Chapter 2

Review of literature
Genotoxicity of coal fly ash:
The environmental impact of coal industry and thermal power plants in India in relation to ecological, radio-ecological and pollution was assessed by Mishra 2004. The problems were found to be formidable.

Fly ash is considered as a serious source of air pollution since it remains air-borne for a long period. It possesses a hazard to the lungs through its potential to cause oxidative stress and inflammation (Donaldson et al., 2005). van Maanen et al., 1999, measured iron release, acellular generation of hydroxyl radicals, and oxidative DNA damage and cytotoxicity in rat lung epithelial (RLE) cells by different coal fly ashes (CFA). Their results indicate an important role for size and iron release in generation and subsequent effects of reactive oxygen species caused by CFA.

The trace elements found in fly ash namely Mn, As, Cu, Cd, Se and Be were found to inhibit lectin-induced lymphocyte division (Shifrine et al., 1984). Kleinjans et al., 1989, demonstrated that DMSO extracts of fly ash significantly increased the sister chromatid exchange (SCE) frequency of human lymphocytes after incubation in vitro in comparison to non-exposed cells. Similar increase was found in peripheral lymphocytes of the occupationally exposed population than the control population. The authors concluded that exposure to fly ash from powder coal combustion implies a moderate genotoxic risk to man. The study by Celik et al., 2007, showed that mean frequencies of chromosonal aberrations, polyplody, sister-chromatid exchanges and micronuclei were significantly higher in the peripheral lymphocytes of workers exposed to coal combustion products like coal ash and gaseous emissions than individuals without exposure to such agents.

Mutagenicity of fly ash particles had been studied by a number of workers (Chrisp et al., 1978; Fisher et al., 1979; Smith-Sonneborn et al., 1981; Griest et al., 1982; Mumford and Lewtas 1982; Li et al., 1983; Kleinjans et al., 1989). Chrisp et al. (1978) reported that cyclohexane-, saline-, and serum-soluble surface components of respirable fly ash particles produced an increased number of revertants in two frame-shift tester strains of S. typhimurium. Their result concluded that both organic and inorganic mutagens are present in coal fly ash. The effect of physical factors on the mutagenicity of coal fly ash was studied by Fisher et al., 1979. They found finer coal fly ash fraction to be more mutagenic than coarser fractions. In addition, the mutagens in coal fly ash were found to be resistant to x-ray or ultraviolet irradiation but all
mutagenic activity was lost when heated to 350 °C. Smith-Sonneborn et al., 1981, monitored the biological effects of the respirable fraction of coal fly ash in Paramecium and found it to be mutagenic. The effects of physical and chemical treatment provided evidence for both heat-stable, heat-labile and acid extractable mutagenic agents in the fly ash. Griest et al., 1982, concluded that the presence of aromatic hydrocarbons and chemically derivatizable polar organic compounds to be associated with mutagenicity of coal fly ash. The mutagenicity and cytotoxicity of coal fly ash from fluidized-bed and conventional combustion was compared by Mumford and Lewtas (1982). They found that both types of coal ash are cytotoxic and contained bioavailable mutagens but that from conventional power plant was less mutagenic. Li et al., 1983, found that the dichloromethane extract of a coal fly ash sample to be directly mutagenic in S. typhimurium strain TA98 and the mutagenicity decreased with addition of exogenous liver S9. The same extract was cytotoxic to Chinese hamster ovary (CHO) cells but was not mutagenic. The addition of exogenous liver S9 decreased the cytotoxicity but increased the mutagenicity at both Na+-K+-ATPase and hypoxanthine-guanine phosphoribosyl transferase (HGPRT) gene loci in CHO cells. The possible explanation given by them was that the bacterial mutagenicity was induced by the nitro-PAHs that are potent bacterial mutagens and mammalian mutagenicity was induced by both PAHs nitro-PAHs that are promutagens. Kleinjans et al., 1989, demonstrated that DMSO extracts of fly ash to be slightly mutagenic to Salmonella tester strains TA97 and TA102.

Thus, fly ash has been tested positive for genotoxicity and mutagenicity and involvement of heavy metals and aromatic hydrocarbons has been implicated for such studies.

With respect to plants, literature review shows that fly ash when mixed with soil acts as a growth promoter at low concentration but has adverse effect at high concentration (Mishra and Shukla, 1986; Wong and Wong, 1990; Khan and Khan, 1996).

**Genotoxicity of coal fly ash leachate:**

There are many published work on the chemistry of leachates generated from these ash ponds, which has been found to contain various trace elements such as Ca, Mg, Na, K, Mn, Cu, Fe, Zn, S, Pb, B etc. (Lee and Spears, 1995; Landsberger et al. 1995; Spears and Lee, 2004; Singh et al., 2007). Heavy metals from fly ash dumpsites may leach into soil and groundwater and pose long-term risk to the environment.
The leachate resulting from the weathering process of coal fly ash was found to be toxic to plants (McMurphy et al., 1996). Ali et al., 2004 demonstrated that fly ash constituents have potential to induce oxidative stress in fish and gills are the most vulnerable organs. Manerikar et al. (2008) reported genotoxicity of fly ash leachate in earthworm coelomocytes.

**Phytoremediation of fly ash dumpsites:**
Phytomanagement of fly ash helps to reduce potential toxic metal mobility and the effects of contaminants on humans and ecosystems (Pandey et al., 2009).

The plant species to be used for successful vegetation of fly ash dumpsites should be able to grow in a highly alkaline environment and enriched levels of trace elements like As, B, Cd, Pb and Se content that limit plant growth. Retardation of growth and development of plants on fly ash landfill can also be because of the unavailability of nitrogen (N is absent because it is oxidized into gaseous constituents during the combustion) and P (although a high concentration of P is present in ash compared with soils, it is not in a form readily available to plants, presumably due to interactions with ash Al, Fe and Ca) (Gupta et al., 2002).

The application of bluegreen algal inoculants enhances the fertility of irrigated fly ash landfills in terms of improvement in physicochemical properties, such as pH, electrical capacity and cation-exchange capacity, as well as increasing total nitrogen, available phosphorus and organic matter. Rai et al., 2000 studied amelioration of fly ash by nitrogen fixing blue green algae (viz., Nostoc muscorum, N. commune, N. calcicola, Anabaena doliolum, Aulosira fertilissima, Gleocapsa magma and Scytonema oscillatum). Of the seven different blue green algal strains under study A. doliolum was found growing well on plates containing fly ash though at the initial stage, the growth of alga was reduced due to the phytotoxicity caused by fly ash. A significant increase (p < 0.05) in total nitrogen, available phosphorus and organic matter of fly ash was also found. Further, the alga reduced the toxicity of fly ash by accumulating metals in their tissues. 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. The main constraint in adopting this technology is that fly ash does not hold water to support algalisation (Rai, 2000).
Vajpayee et al., 2000 reported the potential of *Cassia surattensis* to grow on fly ash without any visible phytotoxic symptoms. During the study, a fly-ash-tolerant *Rhizobium* strain inoculation enhanced the growth of the *C. surattensis* in N limited fly ash. However, a decrease in biomass of plant was recorded which according to the authors may be due to toxic effects of metals present in fly ash. Fly ash also reduced the leaf number and photosynthetic area in *C. surattensis*. Rai et al., 2004 concluded that *Rhizobium* inoculation would be beneficial for revegetation as well as decontamination of fly ash contaminated soil and landfills using *Prosopis juliflora*. Cheung et al., 2000 reported that though either *Acacia auriculiformis* or *Leucaena leucocephala* grew on ameliorated lagoon ash but they exhibited poor growth compared with the agricultural soil, indicating that alkaline conditions and elevated ash-borne trace elements limit not only plant growth but also nodulation with *Rhizobium*. Thus according to the authors even though the physical and chemical properties of lagoon ash can be improved by ameliorant addition, the limiting factor to successful revegetation will be the problem of plant toxicity and low level of macronutrients.

For developing effective revegetation on fly ash landfills/dykes/lagoons some species are recommended by researchers such as *Mesembryanthemum aitlonis, M. nodiflorum, Atriplex holocarpa, A. lindleyi, A. vesicaria, Enchyelaena tomentosa, Halosarcia halocnemoides, H. pergranulata, Scaevola collaris* and *Nitraria billardierei* (Jusaitis and Pillman, 1997); *Acacia auriculiformis, Leucaena leucocephala* (Cheung et al., 2000); *Cassia surattensis* (Vajpayee et al., 2000); *Prosopis juliflora* (Rai et al., 2004); *Cassia siamea* (Tripathi et al., 2004); *Sesbania cannabina* (Sinha and Gupta, 2005); *Brassica juncea* (Gupta and Sinha, 2006); *Calotropis procera, Cassia tora, Chenopodium album, Sida cardifolia, Blumea lacera* (Gupta and Sinha, 2008).

**Vetiver as phytoremediant:**

Vetiver can grow on a wide range of substrates, such as: sandy soil, loamy sand, clay soil, crushed limestone, sandy clay loam, and tuff/peat mixture (Dudai et al., 2006) and is used for erosion control (Dalton et al., 1996). The plant has showed high potential for phytoremediation of metal-contaminated soil (Chen et al., 2004; Rotkittikhun et al., 2007; Paz-Alberto et al., 2007), 2,4,6-trinitrotoluene contamination (Makris et al., 2007), phenol (Singh et al., 2008),
atrazine (Marcacci et al., 2006), and petroleum hydrocarbon-contaminated soils (Brandt et al., 2006).

Vetiver grass has an ability to remove Cd, Pb and Zn from soil (Chen et al., 2004). The potential of three grass species - vetivergrass, cogongrass and carabaograss as phytoremediators of lead-contaminated soil was studied by Paz-Alberto et al. (2007). Vetivergrass yielded the highest biomass, the highest percent plant survival, and had the greatest amount of Pb absorbed in roots and shoots. Rotkittikhun et al., 2007 found that Vetiver had high biomass yield when grown in lead-contaminated soil and fertilizer application had no influence on Pb uptake. They also observed that the plant accumulated Pb mainly in roots and low Pb concentration was transported to shoots. The application of chelating agents like EDTA was reported to increase the amount of metals like Pb, Zn, Cu and As that was phytoextracted by Vetiver and increased the translocation ratio of Pb to shoots (Chen et al., 2004; Chiu et al., 2005; Wilde et al., 2005; Lou et al., 2007; Gupta et al., 2008). Humic acid addition to Vetiver grass can be an effective way to enhance phytoremediation of B and Pb but optimum rates differ depending on soil B and Pb contamination levels (Angin et al., 2008). Chiu et al., 2006 reported that application of manure compost and sewage sludge could significantly reduce Pb uptake and accumulation, but not Cu in Vetiver. The heavy metal accumulation in roots was always higher than that observed in shoots (Antiochia et al., 2007). However, the biomass of vetiver plants was found to decrease when the lead concentration was increased in a simulated field study (Chantachon et al., 2004). Lead addition beyond 45 kg Pb ha\(^{-1}\) decreased Pb uptake mostly due to a yield decline (Angin et al., 2008). Chen et al., 2004, showed that soil matrix with planted Vetiver could adsorb high percentage of initially applied metals like Pb, Cu, Zn, and Cd, which may reduce the risk of heavy metals flowing downwards and entering the groundwater. The high tolerance of Vetiver grass to Pb was attributed to the lead-binding phytochelatins within the plant tissues. Pb induces the synthesis of phytochelatins (PC) and the formation of Pb-PC complexes, alleviating the phytotoxic effects of free Pb ions (Andra et al., 2009). Pang et al., 2003 studied the physiological aspects of vetiver grass when grown on mine wastes. It was observed that high proportions of lead and zinc tailing inhibited the leaf growth, dry matter accumulation, and photosynthesis of leaves, but stimulated the accumulation of praline and abscisic acid (ABA), and enhanced the activities of superoxide dismutase (SOD), peroxide (POD) and catalase (CAT). Thus, different mechanisms to detoxify active oxygen species (AOS) exist in different parts of Vetiver plant.
Wong et al., 2007 showed that inoculation of the Vetiver plants with arbuscular mycorrhizal fungi (AMF) protects them from the potential toxicity caused by increased uptake of Pb and Zn, but the degree of protection varied according to the fungus and host plant combination.

Singh et al., 2008, studied the potential of Vetiver (Vetiveria zizanoides L. Nash) for phytoremediation of phenol. They found phenol removal to be associated with the enhanced levels of antioxidant enzymes like superoxide dismutase and peroxidase.

Vetiver was found to tolerate crude-oil contamination at a concentration of 5 % (w/w) in a study by Brandt et al., 2006. However, there was no evidence of Vetiver enhancing the biodegradation of crude oil under the condition of the trial. However, according to the authors the use of Vetiver grass crude-oil contaminated sites could help by allowing other species to be established and through erosion control.

The mechanism of resistance of Vetiver to atrazine was investigated by Marcacci et al., 2006 and their work indicated that conjugation to glutathione was a major metabolic pathway to detoxify atrazine in Vetiver.

Vetiver could hydroponically grow well in wastewater and successfully remove heavy metals from industrial wastewaters (Roongtanakiat et al., 2007) and from landfill leachate (Roongtanakiat et al., 2003) Vetiver is a dense, bunch-type grass with stiff stem, and an extremely strong root system (up to 4.6 m deep), and grows to the height of over 2 m (The Wealth of India, 1976). For these characters, Vetiver grass is considered an ideal plant for erosion control and for stabilizing steep and unstable slopes. It has been found to be hydraulically feasible to use vetiver hedges to control flood flow and erosion on cropped flood plains and at land slopes between 0.5 % and 2 % (Dalton et al., 1996).
According to our knowledge, there are reports on the use of Vetiver for phytoremediation of sites contaminated with heavy metals and for erosion control, but has never been tried for phytoremediation of fly ash dumpsites.