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Notes & Tips

Tumor inducing potential of agricultural soil samples of Amritsar (India)

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Abstract

Soil is a three dimensional, dynamic natural body occurring on the surface of earth that provides all essential nutrients and mechanical anchorage to the growing plants. But, rapid industrialization, increasing population and inevitable use of xenobiotics have polluted the soil system. Contamination of soil is more prevalent in agricultural lands due to excessive use of agrochemicals like pesticides and inorganic fertilizers to obtain more crop yield. Upon entering the living systems *via* inhalation, ingestion and dermal contact, these chemicals cause a potential health risks. Considering the serious consequences of agricultural soil pollution on human health, the present study was planned to evaluate tumor inducing potential of agricultural soil samples collected from five different zones (Center, North, East, West and South) of Amritsar, Punjab (India) during cultivation of wheat and rice using potato disc tumor assay. It was found that SW-I soil sample induced maximum (24) tumors while Centre and North zone soil samplesinduced least number of tumors (3) during potato disc tumor assay. The present study clearly indicates the high risk to farmers as well as consumers if exposed to such polluted lands and consuming the contaminated crops.

Introduction

Soil is defined as naturally occurring loose covering of broken rock particles that exists on earth's surface that supports plants and other living beings by providing primary requirements of life (1). Soil consists of around 40 % minerals, 23 % air, 6 % organic matter and 8 % living organisms (2). Soil is formed by a process called weathering where mineral rocks are broken down by various physical, chemical and biological processes. Physical processes involve freezing and thawing, heating and cooling, wetting and drying, erosion, chemical decomposition as oxidation, reduction, solution, hydrolysis and carbonation whereas biological actions include action of microorganisms, plants, animals and man(3). The microorganisms also help in formation of soil grains from parent materials by direct or indirect actions(4).The study of soil is of immense importance as it acts as a major ecosystem supporting the survival of all living beings. However, in

recent years, soil has been exposed to various contaminants like application of pesticides (5-7), industrial effluents (8-12), wastewater discharges(13-16), which have threatened the life of various organisms including human beings. Furthermore, different heavy metals like arsenic, cadmium, copper, cobalt, lead, manganese, mercury, nickel and zinc are reported to be accumulated in the soil through these sources and cause genetic damage (17- 22). Many bioassays have been established to assess the genotoxic/mutagenic effects of soil, air and water samples(23- 26). Among various bioassays, potato disc tumor assay has been developed as a very proficient method for evaluation of antitumor induction potential of different plant extracts and drugs in past few decades. Since then, this bioassay has been used for antitumor induction of various drugs and extracts(27, 28).Considering the fidelity of potato disc tumor assay, the current research was planned to evaluate the tumor inducing potential of agricultural soil sample of Amritsar (India) collected during rice and wheat cultivation.

Materials and Methods

Sample collection and soil extract preparation

Soil samples were collected from agricultural field of different regions (East, West, North, South and Central) of Amritsar, Punjab (India) during rice (September) and wheat (March) cultivations. The soil extracts were prepared by adding 100 ml distilled water in 50 g soil sample contained in 250 ml flask (1 : 2 w/v) for each sample. The solution was kept on a mechanical shaker for 12 h. The solution was filtered through nylon filter mesh size 10 - 100 µm. The filtrate was treated as the soil extract.

Bacterial Culture

Bacterial culture (*Agarobacteriumtumifaciens*) strain (MTCC 431) was procured from Institute of Microbial Technology (IMTECH), Chandigarh.*Agrobacterium tumefaciens* cause crown gall disease in plants. The bacterium carries Ti plasmid which possesses tumor causing genes.

Nutrient broth

Nutrient broth medium was prepared by dissolving beef extract (1 g), yeast extract (2 g), peptone (5 g) and sodium chloride (5 g) in double distilled water and final volume was made up to 1000 ml and autoclaved at 121 °C for 20 minutes at 15 lb/in^2 pressure. Medium was then transferred to 25 ml sterile flasks (12 ml in each flask).

Preparation of *Agrobacterium tumefaciens* frozen permanents

Ampule containing lyophilized culture (powder) procured from Institute of Microbial Technology (IMTECH), Chandigarhwas broken under the sterilized conditions and the culture (powder) was poured to 25 ml flask containing 12 ml of nutrient broth. The flask was kept on shaker for 18 h at 225 rpm and at 28 ^oC. The culture was poured to 2 ml vials and was preserved in liquid nitrogen or in -80 ^oC freezer for future use.

Preparation of culture plates

5 g peptone, 1 g beef extract, 2 g yeast extract and 5 g sodium chloride (NaCl) and 15 g of agar was dissolved in 1000 ml of distilled water. The medium was dissolved by boiling on hot plate and autoclaved at 121 °C for 20 minutes at 15 lb/in^2 pressure. Approximately 30 ml of medium was poured to each autoclaved Petri dish and kept for solidification. The culture from the frozen permanents was streaked on the nutrient plates with sterilized inoculation

needles and was kept in BOD incubator at 28 °C for 24 h. The culture plates could be used for 30 days after preservation in refrigerator at 4 °C.

Preparation of working culture

0.05 g peptone, 0.01 g beef extract, 0.02 g yeast extract and 0.05 g NaCl were dissolved in 10 ml distilled water and autoclaved. A loop of culture was taken with the help of inoculation needle from the nutrient plates and was poured into 25 ml flask containing nutrient broth. The flask was kept on shaker for 18 h at 225 rpm and at 28 °C. The fresh culture of *Agrobacterium tumefaciens* was used for further experiment.

Preparation of Agar plates

15 g of agar was dissolved in 1000 ml of distilled water, boiled and autoclaved. Approximately 30 ml of autoclaved agar was poured to each autoclaved Petri dish and was kept for solidification.

Procedure

Fresh russet potatoes were peeled off and cut with cork borer into discs (1.1 × 0.5 cm^3). Discs were sterilized with 10 % bleach solution. Potato discs were embedded to agar plates (Petri dishes) upto 2/3rd of height. The experiment was divided into 4 groups: (i) only soil extract (1 : 1 :: soil extract : double distilled water) was taken in a vial, (ii) only bacterial culture (1 : 1 :: bacterial culture : distilled water) was taken in a vial, (iii) soil culture and bacterial culture (1 : 1) was taken in a vial, (iv) only solvent (double distilled water) was taken in a vial. 50 µl of each was poured on potato discs. Petri plates were covered and sealed with parafilm and kept in BOD incubator at 27 °C for 16 days. After 16th day, discs were stained with Lugol's solution. Unstained and bulged portion of the disc represented the tumors which were counted under 25 x stereomicroscope.

Positive control

50 µl of solution (25 µl of *Agrobacterium* culture + 25 µl sterile distilled water) was used as positive control.

Negative control

50 µl sterile double distilled water and 50 µl of 70 % ethanol were used as negative controls.

Preparation of Lugol's Stain

For preparation of Lugol's stain, 5 g of potassium iodide (KI) and 5 g of iodine (I_2) were dissolved in sterile distilled water. This solution is kept overnight and filtered on next day. It is always kept in dark bottle because Lugol's stain is sensitive to light.

Calculations

The number of tumors was counted per Petri dish.

Statistical Analysis

Level of significance was checked at $p \leq 0.5$ level using Student "t" test.

Results and Discussion

It was seen that most of the centre zone samples (CR-II, CW-I and CW-II) and all north zone (NR-I, NR-II, NW-I and NW-II) soil samples induced least number of tumors (3) whereas SW-I soil sample induced maximum tumors (24) in potato disc tumor assay (Table 1 and Fig. 1). No report could be traced during survey of literature that would evaluate the tumor inducing potential of environmental mixtures. However, the initative to evaluate tumor inducing potential of roadside soil of Amritasr, Punjab (India) was taken by Kaur*et al.*(29). The authors evaluated tumor inducing potential of roadside soils samples of Golden Temple and Putlighar of Amritsar (India). They observed that both samples induced mean number of tumors as 13 (Golden Temple) and 14.4 % (Putlighar) at

maximum concentration of soil extract (100 %). However, potato disc tumor assay has been extensively used for exploring antitumor activities. Amara *et al*. (30)reported the antitumor activity of ethanolic extract of seeds of *Meliaazedarach* following potato disc tumor assay. The antitumor activity of different plants belonging to different families are also well documented (31- 34).

The present study creates a health alarm to the farmers as well as crop consumers. Thedetections of different groups of pesticides (organochlorines, organophosphorous and pyrethroid pesticides) and heavy metals (Cd, Cr, Cu, Fe, Mn, Ni, Pb and Zn) in agricultural soils of Amritsar (India) is the matter of serious concern. As the soil samples were observed to induce tumors during potato disc tumor assay, the present study clearly indicates the high risk to farmers as well as consumers if exposed to such polluted soils and consuming the contaminated crops.

Table 1 Tumor inducing potential of agricultural soil samples of Amritsar (India) using potato disc tumor assay.

Sampling Zone	Sample Code	Mode of treatment	Total no. of tumors induced
Solvent only	**OS**		0±0.0
Disc only	**OD**		0±0.0
Culture only	**OC**		24±0.23*
Negative Control	**CN**	**E**	0±0.23
		E+C	3±0.06
Centre (C)	**CR-I**	**E**	4±0.56*
		E+C	4±0.48*
	CR-II	**E**	3±0.51*
		E+C	4±0.45*
	CW-I	**E**	3±0.21*
		E+C	5±0.31*
	CW-II	**E**	3±0.14*
		E+C	4±0.35*
North (N)	**NR-I**	**E**	3±0.48*
		E+C	4±0.35*
	NR-II	**E**	3±0.31*
		E+C	4±0.64*
	NW-I	**E**	3±0.71*
		E+C	4±0.82*
	NW-II	**E**	3±0.32*
		E+C	4±0.75*

East (E)	**ER-I**	**E**	5±0.78*
		E+C	5±0.42*
	ER-II	**E**	5±0.24*
		E+C	5±0.51*
	EW-I	**E**	5±0.28*
		E+C	7±0.31*
	EW-II	**E**	5±0.29*
		E+C	7±0.98*
West (W)	**WR-I**	**E**	9±0.59*
		E+C	16±0.65*
	WR-II	**E**	9±0.51*
		E+C	15±0.62*
	WW-I	**E**	9±0.45*
		E+C	18±0.78*
	WW-II	**E**	9±0.52*
		E+C	18±0.75*
South (S)	**SR-I**	**E**	17±0.54*
		E+C	24±0.26*
	SR-II	**E**	15±0.82*
		E+C	24±0.98*
	SW-I	**E**	24±1.13*
		E+C	28±0.97*
	SW-II	**E**	22±1.31*
		E+C	28±0.98*

E: Extract only; **E+C:**Extract+Culture; Data shown are Mean±S.E. of three experiments; **R-I:** Soil samples collected during July, 2009; **R-II:** Soil samples collected during July, 2010; **W-I:** Soil samples collected during February, 2010; **W-II:** Soil samples collected during February, 2011; *Statistically significant at P≤0.05 level of significance

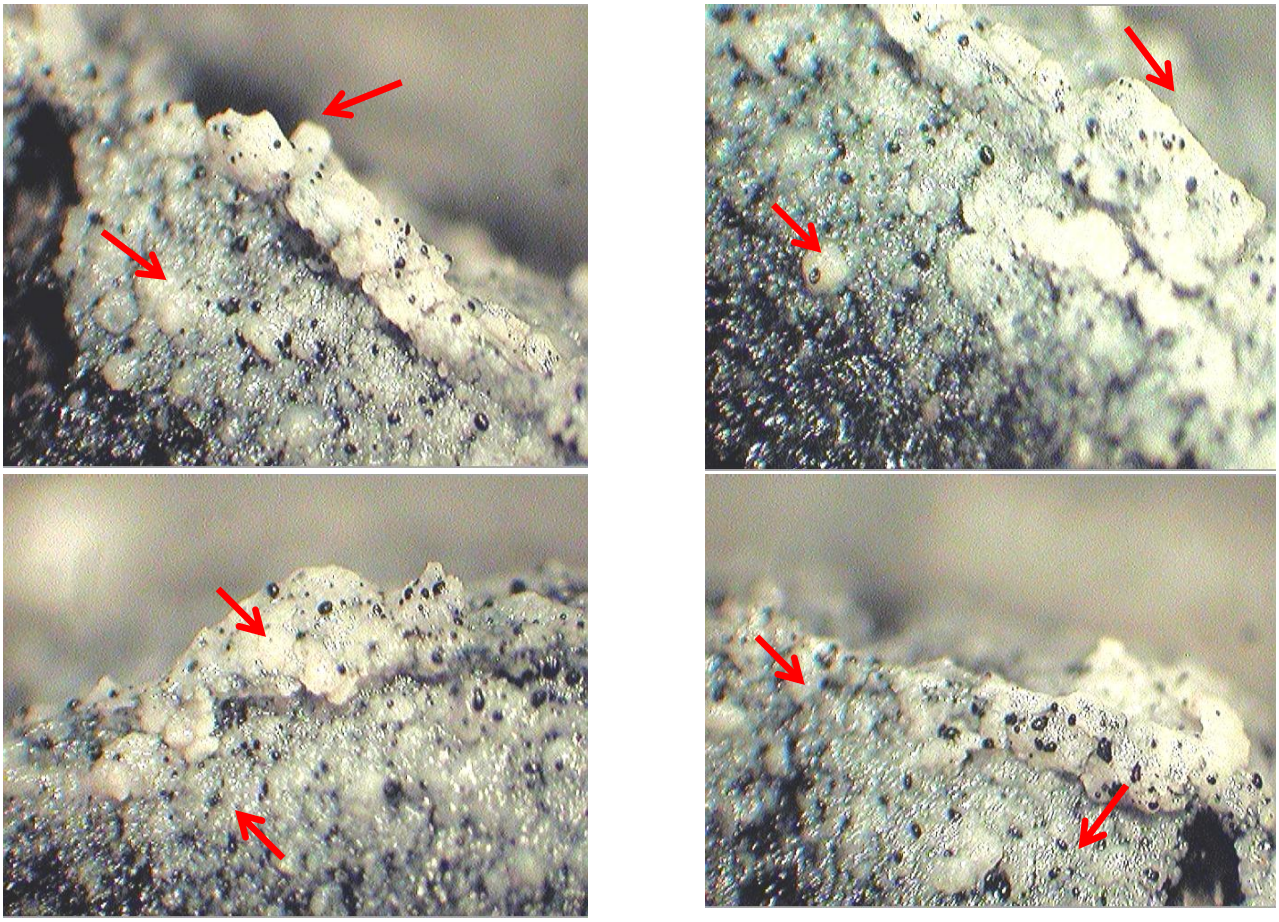

Fig. 1 External morphology of tumors observed under the Stereoscope (25x) during potato disc tumor assay

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Conflict of interest

Authors declares no conflict of interest

Compliance with Ethical Standards

The authors declare that they have no conflict of interest. This article does not contain any studies involving animals or human participants performed by any of the authors

Author contributions

VC: did experiment work and manuscript preparation

UA: manuscript Preparation and editing

JKK: designed the study and prepared manuscript

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