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Municipal solid waste landfill leachate induced cytotoxicity in root tips of *Vicia faba*: Environmental risk posed by non-engineered landfill

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Landfills are considered the main option for dumping of municipal solid waste (MSW) all over the world, but these landfills are mostly non-engineered. The decomposition of solid waste in the landfill and rainwater penetration into the decomposing waste produces leachate that contains dissolved organic and inorganic compounds, heavy metals, suspended particles and hazardous substances. Leachate migration in the environment may pose serious health risks to organisms exposed. Hence, the present study explored the cytotoxic potential of landfill leachate collected in different seasons from the Okhla landfill site, Delhi, India. Cytotoxicity of leachate samples was evaluated by cell apoptosis and ultrastructural observations based on Transmission Electron Microscopic (TEM) observations of the cells of root tips of *Vicia faba* seedlings treated with the leachates collected in summer, winter and monsoon in a time and dose dependent manner. Leachate collected in all the three seasons induced apoptosis in cells of root tips of *Vicia faba* that increased in a time and dose dependent manner when compared to control. The apoptosis was highest in the samples treated with leachate collected in the summer season, followed by winter and monsoon. It was further confirmed with TEM images that there was induction of apoptotic-like morphological changes in the root cells treated with landfill leachate when compared with the control. The present study indicates that municipal solid waste leachate is very toxic and it should be treated before disposing it to the environment. This study may be useful for the assessment of hazardous and toxic effects of pollutants from solid waste landfill sites.

Keywords: Apoptosis, Hazardous, Landfill leachate, Solid waste, Toxicity, Transmission Electron Microscopy (TEM), *Vicia faba*

In the past 75 years, India has witnessed plethora of developmental activities accompanied by rapid urbanization and lifestyle changes. It has resulted in ever increasing generation of solid waste. Landfills are considered the main option for the dumping of municipal solid waste (MSW) all over the world, but these landfills are mostly non-engineered[1]. Compared to other technologies such as incineration and composting, sanitary landfilling is a relatively convenient, inexpensive, and widely employed method for MSW management[2,3]. It is believed that of the total MSW collected worldwide, up to 95% is disposed off in landfills[4].

The decay and decomposition of solid waste in the landfill and rainwater penetration into the decomposing waste produces a dark liquid with an unpleasant odor known as leachate[5,6]. Leachate can be considered as a water-based solution having dissolved organic and inorganic compounds, heavy metals, suspended particles, and hazardous substances[7,8]. Most of the above pollutants have accumulative, threatening, and detrimental effects on the growth of aquatic organisms, ecology, and food chains, thereby leading to enormous problems in public health, *e.g.*, carcinogenic effects, acute toxicity, and genotoxicity[9-11]. Gupta and Rajamani (2017)[12] have reported that the toxicity of landfill leachate was dependent on the

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Abbreviations: µm, micro meter; µS, microsiemens; AAS, Atomic Absorption Spectrophotometer; AN, Ammonical Nitrogen; BOD, Biochemical Oxygen Demand; Chloride, Cl; cm, centimetre; COD, Chemical Oxygen Demand; Cr, Chromium; Cu, Copper; CW, cell wall; DNA, Deoxyribonucleic Acid; EC, Electrical conductivity; ER, Endoplasmic reticulum; Fe, Iron; FITC, Fluorescein isothiocyanate; HEK, Human Embryonic Kidney; IARI, Indian Agricultural Research Institute; M, Mitochondrion; ml, mililiter; MSW, Municipal solid waste; NU, Nucleus; NUE, Nucleolus; OsO_4, Osmium tetra-oxide; Pb, Lead; PBS, Phosphate-buffered saline; PCD, Programmed Cell Death; PD, Plasmodesmata; PI, Propidium Iodide; PL, Plasma lemma; PM, Plasma Membrane; SO_4^{2-}, Sulphate; TDS, Total dissolved solids; TEM, Transmission Electron Microscopy; V, Vacuoles; Zn, Zinc; µg, micro gram concentration of heavy metals (Pb, Cu), conductivity, and organic matter (COD and BOD_5). Wdowczyk and Szymańska-Pulikowska (2021)[13] have reported that the toxic nature of the leachate may have resulted from the occurrence of high concentrations of ammonical nitrogen (AN), copper, and chromium. Gupta and Paulraj (2016)[14] have also reported a very high concentration of ammonical nitrogen in the landfill leachate samples. Research on the composition of the leachate has shown that it contains more than 400 polluting components, while many others have perhaps not yet been analysed[15]. Leachate migrated to surface water can lead to a decrease in the dissolved oxygen content of surface water, whereas long-term exposure to the pollutants like heavy metals present in leachate can lead to their absorption by aquatic organisms and bioaccumulation in the food chain[12,16]. Heavy metals pose a serious health hazard due to their bioaccumulation and biomagnification properties, as shown by various health risk assessment indices in a recent study of the Delhi-NCR region[17].

Leachate migration in the environment may pose serious health risks to those who are exposed[18]. Anand and Pilani (2022)[19] have reported that leachates from both active as well as closed landfills induce phytotoxicity and genotoxicity. Gupta and Paulraj (2015)[20] have reported that landfill leachate collected in three seasons (summer, winter, and monsoon) caused toxicity in *Vicia faba* seedlings by inhibiting growth, antioxidant enzyme activities as well as a reduction in chlorophyll content. The physiological responses varied as a function of leachate concentration, which was further dependent on the season of leachate collection. Previous research has found that leachate causes ecotoxicity. However, less attention has been paid to the cytotoxicity induced due to leachates collected in different seasons. Therefore, the objective of the present study is to explore the cytotoxic potential of landfill leachate collected in different seasons from the Okhla landfill site, Delhi, India. *Vicia faba* has been selected as bioassay for performing the toxicity tests as plant systems have been established to be simple, sensitive, economical, and effective for toxicological evaluation of landfill leachates[21].

Materials and Methods

Landfill site

The Okhla landfill started operation in 1994. The expected life span of Okhla landfill was till 1997-

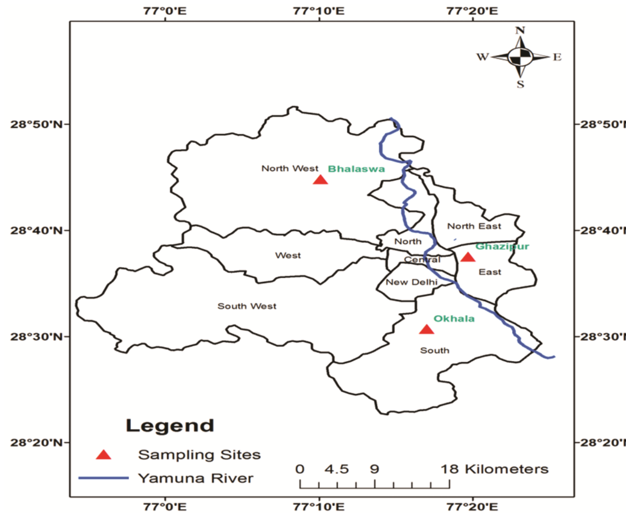


Fig. 1 — Showing Okhla landfill site in Delhi

1998 but the solid waste is dumped into the landfill even now. It is a non-engineered landfill, unscientific method is adopted for the disposal of the waste that is, it lacks base liners, leachate collection and treatment system. It is located in the south of Delhi, which is in proximity to the heavily populated residential area and is one of the biggest industrial areas in close proximity to the Yamuna River bank (Fig. 1).

Landfill Leachate Sample Collection and Analysis

According to multi-spot collection principle[22], the leachate samples were collected from different points of the landfill, mixed and sealed in bottles and transported to laboratory for further analysis without any delay. Samples were collected in three different seasons (Summer, Monsoon and Winter) from the Okhla landfill site. pH, Electrical conductivity (EC), Total dissolved solids (TDS) were measured in the field without any delay by water analysis kit (PC-510, Eutech Instruments). Basic physicochemical properties of the leachate samples collected in different seasons were analysed according to Standard methods[23]. Metals were analysed by Atomic Absorption Spectrophotometer (AAS) (Thermo Scientific). Chemical oxygen demand (COD) was measured by the potassium dichromate oxidation method, Biochemical oxygen demand (BOD) was measured by a 5-day BOD test[23]. Ammonia concentration was measured by ammonia selective electrode.

Plant Bioassay

Dry seeds of *Vicia faba* were supplied by the Indian Agricultural Research Institute (IARI), Pusa, New Delhi. The seeds were soaked for 10 h in distilled water and then allowed to germinate on moist germination sheets. The experiment has been conducted in an incubator at (25 ±1)°C under a dark/ light cycle (14 h:10 h).

Preparation of plant material

Thirty seedlings in each group, which reached 1.4 cm root length, were treated for 24, 48, 72 and 96 h with different doses of leachate collected from landfill site for three seasons. Five different doses *i.e.*, 6.25%, 12.5%, 25%, 50% and 100% of leachates were prepared for the exposure of the seeds by diluting the crude leachate with distilled water while the negative control group was exposed to distilled water.

Cell apoptosis

In order to measure cell apoptosis in the cells of the seedlings treated with leachate samples collected in different seasons from the Okhla landfill site, Flow cytometry was carried out by the method given by Lei *et al* (2002)[24].

Sample preparation

The root tips were cut and snap frozen in liquid -N_2 and then were chopped on ice with a single edged razor blade in a glass petri plate. After the chopped cell suspension was pipetted up and down several times, the suspension was incubated on ice for 5 min and filtered through a 3-tiered Nylon mesh (100, 50 and 30 μM). Single cell suspension was washed in cold PBS and the cell density was adjusted to $\sim 1 \times 10^6$ cells/mL in PBS. Assay was done according to Annexin-VFITC /PI apoptosis kit (Molecular Probe, Invitrogen). In brief, 5 μL of FITC annexin V and 1 μL of the PI solution (100 μg/mL) were added to 100 μL of cell suspension. Then the cells were incubated at room temperature for 15 min. After the incubation period, 400 μL of 1X annexin-binding buffer was added and mixed gently and samples were kept on ice. Samples were acquired for stained cells by flow cytometry and cell sorter (Beckman coulter), using 488 nm excitation with red fluorescence emission at 530 nm (Annexin V) and >575 nm (PI). The cell populations were separated into three groups: live cells, apoptotic cells show green fluorescence and dead cells show both red and green fluorescence.

Transmission Electron Microscopy (TEM)

It was carried out according to the method of Tian *et al*. (2011)[25]. Fresh root tips with the different treatments of the MSW leachate were selected (about 1-3 mM) and fixed in 4% Glutaraldehyde (*v/v*) in 0.2 M Sodium Phosphate Buffer (pH 7.2) for 6-8 h, post fixed in 1% Osmium tetra-oxide (OsO_4) for 1 h and washed in 0.2 M Sodium Phosphate buffer (pH 7.2) for 1-2 h. Dehydration was carried out in a graded ethanol series (50%, 60%, 70%, 80%, 90%, 95% and 100%) followed by acetone (100%). Then the samples were infiltrated and embedded in spurs's resin. Ultra-thin sections (80 nm) were prepared and these sections were mounted on copper grid and viewed under Transmission Electron Microscope (JEOL-JEM-2100F TEM) at an accelerating voltage of 60.0 kV.

Table 1 — Characteristics of crude leachate samples collected in summer, monsoon and winter season from Okhla landfill site in Delhi

Parameter	Summer	Monsoon	Winter
pH	7.9	6.4	7.4
EC (μS/cm)	52,300	44,200	62500
TDS (mg/L)	27,660	23,500	30,550
BOD_5(mg/L)	2340	445	1890
COD (mg/L)	12,150	2385	13,120
NH_4(mg/L)	2150	560	1850
Pb (mg/L)	6.2	0.65	3.9
Cr (mg/L)	9.5	0.56	4.2
Zn (mg/L)	0.79	0.35	2.87
Cu (mg/L)	1.96	0.75	1.20
Fe (mg/L)	110	17.7	82.5
Cl (mg/L)	4250	1725	2680
SO_4^{2-}(mg/L)	6220	1337	6260

Results

Physico-chemical and metal analysis

Basic chemical properties of the leachate collected from the Okhla landfill sites for three seasons are presented in (Table 1). Analysis of the basic properties and chemical composition of the leachate indicates that even though landfill leachate is a mixture and contains different types of contaminants, there are some general underlying pollutants common to all landfill effluents[26]. The leachate samples collected in all the three seasons showed very high concentrations of all the parameters analyzed.

The 5-day Biochemical Oxygen Demand (BOD_5) and Chemical Oxygen Demand (COD) were beyond the permissible levels as per Gazette of India[27] in all three seasons, which showed the high organic strength of the leachates. Both BOD and COD were at their highest in the summer season. Heavy metals, including Cr and Pb, were beyond the permissible limits[27]. High concentration of Fe in the leachate is the evidence of dumping of iron and steel scraps in the dump site[28]. The quantity of Pb in the leachate is attributed to the disposal of wastes such as batteries, paints, and photography processing chemicals at the dump site[29]. The concentration of ammonical nitrogen was very high in the leachates of the three seasons. High concentrations of Chloride and Sulphate were also found in the leachates. Also, chloride is a conservative parameter of the landfill leachate, thus posing a serious threat to the ground water in the vicinity of the landfill[30].

Cell apoptosis by landfill leachate collected in summer

The apoptosis of root cells was tested by flow cytometry, as shown in (Fig. 2A-D). The apoptosis rate of cells was increased from 0.51% in the control group to 13.18, 14.76, 15.96, 14.68, and 17.34% after the plant was treated with leachate concentration (6.25, 12.5, 25, 50, and 100%), respectively, for 24 h (Fig. 2A). After 48 h of leachate exposure, the apoptosis increased to 1.78% in control and 23.56%, 25.63% in 50% and 100%, respectively, (Fig. 2B). Further, 72 h exposure increased apoptosis to 19.85, 24.07, and 29.4% in 25, 50, and 100% leachate, respectively (Fig. 2C), which increased to 31.22, 32.64, and 43.34% in the same concentrations after 96 h exposure (Fig. 2D). The result indicated that the leachate induced apoptosis of cells in a time and dose-dependent manner (Fig. 3).

Cell apoptosis by landfill leachate collected in winter

The apoptosis of root cells treated with leachate from Okhla landfill collected in the winter season was measured by flow cytometry, as shown in (Fig. 4A-D), in which the apoptosis rate of cells was increased from 0.79% in the control group to 7.17, 8.51, 9.1, 7.83 and 13.11% after the plant was exposed to leachate concentration (6.25, 12.5, 25, 50 and 100%), respectively for 24 h (Fig. 4A). After 48 h of leachate exposure, the apoptosis increased to 1.35% in control and 14.93, 15.67, and 13.46% in leachate treatments of 25, 50, and 100%, respectively (Fig. 4B). Further exposure for 72 h increased the apoptosis to 15.69, 20.89, and 22.52% in 25, 50, and 100% leachate (Fig. 4C), which increased further to 24.66, 30.1, and 33.61% respectively in the same concentrations after 96 h of exposure (Fig. 4D). The result indicated that the leachate induced apoptosis of cells in a time and dose-dependent manner (Fig. 5).

Cell apoptosis by landfill leachate collected in monsoon season

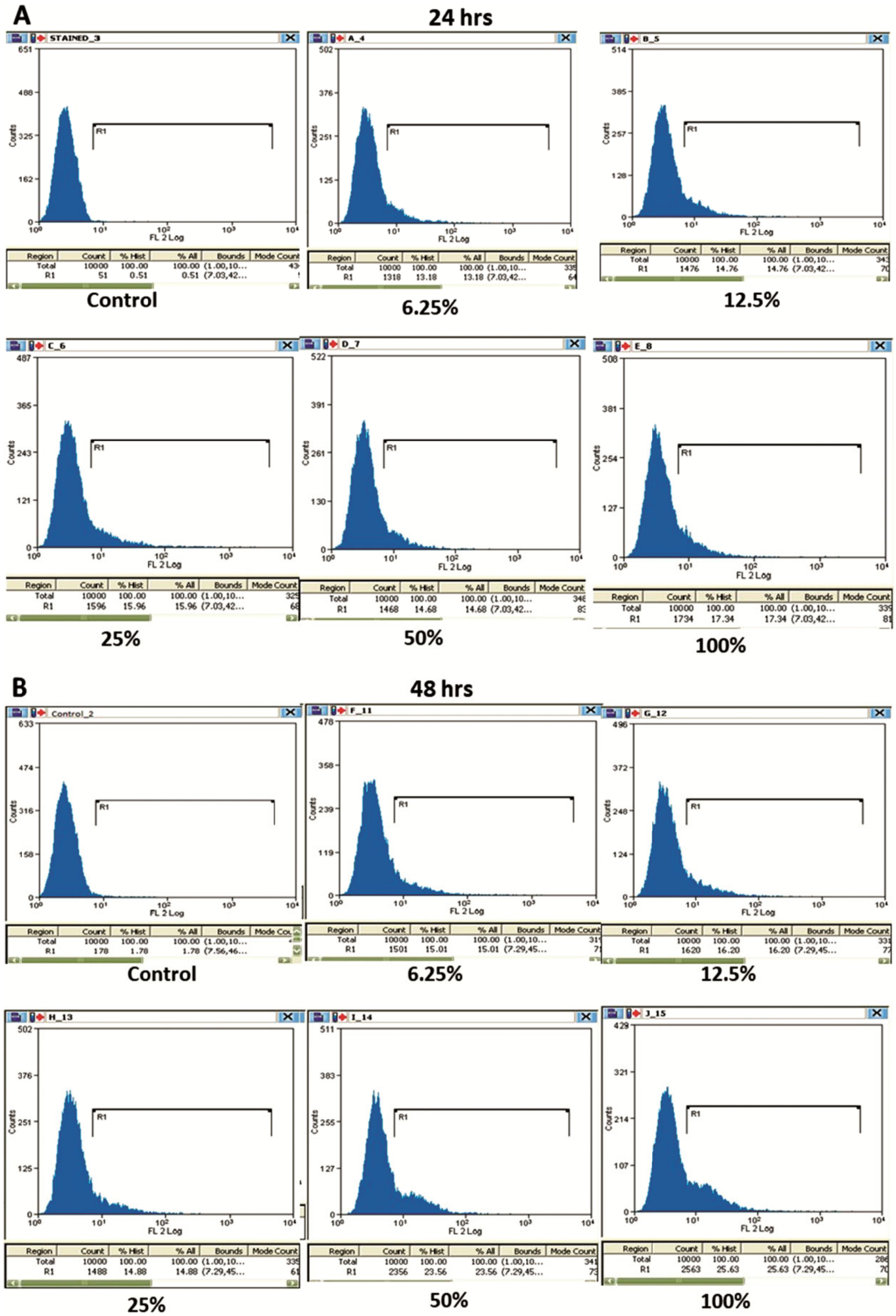
The apoptosis of root cells treated with leachate from Okhla landfill collected in monsoon season was tested by flow cytometry as shown in (Fig. 6A-D), the apoptosis rate of cells was increased from 1.86% in control group to 3.81, 6.99, 7.85, 10.84 and 12.66% after the plant exposed to leachate concentration (6.25, 12.5, 25, 50 and 100%), respectively, for 24 h (Fig. 6A). After 48 h of leachate exposure the apoptosis increased to 2.12% in control and 8.97, 13.33, 16.49% in leachate treatment of 25, 50 and 100% respectively (Fig. 6B). Further exposure for 72 h increased the apoptosis to 12.25, 16.19 and 21.5% in 25, 50 and 100% leachate (Fig. 6C) which increased further to 14.86, 19.80 and 24.54% respectively in the same concentrations after 96 h of exposure (Fig. 6D). The result indicated that the leachate induced apoptosis of cells in a time and dose-dependent manner (Fig. 7).

Leachate collected in all the three seasons induced apoptosis of cells of root tips of *Vicia faba* that increased in a time and dose dependent manner when compared to control. The apoptosis was highest in the samples treated with leachate collected in the summer season, followed by that of the winter and then the monsoon. Gupta and Rajamani (2015)[20] have reported that there is a lower level of pollutants at the lower leachate concentration, whereas, exposure to a higher level of leachate treatment, which contains a higher level of pollutants and induces the production of ROS (Reactive Oxygen Species).

As a result of excessive ROS, it attacks lipids and proteins, and the plant experiences substantial oxidative stress[20,31]. Furthermore, numerous diverse valence state metals such as iron, chromium, copper, and arsenic present in landfill leachate can interact with mitochondrial redox mechanisms and produce superoxide ions[32]. Gupta et al. (2019)[33] have demonstrated that mitochondria, the most versatile organelle, are highly vulnerable to the landfill leachate, leading to the onset of several molecular cascades and potentially oxidative stress *via* directly disturbing mitochondrial machinery, ultimately leading to apoptosis. It has been reported that leachate inhibits cell proliferation and activates cytotoxic events after prolonged exposure[34]. Recent study has further confirmed the presence of cytotoxic and genotoxic effect of pollutants on HEK293 cells that leads to decreased cell viability and DNA damage in dose-dependent manner[35].

Transmission Electron Microscopic observations (TEM)

Transmission Electron Microscopic images of the root tips of *Vicia faba* suggest that the different concentrations of landfill leachate cause significant ultrastructural changes. Well defined cellular organelles,


A
24 hrs
Control
6.25%
12.5%
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B
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6.25%
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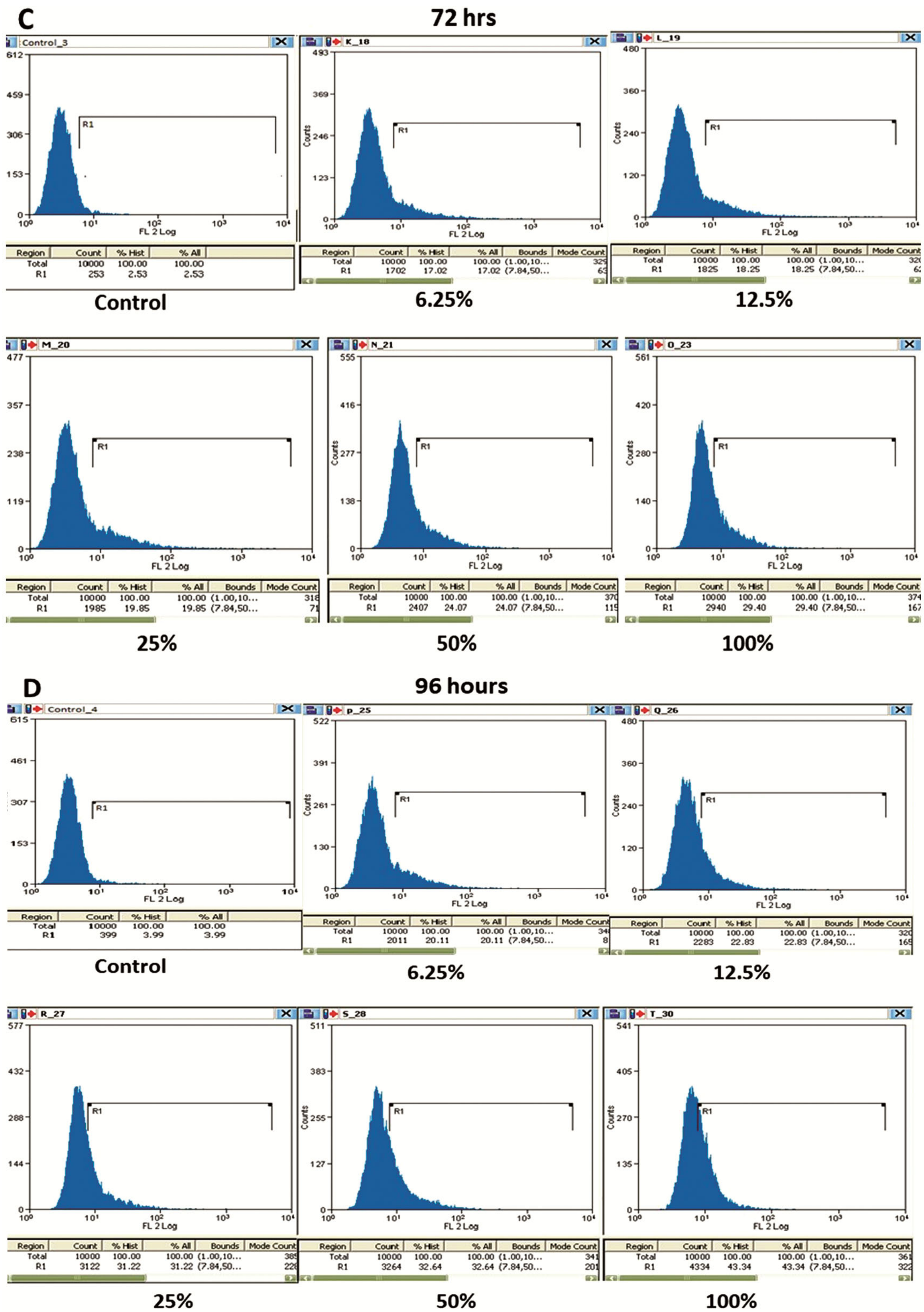


Fig. 2 — (A-D) showing cell apoptosis of *Vicia faba* root cells among the leachate treated groups and control group for 24, 48, 72 & 96 h respectively. Histograph of root cells (treated groups and control ones). X-axis shows the number of events and y-axis indicates fluorescence at Annexin V-FITC. The leachate was collected from Okhla landfill in summer season

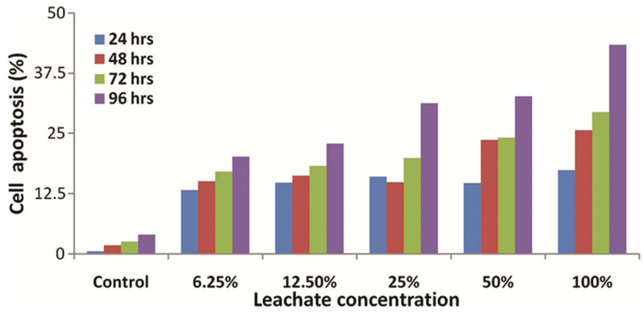
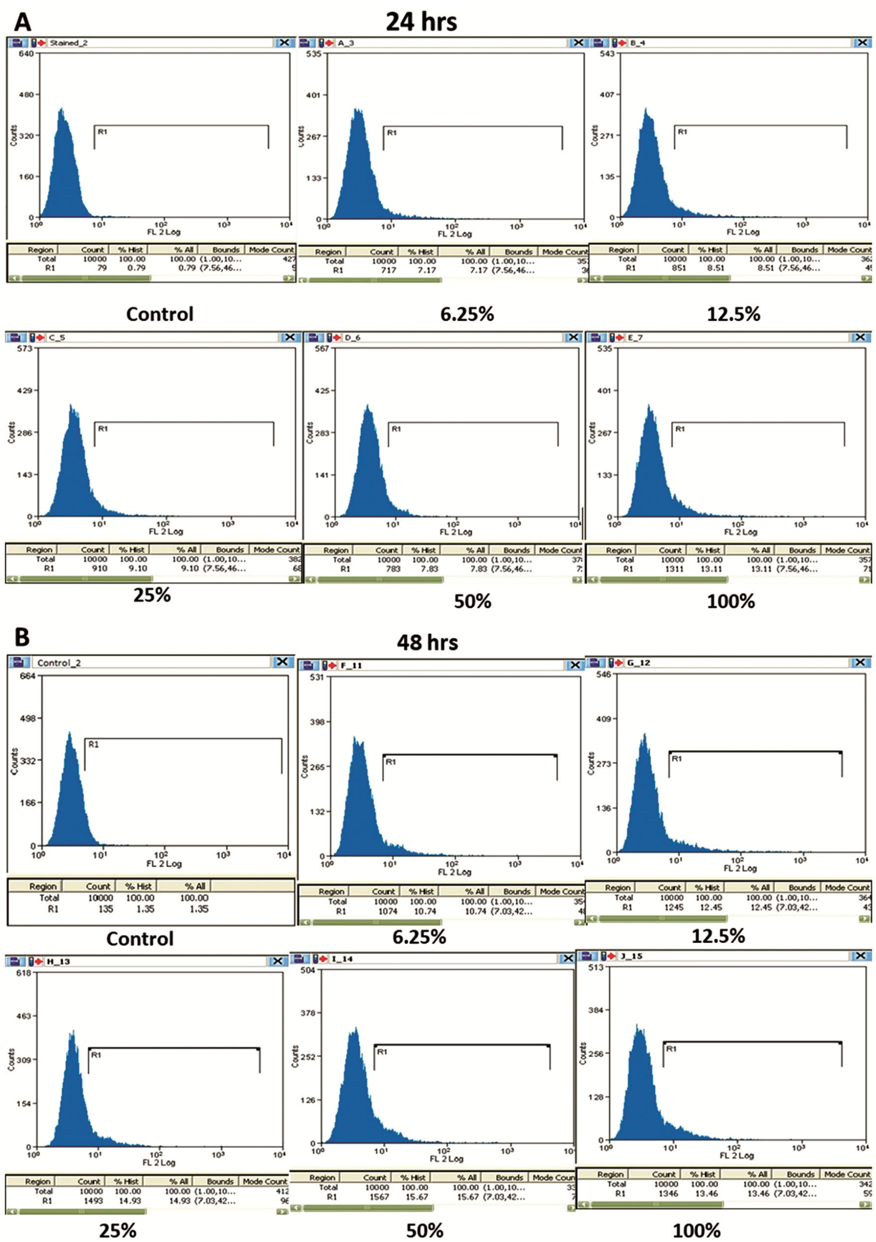


Fig. 3 — Showing the cell apoptosis in the root cells of *Vicia faba* treated with leachate collected from Okhla landfill in summer season for 24, 48, 72 and 96 h, respectively

especially nuclei with prominent nucleolus, were observed in the seedlings treated with only distilled water (control group) (Fig. 8A). Cells treated with a lower concentration (6.25%) of leachate (Fig. 8B) did not show much change as compared to the control. Cells treated with high concentrations (50% and 100% (Fig. 8C & D) of leachate alter the structure of cellular organelles like mitochondria, the nuclear membrane becomes disrupted (Fig. 8D), the nucleolus is degraded, and the membrane starts becoming blebby (Fig. 8C & D). The TEM images provide new evidence for the presence of autophagic vacuoles in



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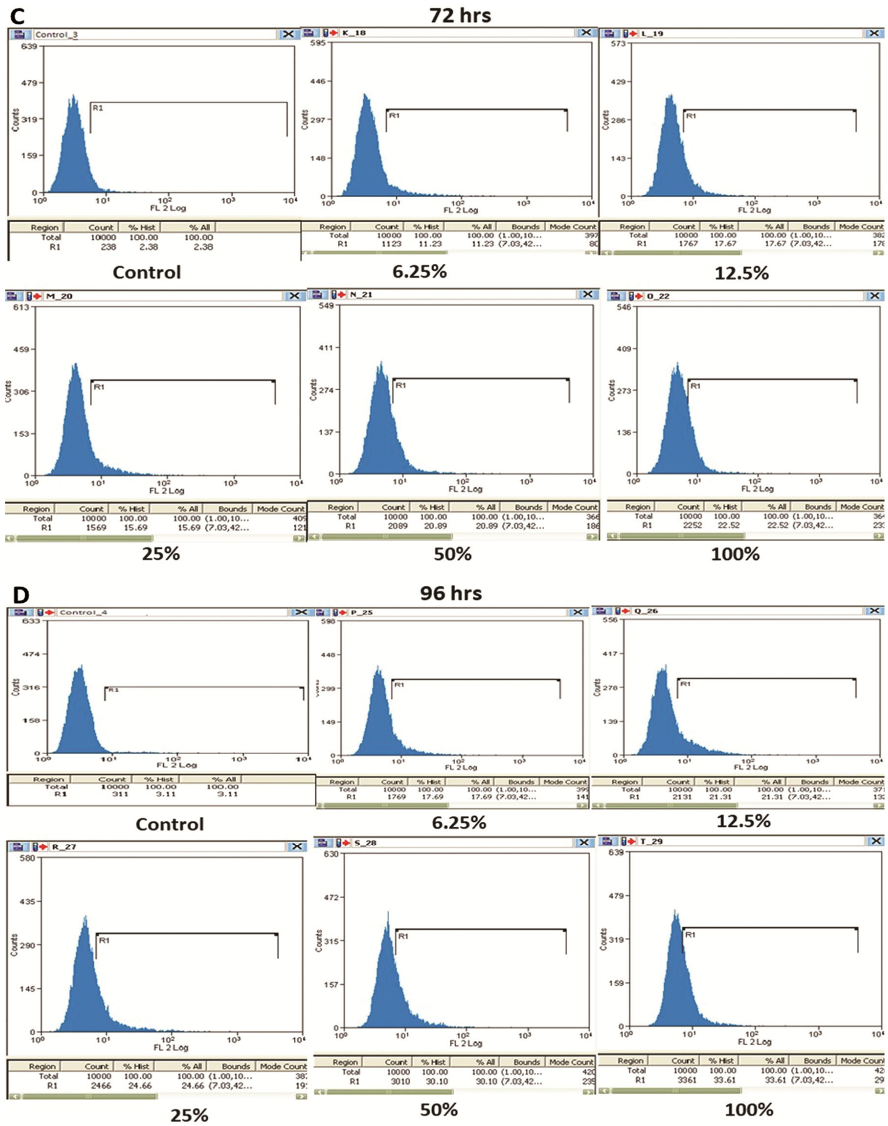


Fig. 4 — (A, B, C, D) showing cell apoptosis of *Vicia faba* root cells among the leachate treated groups and control group for 24, 48, 72 & 96 h, respectively. Histograph of root cells (treated groups and control ones). X-axis shows the number of events and y-axis indicates fluorescence at Annexin V-FITC. The leachate was collected from Okhla landfill in winter season

the cells which are highly stressed by the contaminated leachate.

TEM confirmed the induction of apoptotic-like morphological changes in the root cells treated with landfill leachate when compared with the control. On comparing the ultrastructure of stressed cells with normal cells, the change in plasma membrane was noticeable. At an early stage of apoptosis, plasma membrane (PM) began to detach from the cell wall and dense material containing vacuoles occupied up to 80-90% of the cell volume (Fig. 8C) and the cytoplasm appeared electron opaque. A key process by which eukaryotic cells respond to various stresses is vacuolar autophagy[36]. Whether autophagy works in

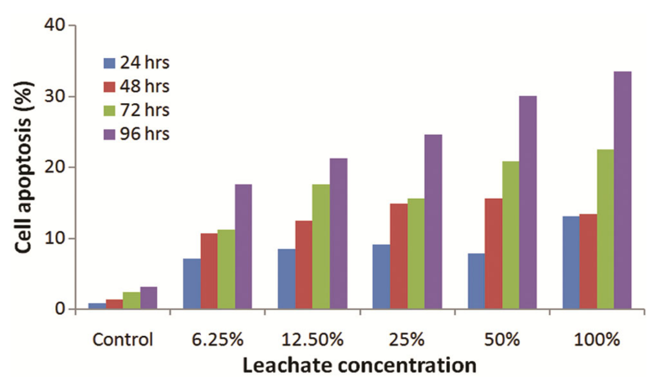
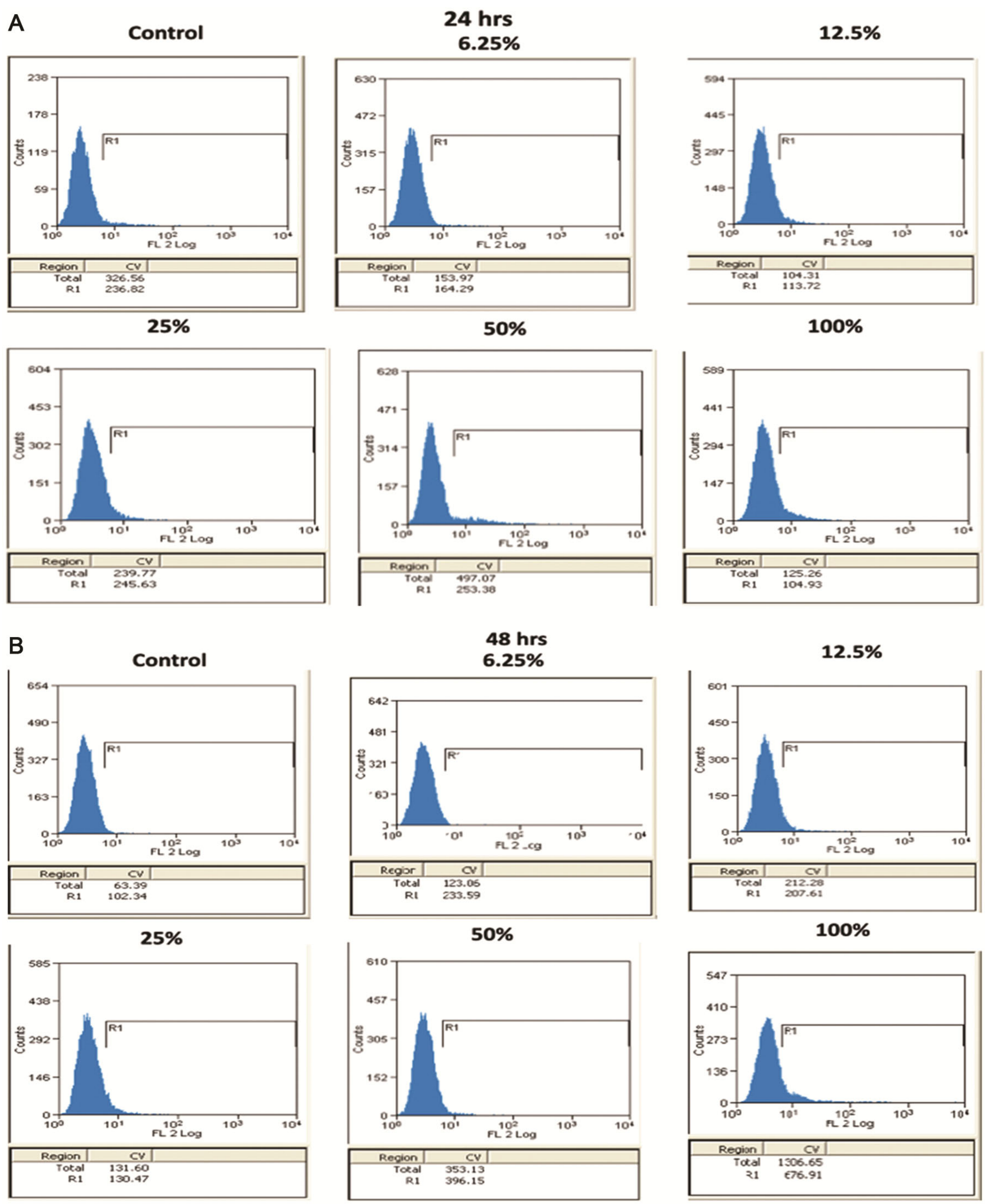


Fig. 5 — Showing the apoptosis in the root cells of *Vicia faba* after exposed to leachate collected from Okhla landfill in winter season for 24, 48, 72 and 96 h, respectively

cell survival or cell death, depends upon the extent of autophagy induced during a physiological condition[37]. In stressed cells, the cell wall retracts from the cytoplasm with abundant vesicles present near the plasma membrane. Wang *et al*. (1996)[38] reported apoptotic like bodies, membrane bound bodies containing cellular organelles and other materials.

TEM confirmed the induction of apoptotic-like morphological changes in the root cells treated with landfill leachate when compared with the control. Mis-shapen nuclei that contain condensed chromatin with sharply defined margins associated with the nuclear envelope, such as seen in the present study under TEM, are the characteristics of programmed cell death (PCD) in plants[39,40], and fragmented nuclei



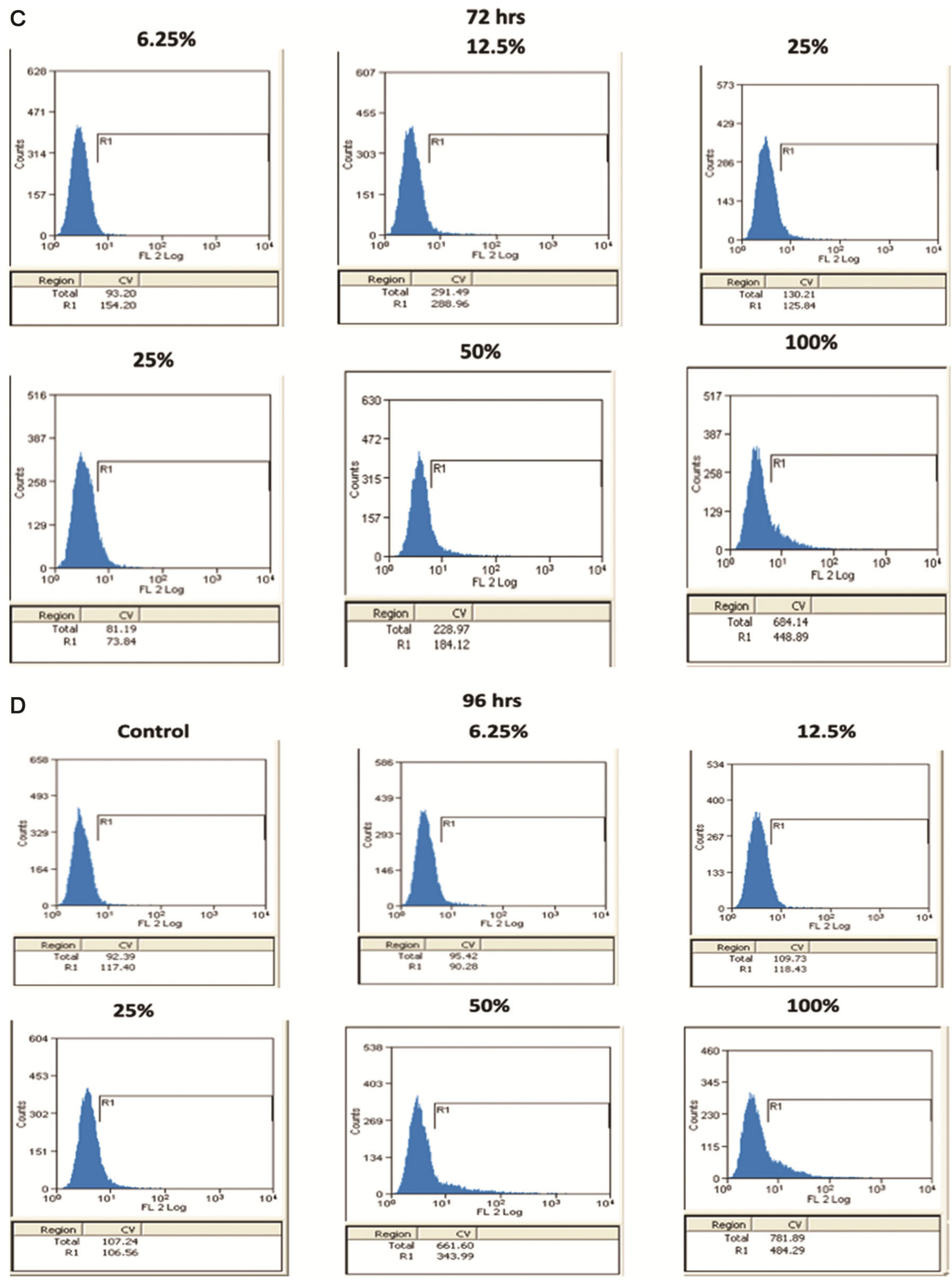


Fig. 6 — (A-D) showing cell apoptosis of *Vicia faba* root cells among the leachate treated groups and control group for 24, 48, 72 & 96 h, respectively. Histograph of root cells (treated groups and control ones). X-axis shows the number of events and y-axis indicates fluorescence at Annexin V-FITC. The leachate was collected from Okhla landfill site in monsoon season

are characteristic of apoptosis, as are shrunken and fragmented cytoplasms[41-43]. The TEM images provide new evidence for the presence of autophagic vacuoles in the cells which are highly stressed by the contaminated leachate.

Gupta *et al.*(2019)[33] have further found that their results evidently indicate the ability of landfill leachate to interrupt mitochondrial redox homeostasis, which might be a likely source for the immunotoxic consequences leading to plausible patho-physiological

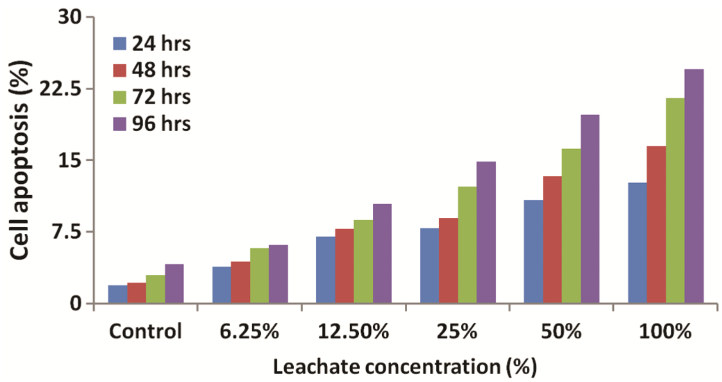


Fig. 7 — Showing the apoptosis in the root cells of *Vicia faba* after exposed to leachate collected from Okhla landfill in monsoon season for 24, 48, 72 and 96 h, respectively

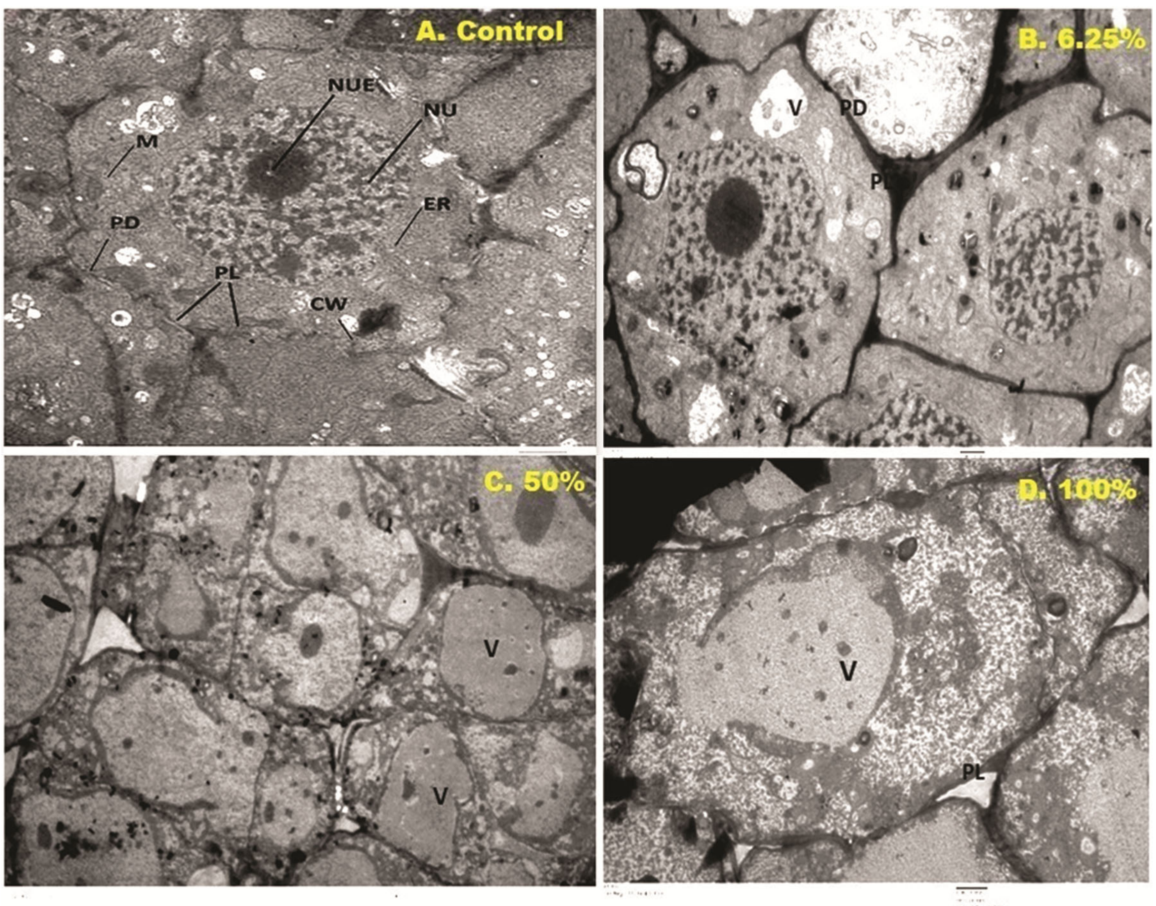


Fig. 8 — Transmission electron micrograph of root meristematic cells from *Vicia faba* seedlings exposed for 72 h to MSW landfill leachate from Okhla landfill site collected in summer. (A) shows the roots treated with Distilled water (control); (B) shows the roots treated with Leachate concentration 6.25%; (C) with leachate concentration 50%; and (D) with 100%. Scale 2 microns. Arrow heads NUE: Nucleolus, NU: Nucleus, CW: cell wall, PD: Plasmodesmata, PL: Plasma lemma, ER: Endoplasmic reticulum, M: Mitochondrion, V: Vacuoles

conditions in humans susceptible to such environmental exposures. In addition, the long-term exposure to toxic agents present in leachate has a strong impact both on the environmental health and on the organisms living in the ecosystem. If exposed for long term, it promotes an increase and accumulation of chromosomal aberrations affecting the cellular mechanisms, leading to a loss of cellular control, neoplasia, and cellular apoptosis[44]. These findings are in line with those described by Bortolotto *et al*. (2009)[21].

Conclusion

Present study confirms that leachate induces cytotoxicity and leachate generated in summer and winter season is more toxic as compared to that generated in monsoon season. It indicates that if leachate is released without treatment, it can lead to contamination of the aquatic environment in the vicinity of the landfill even at dilute concentrations. It also states that the long-term exposure to toxic agents that are present in leachate has a strong impact both on the environmental health and on the organisms living in the ecosystem posing a risk to the organisms exposed. It also suggests that the most important aspect for the treatment of landfill leachate is controlling its concentration which varies with respect to different seasons so that the proper management of landfills is ensured. The present study indicates that municipal solid waste leachate is very toxic and it should be treated before disposing it to the environment. The outcomes of this study may be useful in environmental waste management and for the assessment of the hazardous effects of the pollutants from solid waste landfill sites.

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Conflict of interest

All authors declare no conflict of interest.

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