Article

Effects of heavy metals/metalloids contamination of soils on micronucleus induction in *Tradescantia pallida*

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Abstract

The present study was conducted in GGV campus, Bilaspur in which heavy metals/metalloids speciation of soil (for Cr, Fe, Ni, Cd and Pb) was performed for assessing the genotoxicity of these metals. The metals concentrations were measured with the help of AAS 7000 (Shimadzu) and the standard solution was prepared using standard metal solution of Inorganic Ventures. The concentrations of Cr, Fe, Ni, Cd and Pb (in µg/100 g soil) were 12.4, 33.9, 3.1, 0.07 and 2.4 respectively. The flowers of *Tradescantia pallida* plants growing in this soil were taken and their micronucleus (Trad-MCN) bioassay was performed. Trad-MCN bioassay was performed using the protocols established by Ma (1981). The study revealed that at these concentrations of metals micronuclei (stained objects that were smaller than the nuclei and not connected to the nuclei are classified as MCN) were formed. Therefore it can be inferred from the present study that soil of GGV campus is genotoxic for the *Tradescantia pallida*.

Keywords heavy metals; genotoxicity; *Tradescantia pallida*; soil; micronucleus induction.

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1 Introduction

Contamination of the environment by heavy metals has been increasing every year (Majer et al., 2002; Sayadi and Sayyed, 2011) and the analysis of the cytotoxic effects of such metals has received special attention due to the fact that they are potentially mutagenic and induce the formation of tumors in experimental organisms and humans exposed to them (Garcia- Rodríguez et al., 2001). Environmentally released metals are mainly deposited in soils and are mobilized either by leaching or by uptake into plants (Prajapati et al., 2012). The DNA-damaging effects of metals in soils cannot be monitored by conventional genotoxicity tests with bacteria, no extraction methods are available for experiments with mammalian cell lines, and results obtained in commonly used mammalian cell lines with individual heavy metals are highly controversial. Plant bioassays are cost and time effective and do not require specific equipment, excessive sample manipulation, and concentration procedures and have been used successfully for in situ exposure studies. Plant bioassays are considered relevant because wastewaters from tanneries, in a semi-treated form, are used for irrigation and the sludge is used as cheap manure in the adjacent farms by farmers leading to accumulation of chromium in agricultural soils. Majer et al. (2002) reported that exposure of intact plants (with roots) to contaminated soils enables the detection of DNA damage caused by metal contaminations. *Vicia faba* and *Arabidopsis thaliana* were used to monitor the genotoxic effects (sister chromatid exchanges and micronuclei) in air and soils polluted by industrial factories (Chroust et al., 1997). Among the different plants assays, the *Tradescantia* micronucleus (Trad-MN) assay with pollen tetrads is the most extensively validated procedure (White and Claxton, 2004). This test-system has been used worldwide to evaluate the genotoxic potential of chemicals, air, soil and water (Gong et al., 2003; Monarca and Feretti, 1997; Cotelle and Masfaraud, 1999; Crebelli et al., 2005, Prajapati and Tripathi, 2008). Mutagenicity of sewage sludge has been examined by Hopker et al. (1982) using tests with *Tradescantia palludosa*, *Salmonella*/microsome assay and germ cells of *Zea mays*.

Elevated concentrations of metals in human diet constitute a potential health hazard in the long term. Chromium has many oxidation states, ranging from Cr (II) to Cr (VI) but it mostly exists as Cr (III) and Cr (VI), as these are more stable states. Hexavalent Cr contamination of the environment is a matter of concern because it is a powerful oxidizing agent. Ingestion of very high concentrations of Cr (VI) by humans can result in gastritis, nephrotoxicity, and hepatotoxicity (Paustenbach et al., 2003. Chromium is released to the environment due to careless and improper management practices of effluent discharge, mostly from industries related to metallurgical, electroplating, production of paints and pigments, tanning, and wood preservation (Shanker et al., 2005). In developing countries, the wastewaters from tanneries, in a semi-treated form, are used for irrigation and the sludge is used as cheap manure in the adjacent farms by farmers. This leads to cumulative accumulation of Cr in the top soil over a period of time (Singh et al., 2004; Sinha et al., 2006, 2007), which manifests itself as a decline in agricultural yield. Toxic heavy metals interfere with several metabolic processes, causing toxicity to the plants revealed by reduced root growth and phytomass, chlorosis, photosynthetic impairing, stunting, and finally plant death (Sinha et al., 2007).

Arsenic (As) is ubiquitous in many environments and highly toxic to all forms of life. It occurs predominantly in inorganic form as arsenate (AsV) and arsenite (AsIII). Arsenate is an analogue of phosphate and thus interferes with essential cellular processes such as oxidative phosphorylation and ATP synthesis, whereas the toxicity of AsIII is due to its propensity to bind to sulfhydryl groups, with consequent detrimental effects on general protein functioning. In some areas of India, groundwater As concentration has exceeded 2000 mg l^{-1} (Hossain, 2006). Arsenic can find its way into the grains of plants, such as rice and wheat, and into vegetables and fruit plants (Meharg, 2004; Norra et al., 2005; Zhao et al., 2006) through irrigation with As-contaminated water. Many anthropogenic activities, such as leather processing, electroplating, wood preservation (Shanker et al., 2005) and production of re-chargeable nickel-cadmium batteries (Järup, 2003), have released large amounts of heavy metals like Cr, Cd and Pb into the natural environment, causing widespread heavy metal contamination worldwide (Nriagu, 1988).

Although the genotoxic effects detected by Tradescantia micronucleus (Trad-MCN) bioassay cannot be extrapolated directly to human populations, these bioassays are very useful tools for screening the mutagenic potential in the environment. In the present study Tradescantia micronucleus (Trad-MCN) bioassay was performed to assess the genotoxicity of heavy metals/metalloids.

2 Material and Methods

The present study was conducted in Bilaspur district (C.G), India. Biomonitoring and speciation of road dust for heavy metals using *Calotropis procera* and *Delbergia sissoo* has already been done by Prajapati (2012). *Tradescantia pallida* plant was used for assessing the genotoxicity of the heavy metals/metalloids. The plants were planted in the soil of Guru Ghasidas Vishwavidyalaya near the Botany building. The heavy metal analysis of soil where the plants were planted was estimated with the help of atomic absorption spectrophotometer (AAS) model: AA 7000, SHIMADZU and the standard was prepared using standard metal solution of Inorganic Ventures. Tradescantia micronucleus (Trad-MCN) bioassay was performed using the protocols established by Ma (1981).

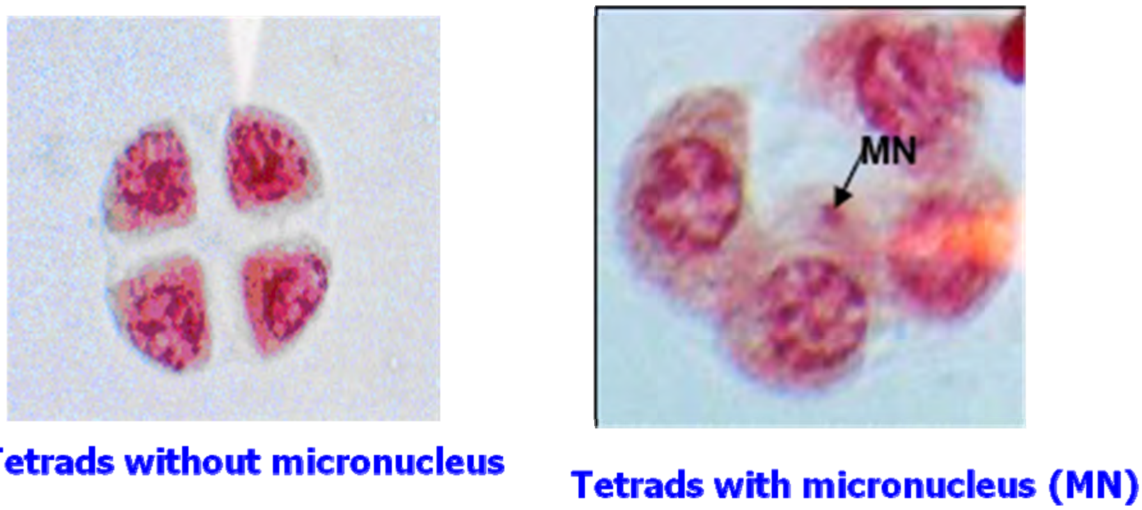

Fig. 1 Pollen tetrads showing the absence and presence of micronuclei.

3 Results and Discussion

The heavy metal speciation was performed for the soil taken from the place where the plants were grown. The concentrations of Cr, Fe, Ni, Cd and Pb (in µg/100 g soil) were 12.4, 33.9, 3.1, 0.07 and 2.4 respectively. The concentration of these heavy metals/metalloids are not very high in the soil, even though they are capable of breaking the chromosomes and capable of forming the micronuclei (MCN) as shown in Fig 1. MCN are DNA-containing extracellular bodies surrounded by a plasma membrane, which are formed as a consequence of chromosomal breakage (clastogenicity) or aneuploidy (Natarajan and Obe, 1982).

Present study showed that in situ biomonitoring using higher plants may be useful for characterizing air pollutants in areas even without any sophisticated instrument. Although mutagenesis in plants and the risk of malignancies in human cannot be equated, Trad- MCN bioassay can be very useful as a proxy for assessing human risk. Short-term assays in field conditions may help to predict the risk of genotoxicity, the estimation of actual risk from metals/metalloids pollutants in malignancies, using epidemiological tools, generally demands long-term studies involving cohort.

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