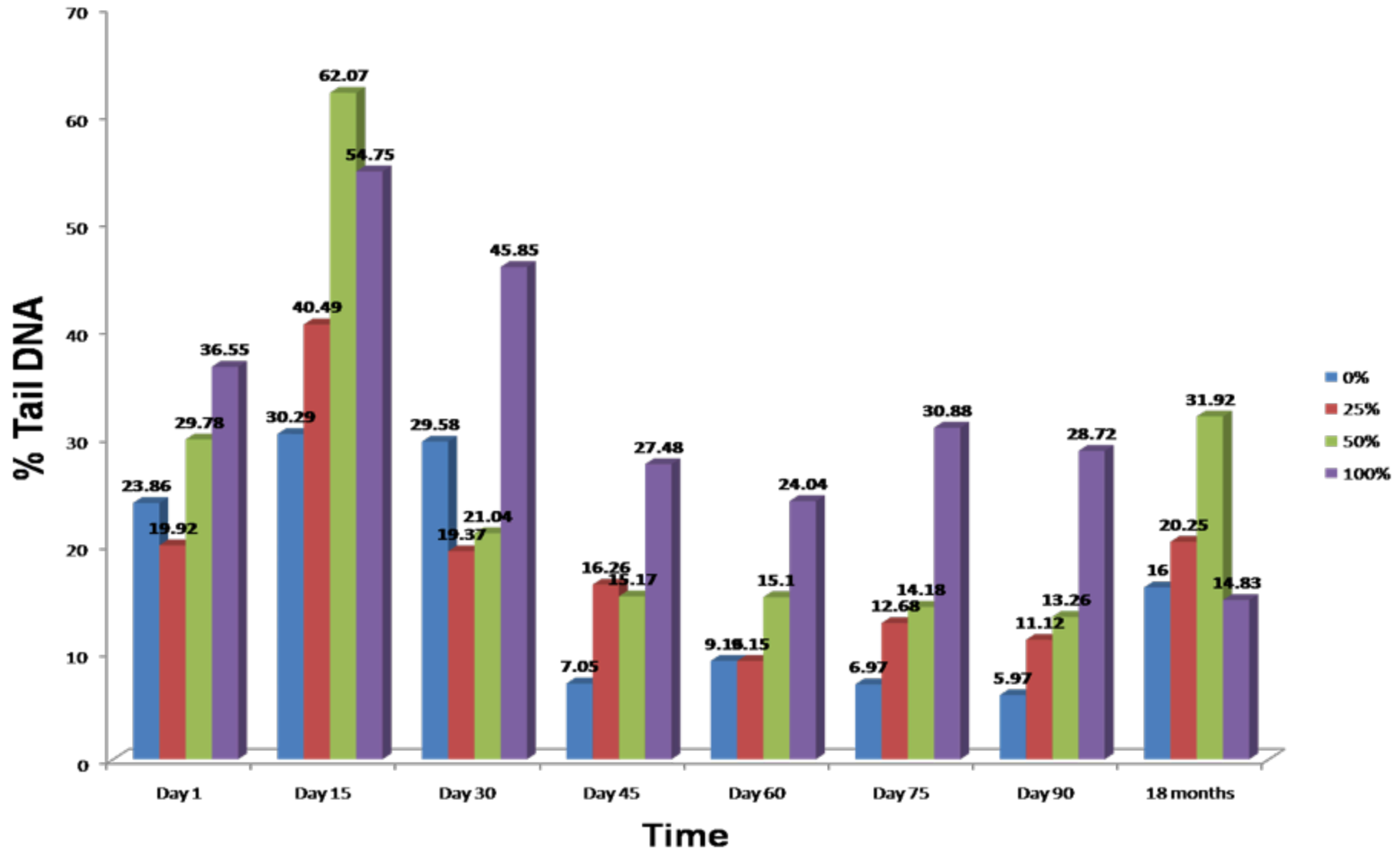
Centre of Advanced Study in Cell and Chromosome Research, Department of Botany, University of Calcutta, Kolkata, India

Plant material	Control ^a [Tail DNA (%)]	Treated ^b [Tail DNA (%)]
<i>A. cepa</i>	15.77 ± 1.63	82.88 ± 2.98*
<i>V. zizanioides</i>	8.36 ± 1.16	8.31 ± 1.13 ^{ns}

^a*A. cepa* grown for 5 days and *V. zizanioides* grown for 3 months in garden soil.

^b*A. cepa* grown for 5 days and *V. zizanioides* grown for 3 months in fly ash sample.

Detection of DNA damage in root cells of *Allium cepa* over a period of 18 months



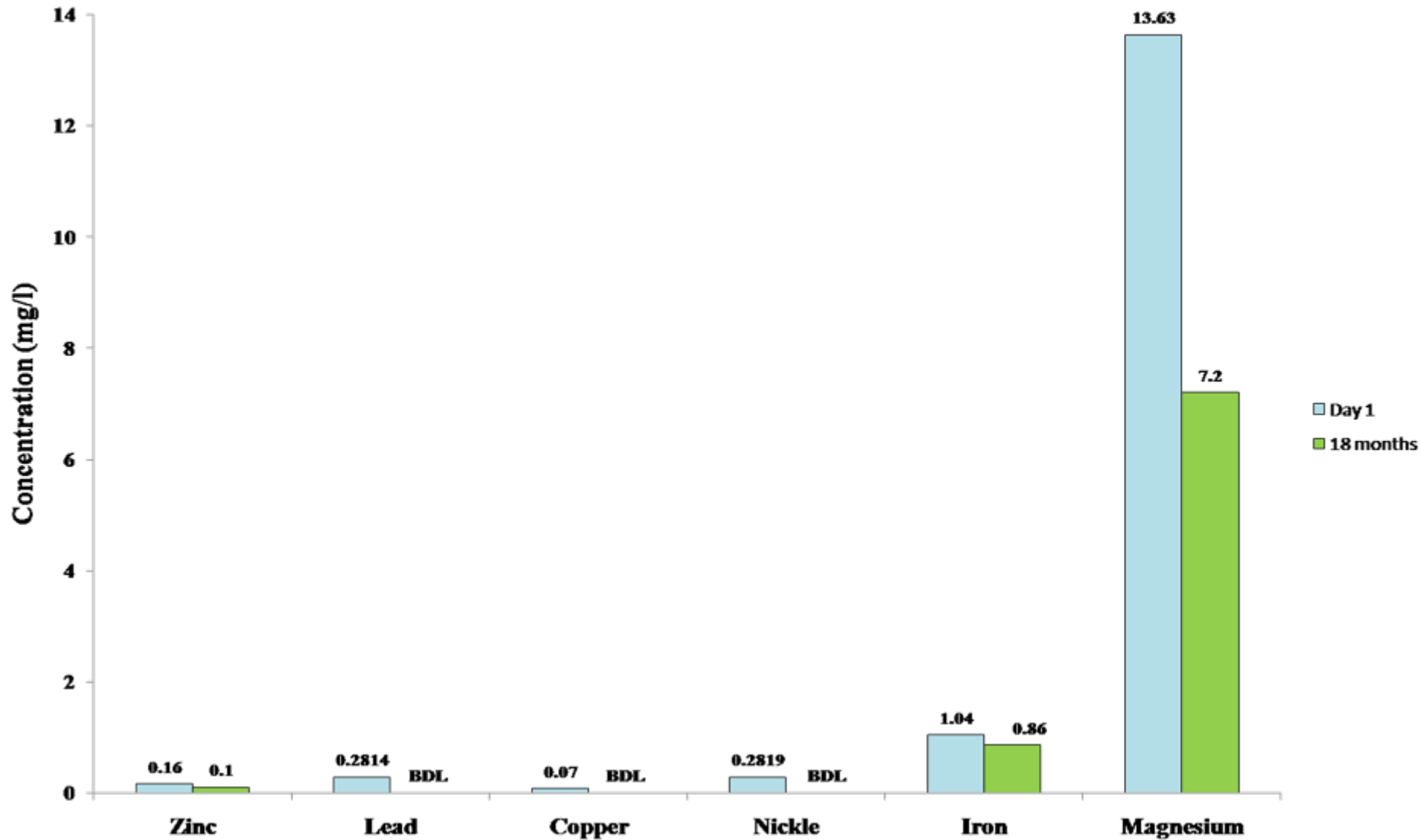
Mitotic index (MI), number of micronuclei / 1000 cells, % binucleate cells and % chromosomal aberrations revealing the genotoxic potential of fly ash-soil amendments in *Allium cepa* roots as analyzed by *Allium* test and *Allium* anaphase - telophase chromosome aberration assay; * significant at $p \leq 0.05$.

fly ash- soil amendments	Mitotic Index (18 months)	Micronuclei/1000 cells		%Binucleate cells		%Chromosomal aberration	
		Day 1	18 months	Day 1	18 months	Day 1	18 months
Garden soil	9.43 ± 1.23	2.25 ± 0.23	1.89 ± 0.46	-	0.09 ± 0.02	-	0.11 ± 0.03
25 % fly ash- soil amendments	8.06 ± 0.83	0.99 ± 0.17	-	-	0.26 ± 0.09	1.09 ± 0.25*	0.27 ± 0.12
50 % fly ash- soil amendments	9.00 ± 1.58	2.93 ± 0.12	-	-	0.64 ± 0.06*	1.12 ± 0.16*	0.29 ± 0.08
100 % fly ash- soil amendments	6.97 ± 0.58*	4.44 ± 0.36*	2.94 ± 0.86*	0.1 ± 0.04	0.58 ± 0.12*	0.80 ± 0.07*	0.18 ± 0.02

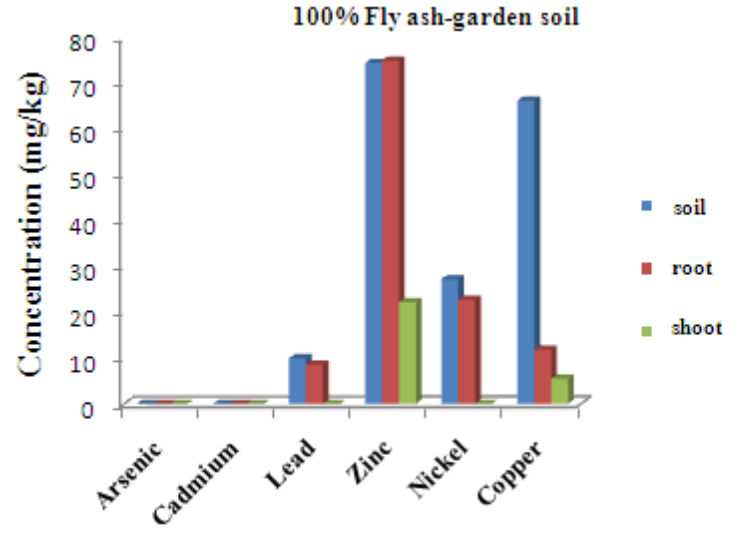
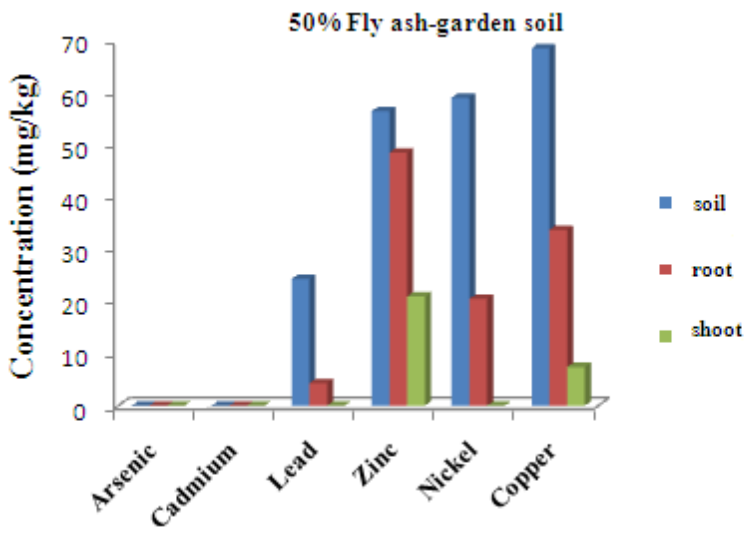
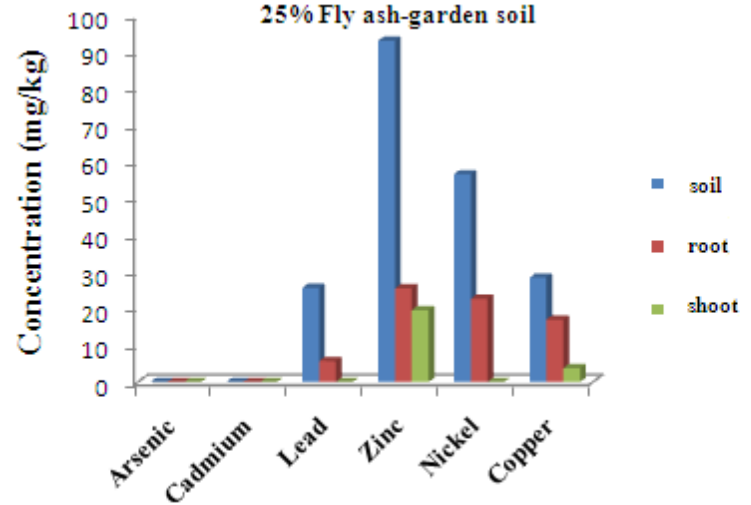
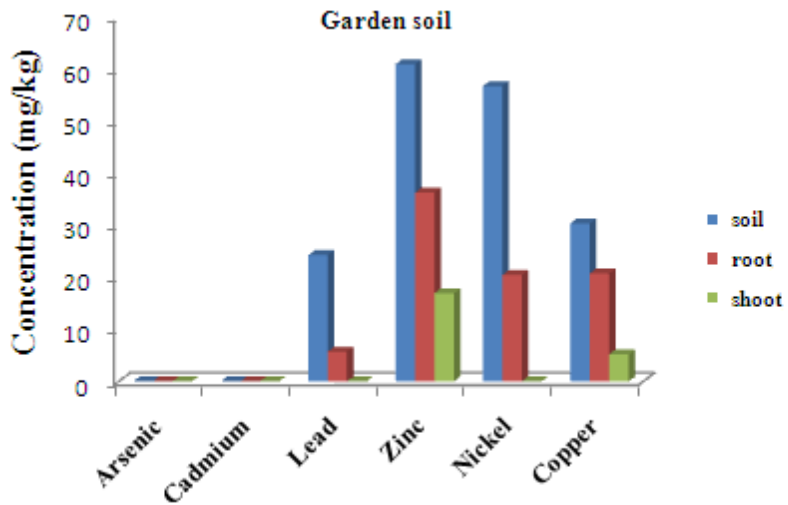
Heavy metal estimation in fly ash on day 1 and at the end of 18 months

Time	Day 1	18 months
Metals		
Zinc	28.9	74.6
Lead	23.39	10
Copper	16.29	66.32
Nickel	14.06	27.27
Cadmium	0.91	BDL
Arsenic	0.51	BDL

Heavy metal estimation in fly ash leachate on day 1 and at the end of 18 months



Comparative distribution of heavy metals in fly ash-soil amendments , and root and shoot of *Vetiver*.



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	0.595148	0.359972	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





Thank you

