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ORIGINAL ARTICLE

DNA damage in *Cicer* plant grown on soil polluted with heavy metals

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Abstract In recent years industrialization is growing rapidly due to which the pollution load in water, air and soil is increasing day by day. Heavy metal pollution of the soil has raised concern in recent years due to its possible impact not only on human health but also on the plant system. To understand the consequences on plant systems, in the present study we cultivated the *Cicer* plant in soil polluted with heavy metals (Cd, Pb, Cr and Zn) collected from the Jhansi City of Uttar Pradesh, India with a geographical area of 502.75 thousand hectares. Seeds of *Cicer* were germinated in polluted soil sites such as T1 (Garden Soil, Control); T2 (Bharat Heavy Electrical Limited (BHEL)-Industrial); T3 (BHEL-Agricultural); T4 (Bijouli-Industrial); T5 (Bijouli-Agricultural). The effect of soil polluted with the heavy metals was analyzed by studying the percentage of seed germination, radicle length (RL), mitotic index (MI) and chromosomal aberrations (CAs) in root tip meristems. The results revealed that polluted soil with heavy metals T2 (BHEL-Industrial site) and T4 (Bijouli-Industrial site) had a significant impeding effect on the root meristem activity in *Cicer* as noticed by the reduction in seed germination percentage and RL compared to the control. Additionally, the variation in the percentage of mitotic abnormalities was observed. In general, increased percentage of chromosomal aberrations was observed in root tip cells of seedlings grown in polluted soil. Among these abnormalities laggards, bridges, stickiness, precocious separation and fragments were the most common. The obtained results demonstrated that heavy metal polluted soils led to a significant MI reduction and CA increase in root tip meristems of *Cicer*.

1. Introduction

Soils are the main reservoirs for heavy metals generated by industrial activities e.g., metal finishing, paint pigment and battery manufacturing, leather tanning, mining activities, municipal wastewater sludges, urban composts, pesticides, phosphate fertilizers, or from atmospheric depositions. Manmade activities are primarily responsible for the increasing concentrations of heavy metals in agricultural land. Soil, especially those found in and around the industrial area are

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usually highly contaminated with the heavy metals, including, Zn, Ni, Cd, Cu, Pb, etc. (Adriano, 1986; Kabata-Pendias and Pendias, 1992). Metals are non- biodegradable and persist for a long period in aquatic as well as terrestrial environments. They may reach ground water through the soil and can be taken by plants causing profound effects on plant physiology and cytology (Balsberg Pahlsson, 1989; Siddiqui, 2013).

Plants are main components as they form the base of the food chain. Food chain contamination by heavy metals has become a serious issue in recent years because of their potential accumulation in biosystems through contaminated water, soil and air. Therefore, plant systems seem to be particularly important to analyze environmental issue on risk assessments (Sharma et al., 2004). In recent years, the consumption of *Cicer arietinum*, legume of the family Fabaceae, as an alternative source of food has been increased due to the fact that this plant contains high levels of proteins, vitamins and carbohydrates (Wang et al., 2010). In the last 20 years, the rapid increase in population in developing countries caused a massive food requirement and as a consequence planting fields for leguminosae and poaceae have continuously increased in the world.

The present investigation was aimed at evaluating the phytotoxicity and genotoxicity effects of different heavy metal contaminated soils on *C. arietinum* plant. For this purpose we analyzed *C. arietinum* Var.-C-18 plants cultivated in heavy metal polluted and in non-polluted soil samples collected from different areas of the Jhansi City, the well-known district of the Bundelkhand region in Uttar Pradesh, India with a geographical area of 502.75 thousand hectares.

2. Materials and methods

2.1. Site description

Soil samples were collected from five different sites of the Jhansi City, Uttar Pradesh, India. Jhansi is located at an elevation of 300 m above mean sea level (msl) and between the latitude of 24011′N–26027′N and longitude of 78017′E–81034′E (Fig. 1). The details of sites from which the soil was collected for the present study are as given below.

T1 = Garden Soil, Bundelkhand University, Main Camps
T2 = BHEL-Industrial
T3 = BHEL-Agricultural
T4 = Bijouli-Industrial
T5 = Bijouli-Agricultural

The distance between the site T1 and T2 is 17 km; the distance between the T2 and T3 is 1 km; distance between site T1 and site T4 is 12 km; and the distance between site T4 and T5 is 1\2 km.

2.2. Collection of seeds

Healthy and uniform seeds of *C. arietinum* Var.-C-18 were collected from the Crop Research Farm, Govt. of Uttar Pradesh, Mauranipur, Jhansi, India.

2.3. Soil collection and analysis

The soil samples were collected from various parts of Jhansi as described above, air-dried at 20 °C, ground in a mortar, and then passed through a 2 mm plastic sieve. Well-fixed samples of 2 g each were digested with 8 ml of Aqua Regia on a sand bath for 2 h. After the samples were completely dried, the samples were dissolved with 10 ml of 2% nitric acid, filtered and then diluted to 50 ml with distilled water (Chen and Ma, 2001).Concentration of Cd, Pb, Cr, Zn was determined by using atomic absorption spectroscopy (iCE^{tm} 3000, Waltham, MA, USA) and the results are shown in (Table 1).

2.4. Morphological analysis

Soils were collected from various sites with plastic spatulas and stored in polypropylene boxes. After removing the pebbles and twigs the soil samples were air-dried. Then they were passed through a 2 mm sieve and the soil was uniformly poured into sterilized Petri Plate. 30 seeds of *C. arietinum* were sown in duplicate. 30 seeds were surface sterilized with 0.5% sodium hypochlorite for 10 min, washed with distilled water several times to remove any excess of chemicals. Seeds were then soaked in double distilled water (DDW) for 12 h and then transferred to Petri Plates (15 cm diameter) containing 260 g of soil from the five different sites and placed in a Biological Oxygen Demand incubator (BOD-170, Pulse Life Science, Maharashtra, India) maintained at 24 ± 2 °C temperatures for further observation. Germination of seeds and radicle length (measured using a millimeter ruler) were analyzed at every 24 h interval. The experiment was repeated three times under the same conditions.

2.5. Cytogenetic analysis

Cytogenetic analysis was performed on *Cicer* root tip meristems fixed after seven days from seed germination on the different contaminated soils. Briefly, root tips were washed in water, fixed in Carnoy's solution of (100% ethanol:glacial acetic acid 3:1) for 24 h and stored in 70% ethanol until further use. The fixed roots were hydrolyzed at 60 °C for 15 min, in 0.1 N HCl and stained in Leuco Basic Fuchsin for 10–20 min as described earlier (Siddiqui et al., 2007).

Root tips were squashed in 2% acetocarmine on slides, mounted and observed under oil immersion objective using a light microscope from Leica DMi1, Germany. All the slides were coded and examined unsighted. Mitotic index (MI) and chromosomal aberrations (Abs) in metaphase and anaphase plates were studied using a light microscope at a higher magnification (100×). From each slide, minimum of 1000 cells were scored and mitotic index was calculated. Chromosomal aberrations such as sticky chromosomes, precocious separation, fragments, and laggard were studied in a minimum of 50 metaphase and anaphase plates per slide and expressed in percentage.

2.6. Statistical analysis

Statistical analysis was performed employing a one way ANOVA test using the GPIS software 1.13 (Graph PAD,