

## **GENOTOXICITY EVALUATION OF ATMOSPHERIC ENVIRONMENTS OF SUBWAY STATIONS IN SEOUL USING TRADESCANTIA MICRONUCLEUS ASSAY**

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### **ABSTRACT**

Airborne pollutants in the subway facilities can be potentially harmful to the health of passengers. This study was designed to check whether the suspended particulates have mutagenic or carcinogenic effects on the plant cell systems. Total suspended particulates were collected with a high volume air sampler, in the entrance, the waiting room, and the platform of each subway station. The biological end-points in this experiment were the micronuclei in the pollen mother cells of *Tradescantia*. The exudates were prepared by shaking the filter paper from the sampler in distilled water for 30 minutes. The differences in genotoxicity between air samples from the subway station and the control value was statistically significant. Almost every plant cutting exposed to the exudates resulted in a positively clastogenic response. The chemical analysis showed that the aqueous extracts from the air samples contained several heavy metals. Relationship between the induced genotoxicity and heavy metal concentration could not be clearly defined. The meiotic pollen mother cells of *Tradescantia* clone 4430 are particularly sensitive to genotoxic pollutants. Thus the *Tradescantia* micronucleus (Trad-MCN) assay was selected as a biological test system to evaluate airborne particulates collected in the subway facilities. A significant correlation was observed between the intensity of the pollution and the frequency of micronuclei appearing at the tetrad stage of meiosis.

**Key Words:** *Tradescantia* micronucleus assay; Air particulates, Genotoxicity, Subway station

## **1. INTRODUCTION**

Airborne pollutants in the subway facilities can be potentially harmful to the health of passengers. Experimental studies have shown that extracts of urban air particulates can induce cancer in animals and are mutagenic in bacteria and mammalian cells (Sasaki et al., 1987, Crebelli et al., 1995, Pagano et al., 1996). Several epidemiological studies have revealed possible health risks related to air pollution and have suggested an association with an increase in lung cancer and respiratory diseases for the urban population (Schwartz, 1991, Pershagen and Simonato, 1993).

The quality of the indoor air we breathe and the attendant consequences for human health are influenced by a variety of factors. These include hazardous material discharges indoor and outdoors, meteorological and ventilation conditions, and pollutant decay and removal processes. Over 80% of our time is spent in indoor environment so that concentration of indoor air pollutants, exposure and ventilation are important consideration for evaluation of human risks (Dockery and Spengler, 1981). It is important to identify the level of genotoxic activity in the environment and to relate it to biomarkers of cancer risk in humans.

The Tradescantia-micronucleus test has been used in studies on air pollution because this plant is particularly sensitive to chemical mutagens and can be used for in situ monitoring programs (Ma, 1983, Ma et al., 1994).

This study was designed to check whether the suspended particulates have mutagenic or carcinogenic effects on the plant cell systems. Total suspended particulates were collected with a high volume air sampler, in the entrance, the waiting room, and the platform of three subway stations in Seoul.

## **2. MAIN TEXT**

Collection of subway particulate sample

Suspended particles were collected on fiber glass filter (8" x 10", Whatman, PM2000) by high-volume air sampler (Sierra Anderson, Model 305, USA) operated at a flow rate of 1.2 m<sup>3</sup>/min for 20h. The air volume pulled through filter was 1221.7 – 1682.4 m<sup>3</sup>. Air samples were collected with a high volume air sampler, in the entrance, the waiting room, and the platform of each subway station. The

biological end-points in this experiment were the micronuclei in the pollen mother cells of *Tradescantia*. The exudates were prepared by shaking the filter paper from the sampler in distilled water for 30 minutes. The aqueous extracts for heavy metals from these glass filters were extracted by sonicated for 30 min using Branson 5210 sonicator. The aqueous extracts were tested for Trad-MCN assay after extraction.

#### **Tradescantia-micronucleus test**

The aqueous filter extracts for heavy metals were tested according to the procedure used by Ma et al. (1994). Briefly, 10 - 15 plant cuttings bearing young inflorescence of *Tradescantia* clone 4430 were exposed to 100 ml aqueous filter extracts in Erlenmeyer flasks with various sampling location samples. The duration of young inflorescence treatment was 24-h, followed by a 24-h recovery in Hoagland solutions. Next young inflorescences were fixed for 24-h in prepared 1:3 aceto-ethanol solutions and then transferred to 70% ethanol for storage to reuse.

For scoring of Trad-MCN, five slides per treatment were prepared using the aceto-carmin technique. The genetic damage was expressed as Trad-MCN /100 tetrads. Negative controls were performed in Erlenmeyer flasks with deionized water.

The concentration of the total suspended air particulates and genotoxicity induced in the Trad-MCN assay are given in Table 1. The micronucleus frequencies in the *Tradescantia* inflorescence exposed to the extracts from the total suspended air particulates were from the lowest of  $5.27 \pm 0.27$  (waiting room of City hall subway station) to the highest of  $10.13 \pm 0.37$  (platform of Shindorim station).

Almost every plant cutting exposed to the exudates resulted in a positive response. The differences in the genotoxicity between the air samples from the subway station and the control value ( $2.41 \pm 0.32$ ) were statistically significant. In case of Shindorim station, the Trad-MCN frequencies were  $7.27 \pm 0.43$  MCN/100 tetrads ( $p < 0.001$ ) for the entrance,  $8.53 \pm 0.47$  MCN/100 tetrads ( $p < 0.001$ ) for the waiting room, and  $10.13 \pm 0.37$  ( $p < 0.001$ ) for the platform of the subway station.

Table 1. The frequency of micronuclei in *Tradescantia* inflorescences exposed to the extracts from the total suspended airborne particulates

Sampling sites	TSP Conc. (ug/m <sup>3</sup> )	Exposure (h)	MCN / 100 tetrads	Significance
Sindorim	A 219.4	24	7.27 ± 0.43	***
	B 278.8	24	8.53 ± 0.47	***
	C 269.5	24	10.13 ± 0.37	***
City Hall	A 141.5	24	9.80 ± 0.65	***
	B 188.2	24	5.27 ± 0.27	***
	C 238.6	24	8.07 ± 0.36	***
Shincho n	A 138.7	24	8.27 ± 0.55	***
	B 282	24	7.87 ± 0.40	***
	C 252.5	24	9.87 ± 0.62	***
N.C.	D	24	2.41 ± 0.32	

A) Entrance, B) Waiting room, C) Platform, D) D.W., N.C. : Negative Control, \* : Duncan's t-test

### 3. CONCLUSION

The chemical analysis showed that the aqueous extracts from the air samples contained heavy metals such as copper, iron, antimony, barium, lead, manganese, etc. However their concentrations were relatively lower than those of any other environmental samples such as sediments, soils and river waters. And thus relationship between the induced genotoxicity and heavy metal concentration could not be clearly defined in this study.

It has been reported in several studies that the micronucleus assay proved more reliable and sensitive to the test than the stamen hair assay (Monarca et al., 1999, Kim et al., 2003). The results indicate that the air particulates can give an adverse effect on the health of subway passengers. The Trad-MCN assay was selected as a biological test system to evaluate airborne particulates collected in the subway facilities since the meiotic pollen mother cells of *Tradescantia* clone 4430 are particularly sensitive to genotoxic pollutants. A significant correlation is observed between the intensity of the pollution and the frequency of micronuclei appearing at the tetrad stage of meiosis. It was reported that the *Tradescantia*-MCN assay was

sensitive, reproducible and well standardized (Ma et al, 1994) and our results also support that the assay is easy and inexpensive to use. As found in the previous researches on air pollution and indoor pollution monitoring (Monarca et al., 1999, Kim et al., 2003), the Trad-MCN assay is sensitive to environmental genotoxins and a promising test tool for monitoring airborne genotoxins in the environments.

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