CYTOGENETIC AFTER-EFFECTS OF MUTAGEN SOIL CONTEMINATION WITH EMISSIONS OF BURSHTYNSKA TPS

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Abstract

A cytogenetic analysis of the root meristem cells of winter wheat rootlets, which were affected by soil contaminants of Burshtynska TPS and ash-disposal area № 1, was made. An increase of chromosome aberrations was recorded: 1.5-2.5 and 1.8-3.0, respectively. Their highest frequency was observed when soil mutagen effect occurred in 3- and 5-km zone and an ash-disposal area. The spectrum of chromosome alterations is mostly presented by singular and double fragments and dicentric bridges. An increased mutation level, found in the vicinity of Burshtynska TPS, proves the fact of possible negative genetic consequences of the effect of emissions from stationary sources of heat power engineering on organisms, and it requires further monitoring of the areas adjacent to thermal power stations of Ukraine.

Key words: chromosome aberrations, genetic consequences, mutagens, Triticum aestivum L., mitosis disorder.

Introduction

Current ecological situation in Ukraine is characterized by a considerable anthropogenic burden in the form of mutagens of physical and chemical nature. Total xenobiotic contamination of atmospheric air, soil, drinking-water and foodstuff was caused by genetically-explained pathology; the latter leads to birth defects of the development and cytogenetic disorders in gametal and somatic cells [1, 2].

One of the main contaminants of the bio-sphere of industrial cities is heat power engineering; its share is 27-32% of the total volume of contaminating emissions, recently they have been 100 mln. tons [3]. Thermal electric stations emit mutagens into free air, including heavy metals, radio-isotopes, benz(a)pyrene, dioxins and other chemical carcinogens [4]. The analysis of the contaminating sources confirms that the major contaminant of the biosphere with heavy metals, which come in an aero-industrial way, is TPS; their emissions contain Cu, Zn, Pb, As, Hg, Ni, V, Cr, Al [5]. Despite the fact that they play an active role in bio-chemical reactions in trace amounts, they are toxic and capable of reducing natural resistance of biological objects to biotic and abiotic factors of the environment when they are in large amounts [6]. The most important depositor of heavy metals both in natural and artificially created eco-systems is soil. Heavy metals stay much longer in the soils than in other natural bodies. Thus, conditionally the soil contamination with heavy metals can be considered as “everlasting” (metals are not destroyed; they shift into another form of existence, including the composition of salts, oxides, metal-organic compounds). The period of semi removal for copper is 310-1500 years, that for zinc – 70-510 years, for lead – 740-5900 years, for cadmium – 13-110 years [7].

Thermal power stations as compared with properly functioning nuclear power stations are more dangerous sources of radiation contamination, because additional radiation doses, which people who live near TPS receive, are 40 times higher than those caused by nuclear reactors [8-10]. Cytogenetic studies of the lymphocytes of peripheral blood of the workers of Kemerovska TPS (Siberia) showed certain increase of chromosome aberration frequency comparing to a control group [11]. The highest chromosome aberration frequency was typical for the workers of the chemical workshops, the lowest one – for the workers of the repair shops.

The coal used at thermal power stations contains a relatively small quantity of primary radio nuclides: on average, potassium – 40-50 Bk · kg^{-1}, uranium-238 and thorium-232 – 20 Bk · kg^{-1}, and its contribution to a radiation dose of people is relatively small. Disproportionation/redistribution of radio nuclides from the ground into a biosphere takes place during coal getting and burning, and using coal ash for building materials, which explains radiation increase of the population [8].

To solve ecological problems concerning environmental pollution with TPS emissions requires the development and application of the monitoring system of different kinds in the areas adjacent to stationary sources of thermal power engineering. Most of the research is aimed at studying the accumulation level of heavy metals [12], radio isotopes [13] in the soil, water reservoirs and plants depending on the distance to the pollution source. Genetic studies are very important in the system of biological monitoring, as they make it possible to evaluate the consequences of the simultaneous effect of several stress-factors for the consecutive generations at cellular and molecular levels [1, 14, 15]. To systematically and efficiently remove or reduce harmful anthropogenic after-effects of the polluted areas, it is necessary to carry out a systematic diagnostics of a soil surface condition which records the main tendencies of long-term pollution processes [16].

With this end in view, mutagen activity of soil contaminants of the area adjacent to Burshtynska TPS as to the frequency and spectrum of chromosome aberrations was studied.

Materials and methodology

To determine mutagen activity of soil pollutants of the area adjacent to Burshtynska TPS (Ivano-Frankivsk rgn.), a cytogenetic analysis of meristem cells of primary roots of winter wheat plantlets (*T. aestivum L.*) of Albatross odeskyi and Zymoiarka cultivars was made. Seeds were kept for 40 minutes in a moist soil, taken from the place which is 1, 3, 4 and 12 km from a pollution source on the axis of air mass transfer and near ash-disposal area № 1. Soil sampling was done in accordance with standard techniques [17] and the requirements of National standard № 17.04.3.01.83, № 17.4.4.02.84.

Taking into consideration the fact that the soils of Poltava region were not polluted with radio nuclides as a result of Chornobyl catastrophe and heavy metal content was much lower than permissible concentrations [16], the soil sampled from the area near Svatky, Hadiach district, Poltava region was taken as control. The analysis aimed at identifying heavy metal content in soil samples was made in the department of agro-ecology and analytical research of the NSC “Institute of arable farming of the National Academy of Agrarian Sciences of Ukraine”. The concentration of mobile forms of lead in the soil samples taken from the area adjacent to TPS and ash-disposable area № 1 was 1,0-1,5 maximum allowable concentrations and exceeded control level by 1.6-3.3 times.

Seeds were germinated at 24-26^{0}C. Primary roots (0.8-1.0 cm long) were kept in “vinegar alcohol” and exposed to maceration in muriatic acid solution. Crushed temporary preparations were made from the roots of apical meristem, colored with acetoorsin. When mitosis disorder frequency and chromosome aberration were being determined, the cells in an anaphase and early telophase were taking into account. The sample consisted of at least 1000 cells for each variant.

Results and discussions

Frequency of chromosome aberrations

Ukraine holds one of the first places in Europe as to the number of harmful emissions. In Ivano-Frankivsk region their amounts are 0.1-0.4 t/ha per year, which makes the region equal to highly industrialized regions of the south-east area of the country as to environmental pollution [18]. The process of coal burning is the main source of heavy metals coming into a biosphere and an additional factor of the increase of natural radiation background. Resultant concentration of metals per fuel ton is 500 g. Considering the fact that during the whole history of mankind 130 billion tons of coal were burnt [19], 65 million tons of metals were emitted and additionally added to circulation. Coal, slag and ash resulted from pyrolisis contain 7-10 times more of primary radio nuclides than soil (potassium – 40-400 Bk · kg^{-1} , uranium-238 and 235 – 150 Bk · kg^{-1}) [8]. Volatile ash is taken up by hot gases and it partially enters the atmosphere and is absorbed by soil surface [13]. So we can assume that the main mutagens which contaminate adjacent areas of Burshtynska TPS are heavy metals and natural radio isotopes. A recently developed system of ecologic-genetic monitoring envisages bio-indication of mutagens of the environment with help of a cytogenetic analysis. Its advantage is the feasibility of getting mutagen estimation of natural environment regardless of the composition of contaminating substances [15]. To perform monitoring of real environmental pollution with potentially harmful compounds (in genetic sense), plant test-systems, to which soft wheat was included, are the most appropriate (*T. aestivum L.*) [20, 21].

The analysis of chromosome aberration frequency in meristem cells of winter wheat roots, whose seeds were germinated in soil samples taken near Burshtynska

TPS, showed its growth by 1.9-2.5 times (as compared with control) for cultivar Albatross odeskyi and by 1.5-2.4 times for Zymoiarka cultivar (table 1). The largest number of chromosome aberrations was recorded with the contamination effect of the soil in 3-6-km zone. There was no frequency excess of aberrated cells of plantlets, which were affected by soil factors comparing to control level (a soil sample was taken at a distance of 1-12 km from the source of pollution). However, soil pollution

Table 1. Frequency of chromosome aberrations in winter wheat when mutagen soil pollution occurs in the area of Burshtynska TPS

Sampling location	Studied		Mitosis with disorders and chromosome aberrations	
	roots, pcs	mitosis anatelophase, pcs	pieces, pcs	%
Albatross odesskyi				
Svatky Poltava rgn. (control)	24	1291	7	0.54±0.22
1 km from TPS	18	1195	12	1.00±0,29
3 km from TPS	18	1282	13	1.01±0.28
5 km from TPS	24	1248	17	1.36±0.33*
12 km from TPS	18	1328	15	1.12±0.29
Ash-disposal area № 1	15	1101	18	1.63±0.38*
Zymoiarka				
Svatky Poltava rgn. (control)	24	1200	9	0.75±0,25
1 km from TPS	15	1496	22	1.47±0.10*
3 km from TPS	16	1047	19	1.82±0.41*
5 km from TPS	18	1350	23	1.70±0.35*
12 km from TPS	18	1098	12	1.10±0.31
Ash-disposal area № 1	15	1063	14	1.32±0.35

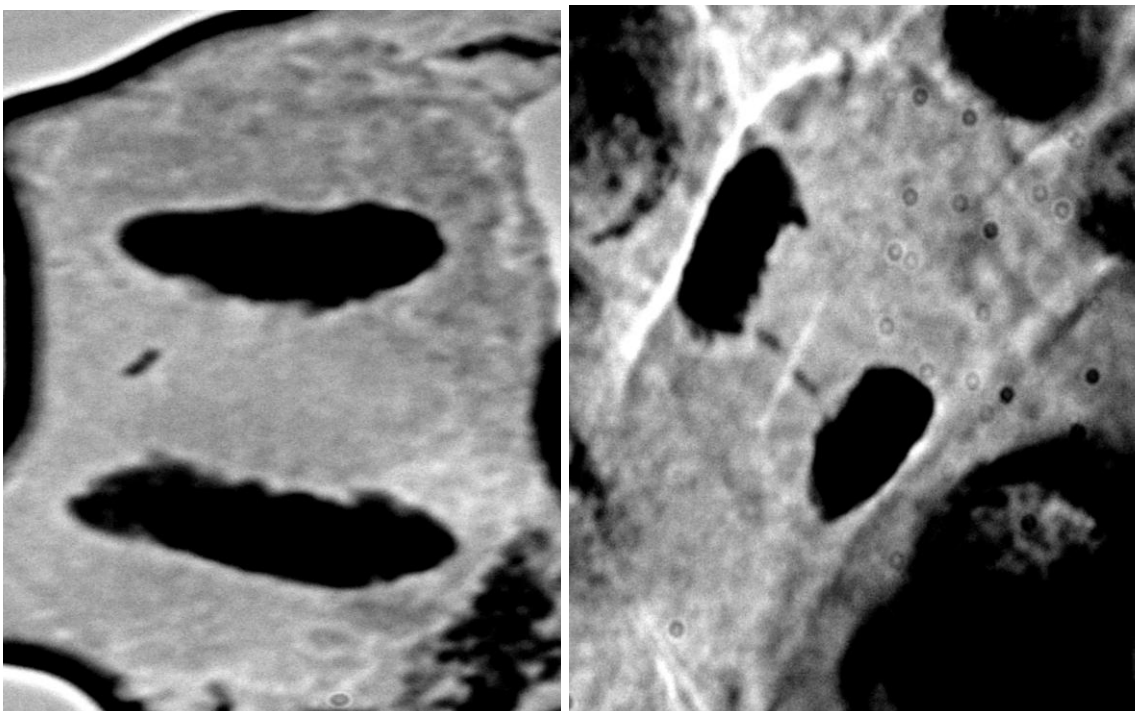
in 1km area from TPS caused 1.47±0.10% cells with chromosome disorders in meristem roots of Zymoiarka plantlets, which certainly exceeded control indices (0.75±0.25 %). The analyses of toxic element content in the soil near Burshtynska TPS (according to the data of other authors [13, 22] showed their maximal accumulation in the area of 3-8 km from the source of contamination toward prevailing winds. Lead, even in small concentrations, is one of the most dangerous pollutants of the environment, which can depress reparatory processes, enhancing in turn genetic after-effects of low radiation doses [23-25]. Based on the results received, we can assume that an increased level of chromosome aberrations caused by soil contaminants is the outcome of a synergistic effect of at least two mutagen factors – lead and primary radio nuclides.

The increase of a cytogenetic disorder level was observed with the effect of soil pollutants in the area of ash-disposal № 1. The frequency of the cells with chromosome disorders was at the level of 1.63±0.38 % in wheat of Albatross odeskyi cultivar and 1.32±0.35 % in Zymoiarka, which exceeded control level by 3.0 and 1.8 times, correspondingly.

Spectrum of chromosome aberrations

The spectrum of chromosome aberrations which were induced by emissions of Burshtynska TPS, absorbed by the soil in 3- and 5-km zone, included mostly singular and pair/double acentric fragments and dicentric bridges (Figure 1, 2). Types of chromosome disorders with the effect of the soil taken at a distance of 1 and 12 km from the contamination source were presented by singular and double/pair fragments and chromatic bridges (table 2). Cells with pair fragments and chromosome bridges occurred very rarely. The frequency of the cells with plural aberrations was 0.07-0.29 %, and their emergence was due to the factors of soil pollution in a 3-km zone (Figure 3).

Along with the mentioned chromosome aberrations in the cells of the samples under study, contrary to control, micro-nuclei and lagging chromosomes were recorded (Figure 4). The latter are the indicators of mitosis anomaly, and they confirm the aneugenetic effect of contaminating factors [26, 27]. The frequency of their fixation ranged 0.08-0.19 %. The germination of wheat seeds (Zymoiarka cultivar) in the soil taken from a 1-km zone resulted in the appearance of the cells with tripolar mitosis.



A B

Figure 1. Singular (A) and pair/double (B) acentric fragments

Figure 2. Dicentric bridge

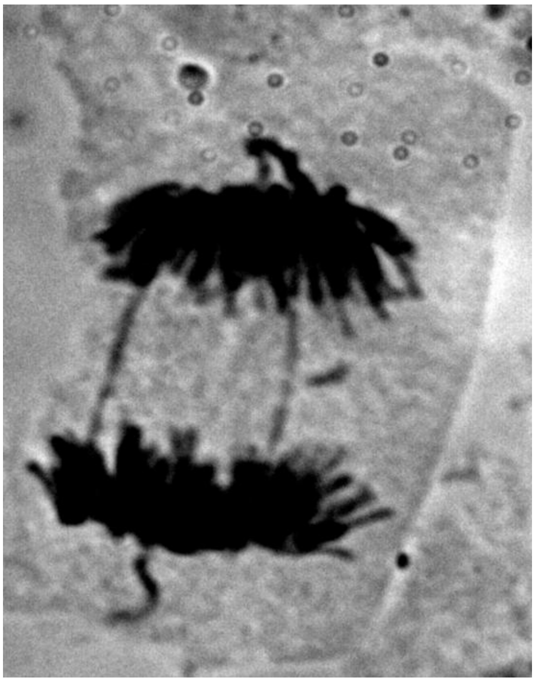


Figure 3. Cell with plural aberrations

Table 2. Spectrum of chromosome aberrations in winter wheat when mutagen soil pollution occurs in the area of Burshtynska TPS

Sampling location	Spectrum of mitosis disorders and chromosome aberrations									
	fragments		bridges		bridges + fragments		micro-nuclei		lagging chromosomes	
	pcs	%	pcs	%	pcs	%	pcs.	%	pcs	%
Albatross odesskyi										
Svatky Poltava rgn. (control)	1	0.08	6	0.47	0	0.00	0	0.00	0	0.00
1 km from TPS	3	0.25	7	0.59	0	0.00	2	0.17	0	0.00
3 km from TPS	5	0.39	5	0.39	1	0.08	1	0.08	1	0.08
5 km from TPS	4	0.32	13	1.04	0	0.00	0	0.00	0	0.00
12 km from TPS	9	0.68	4	0.30	0	0.00	0	0.00	2	0.15
Ash-disposal area № 1	6	0.55	11	1.00	0	0.00	0	0.00	1	0.09
Zymoiarka										
Svatky Poltava rgn. (control)	5	0.42	4	0.33	0	0.00	0	0.00	0	0.00
1 km from TPS	5	0.33	13	0.87	1	0.07	1	0.07	2	0.13
3 km from TPS	5	0.48	10	0.96	3	0.29	0	0.00	2	0.19
5 km from TPS	11	0.82	9	0.67	0	0.00	2	0.15	1	0.07
12 km from TPS	6	0.55	5	0.46	0	0.00	0	0.00	1	0.09
Ash-disposal area № 1	8	0.75	6	0.56	0	0.00	0	0.00	0	0.00

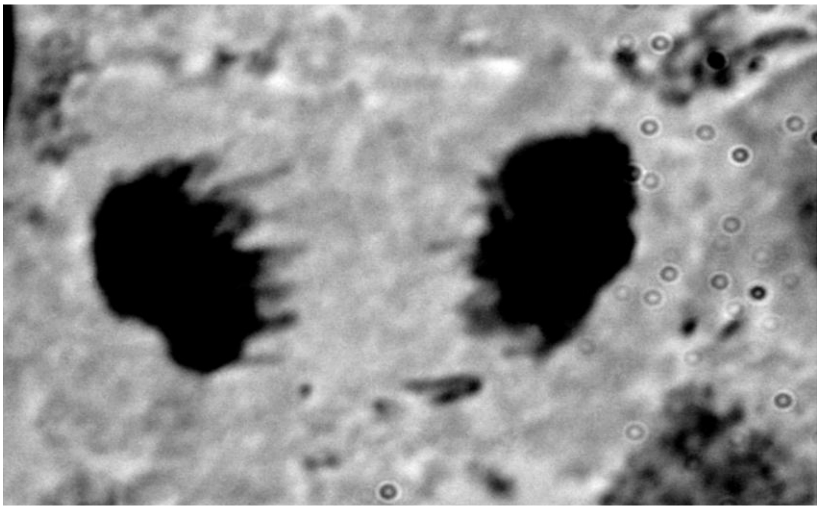


Figure 4. Lagging chromosome

A high level of chromosome alteration, caused by soil pollution near ash-disposable № 1 of Burshtynska TPS, did not show a wide spectrum of their types and contained mostly acentric fragments and dicentric bridges. In meristem cells of wheat plantlets of Albatross odeskyi, contrary to Zymoiarka cultivar, a dominating aberration type was pair fragments and chromosome bridges. An anaphase cell with a lagging chromosome was identified among them.

Conclusions

Thus, a complex effect of soil pollutants of the areas adjacent to Burshtynska TPS and ash-disposal area № 1 causes the increase of the cytogenetic disorder level in meristem root cells of winter wheat plantlets by 1.5-2.5 and 1.8-3.0 times, correspondingly. The highest chromosome aberration frequency was recorded with the mutagen effect of the soil taken at a distance of 3 and 5 km from the contamination source. Their spectrum is mostly presented by singular and pair fragments, and dicentric bridges. Harmful emissions of Burshtynska TPS, absorbed by the soil, result in mitosis anomaly, which is seen in blocking the threads of division spindle and the emergence of the cells with lagging chromosomes. The increased mutation level, found in the vicinity of Burshtynska TPS, confirms the fact of potential negative genetic after-effects of the emissions from the stationary sources of thermal power engineering on the organisms; and it requires further implementation of the monitoring of the areas adjacent to thermal power stations of Ukraine.

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