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Cytogenetic disorders in *Triticum aestivum* L. cells, induced by heavy metal releases from industrial production

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A cytogenetic analysis of meristem cells of *Triticum aestivum* L. of primary rootlets, affected by soil heavy metal (HM) contamination in the industrial zones of SC "Poltavachimmash" in Poltava city, PPC "Specialized company for thermal processing of firm household garbage" (SCTPFHG) in Khrakiv city, PrSC "Avtoradiator" in Mariupol city, CC (commercial company) "Poltava concrete product plant" (PCPP) in Poltava city, SJC "Luhansk accumulator producing company" in Luhansk city, UC "Lubny water supply station" in Lubny city and the territory near B. Khmelnytskyi Street in Kostiantynivka, was made. The frequency of aberrant cells of sprout root meristem of cultivar Al'batros odes'kyi was 1.37-2.30 % and that of cultivar Zymoiarka – 1.22-1.84 %, which exceeded the indicators of a background level by 1.9-3 and 1.6-2,5 times, correspondingly (Svatky village, Poltava region). The highest cytogenetic activity was recorded within the territory of the industrial zones of PrSC "Avtoradiator", SC "Poltavachimmash", SJC "Luhansk accumulator producing company", the soils there were characterized by intensive Cd, Pb i Zn contamination. Significant differences in the levels of cytogenetic disorders depending on a wheat cultivar genotype, induced by high concentrations of soil toxic metals in the industrial zone of PPC "Specialized company for thermal processing of firm household garbage" and the territory near B. Khmel'nytskyi Street in Kostiantynivka, can be caused by physiological-genetic peculiarities of the plants and a possible effect of non-differentiated factors, specific for each territory. Complex effect of low HM concentrations, typical for soil contamination of the industrial zone of CC "Poltava concrete product plant", showed the lowest cytogenetic activity. Soil contamination with HM in the industrial zones and the areas affected by industrial releases resulted in a wide spectrum of cytogenetic disorders: fragmentation, advance and lagging chromosomes, formation of chromosome and chromatid bridges, ring chromosomes, micronuclei. A significant increase of the cells with advance and lagging chromosomes was seen under the effect of HM soil contamination of the industrial zones of PrSC "Avtoradiator", SC "Poltavachimmash", UC "Lubny water supply station" and the territory near B. Khmel'nytskyi Street in Kostiantynivka, Cd, Pb i Zn predominating among them. The induction with high frequency of aneuploid cells shows active interaction of HM with the threads of spindle division which allows using the violation fact of chromosome segregation as an indicator of HM contamination of the environment. As a result of wheat seed sprouting in the soil with the highest HM content (PPC "Specialized Company for thermal processing of firm household garbage", PrSC "Avtoradiator", SC "Poltavachimmash", UC "Lubny water supply station" and the territory near B. Khmelnytskyi Street in Kostiantynivka), besides mentioned-above chromosome aberrations and mitosis pathology in meristem cells, the following complicated cytogenetic disorders occurred: single/pair fragments and bridge; pair fragments and lagging chromosomes; pair fragments, bridge and ring chromosome; pair fragments, chromosome bridge and micronucleus. High cytogenetic activity of HM soil contamination, caused by industrial releases, confirms genetic threat for the organisms of above-ground ecosystems and suggests the necessity to work out a national program of large-scale genetic monitoring over techno-genic contamination of the territories in the industrial regions of Ukraine.
Key words: *T. Aestivum* L.; cytogenetic activity; heavy metals; cytogenetic disorders; chromosome aberrations

Introduction

One of the most dangerous pollutants of the environment of urbanized and techno-genic territories in the world is heavy metals (HM), which are second only to radionuclides and pesticides as to their toxicity (Mylenka, 2009; Belousov et al., 2010; Dovhaliuk, 2013; Kumar & Tripathi, 2008). HM soil contamination is associated with the availability of different sources of industrial pollutant emissions: industrial facilities of mining-metallurgical, chemical, fuel-power complexes, machine-building companies,

a ramified transport system, etc. High HM concentrations have been found within the urbanized territories of almost the whole central and south-east Ukraine, where 1.5 thousand industrial and mining companies are concentrated.
Low concentrations of some HM in the soils are an important condition for natural vital functions of the organisms. However, as soon as their level exceeds physiologically admissible meanings, they become toxic for cells. The reason for it is the ability of HM to intensively join functional enzyme areas, to change conformation of protein molecules, nucleic acids, to break cell membrane integrity, to split important metabolites (Tabrez & Ahmad, 2011; Aslam et al., 2014). But the mentioned pollutants have both a distinct general toxic effect on living organisms (Horova & Kulyna, 2008; Dovhaliuk, 2013) and a mutagenic one (Michailova & Petrova, 2005), which is confirmed by a suppressed mitotic division, the increase of chromosome aberration frequency, a disorder of chromosome segregation, development defects of organisms, the increase of cancer incidence (Korshykov et al., 2013; Kumar et al., 2008; Ritambhara & Kumar, 2010; Kumar & Pandey, 2015).
According to WHO (World Health Organization) (Human Exposure…, 2000), at present acute poisoning with metals occurs very rarely, which is why the issue of mutagenic effect of relatively low doses of their compounds and distant-in-time consequences of such effects on the organisms becomes very urgent. Considering the fact that mutagenicity is seen at much lower concentrations than toxicity, and population-genetic consequences of its effect are a great threat, it is expedient to monitor gene-toxicity of metals, which are accumulated in the zones of ecological stress (Dovhaliuk, 2013). Most of the researches concentrate only on studying ecological indicators of the contaminated territories, correlative links between doses of individual HM compounds and their mutagenic effect (Ritambhara et al., 2010; Kumar et al., 2015; Ahmad, Wahid & Ahmad, 2011; Vasiliev, 2017), and genetic consequences, caused by their complex effect, are not taken into account.
Studying metal-induced mutagenesis, cytogenetic test-systems of the plants are believed to be the most informative ones; they are fast, simple, reliable and cheap, and the similarity between plants and mammals as to morphology and chromosome sensitivity makes it possible to estimate the risks of potential mutagenic effect and even the degree of carcinogenicity (Vasiliev, 2017; Dovhaliuk, 2013; Mesi et al., 2014). An apical part of plant roots is the first to contact soil toxicants. It contains enzymes (oxidases with mixed functions) which activate promutagens turning them into mutagens (Dovhaliuk, 2013). So the use of cytogenetic analysis of meristem cells of sprout primary rootlets is a reliable and sensitive method to study mutagenic activity of the soil, contaminated with HM, which resulted from industrial discharges.

Materials and Methods

To study mutagenic activity of the HM contaminated soil resulted from industrial discharges, a cytogenetic analysis of meristem cells of sprout primary rootlets of soft winter wheat (*Triticum aestivum* L.) – cultivars Al'batros odes'kyi and Zymoiarka – whose seeds were sprouted in soil samples taken from the industrial zones and adjacent territories of the businesses with intensive releases of contaminating substances into the atmosphere: SC "Poltavachimmash" in Poltava city, PPC "Specialized company for thermal processing of firm household garbage" in Khrakiv city, PrSC "Avtoradiator" in Mariupol city, CC "Poltava concrete product plant" in Poltava city, SJC "Luhansk accumulator producing company" in Luhansk city, UC "Lubny water supply station" in Lubny city and the territory near B. Khmelnytskyi Street in Kostiantynivka. The analysis to determine heavy metal content in soil samples was made in the department of agro-ecology and analytical research at NSC "Institute of Arable Farming of Ukraine's NAAS" using the methods which correspond to the legislative enactments of Ukraine. The concentration of cadmium, copper, nickel, lead and zinc labile forms in the soils of industrial business zones and adjacent areas was: SC "Poltavachimmash" – 1.7, 2.1, 0.4, 12.8, 40.9 CPC (critical permissible concentration); PPC "SCTPFHG" – 13.0, 2.1, 0.4, 2.2, 1.3 CPC; PrSC "Avtoradiator" – 4.3, 1.5, 0.7, 28.7, 8.1 CPC; CC "PCPP" – 3.3, 2.1, 0.5, 6.4, 25.3 CPC; SJC "Luhansk accumulator producing company" – 13.0, 5.8, 4.4, 5.5, 5.6 CPC; UC "Lubny water supply station" – 9.8, 1.2, 0.6, 1.9, 1.3 CPC; near B. Khmel'nytskyi Street in Kostiantynivka – 10.5, 0.6, 0.4, 17.0, 20.2 CPC. Taking into account the fact that Poltava region belongs to nominally clean regions of Ukraine, as its soils did not undergo significant radionuclide pollution as a result of the accident at Chornobyl TPS, and they do not contain remains of chlorine-organic and phosphorus-organic pesticides, and HM content is several times lower than CPC (Shvyd et al., 2010), soil samples from Svatky village, Hadiach district, Poltava region, were taken as the control. Soil sampling was done using standard methods (Bekker & Agaiev, 1989) – from the upper 5-cm horizon which has the highest accumulating ability, performs the function of mechanical (for firm industrial particles) and biochemical sorption and chemo-sorption barriers (Alekseev, 1987).
Seeds – 50 pieces per variant of the trial – were sprouted at 24-26 ºC in Petri dishes in soil samples taken from the studied plots and moistened with distilled water. Primary rootlets - 0.8-1.0 cm long - were fixed for 4 hours in Clark holder which consisted of 96% of ethanol solution and icy acetic acid in correlation 3:1. Chemical maceration of rootlets was done during 1minute in 1n solution of hydrochloric acid. After maceration rootlets were placed into aceto-orcein solution for 24 hours at temperature 23-25 ºC to analyze chromosome aberrations and mitosis disorders.
Temporarily crushed cytological preparations were made for a microscopic analysis using conventional methods (Pausheva, 1988). Microscopic studying of rootlet meristem zone was carried out with the use of «JENAVAL» (Carl Zeiss Jena) microscope at magnification 600x. Micro-photographing was done with help of extension Olympus SP–500 UZ integrated into a microscope at magnification 900x and software Quick PHOTO MICRO 2.3 for Windows (Olympus). When determining the frequency of chromosome aberrations and mitosis disorders, the cells, which were in anaphase and telophase, were taken into consideration. A sample for each variant was at least 1000 cells which were studied in 20 and more primary rootlets. The frequency of aberrant cells was taken as the percentage of cells in anaphase and early telophase which contained chromosome disorders. Statistic processing of experimental data was carried out by conventional methods (Lakin, 1990), reliability of the difference was estimated with Student t-criterion. Zero hypothesis was rejected at $P \leq 0.05$. Editor MS Excel 2003 and software package Statistica 6.0 were used to make all calculations. Percentage shares of chromosome aberrations and their errors were given in Tables 1-2.

Results and Discussions

Higher HM concentrations in the soil of the studied territories caused the increase of cytogenetic disorder level in root meristem cells of winter wheat. The frequency of aberrant cells in primary sprout rootlets was 1.37-2.30 % for cultivar Al'batros odes'kyi and 1.22-1.84 % for cultivar Zymoiarka, which exceeded the indicators of a background level by 1.9-3 and 1.6-2.5 times, respectively (Table 1). Regardless of a cultivar genotype, HM in the soil of the industrial zones of PrSC "Avtoradiator", SC "Poltavachimmash", SJC "Luhansk accumulator producing company", UC "Lubny water supply station" showed a high level of cytogenetic activity, which probably exceeded control indicators statistically. Predominant pollutants of the mentioned territories were Cd and Pb, which according to the classification (Mesi & Kopliku, 2014) are extremely cyto- and genotoxic metals, and also Zn; the latter is characterized as relatively non-toxic. Considering the need of Zn as a microelement for plant metabolism, cytogenetic consequences of its effect can be seen only when its concentrations are much higher than a vitally required level (Garipova & Kaliiev, 2009; Vasiliev, 2017; Ritambhara et al., 2010). Sprouting of winter wheat seeds in soil samples with the highest Zn content (industrial zone of SC "Poltavachimmash") resulted in the induction of chromosome aberrations with frequency 1.56% for cultivar Al'batros odes'kyi and 1.71% for cultivar Zymoiarka in meristem cells of primary rootlets, which exceeded the control level by 2 and 2.3 times, respectively (Fig. 1).

Higher genotoxicity is typical for Pb and Cd than for Cn (Ritambhara et al., 2010; Kumar et al., 2015). When in plant tissue, Pb results in chromosome aberrations and can hinder a cell cycle in G_2-period of an inter-phase, which requires additional time for the reparation of heavy DNA disorders or the activation of apoptosis. It can substitute Ca and Zn in the enzymes which are involved in DNA reparation, and it can become more genotoxic when it is combined with other risky agents for DNA (Garcia-Leston, Mendez, Pasaro & Laffon, 2010). Cd genotoxicity is connected with its direct effect on the structure and functions of DNA (Cambier, Gonzalez, Durrieu & Bourdineaud, 2009). Most likely chromosome anomalies occur as a result of the interaction between Cd and non-histoned chromatin proteins, which control chromosome organization. It can induce active oxygen forms, suppress DNA synthesis, break transcription control or, as well as Pb, hinder cells in G_2-phase of a cell cycle (Aslam et al., 2014).

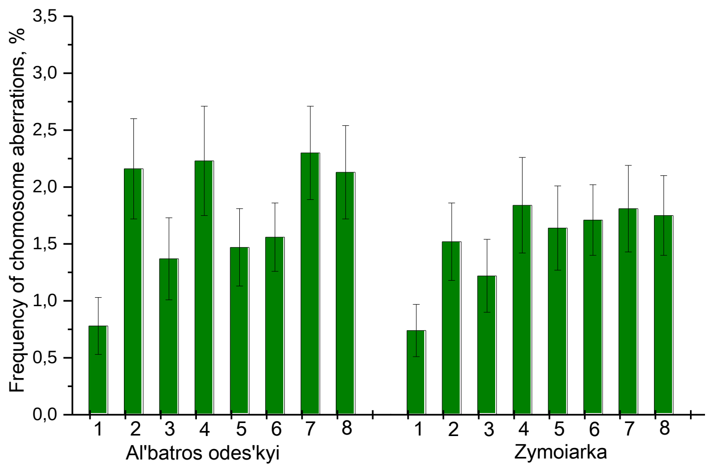

Fig. 1. Frequency of cytogenetic disorders in meristem cells of primary rootlets of winter wheat under the effect of heavy metal soil contamination resulted from industrial discharges: 1 – Svatky village, Poltava rgn. control), 2 – PPC "SCTPFHG", 3 – CC "PCPP", 4 – PrSC "Avtoradiator", 5 – B. Khmel'nytskyi Str., Kostiantynivka, 6 – SC "Poltavachimmash", 7 – SJC "Luhansk accumulator producing company", 8 – UC "Lubny water supply station".

The level of cytogenetic disorders, induced at the highest Pb soil contamination in the industrial zone of PrSC «Avtoradiator» in combination with Cd and Zn ions was 2.33% for sprout meristem cells of cultivar Al'batros odes'kyi and 1.84% for cultivar Zymoiarka, and it exceeded background indicators by 2.4-2.9 times. The increase of the number of cells with chromosome reconstructions by 2.5-3 times, recorded at the most intensive Cd soil contamination of the industrial zone of SJC «Luhansk accumulator producing company», can be additionally caused by high content of Pb, Zn ions and Cui Ni which are extremely toxic in large concentrations (Mesi et al., 2014). Microelement Cu is in phytohormones, plant enzymes, some structural cell proteins, and it is characterized by enhanced biogenic activity (Garipova et al., 2009; Mironov, 2012). Equally with Ni, whose soil content exceeded CPC by 4.4 times, Cu caused oxidative stress through the formation of reactive oxygen forms which damaged main polymers in a cell – lipids, proteins and nucleic acids (Stoliar & Lushchak, 2012). It is assumed that the mechanism of its genotoxic effect, as well as that of Pb, is connected with the process of slowing down DNA reparation, however mutagenic efficiency of Cu effect even in small concentrations is much higher (Gomes, 2011). The increase of Cu content in the soils can break a barrier potential of a root system and favor cumulation of other toxins in tissues, and as a result, it can be a conductor and an inductor of pre-mutation processes in plant tissues (Garipova et al., 2009; Mironov, 2012).

According to their toxicity HM are placed as follows: Cu>Ni>Cd>Zn>Pb>Hg>Fe>Mo>Mn (Dovhaliuk, 2013; Al-Qurainy et al., 2010; Mesi et al., 2014).

Table 1. Frequency and spectrum of chromosome aberrations in meristem cells of primary rootlets of winter wheat under the effect of HM soil contamination resulted from industrial discharges.

Sampling site	Studied anatelophases of mitoses, pcs.	Mitoses with disorders and chromosome aberrations		Spectrum of mitosis disorders and chromosome aberrations											
		pcs	%	Fragments		Bridges		Bridges + fragments		Lagging chromosomes		Micro nuclei		Ring chromosomes	
				pcs.	%	pcs.	%	pcs.	%	pcs.	%	pcs.	%	p	%
Al'batros odes'kyi															
Svatky village, Poltava rgn.(control)	1282	10	0,78±0,25	6	0,47	4	0,31	0	0,00	0	0,00	0	0,00	0	0,00
PPC "SCTPFHG", Kharkiv	1111	24	2,16±0,44*	14	1,26*	5	0,45	2	0,18	2	0,18	0	0,00	1	0,09
CC "PCPP", Poltava	1025	14	1,37±0,36	9	0,88	4	0,39	0	0,00	1	0,10	0	0,00	0	0,00
PrSC "Avtoradiator", Mariupol	941	21	2,23±0,48*	9	0,96	4	0,42	2	0,21	3	0,32	2	0,21	1	0,11
B. Khmel'nytskyi Str., Kostiantynivka	1222	18	1,47±0,34	10	0,81	3	0,25	1	0,08	3	0,25	0	0,00	1	0,08
SC "Poltavachimmash", Poltava	1662	26	1,56±0,30*	10	0,60	6	0,36	1	0,06	5	0,30*	3	0,18	1	0,06
SJC "Luhansk accumulator producing company", Luhansk	1348	31	2,30±0,41*	15	1,11	7	0,52	0	0,00	3	0,22	4	0,30	2	0,15
UC "Lubny water supply station", Lubny	1218	26	2,13±0,41*	13	1,07	5	0,41	2	0,16	4	0,33	0	0,00	2	0,16
Zymoiarka															
Svatky village, Poltava rgn.(control)	1354	10	0,74±0,23	6	0,44	3	0,22	0	0,00	0	0,00	1	0,08	0	0,00
PPC "SCTPFHG", Kharkiv	1316	20	1,52±0,34	9	0,68	8	0,61	0	0,00	2	0,15	0	0,00	1	0,08
CC "PCPP", Poltava	1145	14	1,22±0,32	5	0,44	4	0,35	0	0,00	3	0,26	0	0,00	2	0,17
PrSC "Avtoradiator", Mariupol	1031	19	1,84±0,42*	8	0,78	6	0,58	0	0,00	5	0,48*	0	0,00	0	0,00
B. Khmel'nytskyi Str., Kostiantynivka	1159	19	1,64±0,37*	7	0,60	4	0,35	0	0,00	7	0,60*	0	0,00	1	0,09
SC "Poltavachimmash", Poltava	1755	30	1,71±0,31*	13	0,74	7	0,40	1	0,06	6	0,34*	3	0,17	0	0,00
SJC "Luhansk accumulator producing company", Luhansk	1214	22	1,81±0,38*	12	0,99	5	0,41	0	0,00	3	0,25	2	0,16	0	0,00
UC "Lubny water supply station", Lubny	1372	24	1,75±0,35*	12	0,87	6	0,44	1	0,07	4	0,29	0	0,00	1	0,07

However, this rank can be changed because of different sedimentation rates of the elements by the soil and their transfer into inaccessible state for plants, cultivation conditions and physiological-genetic peculiarities of the plants themselves.

The level of cytogenetic activity of a complex effect of toxic metals in the soil of the territories of some facilities under investigation depended on wheat genotype and potential influence of urbogenic undifferentiated factors, specific for each territory. In particular, under the effect of HM contamination of the zone of PPC «SCTPFHG», statistically possible increase of aberration frequency was found only in the cells of sprout root meristem of cultivar Al'batros odes'kyi (2.16%), and the increased soil metal content in the territory near B. Khmelnytskyi Street in Kostiantynivka induced statistically potential increase of aberrant cells of rootlet apical meristem of cultivar Zymoiarka (1.64%). The spread of heavy metals within Kostiantynivka city is associated with the business of company «Ukrzinc», which leads to moderate and very dangerous contamination levels in some of its districts. Statistically potential increase of chromosome aberration frequency was not recorded under the joint effect of low concentrations of labile forms of soil HM in the zone of CC «PCPP». The lowest genotoxic effect was found in *Allium cepa L.* affected by river contamination with low salt concentrations such as Cr, Cu and Pb in Albania (Mesi et al., 2014).

Based on the mitosis disorders one can make conclusions concerning the intensity of mutation process in cells of organism populations, according to disorder spectrum – about the damage degree of genetic material, i.e., compatibility of life with occurring injuries (Belousov et al., 2010). Neither of techno-genic pollutants can create new mutation types which are not seen in the control. But the correlation between various mutation types under the effect of the factors of different nature can change considerably (Vasiliev, 2017). Soil HM contamination of the industrial zones and territories affected by industrial discharges caused a wide spectrum of cytogenetic disorders: fragmentation, advance and lagging chromosomes (fig. 2, a-c), formation of chromosome and chromatide bridges, ring chromosomes, micronuclei. Herewith, some specificity of the manifestation of these disorders depending on the intensity and composition of contamination components was observed. Statistically potential increase in the number of mitoses by 2.7 times with single and pair fragments was recorded under the effect of soil contamination with metals of the industrial zone of PPC «SCTPFHG» on sprout rootlet meristem of cultivar Al'batros odes'kyi. Under the effect of HM ion complex in the soils of the territory of PrSC «Avtoradiator», SJC «Luhansk accumulator producing company», UC «Lubny water supply station», the frequency of fragment appearance was 0.96 %, 1.11 % and 1.07 %, respectively, which was twice as much as the control indicators (0.47%) - Fig. 3. According to the frequency of acentric fragments in the cells of root meristem of cultivar Zymoiarka (control is 0.44%), double effect was found only under the effect of HM soil contamination in the industrial zone of SJC «Luhansk accumulator producing company» (0.99%). Fragmentation could occur due to chromosome adhesiveness, caused by the interaction of HM ions with histone and non-histone proteins, and in turn, the failure to divide chromatides when they move towards poles (Fusconi et al., 2007; Kumar et al., 2015).

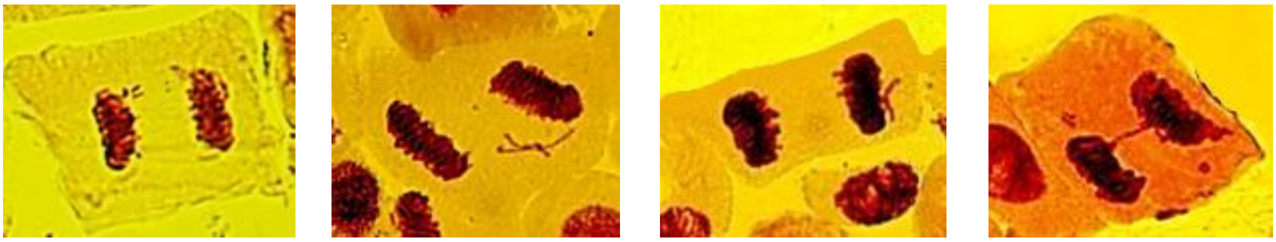

Fig. 2. Types of chromosome aberrations and mitosis anomalies: a – pair acentirc fragments, b – lagging chromosomes, c – advance chromosome, d – pair acentirc fragments, dicentric chromosome and micronucleus.

The frequency increase of aberrant cells of sprout rootles of cultivar Zymoiarka depended on the induction level of dicentrics of predominantly chromatide type considerably. Affected by HM, bridges can be formed due to chromosome adhesiveness and further disorder of free anaphase division, uneven translocation or inversion of chromosome segments (Siddiqui, 2012; Aslam et al., 2014). They occurred with the highest frequency (0.44-0.61 %) during seed sprouting in the soil in the territories of PPC «SCTPFHG», PrSC «Avtoradiator», UC «Lubny water supply station», where the most intensive soil contamination with Cd and Pb was recorded.

The expansion of the spectrum of chromosome aberration types affected by HM soil contamination took place due to the ring chromosome induction. The frequency of meristem cells of wheat primary sprout rootlets with these unstable chromosome reconstructions, which can be lost in the cell division process (Dubinin, 1986), ranged from 0.06-0.16 % in cultivar Al'batros odes'kyi and 0.07-0.17 % in cultivar Zymoiarka.

Cytoskeleton structures of plant cells, in particular microtubes, which show high sensitivity to different endogenous and exogenous stimuli, are very susceptible among potential intra-cell targets of toxic metals. These dynamic formations of cytoskeleton ensure the process of a cell cycle, including mitosis and cytokinesis (Fahr et al., 2013). According to the results of a cytogenetic analysis, it was found out that in the territory of the majority of the studied facilities with HM soil contamination aneugenic anomalies along with clastogenic damages in the cells of root apex were induced with high frequency in cultivar Al'batros odes'kyi and Zymoiarka – 0.10-0.33 % and 0.15-0.60 % respectively. There were no disorders of this type in the control. A serious increase in the number of cells with lagging and advance chromosomes (Figs 2b, 2c) was found under the effect of HM soil contamination in the zones of PrSC «Avtoradiator», SC «Poltavachimmash», UC «Lubny water supply station» and near B. Khmel'nytskyi Street in Kostiantynivka, and Cd, Pb i Zn had the largest share among them. Breaking of chromosome segregation affected by HM, which was confirmed by numerous researches on other plant objects (Garipova et al., 2009; Belousov et al., 2010; Ritambhara et al., 2010; Oladele, Odeigah & Taiwo, 2013), can prove their potential carcinogenicity (Žegura, Heath, Černoša & Filipič, 2009; Tokar, Benbrahim-Talaa & Waalkes, 2011). According to (Kumar et al., 2015), Cd and Pb compounds due to their ability to bind with tubulin protein, causing the dysfunction of division spindle threads, can be classified as colchicine analogues.

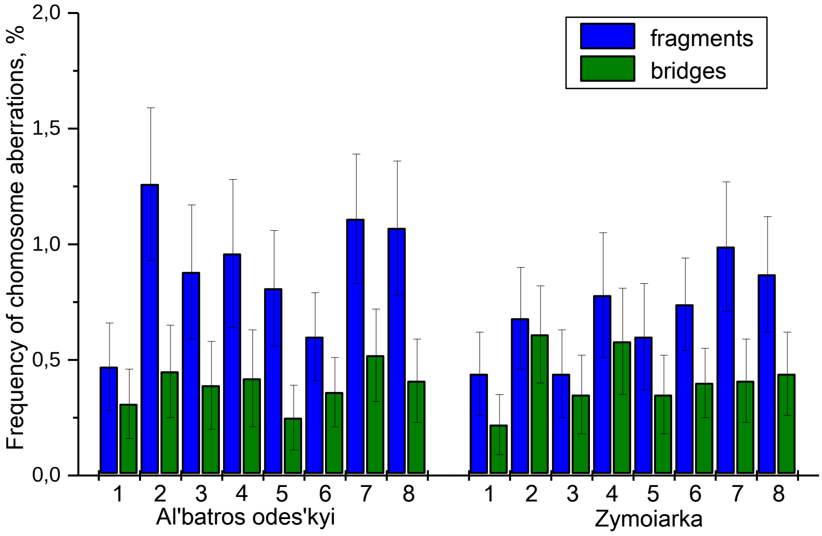

Fig. 3. Frequency of the formation of acentric fragments and dicentric chromosomes in meristem cells of primary rootlets of winter wheat under the effect of HM soil contamination resulted from industrial discharges: 1 – Svatky village, Poltava rgn. (control), 2 – PPC "SCTPFHG", 3 – CC "PCPP", 4 – PrSC "Avtoradiator", 5 – B. Khmel'nytskyi Str., Kostiantynivka,
6 – SC "Poltavachimmash", 7 – SJC "Luhansk accumulator producing company", 8 – UC "Lubny water supply station".

Micronuclei were found in mitosis telophase among the types of cytogenetic disorders, induced by joint effect of high doses of HM (Cd, Pb, Zn) in the soil of the industrial zones of PrSC "Avtoradiator", SC "Poltavachimmash", SJC "Luhansk accumulator producing company". Their formation on the basis of fragments or whole chromosomes, which did not enter any of daughter nuclei, occurs with partial chromatin loss (Ritambhara et al., 2010; Mouna et al., 2013). A positive correlation between high Cd, Pb and Zn doses and the induction of micronuclei in plant tissues was also discovered by other researchers (Ritambhara et al., 2010; Kumar et al., 2015).

As a result of wheat seed sprouting in the soil with the highest content of HM (industrial zones of PPC "SCTPFHG", PrSC "Avtoradiator", SC "Poltavachimmash", UC "Lubny water supply station", the territory near B. Khmel'nytskyi Street in Kostiantynivka), in addition to already mentioned chromosome aberrations and mitosis pathology in meristem cells, complicated cytogenetic disorders also occurred: single/pair fragments and bridge; pair fragments and lagging chromosomes; pair fragments, bridge and ring chromosome; pair fragments, chromosome bridge and micronucleus (Fig. 3d). The formation of multiple aberrations confirms heavy cytogenetic cell damage and high mutagenic activity of complex effect of HM soil contamination.

Conclusions

HM soil contamination in the territories affected by industrial releases causes the increase of cytogenetic disorder level in winter wheat by 1.6-3 times. The highest mutagenic activity was recorded in the territories of industrial zones of PrSC "Avtoradiator", SC "Poltavachimmash", SJC "Luhansk accumulator producing company", UC "Lubny water supply station", their soils were characterized by intensive Cd, Pb and Zn contamination. Significant differences in the levels of cytogenetic disorders depending on a wheat cultivar genotype, induced by high concentrations of soil toxic metals in the industrial zone of PPC "SCTPFHG" and the territory near B. Khmel'nytskyi Street in Kostiantynivka, can be caused by physiological-genetic peculiarities of the plants and a possible effect of non-differentiated factors, specific for each territory. Complex effect of low HM concentrations, typical for soil specific contamination of the industrial zone of CC "PCPP", showed the lowest cytogenetic activity.

A spectrum of cytogenetic disorder types included mostly single and pair acentric fragments, bridges of chromatide type. Its expansion occurred due to the formation of ring chromosomes, micronuclei and mitosis anomaly. The induction with high frequency of advance and lagging chromosomes underlines active interaction between HM and threads of division spindle, which makes it possible to use the fact of chromosome segregation disorder as an indicator of HM environment pollution.

High cytogenetic activity of HM soil contamination, caused by industrial releases, confirms genetic threat for the organisms of above-ground ecosystems and suggests the necessity to work out a national program of large-scale genetic monitoring over techno-genic contamination of the territories in the industrial regions of Ukraine.

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