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Genotoxicity and tumor inducing potential of roadside soil samples exposed to heavy traffic emissions at Amritsar (Punjab), India

Rajwant Kaur[1], Yogesh B. Pakade[2] and Jatinder Kaur Katnoria[1*]

[1]Department of Botanical and Environmental Sciences, Guru Nanak Dev University, Amritsar, (Punjab), INDIA
[2]Hill Area Tea Science Division, CSIR-Institute of Himalayan Bioresources and Technology, Palampur (Himachal Pradesh), INDIA

*Corresponding author. E-mail: jkat08@yahoo.com

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Abstract: Noxious gases released from vehicles adversely affect the microbial population of the roadside soil as well as exerts influence on plant growth and development. Considering the increasing vehicular pollution in Amritsar, a holy city of Punjab (India) and their serious consequences in terms of health problems, the present study was planned to estimate genotoxic and tumor inducing potentials of soil samples from Golden temple (GT) and Putlighar Chowk (PG) by employing *Allium cepa* root chromosomal aberration assay (*Al*RCAA) and Potato disc tumor assay (PDTA), respectively. The genotoxic potential in terms of percent aberrant cells in *A. cepa* was found to be 29.24 % (GT) and 29.24 % (PG) during *in situ* treatment and 39.00 % (GT) and 39.48 % (PG) during root dip treatment. The average number of tumors was found to be 14.4 in PG sample, followed by 13.0 in GT sample. Both samples have shown high content of lead *viz.,* 39.81 (GT) and 56.48 (PG) during physicochemical analysis of the samples.

Keywords: Automobiles, Genotoxic, Heavy metals, Physico-chemical analysis

INTRODUCTION

Transportation is one of the important components of any country's economics growth, yet it has many negative impacts on environment. Vehicles play a significant role in aggravating the pollution of different ecosystems (Plakhotnik *et al.*, 2005; Abechi *et al.*, 2010). The vehicular emissions are the major cause for soil pollution because the air pollutants released into the atmosphere ultimately settle on the ground surface. Therefore, roadside soil contains huge concentrations of pollutants that may deteriorate environmental quality and have toxicological effects on human health. The different types of pollutants released from vehicles are heavy metals, polycyclic aromatic hydrocarbons, oxides of nitrogen and sulphur (Jaradat and Momani, 1999; Imperato *et al.*, 2003; Akbar *et al.*, 2006; Abechi *et al.*, 2010; Evagelopoulos *et al.*, 2010). The pollutants either get accumulated in the surface soil or are transported to the deeper layers of soil leading to its physicochemical alterations (Mbah and Anikwe, 2010). Moreover, the contaminants released from vehicular emissions enter the biological system and cause health hazards to plants, animals and human (Ozaki *et al.*, 2004; Makokha *et al.*, 2008; Sujetoviene and Griauslyte, 2008; Joshi *et al.*, 2010).

To explore the consequences of various pollutants, different bioassays have been recommended (Conder *et al.*, 2001; Sujetoviene and Griauslyte, 2008; Katnoria *et al.*, 2011). A number of studies have shown the presence of DNA damaging pollutants in different samples using a number of bioassays *viz., Allium cepa* root chromosomal aberration assay (Rank and Nielsen 1998; Chakraborty *et al.*, 2009; Katnoria *et al.*, 2011; Abu and Mba, 2011), *Allium sativum* root chromosomal aberration assay (Yi and Meng, 2003; Liu *et al.*, 2009; Saxena *et al.*, 2010), *Tadescantia* stamen hair mutation assay (Arutyunyan, *et al.*, 1999; Marciulioniene *et al.*, 2004), *Vicia faba* chromosomal aberration/micronuclei assay (Misik and Mmicieta, 2002; Kontek *et al.*, 2007; Gulfishan *et al.*, 2010) and Potato disc tumor assay (Coker *et al.*, 2003).

However, to have the exact magnitude of the pollution of environment, the physico-chemical analysis has its own importance. Some scientists have, therefore, studied the soil ecosystem by analysis of various physico-chemical properties like pH, alkalinity, soil texture, water holding capacity, bulk density, nitrates, phosphates, potassium, calcium, sodium, magnesium, chlorides and lead (Garcia *et al.*, 2001; Garg, 2002; Kulkarni and aggarwal, 2003; Aseri and Tarafdar, 2006; Glori *et al.*, 2008 and Katnoria *et al.*, 2011). Considering the increased exposure of people to traffic emissions in Amritsar, Punjab (India), the present study was planned to estimate the genotoxic and tumor inducing potential

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of surface soil samples exposed to vehicular emissions.

MATERIALS AND METHODS

Collection of soil samples: Soil samples were collected from two different road junctions of Amritsar *viz.,* Golden temple (GT) and Putlighar (PG) by scraping of surface soil. The soil samples were brought to laboratory and dried for 72 h (Cabrera and Rodriguez, 1999). The samples were used to analyze the physico-chemical characteristics, genotoxic and tumor inducing potential.

Analysis of soil samples: The physico-chemical parameters *viz.,* pH, alkalinity, bulk density, water holding capacity, soil texture, chlorides, calcium, magnesium, nitrates, phosphates, potassium, sodium and lead of soil samples were analyzed by using standard protocols (Trivedy *et al.,* 1987; Akbar *et al.,* 2006 and Katnoria *et al.,* 2011). The estimation of lead was carried out at Institute of Himayan Bioresourse Technology (IHBT), Palampur using Atomic absorption spectrophotometer with graphite furnace.

Estimation of genotoxic potential: The genotoxic potential of soil samples was estimated by using *A. cepa* root chromosomal aberration assay. *A. cepa* root chromosomal aberration assay performed in two modes *viz., in situ* treatment and root dip treatment to evaluate the genotoxic potential of soil samples During root dip treatment, peeled onion bulbs were kept for root germination in distilled water for 24-36 h. The emerged roots (0.5 - 1.0 cm) were treated with different concentrations (25%, 50%, 75% and 100%) of soil extracts (1 : 2, w/v; soil : distilled water) for 3 h. During *in situ* treatment, the peeled onion bulbs were directly placed on soil contained in small pots and were allowed to root for 24-36 h. then the root tips were washed thoroughly with distille water, cut and fixed in farmer's fluid (3 : 1: : ethanol : glacial acetic acid). Slides were prepared by squashing in aceto-orcein stain and scanned under microscope for scoring different types of aberrations. Distilled water and acid washed sand were treated as negative control during root dip and *in situ* treatment, respectively.

Estimation of tumor inducing potential: The tumor inducing potential of soil samples was estimated using potato disc tumor assay (Cooker *et al.,* 2003). The grown gall bacteria *Agrobacterium tumifaciens* strain MTCC (Microbial Type Culture Collection and Gene Bank) No. 431 was purchased from Institute of Microbial Technology (IMTECH), Chandigarh and was used for the experiment. The Potato disc tumor assay was performed in two modes *viz.,* with culture and without culture and two types of soil extracts i.e. aqueous and Dimethyl sulfoxide (DMSO) extract were used. The lab area was sterilized using 20% Bleach solution. Fresh Russet potatoes purchased from local grocery store were washed thoroughly under running tap water for 2-3 min and were peeled off. The potatoes were cut with the help of cork borer (1 cm) and discs of 0.5 cm height prepared. The discs were sterilized with 10% Bleach solution. The potato discs were imbedded in agar plates (Petridish) up to 2/3 rd of the height. 400 µl of soil extract and 400 µl of culture of *A. tumefaciens* were mixed in a vial and 50 µl of the mixture was put on each disc. In another set, 50 µl of only soil extracts was poured on to the discs. 50 µl of only culture was used as positive control while 50 µl of distilled water and 50 µl of DMSO were used as respective negative controls. Petri plates were covered, sealed with parafilm and incubated in B.O.D. incubator for 21 days to induce tumors. After inoculation period, potato discs were analyzed for scoring number of induced tumors using stereomicroscope at 25X magnification after staining with Lugol's Solution (5 % KI + 5 % I_2).

RESULTS AND DISCUSSION

Diverse contaminants breakthrough the soil ecosystem either naturally from soil parent material, volcanic eruptions, forest fires etc. or arthropogenically by use of pesticides and activities such as mining, smelting, transportation, refining etc. Apart from this, heavy metals enter the soil through wear and tear of tyres, construction of railway and road lines (Haal *et al.,* 2008). This is the reason that most of the surface roadside soils are reported to be polluted with heavy metals. The polluted soils are of concern due to the fact that under supersaturated conditions through rainfalls or surface water runoff, the pollutants percolate through the soil profile and contaminate the ground water. Moreover, the movement of vehicles can re-suspend the pollutants by causing the highly turbulent conditions which expose the people to these pollutants via inhalation of dust particles.

In the present study, lead content was found to be 39.81 mg/Kg in GT and 56.48 mg/Kg in PG sites which were under the exposure to heavy metal transportation. Although there are no permissible limits defined for soil ecosystem, yet, Aggarwal (2009) has given the typical concentration of Pb in lithosphere as 10 µg/g while its range as 2-200 µg/g. The concentration of Pb in both the samples were found to be higher than the typical concentration of Pb as given by Aggarwal (2009). Similar studies have been conducted worldwide and many reports support the fact of increased lead contents along the roadside soils (Jaradat and Momani, 1999; Onder *et al.,* 2007; Abechi *et al.,* 2010). The other physico-chemical parameters were also analyzed and results are shown in Table 1. Both samples have alkaline pH while bulk density was found to be slightly higher i.e. 1.92 (GT) and 1.834 (PG) than the normal bulk density (1.3 – 1.6) of the agriculture soil samples while the other parameters were

Table 1. Physico-chemical characteristics of roadside soil samples of Amritsar, Punjab (India).

Parameters		Golden temple (GT)	Putiighar (PG)
pH		7.66 ± 0.01	8.43 ± 0.01
Alkalinity (meq/100g)		0.40 ± 0.05	0.560 ± 0.03
Bulk density (g/cc)		1.92 ± 0.00	1.834 ± 0.00
Water holding capacity (%)		24.11 ± 0.12	37.91 ± 0.34
Soil texture	Sand (%)	43.07 ± 1.00	41.49 ± 0.19
	Silt (%)	29.50 ± 0.04	35.99 ± 0.64
	Clay (%)	26.89 ± 0.86	22.33 ± 0.75
Chlorides (mg/g)		0.02 ± 0.00	0.02 ± 0.00
Calcium (mg/g)		4.81 ± 0.46	2.14 ± 0.26
Magnesium (mg/g)		11.19 ± 1.66	27.20 ± 0.40
Nitrates (mg/g)		0.22 ± 0.00	0.01 ± 0.00
Phosphates (mg/g)		0.03 ± 0.00	0.09 ± 0.00
Potassium (mg/g)		8.10 ± 0.06	11.20 ± 0.00
Sodium (mg/g)		33.66 ± 0.21	29.50 ± 0.23
Lead (mg/Kg)		39.81 ± 0.00	56.48 ± 0.00

within the range as earlier reported by Warhate *et al.* (2006). The study was further carried out to explore the genotoxic and tumor inducing potential of both soil samples using bioassays. It was observed that both soil samples induced genotoxicity in *A. cepa* root tips cells and showed the tumor inducing potential during Potato disc tumor assay. During genotoxicity assay, the percentage of different 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 in root tip cells of *A. cepa* exposed to both soil samples (Fig. 3). The Golden temple sample showed 23.85 % physiological aberrations while 5.39% clastogenic aberrations during *in situ* treatment. The aberrant cell in GT was found to be 39.00 %. The frequency of physiological and clastogenic aberrations at different concentrations *viz.*, 25%, 50%, 75% and 100% were 18.68%, 27.48%, 33.40% and 34.08% and 2.85%, 2.53%, 4.09% and 4.92%, respectively. In PG sample, the percentage of various physiological aberrations was found to be 25.63% while the percentage of clastogenic aberrations was 5.46% during *in situ* treatment. 25.04%, 27.53%, 33.18% and 39.48% of total aberrant cells were found at 25 %, 50 %, 75 % and 100 %, respectively during root dip treatment in PG sample. Many authors consider the plant bioassays as first alerts/biological indicators to explore the harmful consequences of environmental complex mixtures (Sujetoviene and Griauslyte, 2008; Leme *et al.,* 2008; Hoshina and Marin-Morales, 2009). The clastogenic damages in terms of tumor induction were further observed in the second bioassay *i.e.* Potato Disc Tumor assay. The tumor inducing potential of soil samples is shown in Fig.4. When the potato disc assay was performed without *A. tumifaciens* culture, the aqueous golden temple extract (AGT), the maximum numbers of tumors (12.0) were found at 100 %, while minimum (1.4) were found at 25 %. In the DMSO golden temple extract (DGT), the number of tumors were ranged from 1.6 (25 %) to 3.6 (100%). The mean number of tumors in aqueous extract Putlighar (APG) varied from 1.0 (25%) to 4.8 (100%) while in DMSO extract Putlighar (DPG), the number of tumors varied from 1.6 (25%) to 3.2 (100%). When the potato disc assay was performed with *Agrobacterium tumifaciens* culture, the aqueous extract with culture (AGT+C), the mean number of tumors varied from 5.4 (25%) to 13.0 (100%) whereas in DMSO extract with culture (DMSO+C), the mean number of tumors varied from 3.4 (25%) to 5.4 (100 %). The mean number of tumors at 25%, 50%, 75% and 100% of aqueous extract Putlighar with culture (APG+C) were found to be 4.6, 5.4, 6.8 and 14.4, respectively while mean number of tumors at 25%, 50%, 75% and 100% of DMSO Putlighar extract (DMSO+C) were 4.4, 5.4, 6.6 and 6.8, respectively.

Although there are many studies related to use of this bioassay as antitumor activities of various plant extracts (Hadizadeh *et al.,* 2007; Silva *et al.,* 2012; Karakas *et al.,* 2012), very few studies reported its use in direct clastogenic effect. During infection of a plant with *A. tumefaciens*, a tumor producing plasmid (Ti) is incorporated with the plants chromosomal DNA which activates if phenols are released (Coker *et al.,* 2003). Ti plasmid causes the plants cell to multiply rapidly without going through apoptosis results in tumor formation that histologically is similar to human and animal cancers (Agrios, 1997).

Table 2. Genotoxic potential of roadside soil of Amritsar, Punjab (India) in Allium cepa root chromosomal aberration assay.

Sample code	Treatment	TC	TDC	MI	Physiological aberrations (PA)							Total cells with PA		Clastogenic Aberrations (CA)			Total cells with CA		TAC	
					Cm	Aa	Am	Da	Vg	Lg	St	No.	%	Bk	Rc	Cb	No.	%	No.	%
NC1	-	4705	439	9.33	9	1	-	1	-	-	-	11	2.50	-	-	-	-	-	11	2.50
NC2	-	4769	474	9.93	1	1	0	3	2	0	0	7	1.476	3	-	1	4	0.843	11	2.319
GT	25	4734	455	9.61	25	13	8	23	10	-	6	85	18.68	12	-	1	13	2.85	98	21.53
	50	4869	473	9.71	52	26	12	11	18	1	10	130	27.48	11	-	1	12	2.53	142	30.02
	75	5159	464	8.99	51	32	20	18	15	1	18	155	33.40	15	1	3	19	4.09	174	37.49
	100	5152	487	9.45	68	24	23	18	13	2	18	166	34.08	19	2	3	24	4.92	190	39.00
	in situ	4783	482	10.07	19	26	10	16	27	1	16	115	23.85	15	5	6	26	5.39	141	29.24
PG	25	4860	479	9.85	32	23	4	20	15	-	12	106	22.12	11	1	2	14	2.92	120	25.04
	50	4940	483	9.77	36	23	13	19	13	3	11	118	24.43	11	-	4	15	3.10	133	27.53
	75	4494	458	10.19	51	33	18	10	12	2	11	137	29.91	12	1	2	15	3.27	152	33.18
	100	4695	466	9.92	69	35	33	12	13	1	10	173	37.12	11	-	-	11	2.36	184	39.48
	in situ	4765	476	9.98	31	11	11	30	16	3	19	121	25.63	19	1	6	26	5.46	147	31.09

NC1: Negative control (Distilled water); NC2: Negative control (acid washed sand); GT: Golden temple; PG: putlighar; TC: Total cells; TDC: Total dividing cells; PA: Physiological aberrations; Cm: c-mitosis; Aa: Abnormal anaphase; Da: Delayed anaphase; Vg: Vagrant/s; Lg: Laggard/s; St: Stickiness; Am: Abnormal metaphase; CA: Clastogenic aberrations; Bk: Chromosomal break/s; Rc: Ring chromosome/s; Cb: Chromatin bridge/s; TAC: total aberrant cell.

Table 3. Tumor including potential of soil samples of Amritsar, Punjab (India) in potato disc tumor assay.

Sample code	Treatment	Induction of tumor at different concentrations							
		25%		50%		75%		100%	
		ST	LT	ST	LT	ST	LT	ST	LT
GT	AGT+C	5.4±1.077	2	6.0±2.145	-	6.4±1.208	-	13.0±2.460	-
	DGT+C	3.4±1.470	2	3.8±2.653	-	3.8±1.114	-	5.4±0.748	-
	AGT	1.4±0.245	-	3.4±1.122	-	4.8±0.663	1	12.0±1.761	-
	DGT	1.6±0.400	-	2.4±0.510	-	2.6±0.510	1	3.6±0.678	-
PG	APG+C	4.6±1.364	4	5.4±1.030	-	6.8±1.020	-	14.4±3.820	-
	DPG+C	4.4±0.748	1	5.4±1.400	1	6.6±1.249	-	6.8±1.497	-
	APG	1.0±0.632	-	4.0±1.140	1	4.2±1.068	2	4.8±1.828	1
	DPG	1.6±0.400	1	1.8±0.374	-	1.8±0.374	1	3.2±0.583	-

GT: Golden temple, PG:Putlighar;, AGT+C: Aqueous extract golden temple+culture; DGT+C: DMSO extract golden temple+culture; AGT: Aqueous extract golden temple; DGT: DMSO extract golden temple; APG+C: Aqueous extract putlighar + culture; DPG+C: DMSO extract putlighar + culture; APG: Aqueous extract putlighar; DPG: DMSO extract putlighar; ST: Small tumor; LT: Large tumor

Conclusion

Average increase of concentration of lead in roadside soil was directly correlated with increase of vehicular pollution in the city. Lead is widely documented to be one of the major metal pollutants released from vehicles in the roadside environments and has to induce potential genotoxicity/carcinogenicity. There was an increase Pb concentration in roadside soil samples of Amritsar city and also showed genotoxicity/carcinogenicity in both the bioassays used.

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