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A Study on Physicochemical Analysis of Road and Railway Track Side Soil Samples of Amritsar (Punjab) and Their Genotoxic Effects

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***Abstract*—**Considering the serious health hazards of air pollutants from automobiles, the present study was aimed to estimate the genotoxic/tumor inducing potential of three soil samples collected from junctions of Bus stand (BS), Crystal (CT) and Railway station (RS) of Amritsar, Punjab (India) using *Allium cepa* root chromosomal aberration assay (*Al*RCAA) and potato disc tumor assay (PDTA). The genotoxic potential in *Al*RCAA was 41.27% and 41.26% for BS; 37.89% and 43.38% for RS and 33.76% and 37.83% for CT during *in situ* and root dip treatments, respectively. The maximum number of tumors were induced in RS sample (64) followed by BS (21) and CT (9) during PDTA. The physicochemical parameters of soil sample were also studied and the concentration of lead was found to be 95.21 mg/Kg in RS, 35.30 mg/Kg in BS and 24.59 mg/Kg in CT samples.

***Keywords*—**Automobiles, genotoxicity, Physicochemical parameters, pollutants.

I. Introduction

SOIL is the top layer of the earth's crust that performs many vital functions such as food and biomass production, storage, filtration and transformation of many macro and micro nutrients. However, in recent years, the composition of soil has been altered due to various natural (weathering and erosion) and anthropogenic activities (chemical and industrial process) [1]. Soil pollution has been documented to increase at alarming rates, especially, in developing countries including India [2]. The surface soil not only gets contaminated with discharges/effluents from industries but also by emissions from automobile. Although the emitted pollutants from vehicles disperse into the air but they ultimately reach and settle in the soil ecosystem. The surface soil thus seems to be the promising material for analysis of vehicular pollution. Moreover, it is easy to obtain the adequate soil samples for both biological and chemical analysis. At present, identifying the sources of contaminants in the environment is of key importance to understand the pollution patterns and to make decisions concerning the polluted and non polluted areas/sites [3].

The regulations concerning contaminated soils so for are mainly based upon the physicochemical analysis. However, this approach is not sufficient to evaluate the toxic effects of contaminants and to characterize the contaminated environment. Apart from this, the chemical analysis alone is not able to provide information on effects of chemical compounds and does not take into consideration the interactions between environmental matrix and biota [4]. Bioassays, on other the hand, can mitigate these constraints and therefore are recommended for the assessment of ecological risks in soils or other matrices [5].

Human beings along with other living organisms get directly exposed to the contaminants (diverse mutagens/carcinogens) of soil ecosystem via inhalation or direct contact which results in serious health problems including damages to gene pool. Al-Chalabi and Hawker [6] reported distribution of vehicular lead in roadside soils of Brisbane, Australia while Abechi [7] documented the occurrence of different heavy metals in order of Fe>Zn>Mn>Pb>Cd>Cu in roadside soil sample of major streets in Jos metropolis, Nigeria. A number of studies have shown the presence of DNA damaging pollutants in different samples using different bioassays [8], [9].

The main objective of present study was to estimate the genotoxic and tumor inducing potential of three soil samples collected from different sites of Amritsar, Punjab (India). The study also included on the estimation of physico-chemical parameters (pH, electrical conductance, alkalinity, bulk density, water holding capacity, nitrate, phosphate, potassium, sodium, calcium and magnesium).

II. Materials and Methods

Soil samples were collected from three different junctions of Amritsar *viz.*, Bus stand (BS), Crystal (CT) and Railway station (RS) by scraping of surface soil from roadside/railway trackside with the help of spatula. 4-5 soil samples were collected from each junction and were pooled to comprise the sample of the specific site. The soil samples were brought to laboratory and dried for 72 h. After drying, the samples were sieved through sieve of 200 µm pore size, packed in bags. The samples were analyzed for physicochemical characteristics, genotoxicity and tumor inducing potential.

The soil samples were analyzed for various physicochemical parameters like pH, alkalinity, bulk density, water holding capacity, soil texture, chlorides, calcium,

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magnesium, nitrates, phosphates, potassium, sodium and lead by using standard protocol [6], [10].

For Pb analysis, 1 g soil sample was digested in glass digestion tube of 250 ml with 15 ml of nitric acid (HNO_3) at 140°C for 2 h in digestion chamber. The content was evaporated to dryness. The dried sample was treated with 3 ml of perchloric acid ($HClO_4$) for further oxidation at 240°C for 1h. After digestion, the content was cooled; filtered and final volume was made up to 50 ml with double distilled water. Heavy metals were analyzed using Atomic Absorption Spectrophotometer (Shimadzu model AA 6300, Tokyo, Japan) with reference to standard graph curve of lead in the range of 1 – 10 mg/l.

Allium cepa root chromosomal aberration assay was used to evaluate the genotoxic potential of soil samples. This assay was performed using two modes *viz.*, *in situ* treatment and root dip treatment. During *in situ* treatment, the onion bulbs were directly placed on soil contained in small pots and allowed for root germination for 24-36 h and during root dip treatment, the 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 extract (1 : 2, w/v; soil : distilled water) for 3 h. After this, the root tips were cut and fixed in Farmer's fluid (3 : 1; ethanol : glacial acetic acid). These root tips are squashed in aceto-orcein stain and slides are scored for different types of aberrations under microscope.

For analysis of tumor inducing potential, two types of soil extracts *viz.*, one in distilled water and other in DMSO (Dimethyl Sulphoxide) were prepared. To prepare soil extract, 50 g of sample was dissolved in 100 ml distilled water and 100 ml of DMSO (1:2 w/v) separately and both the flasks were kept on shaker for 12 h. The solutions are filtered through Whatman no.1 filter paper and filtrates were treated as aqueous and DMSO soil extracts. Different concentrations viz., 25%, 50%, 75% and 100% of extracts were prepared by diluting the extracts with distilled water. Potato disc tumor bioassay is based on *Agrobacterium tumefaciens* infection on potato disc which is used to check tumor induction activity of different carcinogens. The laminar air flow chamber 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 diameter) 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 *Agrobacterium tumefaciens* were mixed in a vial and 50 µl of the mixture was poured on each disc. In another set, 50 µl of only soil extracts (25 µl soil extracts + 25 µl distilled water) was poured on to the discs. 50 µl of only culture (25 µl culture + 25 µl distilled water) was used as positive control while 50 µl of distilled water and 50 µl of DMSO were used as negative controls. Petri plates were covered, sealed with parafilm and incubated in B.O.D. incubator for 21 days to induce tumors. After incubation period, potato discs were analyzed after staining with Lugol's Solution (5 % KI + 5 % I_2). Number of induced tumors was scored using stereomicroscope at 2.5X magnification.

III. Results and Discussion

The results of physicochemical parameters of soil are given in Table I. pH all samples studied were found to be alkaline ranging 7.113 (RS) to 8.123 (BS). The soil samples *viz.*, Bus Stand (BS), Crystal (CT) and Railway Station (RS) have shown the contents of chlorides as 0.0141 mg/g, 0.020 mg/g and 0.0059 mg/g, respectively. It was observed that contents of calcium and magnesium were in the range of 2.645 mg/g (CT) to 4.274 mg/g (RS) sample and 17.330 mg/g (CT) to 47.7304.274 mg/g (RS) sample, respectively. Among all the samples studied, the contents of nitrates, phosphates and potassium ranged from 0.0047 mg/g (BS) to 0.160 mg/g (CT), 0.029 mg/g (BS) to 0.177 mg/g (RS) and 0.766 mg/g (RS) to 19.26 mg/g (CT), respectively. The content of sodium for different soil samples *viz.*, Bus Stand (BS), Crystal (CT) and Railway Station (RS) was observed to be 9.20 mg/g, 30.66 mg/g and 21.53 mg/g, respectively. The content of lead was found to be maximum 95.21 mg/Kg for RS sample followed by 35.30 mg/Kg for BS and 25.59 mg/Kg for CT sample.

Several studies on the pollution of soil along the highways and railway tracks indicated the presence of carcinogenic heavy metals [6], [11]. Some authors stated that increasing distance from railway/roadway edge decreased the background values of contaminants [6], [12]. However, maximum concentration of heavy metals was observed to be at 10 – 30 m distance from road or railway track [13]. Lu [14] stated that railroad transportation had an impact on soil heavy metal concentrations. The analysis of roadside/railway track side soils and the plants revealed that they contain elevated levels of various pollutants [11].

In a report, Sharma and Prasad [15] found alkaline pH of 10 roadside soils of Agra, India in the range of 7.44 to 9.20. Contrary to this, Ramakrishnaiah and Somashekar [13] had reported acidic nature of roadside soil samples of Bangalore, India with a pH range of 5.40 – 6.88. Many other authors have analyzed the pH of soil samples and have shown variations in pH [16]. Soil alkalinity is associated with the presence of carbonate in the soil which may results from the natural weathering of the soil particles. A numbers of studies have reported the wide variation in different physicochemical parametes *viz.*, alkalinity [17], soil texture [6], lead [6], [7], [14], phosphorous [18], nitrates [10] and magnesium [18] in of soil samples.

The genotoxic potential of soil at different concentrations of extract is given in Fig. 1. The maximum percent aberrant cells were (41.27%) found in BS sample, followed by RS (37.89%) and CT (33.76%) samples during *in situ* treatment. BS soil sample have shown maximum (36.38%) of physiological aberrations. During root dip treatment; the genotoxic potential was found to be 43.38% (RS), 41.26% (BS) and 37.83% (CT). Among all the samples studied, the CT soil sample has induced maximum clastogenic aberrations (8.33%) comprising chromosomal breaks (5.26%) and chromatin bridge (3.07%). C-mitosis (7.25%) and delayed anaphase (6.45%) were found

to be high among total physiological aberration (32.05%) in CT soil sample. During the root dip treatment, the frequencies of aberrant cells for BS sample at 25%, 50%, 75% and 100% concentrations of soil extract were found to be 16.87%, 24.14%, 33.76% and 41.26%, respectively. The maximum frequency of aberrant cells was observed in RS sample. The percentage of physiological aberrations at 25%, 50%, 75% and 100% were found to be 24.51%, 27.74%, 37.40% and 39.93%, respectively while the frequency of clastogenic aberrations at 25%, 50%, 75% and 100% were 3.69%, 2.50%, 2.63% and 3.45%, respectively.

TABLE I
PHYSICOCHEMICAL CHARACTERISTICS OF ROADSIDE AND RAILWAY TRACK SOIL SAMPLES OF AMRITSAR, PUNJAB (INDIA)

Parameters		Bus stand (BS)	Crystal (CT)	Railway station (RS)
pH		8.123 ± 0.056	7.745 ± 0.014	7.113 ± 0.034
Alkalinity (meq/100g)		0.460 ± 0.033	0.230 ± 0.023	0.300 ± 0.058
Bulk density (g/cc)		1.929 ± 0.006	1.941 ± 0.000	1.840 ± 0.006
Water holding capacity (%)		26.867 ± 0.435	23.627 ± 0.497	35.73 ± 0.374
Soil texture	**Sand (%)**	24.430 ± 0.569	35.988 ± 0.704	54.74 ± 1.855
	Silt (%)	46.211± 0.155	43.668 ± 0.823	26.75 ± 1.398
	Clay (%)	28.83 ± 0.505	20.116± 1.113	18.40 ± 0.628
Chlorides (mg/g)		0.0141 ± 0.004	0.020 ± 0.000	0.005 ± 0.000
Calcium (mg/g)		3.206 ± 0.463	2.645 ± 0.268	4.274 ± 0.534
Magnesium (mg/g)		42.130 ± 1.092	17.330 ± 0.267	47.73 ± 1.639
Nitrates (mg/g)		0.0047 ± 0.000	0.160 ± 0.000	0.116 ± 0.000
Phosphates (mg/g)		0.029 ± 0.000	0.030 ± 0.000	0.177 ± 0.000
Potassium (mg/g)		2.060 ± 0.067	19.26 ± 0.088	0.766 ± 0.033
Sodium (mg/g)		9.20 ± 0.1200	30.66 ± 0.348	21.53 ± 0.088
Lead (mg/Kg)		35.30 ± 0.000	25.59 ± 0.000	95.21 ± 0.000

The result for tumor inducing potential is shown in Fig. 2. Among all sample studied, the number of tumors were found to be maximum (64 tumors at 100 % concentration) in Railway Station sample. The average number of tumors was found to be maximum in RS sample (64) followed by BS (21) and CT (9).

Though the bioassays are the first alert to indicate the pollution of ecosystems while the physico-chemical analysis sometimes takes series of experiments to reach to the similar conclusion, only few reports indicate the use of bioassays to monitor the environmental complex mixtures like soil [19]. Although no reports could be explored on genotoxicity of roadside soil samples using *Allium cepa* root chromosomal aberration as well as PDTA but some reports on mutagenic and genotoxic effects of roadside soil have been reported using other bioassay [20]. Hence, the present study has a great significance in field of environment as being the first study to indicate the use of potato disc tumor assay to explore the tumor inducing potential of soil samples contaminated with vehicular emissions.

IV. CONCLUSION

In the present study the content of lead was found to be maximum (95. 21 mg/Kg) in RS sample which indicated that the lead was the major metal pollutant released from vehicles in the roadside environments. The content of lead directly correlated with the genotoxic and tumor inducing potential, as among all samples RS sample shown maximum lead content and maximum genotoxic and tumor inducing potential. The present study is a clear indication of increased soil pollution by vehicular emissions. Moreover, it is first of its kind because despite the use of potato disc antitumor assay for estimation of antitumor inducing potential; this bioassay has not been reported to be used for monitoring the pollution of any ecosystems.

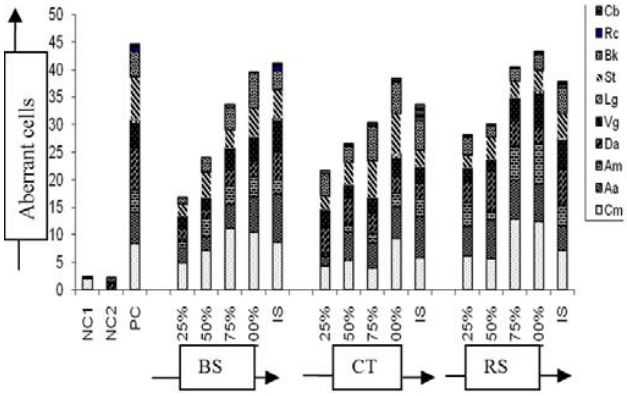

Fig. 1 Genotoxic potential of roadside and railway trackside soil samples of Amritsar following *Allium cepa* root chromosomal aberration assay

BS: Bus stand; CT: Crystal; RS: Railway Station; NC1: Negative control (distilled water); NC2: Negative control (acid washed sand); PC: Positive control (0.5 ppm lead acetate); Cb: Chromatin bridge; Rc: Ring chromosome; Bk: Chromosomal break; St: Stikiness: Lg; Laggard; Vg: Vagarant; Da: Delayed anaphase; Am: Abnormal metaphase; Aa: Abnormal anaphase; Cm: C-mitosis

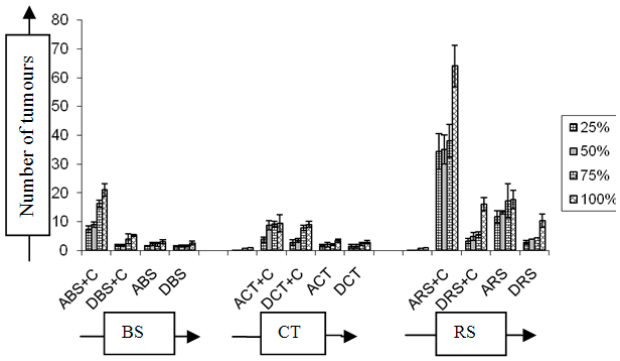

Fig. 2 Tumor inducing potential of roadside and railway trackside soil samples of Amritsar, Punjab (India) following Potato disc tumor assay

BS: Bus stand; CT: Crystal; RS: Railway station; ABS+C: Aqueous extract bus stand + culture; DBS+C: DMSO extract bus stand + Culture; ABS: Aqueous extract bus stand; DBS: DMSO extract bus stand; ACT+C: Aqueous crystal extract + culture; DCT+C: DMSO crystal extract; ACT: Aqueous crystal extract; DCT: DMSO crystal extract; ARS+C: Aqueous extract railway station + culture; DRS+C: DMSO extract railway station + culture; ARS: Aqueous extract railway station; DRS: DMSO extract railway station

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