Comparison of Gene Mutation Frequency in *Tradescantia* Stamen Hair Cells Detected after Chernobyl and Fukushima Nuclear Power Plant Accidents

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Abstract − Our aim was to investigate the genotoxicity of ambient air in the Kraków area after Fukushima Nuclear Power Plant (NPP) accident and compare with results from Chernobyl fallout. For the detection of ambient air genotoxicity the technique for screening gene mutation frequency in somatic cells of the *Tradescantia* stamen hairs (*Trad*-SH assay) was used. Since 11th of March 2011 (Fukushima NPP accident), several pots containing at least 15 shoots of bioindicating plants were exposed to ambient air at 2 sites in the Kraków surrounding area, one in the city center, and about 100 pots in a control site (in the glasshouse of the Institute of Nuclear Physics) Continuous screening of mutations was performed. Progenies of 371,090 cells exposed were analyzed. Mutation frequency obtained in the first 10 days has shown a mean control level (GMF∗100=0.06±0.01). At scoring period related to influence of a potential Fukushima fallout, a significant increase of gene mutation frequencies above the control level was observed at each site in the range, 0.10~0.33 depending on the location, (mean value for all sites GMF∗100=0.19±0.05) that was associated with a strong expression of toxic effects. In the reported studies following the Chernobyl NPP accident monitoring *in situ* of the ambient air genotoxicity was performed in the period since April 29th till June 3rd 1986 also with Trad-SH bioindicator. In general, mutation frequency increases due to Chernobyl fallout (GMF∗100=0.43±0.02) were corresponding to fluctuation of radioactivity in the air reported from physical measures, and to published reports about increase in chromosome aberration levels. Although, recent data obtained from monitoring of the ambient air quality in the Kraków and surroundings are lower when compared to results reported after Chernobyl NPP accident, though results express a significant increase above the control level and also are corresponding with increased air radioactivity reported from physical measurements. Statistically significant in comparison to control increase in gene mutation rates and more prolonged than that after Chernobyl fallout increase of GMF was observed during the period following the Fukushima NPP failure.

Key words : gene mutation, *Trad*-SH assay, Nuclear Power Plant accident

INTRODUCTION

On 11 March 2011 following the Tohoku earthquake and tsunami in the Fukushima Nuclear Power Plant were series of equipment failures, nuclear meltdowns, and releases of radioactive materials. The Fukushima disaster is the largest

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of the 2011 Japanese nuclear accidents and is the largest nuclear accident since the 1986 Chernobyl disaster. The Fukushima accident has led to trace amounts of radiation, including iodine-131, caesium-134 and caesium-137, being observed around the world. The total amount of iodine-131 and caesium-137 released into the atmosphere has been estimated to exceed 10% of the emissions from the 1986 Chernobyl disaster. Among the various radionuclides released, iodine-131 (^{131}I) and cesium isotopes (^{137}Cs and ^{134}Cs) were transported across the Pacific toward the North American continent and reached Europe despite dispersion and washout along the route of the contaminated air masses (Masson *et al.* 2011). Accordingly, on March 12 we started monitoring the air contaminates in the surroundings Kraków. For the detection of ambient air genotoxicity similarly as in the case of Chernobyl disaster *Tradescantia* hybrid clones were used. The technique for screening gene mutation frequency in somatic cells of the *Tradescantia* stamen-hairs (*Trad*-SH assay), have been developed many years ago (Underbrink *et al.* 1973) and applied specifically for radiobiological and radiation protection studies (Cebulska-Wasilewska *et al.* 1981). *Tradescantia* hybrid clones are highly sensitive to chemical mutagens as well as to radiation. This facts make Trad-SH assay particularly suitable for the environmental studies and for the detection of ambient air genotoxicity (Cebulska-Wasilewska 1988).

MATERIALS AND METHODS

The present studies were carried out on clone T-02 from a glasshouse of the Institute of Nuclear Physics (INP). Since 11th of March 2011 (Fukushima NPP accident), in the Kraków area several pots containing at least 15 shoots of bioindicating plants were exposed to ambient air in each of the 3 sites out of the INP. Site no.1 set in INP (plantation in glasshouse) situated 7 km to the North-West from the city center, no. 2 was in the city center (Kazimierz district) while 3 and 4 were set in the surrounding villages in various geographical directions from Kraków city; 18 km to West-North (Karniowice) and 19 km to the South-East (Lyczanka) respectively. The monitoring of air genotoxity was performed since 12th of March to mid July. Progenies of 371,090 cells exposed were analyzed to find out the mutated cells for the determination of GMF. Counts of stunted hairs (lethal mutations) and pink events (gene mutations) were made in the stamen hairs of *Tradescantia* flowers according to the method described earlier (Cebulska-Wasilewska *et al.* 1981; Cebulska-Wasilewska 1988, 1989, 1992; Cebulska-Wasilewska and Korzeniovski 1989). Mutations scored from the 11th day of exposure and expressed as mutations per 100 hairs were the measure of the biological effects of the ambient air genotoxic pollutants.

RESULTS

Figs. 1a-1d present the variation of gene mutation frequency detected in plants exposed to ambient air at investigated sites from mid March (11th day of nuclear plant accident) to the end of the first week of July. In figures are also displayed numbers of progenies of exposed cells that were analyzed for the presence of mutation events. The range of detected pink mutation frequency in monitored time was fluctuating between 0 and 1.1 per 100 hairs. The mean value of mutation frequency observed in plants growing in glasshouse before the Fukushima NPP accident, (obtained as the average value of mutation frequencies detected in the first 10 days) are presented in Figs. 1a to 1d as control levels (GMF$*$=0.06 $\pm$0.01. During the period between 22nd of March and 3rd of May, majority of measurement were significantly elevated above the control level of gene mutations frequencies observed before. The highest level of mutation frequency was noted in the site no.4 on 4th of April. The average of gene mutations frequency resulting from scoring at all four investigated in this study sites are summarized in Table 1. In general, statistically significant increases of gene mutation frequency from mid March to the end of the first week of May (GMF$*$ 100=0.19$\pm$0.05, the range, 0.10~0.33 depending on the location) was reported. The highest mean level of gene mutation frequency was observed in the city center (site no. 2). The lower gene mutation frequency level was noticed in the surrounding villages of Lyczanka.

The lowest but still statistically significantly elavated in comparison with control level (the mean of gene mutation frequency for glasshouse plantation in the Institute of Nuclear Physics) was obtained at site 3 Karniowice and in the glashous of INP (site 1). Significant increase of gene mutation frequency in all sites was associated with a strong expression of toxic effects (data not shown). Follow-up measurements in investigated sites (since the end of first week of May till

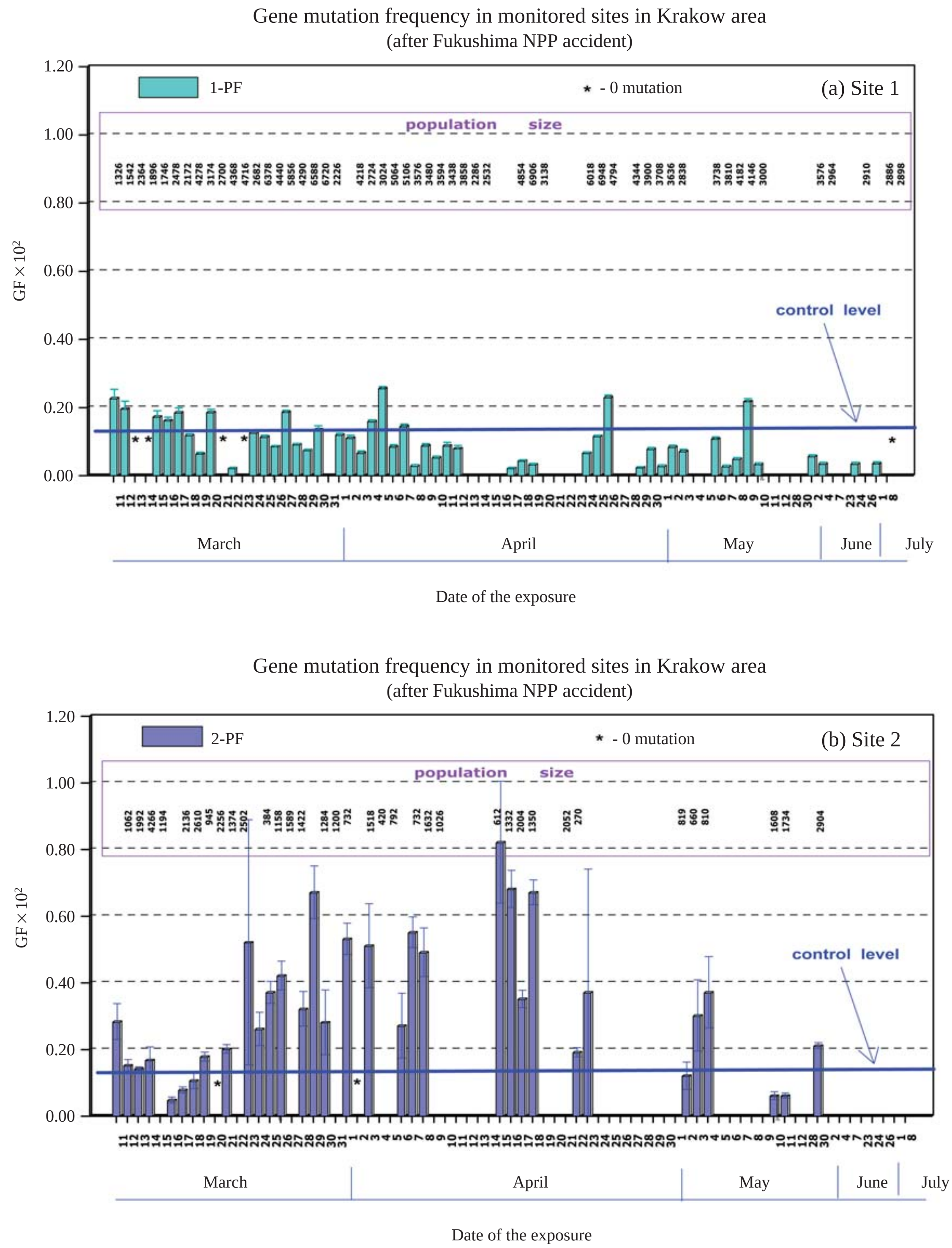
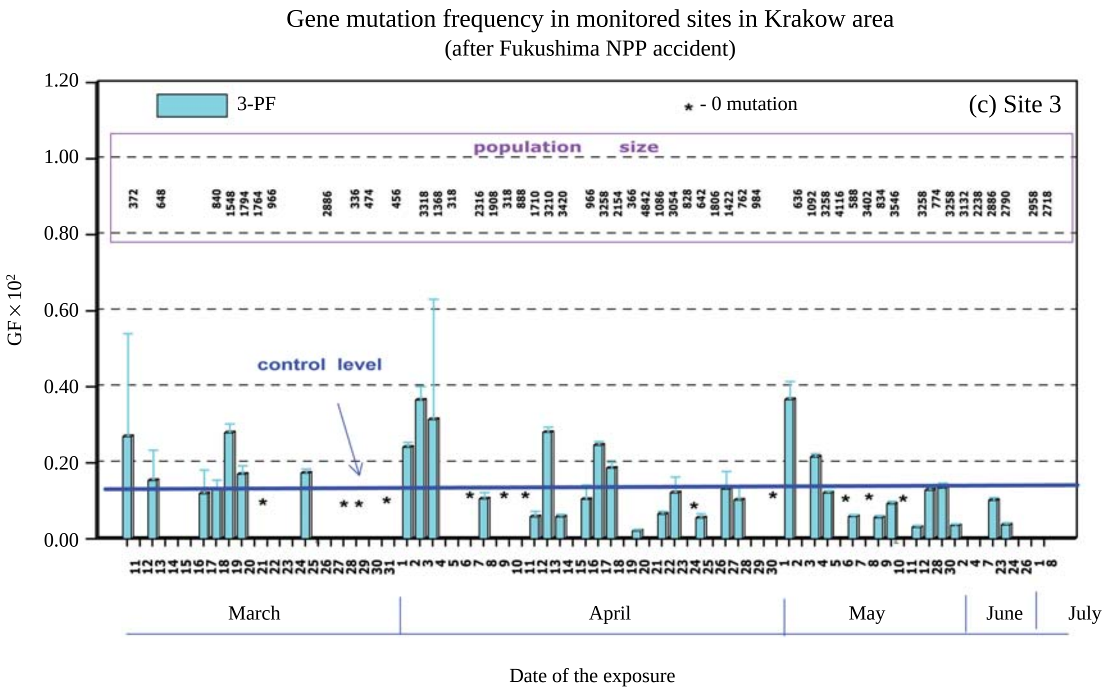


Fig. 1. The variation of mutation frequency observed in monitored sites (no.1-4) in Kraków area after the Fukushima NPP accident.

mid-July) showed a systematic decline in the gene mutation frequency. The mean value of gene mutation frequency in this monitored period was 0.068 ± 0.002.

Results of applications of the bioindicator for *in situ* monitoring gentoxicity of the ambient air pollution including ionizing radiation from Fukushima Nuclear Power Plant accident are compared to results of monitoring of ambient air mutagenicity after the Chernobyl accident (Table 2). Following



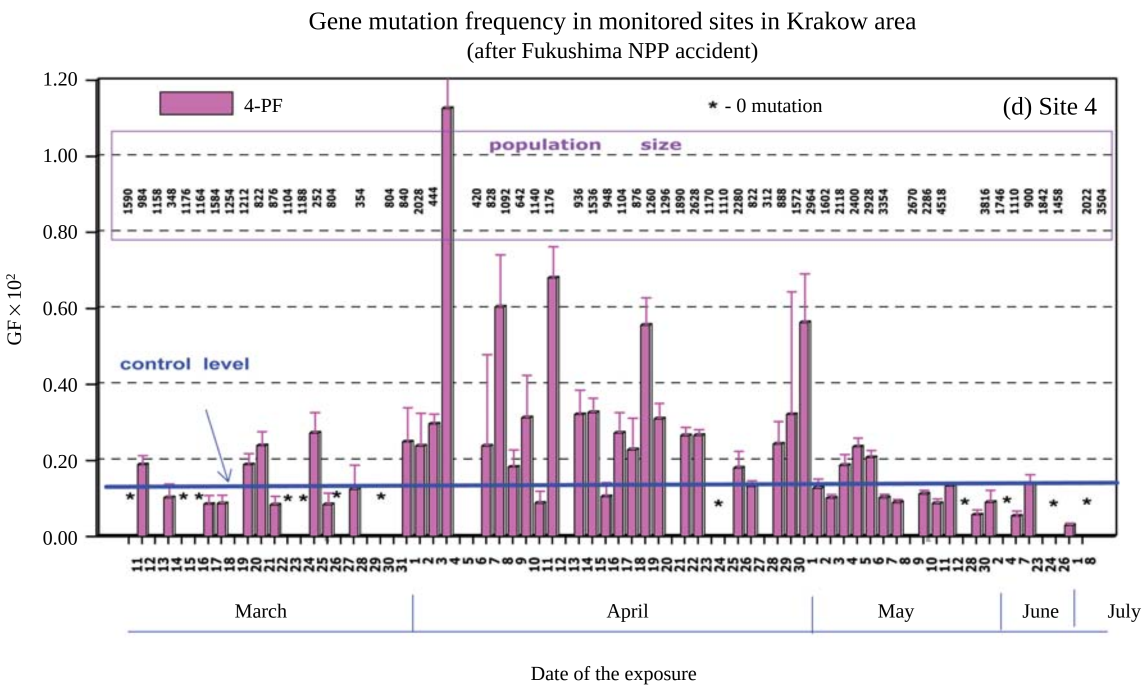


Fig. 1. Continued.

the Chernobyl accident studies of mutagenicity of ambient air in the period since April 29th till June 3rd 1986 were performed by the author (Cebulska-Wasilewska 1992). Statistical analyses show lower but more prolonged than that after Chernobyl fallout increase of GMF.

DISCUSSION

The *Tradescantia*-stamen-hair mutation test (*Trad*-SH) was used successfully to evaluate the genotoxicity of radiations (Cebulska-Wasilewska *et al.* 2001; Do Santos *et al.* 2005)

Table 1. Comparison of the mean value of gene mutation frequency observed from mid March to the end of the first week of May

Site number	Fukushima fallout March 11th-June 8th 2011, sites with bioindicating plants	NS	NOH	NGM	GMF_P*10^2
0*	INP glasshouse (before 11th of March)	40	48675	29	0.06±0.01
1	INP glasshouse	40	166344	117	0.10±0.02
2	Kazimierz (city center)	16	35621	112	0.33±0.04
3	Karniowice (village)	12	65100	54	0.11±0.02
4	Łyczanka (village)	8	55350	91	0.22±0.05

0* - control level, NS- number of shoots exposed, NOH - number of progenies analyzed, NGM - number of gene mutation events detected, GMF_P*10^2 - gene mutation frequency per 100 hairs

Table 2. Comparison of results of ambient air mutagenicity monitoring performed after the Chernobyl and Fukushima NPP accidents

Exposure period	NS	NOH	NGM	GMF_P*10^2
Chernobyl fallout * 29th of April-3rd of June, 1986	22	10 259	40	0.43±0.02
Fukushima fallout 11th of March-8th of May, 2011	38	321 665	348	0.19±0.02
May 9th-8 July 2011	38	107 022	52	0.07±0.02

*date from publication (Cebulska-Wasilewska 1992). Abbreviations as in the Table 1

and chemical pollutants (Ferreira *et al.* 2007). This technique has been used for example to detect the effects of environmental radionuclides (Cebulska-Wasilewska 1992; Minouflet *et al.* 2005) and to study the toxic effects of air pollution (Cebulska-Wasilewska 1988). Research carried out by the author after the Chernobyl disaster showed that the results of mutation frequency obtained by using the *Trad*-SH test corresponding to fluctuation of radioactivity in the air reported from physical measures (Cebulska-Wasilewska 1992). Results of applications of the bioindicator for *in situ* monitoring gentoxicity of the ambient air pollution including ionizing radiation from Fukushima Nuclear Power Plant accident are compared to recent data from monitoring the ambient air quality (the ratio of the gaseous ^{131}I fraction to total ^{131}I) in the Kraków and surroundings performed by Department of Nuclear Physical Chemistry INP. As is apparent from physical measurements, in Europe, the first signs of the releases were detected 7 days later while the first peak of activity level was observed between March 28th and March 30th. Time variations over a 20-day period rough estimate of the total ^{131}I inventory that has passed over Europe during this period was $<1\%$ of the released amount (Masson *et al.* 2011). Our results from *Tradescantia* stamen-hairs (*Trad*-SH assay) monitoring show that an increase in mutation frequencies is corresponding with increase of physical measured air radioactivity. Relatively higher plants' response at low measured radioactivity might be explain that the mutation level is reflecting the genotoxity of the whole complex mixture of interacting all ambient air pollutants: from radiation and from chemicals. This suggestion seems to confirm the fact that the site with highest mean value of gene mutation frequency is in the center of city (high urban and traffic pollutions). The explanation for higher plants' response is based on studies by Cebulska-Wasilewska, which showed synergistic interaction between chemical pollutants and radiation (Cebulska-Wasilewska *et al.* 1981).

The lowest level of mutation was observed at site no.1 (for a plantation in greenhouse) where plants had limited contact with contaminated air. Comparison of the mutation frequencies obtained during biomonitoring of the Fukushima Nuclear Power Plant accident with results obtained after the Charnobyl fallout indicated the elevated levels of GMF in comparison to control but lower about 66% in relation to the levels in 1986 reported by Cebulska-Wasilewska (Cebulska-Wasilewska 1992). The lower level of the mutation frequencies might be probably associated with a lower air radioactivity resulting from the much higher distance from the site of release of radioactive substances. The distance from Kraków to Chernobyl is about 700 km while to the Fukushima about 13,500 km. On the other hand, Ichikawa *et al.* study (Ichikawa *et al.* 1996), which showed the Chernobyl disaster effect in Japan, confirms that even at such a distance is possible to detect radioactive contamination by the *Trad*-SH assay.

In conclusion our result show statistically significant in comparison to control and more prolonged than that after Chernobyl fallout increase of GMF. It was associated to the strong expression of toxic effects. An increase in mutation rates is corresponding with fluctuation of radioactivity in the air reported from physical measures.

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