



**Research Article**

**Analysis Of Aggravating  
Factors Of Heavy Metal  
Contents And  
Genotoxicity Of Soil  
Samples In Relation To  
Vehicular Emissions**

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Date Received: 5<sup>th</sup> February 2018; Date accepted:  
10<sup>th</sup> February 2018; Date Published: 11<sup>th</sup> February  
2018

**Abstract**

Genotoxicity of soil samples under exposure to vehicular emissions was evaluated using *Trigonella foenum-graecum* root chromosomal aberration assay (TfRAA) and pollen viability assay (PVA) for *in vitro* and *in situ* studies, respectively. For PVA, pollen grains of four common plant species (*Cannabis sativa*, *Hamelia patens*, *Jatropha panduraefolia* and *Thevetia peruviana*) growing at sites under study were stained using Alexander's, Aceto-orcein, Lugol's and 2,3,5-triphenyltetrazolium chloride (TTC) stains. TfRAA has shown the induction of physiological as well as clastogenic aberrations while PVA revealed that TTC stain induced maximum pollen variability. Heavy metals analysis revealed that all soil samples contained Cd, Cr, Co, Cu, Fe, Pb, Mn and Zn. Statistical analysis has shown that genotoxicity was found to be positively correlated to

contents of Cr, Cu, Fe, and Pb as well as the pollen viability using Lugol staining method. Scanning Electron Microscope (SEM) analysis of collected pollens did not show any significant structural variations.

**Key words:** Genotoxicity, Pollen viability assay, Scanning electron microscope, Heavy metals.

**INTRODUCTION**

Rapid increase in automobiles for daily transportation along with lack of emission standards in several countries has further contributed to environmental pollution. Nowadays, air contaminants have gained lots of public and scientific attention because these contaminants can directly enter the human system through inhalation or can get settled on the ground surfaces. Contaminated surface roadside soil recycles these particulates back to air by moving automobiles in dry season as well as results in leaching towards ground water during rainy season. Plants, being sessile in nature, continuously get exposed to these contaminants. In order to explore the effects of air contaminants from vehicular emissions, both surface soil and plants of roadside turned out as a promising experimental material.

At present, the most of regulations related to pollution evaluation of any ecosystem are relied upon the physico-chemical analysis. However, this analysis unaccompanied by toxicological studies is not able to provide information on the interactions between environmental matrix and biota<sup>1</sup>. Bioassays, on other hand, can mitigate these constraints and are recommended for the assessment of ecological risks for soils and other matrices<sup>2</sup>. Bioassay or ecotoxicity assay is a method in which living test species *viz.*, bacteria, plants and animals are exposed directly to an environmental sample (soil, sediment, water, air) or extract/s of environmental samples to measure their toxic effects. Among different bioassays, plant bioassays are proven to be efficient test systems for chemical screening and especially for *in situ* monitoring of genotoxicity of environmental pollutants. *Trigonella foenum-graecum* (also known as "Methi" or "Fenugreek")

has received the global attention as a study material for genotoxicity studies because of low chromosome number ( $2n = 16$ ) and easy root growth at normal temperature<sup>3</sup>.

The constant exposure of plant parts to air pollutants makes the pollen grains as a sensitive bioindicator of air pollution<sup>4</sup>. Three general approaches for evaluating pollen viability *viz.*, *in vivo* approach (germinating pollens on stigmas of emasculated flowers), *in vitro* approach (germinating pollens on artificial media) and histochemical approach (staining the pollens) has been documented<sup>5</sup>. Among three, pollen viability data has been documented as a valuable tool to access the successful adaptation of plant species to inauspicious (stress) conditions while the histochemical approach has been considered as one of the most suitable methods for routine screening as it requires short duration<sup>6</sup>. Hence, both these parameters were taken into consideration to evaluate effects of vehicular emissions. As instrument like Scanning Electron Microscope (SEM) has further offered a rapid means of observing pollen wall surfaces by providing greater resolutions which has extended the scope and precision of pollen analysis as well as pollen morphology, the same was used during present study<sup>7</sup>.

Considering the increased number of vehicles in the urban areas and their harmful consequences in terms of ill health effects, the present study was planned to estimate the physico-chemical characteristics including heavy metal contents of soil samples under constant exposure to automobile emissions and to evaluate their genotoxic effects using *Trigonella foenum-graecum* root chromosomal aberration assay. The study further pertained to conduct the pollen viability assay in plants grown in the vicinity of those contaminated sites.

## MATERIALS AND METHODS

### Collection of samples

Roadside soil samples were collected from four different sites *viz.*, bus stand (BS); Guru Nanak Dev University (GU); railway station (RS); a flyover bridge (FB) of Amritsar city, Punjab (India) by taking 3 samples from each site at the distance of approx. 100 m. These samples were under continuous exposure to vehicular emissions. The samples were collected in polythene bags and were brought to

laboratory for estimation of physico-chemical properties, heavy metal contents and genotoxic effects.

In order to evaluate the effects of vehicular emissions on pollen viability, different plant species *viz.*, *Cannabis sativa*, *Hamelia patens*, *Jatropha panduraefolia* and *Thevetia peruviana* were selected commonly grown along roadside sites from where the soil samples were collected. Flower buds at anthesis stage were collected from the selected sites in the morning time

### Estimation of heavy metal contents

1 g of each soil sample was weighed and taken into separate beakers and were digested using aqua regia (3 : 1; HCl : HNO<sub>3</sub>). The digested product was mixed into 2 ml of distilled water with the help of glass rod. Final volume was made 20 ml after filtration of digested soils. The samples were further diluted 10 times before analysis of heavy metals using Atomic Absorption Spectrophotometer. Different heavy metals *viz.*, cadmium (Cd), copper (Cu), chromium (Cr), cobalt (Co), iron (Fe), manganese (Mn), lead (Pb) and zinc (Zn) were analyzed using atomic absorption spectrophotometer (model 240 FS make Agilent) at Guru Nanak Dev University, Amritsar.

### Estimation of Genotoxicity

Genotoxicity of soil samples was estimated using *Trigonella foenum-graecum* root chromosomal aberration assay. For it, soil extracts (1 : 2; Soil : water; (w/v) ) were prepared by dissolving 100 g of soil in 200 ml distilled water. The solution was kept on mechanical shaker for 12 h and filtered using *Whatman* No. 1 filter paper. The filtrate was considered as soil extract for genotoxicity studies.

*Trigonella foenum-graecum* seeds were purchased from local market. Healthy seeds of uniform size free from mould and mechanical injury were selected for genotoxicity studies. Seeds were surface sterilized with mercuric chloride for the prevention of surface fungal/bacterial contamination. The seeds were then washed with sterilized double distilled water before keeping for germination. For germination of seeds, Petri dishes were first washed and swabbed with spirit. *Whatman* No. 1 filter papers were cut in size and shape of Petri dish, autoclaved and spread on inner side of Petri

dishes. 50 seeds of *Trigonella foenum-graecum* were arranged at equidistance on the filter papers in each Petri dish and seeds in each Petri dish were treated with 5 ml of different soil extracts. Each treatment was given in triplicate. The Petri dishes with treated seeds were placed in seed germinator at  $25 \pm 2$  °C. The Petri dishes were monitored daily for fungal and other inspections.

For mitosis study, from each treated seed, root tips approximately to the size of 0.5 - 1 cm were cut and preserved in Farmer's fixative i.e. 1 : 3 : : glacial acetic acid : ethanol. The root tips were hydrolyzed in 1 N HCl at  $60 \pm 2$  °C for 1 min. After hydrolysis, root tips were transferred to watch glass containing 2 % aceto-orcein stain. The apical tips of these root tips were removed and used for slide preparation. Microscopic examination was done for chromosomal aberrations for each set of treatment and control (distilled water). Total 50 cells were observed for each slide. At least three slides were scored per sample. Calculations were done using the following formula:

$$\text{Percentage of aberrant cells} = \frac{\text{No. of aberrant cells}}{\text{Total no. of dividing cells}} \times 100$$

#### Estimation of pollen viability

The effects of vehicular emissions were analyzed following pollen viability assay. Pollen viability was determined using different stains viz., Aceto-orcein stain, Alexander's stain, Lugol's solution and 2,3,5-triphenyltetrazolium chloride. Flower buds of appropriate size i.e. at anthesis stage were collected from the selected sites in the morning time. Collected buds were wrapped in wet cotton to maintain humidity to prevent buds from desiccation. Buds were then brought to the laboratory where they were cut open to remove anthers. Thereafter, anthers were squashed in a drop of stain. The staining was done following four different staining methods including Aceto-orcein, Alexander's, Lugol's and 2,3,5-triphenyltetrazolium chloride (TTC). Different staining methods resulted in differential coloring of fertile and sterile pollens. Minimum of 1000 pollens were scored for each sample. Pollen viability was estimated using formula:

$$\text{Pollen viability (\%)} = \frac{\text{Number of viable pollens}}{\text{Total number of pollens}} \times 100$$

#### Pollen structure and size

Pollen grains of four plant species were also analyzed using scanning electron microscope (SEM Model: EVO LS10, make ZEISS). Fresh flower buds were taken on petri dishes. With the help of forceps and needles, flower buds were cut open to remove anthers. Discarding the other parts, only anthers were then placed on the stubs coated with double-adhesive tape. Further, the squashing of anthers was done with the help of needle. The debris was then removed carefully. and pollens of *Hamelia patens* and *Cannabis sativa* were analyzed under scanning electron microscope at the magnification of 4.00 KX at 10, 000 kv and 2.50 KX at 10,000 kv for *Jatropha panduraefolia* and *Thevetia peruviana*.

#### RESULTS AND DISCUSSION

Although vehicles have increased the human comfort level, their overuse and improper handling have resulted in increased levels of contaminants including metals in different ecosystems. Metals occur naturally in soil ecosystems but in trace levels. Generally, the content of metals in an uncontaminated soil depends on the geology of parent material/rock from where it is formed. However, the surface roadside soil, on the other hand, gets contaminated due to continuous exposure to emissions from vehicles, wear and tear of vehicles and surface runoffs from the roads as well as other polluted dumping areas near to the road sides. The problem arises when these heavy metals percolate into the soil solution and are taken up by the plant roots or get transported to the ground water. The present study revealed that roadside soil samples collected from different sites coded BS, GU, RS and FB had various heavy metals like cadmium (Cd), chromium (Cr), cobalt (Co), copper (Cu), iron (Fe), lead (Pb), manganese (Mn) and zinc (Zn) (Table 1).

The content of various metals in the soil samples ranged as follows:

- i) Bus stand: Fe (5570.40 - 10460.80 mg/kg), Mn (209.64 - 265.03 mg/kg), Zn (63.95 - 140.28 mg/kg), Cu (35.69 - 77.80 mg/kg), Pb ( 41.74 - 75.87 mg/kg), Cr (16.40 - 20 mg/kg), Co (3.73 - 8.53 mg/kg), Cd (0.79 - 1.03 mg/kg)
- ii) Railway station: Fe (452 - 3903.87 mg/kg), Mn (106.24 - 236.27 mg/kg), Zn (43.89 - 131.76 mg/kg), Pb ( 2.13 - 37.87 mg/kg), Cu (9.55 - 18

- mg/kg), Cr (N.D - 12.61 mg/kg), Co (5.2 - 12.13 mg/kg), Cd (0.79 - 1 mg/kg)
- iii) Flyover bridge: Fe (873.60 - 2017.60 mg/kg), Mn (120 - 402.96 mg/kg), Zn (24.57 - 117.29 mg/kg), Pb (52.13 - 91.87 mg/kg), Cu (16.75 - 39.66 mg/kg), Cr (N.D - 5.71 mg/kg), Co (2.4 - 3.87 mg/kg), Cd (0.95 - 1.23 mg/kg).
- iv) Guru Nanak Dev University campus: Fe (N.D. - 718.40 mg/kg), Mn (160.60 - 262 mg/kg), Zn (21.25 - 57.59 mg/kg), Pb (2 - 31.74 mg/kg), Cu (3.85 - 13.8 mg/kg), Co (5.73 - 9.53 mg/kg), Cr (N.D), Cd (0.78 - 1.01 mg/kg).

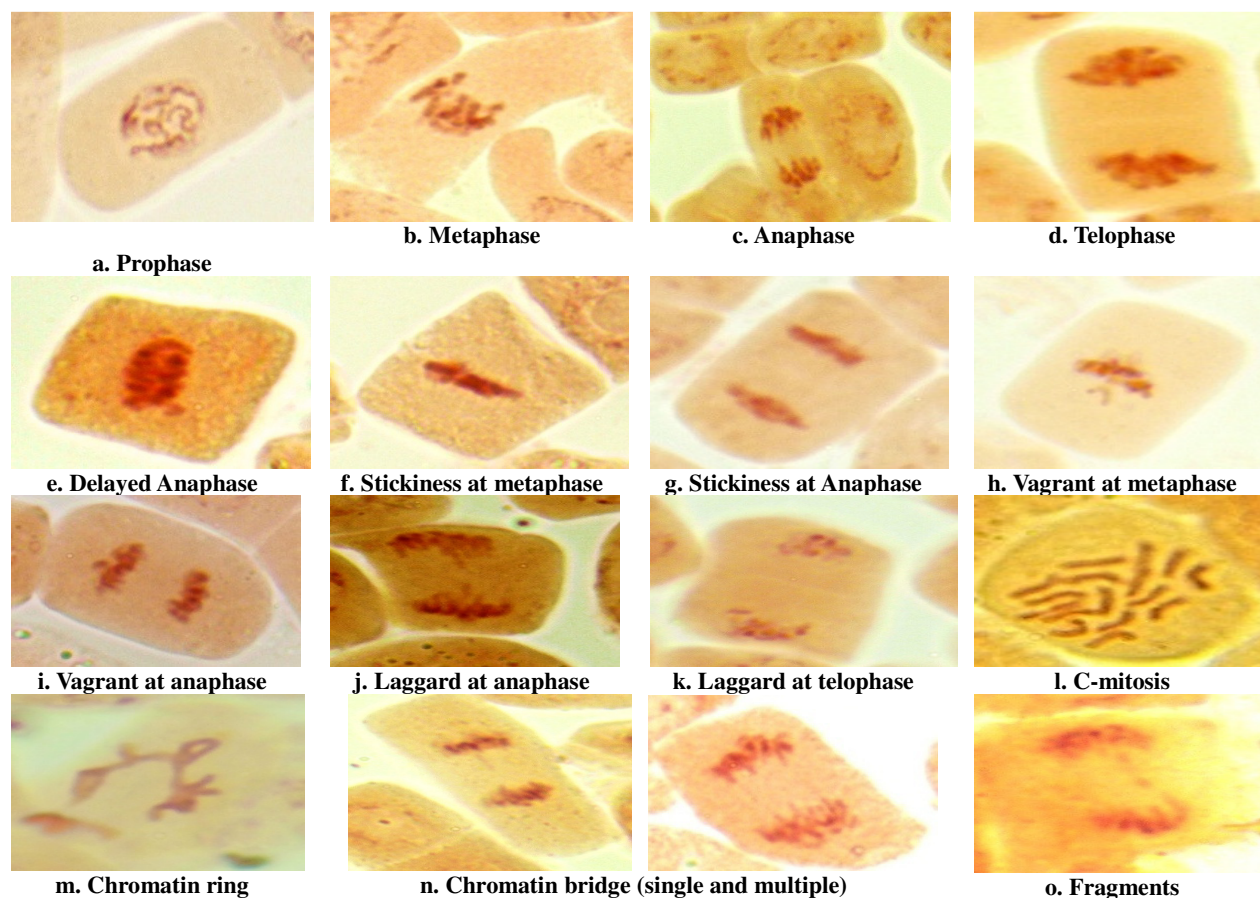
After surveying the surface soils of different parts of world, WHO has given the average value of cadmium content ranging between 0.07 and 1.1 mg/kg; values above 0.5 mg/kg usually reflect anthropogenic inputs while the natural lead content in soil typically ranges from below 10 mg/kg soil up to 30 mg/kg soil<sup>9, 10</sup>. The main content of heavy metals in soils has been documented as: Fe (38000 mg/kg), Mn (600 mg/kg), Cu (30 mg/kg), Cr

(100 mg/kg), Cd (0.06 mg/kg), Zn (50 mg/kg), Pb (10 mg/kg), Co (8 mg/kg)<sup>8</sup>. Fe, Mn and Cr during the present study were found to be low as compared to the values given by Mclean and Bledsoe (1992). Pb, Zn and Cd were detected as major pollutants in the roadside soil samples during the present study. Zakir et al.<sup>11</sup> also have shown high content of these metals in their study. Genotoxic potential of all the samples was analyzed using *Trigonella foenum-graecum* root chromosomal aberration assay. This bioassay has been approved by many authors<sup>12 - 14</sup>. This bioassay has been found to be economic and easy to afford at all the times. Various types of physiological (c-mitosis, abnormal anaphase, abnormal metaphase, delayed anaphase, laggad/s, vagrant/s, stickiness) as well as clastogenic (chromosomal breaks, chromatin bridge and ring chromosomes) aberrations were observed during the present study giving an indication that there is certain cytotoxic/genotoxic substance present in the soil samples (Fig. 1).

**Table 1. Content of heavy metals in different roadside soil samples of Amritsar, Punjab (India).**

Sample code	Content of heavy metal (mg/kg of dried soil (Mean) ± S.E.)							
	Cadmium	Chromium	Cobalt	Copper	Iron	Lead	Manganese	Zinc
BS-I	1.01±0.03	16.40±0.53	3.73±0.41	35.69±1.20	5570.40±23.63	41.74±1.33	265.03±24.92	140.28±1.13
BS-II	1.03±0.04	17.91±0.31	8.53±0.18	77.80±0.73	9020.00±26.04	56.13±0.13	229.88±1.09	67.91±0.35
BS-III	0.79±0.06	20.00±0.52	4.54±0.25	75.35±0.98	10460.80±59.17	75.87±0.72	209.64±4.18	63.95±1.04
GU-I	0.78±0.06	N.D.	5.73±0.07	3.85±0.19	N.D.	2.13±0.58	160.60±3.56	21.25±0.12
GU-II	0.99±0.03	N.D.	8.80±0.23	6.45±0.27	164.00±7.33	2.00±0.50	262.00±6.60	57.59±0.40
GU-III	1.01±0.04	N.D.	9.53±0.58	13.80±0.00	718.40±66.93	31.74±0.64	166.33±42.48	55.72±0.10
RS-I	0.79±0.03	N.D.	5.61±0.21	9.55±0.05	452.00±73.22	11.73±0.50	178.25±5.30	43.89±2.10
RS-II	0.79±0.03	N.D.	5.20±0.12	15.05±0.14	1282.40±6.97	37.87±0.95	106.24±4.30	47.81±0.85
RS-III	1.00±0.04	12.61±0.78	12.13±1.2	18.00±0.23	3903.87±19.86	2.13±0.27	236.27±10.39	131.76±4.44
FB-I	1.00±0.06	N.D.	2.40±0.42	16.75±0.25	1701.60±12.50	52.13±0.96	159.61±2.47	48.51±1.00
FB-II	1.23±0.04	3.30±0.30	3.87±0.47	39.66±0.44	873.60±16.94	91.87±1.98	402.96±2.56	117.29±12.92
FB-III	0.95±0.04	5.71±0.76	3.73±0.41	39.25±0.33	2017.60±64.29	62.47±0.45	120.00±0.45	24.57±0.92

BS: Bus stand; GU: Guru Nanak Dev University; RS: Railway station; FB: Flyover bridge; N.D.: Not detected



**Fig. 1. Root tip cells of *Trigonella foenum-graecum* root with normal stages (a - d) and with aberrations (e - o) following the treatment with extracts of roadside soil samples.**

Among all samples, maximum chromosomal aberrations (29.87 – 37.28 %) were observed in samples collected near bus stand with maximum number of c-mitosis indicating presence of some genotoxin having colchicine like action on spindle apparatus i.e. inactivation/destruction of spindle apparatus (Fig. 2). The inactivation of spindle apparatus connected with delayed division of centromere results in c-mitosis whereas disturbed metaphase, anaphase and telophase occur due to the disturbances of spindle apparatus which allows chromosomes to spread irregularly over the cell resulting in c-mitosis. The presence of chromosome stickiness indicates a highly toxic, irreversible effect that may lead to cell death.

Stickiness is formed due to chromosomal clustering during any phase *viz.*, prophase, metaphase, anaphase or telophase of the cell cycle. The major

contributing factor for induction of stickiness and clumping include genetic and environmental factors. Many authors have reported various agents that cause chromosomal stickiness<sup>15</sup>. Laggard chromosomes results due to failure of the chromosomes to get attached to the spindle fibre consequently. Similarly, vagrant chromosomal abnormality may be caused as a result of unequal distribution of chromosomes with paired chromatids resulted as a result of nondisjunction of chromatids in anaphase. Chromatin bridges and chromosomal fragments are clastogenic effects which results due to clastogen action on the chromosomes involving action on DNA<sup>16</sup>.

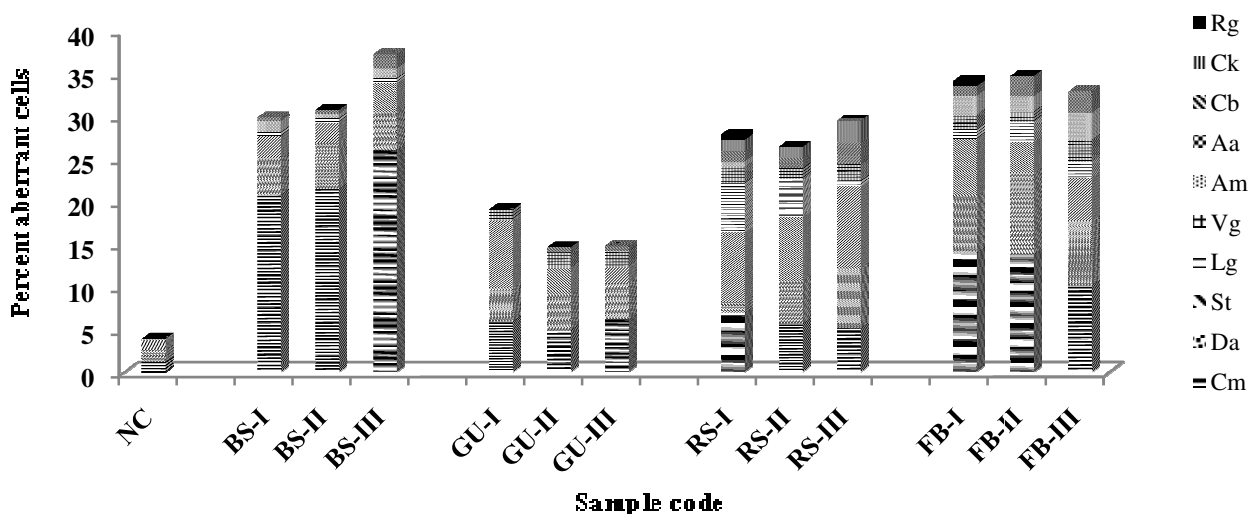
Pollen viability was conducted for four plant species *viz.*, *Cannabis sativa*, *Hamelia patens*, *Jatropha pandurafolia* and *Thevetia peruviana* grown on the roadside soil of sampling sites. The results of pol-

len viability of different plant species used are represented in Table 2.

It was observed that 2,3,5-triphenyltetrazolium chloride showed the best variability of stained and non-stained pollens among the four stains used during the present study. > 90 % pollen viability was seen with Aceto-orcein and Alexander's stain. For Lugol's staining, the pollen viability was observed to be 83.15% to 96.37 %. 2,3,5-triphenyltetrazolium chloride resulted in pollen viability as *Cannabis sativa* (76.86 %), *Hamelia patens* (77.94 %), *Jatropha panduraefolia* (75.58 %), *Thevetia peruviana* (64.64 %). Test with other dyes (Alexander's, Aceto-orcein, Lugol's) overestimated pollen viability when compared to results with TTC. Similar results were reported by Munhoz et al.<sup>17</sup> while testing pollen viability in *Carica papaya* L. Kelen and Demirtas<sup>18</sup> deduced TTC is reliable for testing pollen viability. The percentage of the aborted pollen usually increases with the age of the plant<sup>19</sup>. Alexander's stain does not differentiate between

living and nonliving pollen rather it distinguishes between aborted vs. non aborted or mature vs. immature pollen<sup>20</sup>. He further stated that the Alexander's stain gave a measure of pollen maturity but not viability at the time of anthesis.

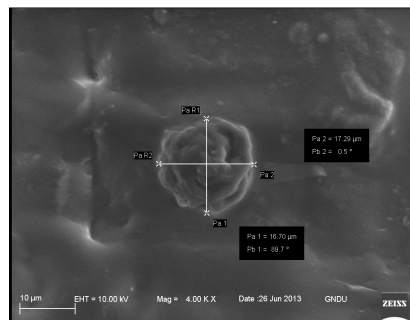
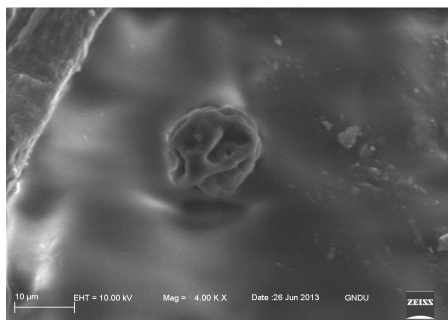
Pollens of selected plant species were also analyzed using Scanning Electron Microscope to study any change in the morphology but no such characteristic variation was observed probably due to the reason that only limited numbers of pollens were scanned. Contrary to this, Rezanejad et al.<sup>21</sup> reported the increased incidence of abnormality and shrinkage in pollens of *Lagerstroemia indica* stressed by air pollutants. Particle agglomeration and cellular material release (exocytosis) were also seen<sup>21</sup>. Moreover, through SEM analysis pollen size of plant species viz., *Cannabis sativa*, *Hamelia patens*, *Jatropha panduraefolia* and *Thevetia peruviana* was found to be as 17.29  $\mu\text{m}$  x 16.70  $\mu\text{m}$ , 20.58  $\mu\text{m}$  x 21.02  $\mu\text{m}$ , 55.55  $\mu\text{m}$  x 58.95  $\mu\text{m}$ , 70.08  $\mu\text{m}$  x 66.09  $\mu\text{m}$ , respectively (Fig. 3).



Cm: C-mitosis; Da: Delayed anaphase; St: Stickiness; Lg: Laggard; Vg: Vagrant; Am: Abnormal metaphase; Aa: Abnormal anaphase; Cb: Chromatin bridge; Ck: Chromosomal break; Rg: Ring chromosome; NC: Negative control (Distilled water)

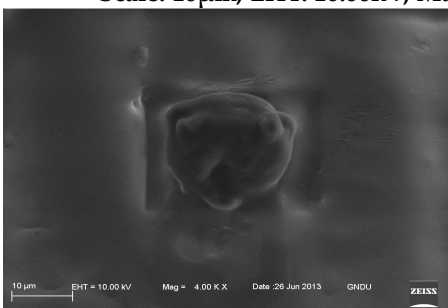
Fig. 2. Spectrum of different types of aberrations induced in *Trigonella foenum-graecum* root tip cells following treatment with different extracts of roadside soil samples of Amritsar, India

*Cannabis sativa*



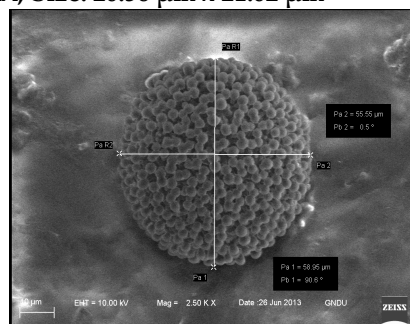
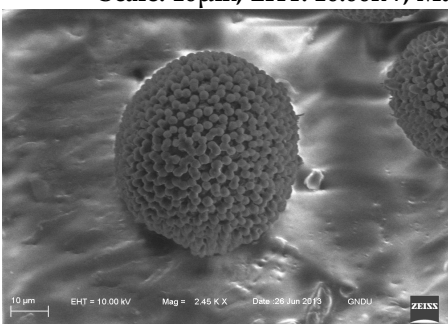
Scale: 10µm; EHT: 10.00kV; Mag.: 4.00KX; Size: 17.29 µm x 16.70 µm

*Hamelia patens*



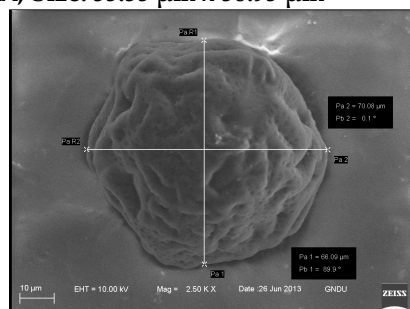
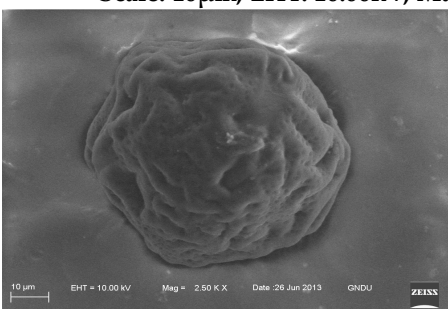
Scale: 10µm; EHT: 10.00kV; Mag.: 4.00KX; Size: 20.58 µm x 21.02 µm

*Jatropha panduraefolia*



Scale: 10µm; EHT: 10.00kV; Mag.: 2.50KX; Size: 55.55 µm x 58.95 µm

*Thevetia peruviana*



Scale: 10µm; EHT: 10.00kV; Mag.: 2.50KX; Size: 70.08 µm x 66.09 µm

Fig. 3. Images of pollen grains of different plant species collected from roadside soils of Amritsar using Scanning Electron Microscope (SEM).

**Table 2. Pollen viability (%) of some plant species grown at the vicinity of sampling sites.**

Plant species	Pollen viability (Mean ± S.E)			
	AO	AL	LG	TTC
<i>Cannabis sativa</i>	97.59 ± 0.91	99.43 ± 0.09	96.37 ± 1.33	76.86 ± 1.98
<i>Hamelia patens</i>	98.08 ± 0.55	97.30 ± 0.04	83.15 ± 1.28	77.94 ± 1.43
<i>Jatropha panduraefolia</i>	99.63 ± 0.13	93.43 ± 1.16	96.01 ± 1.03	75.58 ± 1.72
<i>Thevetia peruviana</i>	99.67 ± 0.12	93.85 ± 1.37	94.80 ± 0.70	64.64 ± 0.46

AO: Aceto-orcein stain; AL: Alexander's stain; LG: Lugol's stain; TTC: 2,3,5-triphenyltetrazolium chloride

### STATISTICAL ANALYSIS

Statistical analysis was carried out using Pearson correlation coefficient. It was seen that lead, copper, iron and chromium were positively correlated to genotoxicity. During pollen viability assay, pollens stained with Alexander's stain showed positive correlation with content of Cr. Further, Lugol's stain was observed to induce positive correlation with that of genotoxicity (Table 3).

	Cd	Cr	Co	Cu	Fe	Pb	Mn	Zn	GENO	PVO	PVA	PVL	PVT
Cd	1												
Cr	0.080	1											
Co	0.090	0.095	1										
Cu	0.172	<b>0.826***</b>	-0.148	1									
Fe	-0.043	<b>0.947***</b>	0.023	<b>0.892***</b>	1								
Pb	0.400	0.349	<b>-0.577*</b>	<b>0.718**</b>	0.426	1							
Mn	<b>0.726**</b>	0.261	0.064	0.251	0.123	0.305	1						
Zn	<b>0.585*</b>	0.509	0.203	0.204	0.303	0.144	<b>0.718**</b>	1					
GENO	0.129	<b>0.566*</b>	-0.499	<b>0.668*</b>	<b>0.568*</b>	<b>0.718**</b>	0.202	0.303	1				
PVO	0.036	-0.356	-0.017	-0.096	-0.259	0.180	-0.050	-0.216	0.267	1			
PVA	0.179	<b>0.564*</b>	0.186	0.470	0.531	0.087	0.516	0.341	-0.089	<b>-0.687**</b>	1		
PVL	0.007	0.536	-0.377	0.449	0.493	0.416	0.159	0.446	<b>0.901***</b>	0.213	-0.159	1	
PVT	-0.508	0.258	0.497	-0.080	0.261	-0.509	-0.201	0.081	-0.450	-0.511	0.437	-0.286	1

\*p≤0.05 level of significance; \*\*p≤0.01 level of significance; \*\*\*p≤0.001 level of significance

Cd: Cadmium; Cr: Chromium; Co: Cobalt; Cu: Copper; Fe: Iron; Pb: Lead; Mn: Manganese; Zn: Zinc; GENO: Genotoxicity; PVO: pollen viability with aceto-orcein stain; PVA: pollen viability with Alexander's stain; PVL: pollen viability with Lugol's stain; PVT: pollen viability with TTC.

## CONCLUSIONS

Heavy metals viz., Pb, Zn and Cd were detected as major soil pollutants. Various chromosomal abnormalities in root tip cells of *Trigonella foenum-graecum* gave an indication of presence of some genotoxic substances in the samples studied. The present study clearly indicated that *Trigonella foenum-graecum* root chromosomal aberration assay can be used as a first alert for the indication of pollution at particular sites. Although SEM studies did not show any variation in pollen size or structure but occurrence of non viable pollens using different staining methods were direct indicative of the harmful consequences of air pollution. Further, among different pollen staining methods, 2,3,5-triphenyltetrazolium chloride has shown the best variability in pollen viability. Hence the present study clearly indicates the use of these bioassays for environmental analysis.

## ACKNOWLEDGEMENTS

Sincere thanks to UGC for providing the financial assistance. Miss Jagdeep Kaur is highly grateful to UGC for awarding her Maulana Azad National Fellowship.

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