Chapter 6
Summary
1. Fly ash is the solid material, which is carried away from the power plant boiler in the flue gas during coal combustion. The mineralogical, physical and chemical properties of fly ash depend on the nature of coal, conditions of combustion, type of emission control devices and storage and handling methods. Fly ash occurs as very fine particles (average diameter of < 10 µm) and is predominantly composed of aluminosilicate particles. Other constituents include Fe, Ca, Mg, Na, K, and nearly all naturally occurring elements in trace quantities. The coal ash also contains organic constituents like a number of polycyclic aromatic hydrocarbons (PAHs) and polychlorinated biphenyls (PCBs).

2. Disposal usually leads to dumping of the fly ash in landfills on open land. Many possible beneficial applications of fly ash are being evaluated to minimize waste, decrease cost of disposal and provide value-added products. Major uses are in cements, concrete, bricks, wood substitute products, soil stabilization, road base, embankments and consolidation of ground, land reclamation and as a soil amendment in agriculture. Despite this, the global utilization rate is 25%. In India, during the year 2006-07 the total amount fly ash which remain unutilized was 70 million tonne per year and is still increasing. Thus, the issue of management of such a large volume of fly ash and mitigation of its likely impact on environment is important and relevant.

3. Fly ash is considered as a serious source of air pollution since it remains air-borne for a long period and possesses a hazard to the lungs. Fly ash itself and extracts of fly ash have been tested positive for mutagenicity and genotoxicity, and involvement of heavy metals and aromatic hydrocarbons has been implicated for such studies. However, there is no comprehensive work on the genotoxic effect of fly ash to plants and the safe limit of use of fly ash when used as fertilizer.

   The prevalent practice of disposal is as slurry of ash and water to storage or ash ponds located near power stations. To prevent the fly ash from being air-borne, the dumping grounds need to be kept wet all the time. This and rainfall on the sites can cause the problem of leaching from the dumpsites. Since leaching is often the cause of off-site contamination and pathway of introduction into the human environment, a study of possible genotoxic effects of fly ash leachate is essential. There are many published work on the chemical character of leachates and the leaching behavior of various trace metals found in the leachates generated from the ash ponds.
in relation to varying factors like pH and temperature. However, comprehensive studies on the genotoxicity of coal fly ash water leachate are limited.

The most eco-friendly alternative for abandoned fly ash disposal sites is to revegetate them. For this, it is important to select an appropriate pioneer plant species. Literature review shows that there are reports on the use of Vetiver (Vetiveria zizanioides) for phytoremediation of sites contaminated with heavy metals and for erosion control but has never been tried for phytoremediation of fly ash dumpsites.

4. The major objectives of the present work are evaluation of genotoxicity of coal fly ash and coal fly ash water leachate, and to assess the of potential of Vetiver for phytoremediation of coal fly ash dumpsites.

5. Fly ash sample was collected from fly ash dumpsite around the Kolaghat Thermal Power Station, in West Bengal, India, dried under sunlight and kept at room temperature (28 ± 1 °C) in a plastic drum.

6. For the evaluation of genotoxicity of fly ash the sample was mixed in varying proportion with commercial sand to obtain the different percent of fly ash in mixtures (0, 5, 10, 25, 50, 75, and 100 %). Allium bulbs were allowed to germinate directly in fly ash and after five days the germinating roots were processed for the Allium test. Additionally, the Allium test was adapted for detecting DNA damage through comet assay.

7. The results from the Allium test indicate that fly ash itself is cytotoxic, inhibits root growth, decreases mitoses, induces binucleate formations and can cause DNA damage to the root meristems of onion. The number of binucleated cells scored in interphase cells was significantly high in fly ash-mixtures. Fly ash thus seems to have an effect on phragmoplast. The results of the mitosis show that the proportion of cells (among total number of cells counted) in metaphases, anaphases and telophases decreased from 25 % of fly ash to 100 % concentration of fly ash. This could be probably due to the arrest in the cell cycle before metaphase to restore the integrity of DNA. In the comet assay, a statistical increase for DNA strand breaks was found only at higher concentrations. The sample was analyzed by flame atomic absorption spectrometer for B, Zn, Pb,
Cu, Ni, Cd and As, whose presence could partly be responsible for the genotoxicity of fly ash. Some physical factors like lack of aeration and physical hindrance to growth may have further added the effect on the root length of plants, particularly those grown at 100 % of fly ash.

8. For studying the genotoxicity coal fly ash leachate, leachate was prepared from the fly ash sample obtained, which was analyzed for metal content, and tested for mutagenicity and genotoxicity. The Ames Salmonella mutagenicity assay, a short-term bacterial reverse mutation assay, was conducted on two-tester strains of Salmonella typhimurium (TA97a and TA102). For genotoxicity, the alkaline version of comet assay on fly ash leachate was carried in vitro on human blood cells and in vivo on Nicotiana plants. The apoptotic potential of fly ash leachate was evaluated in “DNA diffusion assay” on Nicotiana leaf cells and human lymphocytes.

9. Metal analysis of the fly ash leachate showed that among the 15 metals analyzed, the amount of Na content was highest followed by Si, K, Ca, Mg, Fe, Mn, and Zn. The amounts of the metals - Cd, Ni, Cu, Pb, Al, F, and As were below the detection limits for these individual metals. The leachate was high in sulphate (SO₄) concentration and the pH of the leachate was 7.53.

In the Ames test, spontaneous revertants were within the normal values for the two strains. Fly ash leachate was found to be non-mutagenic when it was autoclaved, and mutagenic when not autoclaved. Autoclaving probably precipitated some metals and hence were not available for the Ames test. Moreover, the chances of elimination of the volatile organic compounds in the autoclaved samples might have also contributed to its non-mutagenicity.

Results of comet assay on Nicotiana plants revealed that the increase in comet parameters [tail DNA (%), tail length (µm) and Olive tail moment-OTM (arbitrary units)] was significant compared to the control (P < 0.05) at 3:16 and 1:4 w/v fly ash–water leachate concentration. Results of comet assay on human whole blood cells and lymphocytes showed that 1:4 and 3:16 w/v fly ash–water leachate induced significant DNA damage compared to negative control but the values of the comet parameters were higher (in treatment as well as control groups) in case of whole blood than those of isolated lymphocytes.

Fly ash leachate significantly (P < 0.05) induced apoptosis in both types of cells i.e. Nicotiana leaf cells and human lymphocytes as measured by DNA diffusion assay.
10. Phytoremediation is considered a practical, economical, and environmentally compatible solution for remediating heavy metal contaminated sites on a large scale. It is important to select an appropriate pioneer plant species for successful site reclamation and to ensure a self-sustainable vegetative cover. The objective of the work on Vetiver was two-fold. One was to establish whether Vetiver could be used as a phytoremediant for coal fly ash dumpsites. The second was to find the efficacy of comet assay as a molecular technique for initial rapid screening of plants for an appropriate phytoremediant in a particular polluted site. This was done by comparing the results of comet assay on root nuclei in a known phytoremediant (Vetiver) and in a sensitive plant (*Allium cepa*) grown on the same material (coal fly ash).

Healthy Vetiver plants were planted and grown in pots containing fly ash or garden soil (3 pots/sample) under normal environmental conditions. Equal-sized bulbs were chosen from a population of a local market variety of the common onion *Allium cepa* L. The onions were positioned for germination and growth for 5 days on fly ash and garden soil.

11. The results of comet assay show that *Allium* grown in fly ash for 5 days gave significantly (P ≤ 0.001) higher degree of DNA damage. On the other hand, the DNA damage in Vetiver grown in fly ash for three months was not significantly different from that of the plants grown in garden soil. Absence of damage in nuclear DNA of Vetiver implies its long-term survival on the coal fly ash dumpsite. It also demonstrated that comet assay could suitably help to segregate between a tolerant plant having the potential of a successful phytoremediant and a non-tolerant sensitive plant.

There was dense mesh like growth of root that was found entangling the fly ash in all the pots used for study showing the characters of a good phytostabilizer. This is particularly important in the case of fly ash since due to its lightweight it is easily carried away by wind and cause air pollution. Dense root system and vegetative cover may also help to retard the formation of hazardous leachate from fly ash.

Data from metal analysis of plants shows that the concentration of Zn in the roots of Vetiver plants grown on fly ash (100.66 ± 5.96µg/g) was higher than those grown on garden soil (59.54 ± 2.41µg/g). The other metals namely Pb, Cu, Ni, Cd and As, did not show any increased values in the plant body when grown on fly ash as compared to that grown in garden soil. The
important implication of these findings is that when Vetiver is used for phytoremediation of fly ash dumpsites, animals can safely graze the shoots as very little of these metals are translocated to the shoots.

Result of the metal contents in plant tissues and root growth indicated that Vetiver was more suitable for phytostabilization than phytoextraction in case of coal fly ash.

12. In conclusion, we can state that the classical *Allium* test can give a more comprehensive data when done in combination with the comet assay, which is faster, simpler and independent of mitosis. In addition, when fly ash is used as fertilizer or soil ameliorant, it should be judiciously used at very low concentrations since fly ash at 10% or more induces different types of genetic abnormalities. The results of the Ames test (point mutations), comet assay (DNA breaks) and DNA diffusion assay (apoptosis) all lead us to confirm that the coal fly ash leachate has genotoxic potential and may lead to adverse impact on vegetation and environment. The results may raise general awareness about fly ash leaching that can take place when dumpsites are not properly lined. This can positively influence concerned authorities towards effective management of the problem. Apart from traditional end-points viz. growth parameters like root length, shoot length and dry weight, comet assay could also be included in a battery of tests for initial, rapid and effective selection of plants for restoration and phytoremediation of polluted sites. Vetiver (*Vetiveria zizanioides*) can successfully grow on fly ash. The plant has the potential to phytoremediate coal fly ash dumpsites (may reduce off-site contamination) mainly through the process of phytostabilization and give the added advantage of increased greenery.