USING OF *POPULUS SIMONII* CARR. FOR THE ESTIMATION OF THE URBAN SYSTEM'S MUTAGENIC BACKGROUND

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A differential evaluation of the mutagenic background of the Ivano-Frankivsk urban system has been carried out. To do this, we used one-type (in age, sex and sanitary state) trees of *Populus simonii* Carr. This material was investigated for the contents of trace elements: Fe, Mn, Cu, Al, Pb, Zn, Cr, Cd, Ni (method of energy-dispersion X-ray microanalysis), as well as for mitotic activity and the chromosome aberration levels (anaphase method) in meristem cells of rudimentary leaves. The research of pollen sterility was made in the same trees in spring. The concentration of the majority of heavy metals in vegetative tissues increased with augmentation of an industrial-transport load. In some areas, an increase in the degree of chromosomal apparatus damage, a pronounced mitotic inhibition and a rise of pollen sterility level were observed. Basing on the carried out research, an estimation of the studied areas state according to its mutagenic background was made.

Key words: biomonitoring, environmental mutagens, urban system, *Populus simonii* Carr.

City territories are characterized by the availability of a great deal of contamination sources, their irregular distribution, and composite distributional pattern of pollutants [14]. Many of them are mutagens. Toxic and non-toxic components, which can be promutagens, comutagens, desmutagens or antimutagens, are capable to interact among themselves and to be transformed into new products, both in external surroundings, and in live organisms. Practically, it is not possible to prognosticate such chemical transformations.

Heavy metals are one of the priority pollutants of urban environment. The toxicity's broad spectrum and degree, including the cytogenetic one, characterize their mixtures. In the soil of the city Ivano-Frankivsk, the level of mobile forms of some heavy metals exceeding boundary-permissible concentrations by 1.5-3.0 times [11].

In most cases for investigation of the cytogenetics effects of anthropogenic pollutants grassy plants [17, 18, 20] less often – coniferous species [16, 19] are used.

The high gas-absorbing and metal-accumulating capacity of leaves of some species of *Populus* [9, 13] was determined. Research as for the accumulation of pollutants in the buds,
except for some scientists, is practically not available [1]. There are few reports in the literature presenting cytogenetic effects, caused by contaminants of urban surroundings in woody plants [2, 6]. At the same time, Populus species are characterized by firmness to the urban surroundings factors, they are widespread in urban flora and, as perennial plants, are suitable objects for biological screening and monitoring.

In this connection, in our research we aimed at establishing the degree of cytogenetic activity and the level of the environmental pollution by heavy metals in different areas of the city soil as shown by respective indices of vegetative P. berolinensis buds.

The researches were conducted in the city of Ivano-Frankivsk. The zone of its compact urban buildings has the area 92.8 km², 25 % of which is an industrial territory, 60 % – housing and 15 % – municipal area. The population is 252000.

While determining zones of research we were guided by the general architectural plan of urban buildings and the data of the state department of ecological security in the Ivano-Frankivsk region. The territory of the city was divided into five districts: North, Northeast, Central, South, Southeast and the Northeast industrial suburb – village Yamnytsa. The relatively non-polluted terrain, approximating in soil-climatic conditions (settlement Rozhniativ) served as control.

According to the data of the state department of ecological security in the Ivano-Frankivsk region in the urban system terrain there are 65 firms, basically mechanical engineering, radio-electronic and a chemical industry, which together with transportation, discharge into the atmosphere annually approximately 40000 tons of toxic substances. In the Northeast industrial suburb, there are major enterprises of chemical (plants of fine organic synthesis) and building (cement-slate works, asphalt-concrete works) industries, the annual general discharge of manufacturing waste compounds of which is to about 110000 tons.

In each respective area not less than 10 meters from highways, we picked out five trees of the indicated species of poplar: of the same age (60-80 years), sex and satisfactory sanitary state. For the prospective research, we took 3rd – 5th vegetative buds from the inferior layer of the top of each selected tree on the northwest side on one-year shoots. This procedure was performed in the period of vernal sap moving.

That part of buds, which was planned to be used for cytogenetic analyses, was fixed in Carnoy mixture at t=0-4°C for 24 hours. 4 % acetoironhematoxylin was used for staining. Subsequent clarification, preservation and making squash-slides were performed in Goyer's mixture [15]. Not less than 300 anaphases (test for chromosome aberrations) and not less than 1000 dividable cells (for quantitative and qualitative estimation of their mitotic activity) of each tree were analysed.

Vegetative buds, planned for energy-dispersion X-ray microanalysis, were washed in a mixture of detergents (EDTK), then in deionized water and incinerated [5]. The ash was formed into a tablet. It was mounted on a copper stage with the help of electrically conductive glue and evaporated by a layer of carbon in vacuum. Then the preparation was subjected to bombardment with an electronic beam in a scanning electron microscope REMMA-102 E. Microanalysis was made using an installation for energy-dispersion X-ray microanalysis on a micron level and the method of registration of energy and intensity of a characteristic X-radiation in a definite range. A concentration of the respective chemical elements (mg/kg of ash) was determined in each version. Using the results of quantitative determination the individual (the ratio of the quantitative contents of a contaminant in experiment to monitoring value) and summarised (sum total of individual) coefficients of heavy metals accumulation were calculated.
For the carrying out of test on sterility of pollen, we took flowering inflorescences during the period of mass flowering from the inferior layer of the top of each choose tree on the leeward side on one-year shoots. Then it fixed in Carnoy mixture at \( t=0-4^\circ C \) for 24 hours. Calculation of sterile pollens realised by the acetocarmine method [12]. 600-1000 pollens were analysed from each tree. Further, the coefficient of pollen's sterility was calculated.

All the findings obtained were statistically evaluated (Student's t-test, \( P<0.05 \)). Correlation analysis was carried out too.

As a result of the conducted researches we have determined that absolute values of the contents of the majority of the studied heavy metals in buds of plants growing in urban system conditions are much higher than in the control (Figure 1). Trees from Northeast, Central and North districts of the city accumulated the maximum quantity. Nickel and Zinc contents increased, most of all – 2.5-4.0 and 2.2-4.0 times. Pb, Cu and Cd content were somewhat less – 2.0-3.0, 1.5-3.0 and 2.0-3.2 times, accordingly. The amounts of the other metals did not differ from monitoring parameters significantly. Thus, it is possible to present the change of accumulation coefficients, and, accordingly, the ratio of each trace element under the effect of the urban system in such series: Ni > Zn > Pb > Cu > Cd > Cr > Al > Fe = Mn.

In the urban conditions, we observed an increase in the damage level of \( P. \) simonii buds rudimentary leaves meristem cells chromosome apparatus (Table). Especially, a large percent of aberrant anaphases was observed in plants of the Central region and the industrial suburb, respectively, 4.0 and 6.8 times more than in the control. Somewhat smaller amounts of cells with rearrangements were observed in Northeast and North regions. There, this parameter exceeded the control values by 87% and 136%. In the above regions, the authentic augmentation of aberrations quantity, per one investigated cell, was observed too. In other regions (Southeast and South) statistically significant differences as to the used cytogenetic characteristics were not revealed.
Table

<table>
<thead>
<tr>
<th>District of research</th>
<th>Number of anaphases</th>
<th>Percentage of anaphases with aberrations (M±m)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Total</td>
<td>With rearrangements</td>
</tr>
<tr>
<td>The control</td>
<td>1539</td>
<td>16</td>
</tr>
<tr>
<td>Southeast</td>
<td>1519</td>
<td>18</td>
</tr>
<tr>
<td>South</td>
<td>1518</td>
<td>21</td>
</tr>
<tr>
<td>Northeast</td>
<td>1536</td>
<td>30</td>
</tr>
<tr>
<td>North</td>
<td>1545</td>
<td>38</td>
</tr>
<tr>
<td>Central</td>
<td>1552</td>
<td>64</td>
</tr>
<tr>
<td>Industrial suburb</td>
<td>1519</td>
<td>107</td>
</tr>
</tbody>
</table>

Notice: * - significant differences in comparison with the control

The analysis of mitotic activity has shown (Figure 2), that in the Northeast and North city's districts and in the industrial suburb it is significantly different from the control. While analysing the distribution of cells in mitotic phases, we found out, that in the Southeast and South areas of the city it was practically the same as in the control. In all other remaining areas, we observed definite changes (Figure 3). First, it is necessary to point out, that we observe a significant decrease in the quantity of prophase cells and an increase in metaphase and telophase queues. In all this areas of the city, except for Northeast district, the very process of a mitotic division (the prophase index becomes less than the metaphase one) was observed.

Fig.2. Mitotic index of rudimentary leaves meristem of *P. simonii* at experimental sites of Ivano-Frankivsk city.
According to got results (Figure 4), pollen's sterility does not differ from control in all investigated districts of the Ivano-Frankivsk city, except for Central and industrial suburb where it exceeded significantly in 4.1 and 3.0 times, accordingly.

Fig. 3. Distribution of cells in mitotic phases of rudimentary leaves meristem of *P. berolinensis* in different urban system's areas.

Fig. 4. Sterility of pollen (%) of *P. simonii* in different districts of Ivano-Frankivsk city.
The findings, obtained by us, concerning accumulation of Zn, Cd, Ni and Pb in plants, growing in city conditions, are evidently connected with a greater transport load than in the industrial suburb. It is said that aerosols entering through the surface of the leaf [14] or dilated stomas of leaves in winter [21] determine an increase in the contents of contaminants in shoots of urban plantations, mainly. To a definite degree, it can be connected with their excessive absorption by the root [7]. The latter is due to the fact that though in city conditions the upper layer of soil (0-10 cm) usually gets polluted, and migration of heavy metals in underlayers of soil is hindered. It is found in Ivano-Frankivsk that the number of their mobile forms exceeds boundary-permissible concentrations 1.5-3.0 times [11]. Their mutual antagonism as well as that may account for the absence of an accumulation effect of iron and manganese with zinc, cuprum and nickel [7].

The significant amounts of aberrant anaphases in the industrial suburb, to all appearances, are connected with the emission from the major industrial firms located there. Such treatment agrees with the data research results of other writers concerning the evaluation of genetical tension in other industrially transformed areas [2]. A high level of karyokinesis pathology, typical of the Central and Northeast areas of the city, must be accounted for by discharges of a number of industrial enterprises located there as well as intensive movement of motor vehicles. Similar results from trees of the urban environment were obtained by other scientists [6]. The authentic augmentation, observed by us, of the anaphases ratio with rearrangements in the North and Northeast districts was surprising to some extent. These terrains from the results of a contamination bioindicator using lichens were referred to as rather non-polluted [8]. At the same time, previously Kurinny et al., indicated a possibility of absence of direct dependency between the contamination rate and the mutagenic strain in definite terrains [10]. This is in line with the correlation analysis made by us. Close, in value, the parameters of chromosome apparatus damage in the control and in Southeast and South districts of the city, possibly, indicate the fact that the contamination of the indicated terrains does not exceed "buffer" capabilities of the ecosystems formed there.

A decrease of the mitotic index in the village Yamnysa should be treated as a non-specific reaction of a vegetative organism to intensive stress factors (chemical contamination), associated with adaptive process [4]. The accumulation of metaphase cells in *P. simonii* in the Central and North districts and industrial suburb can indicate that the environmental pollutants inhibit processes of formation and/or of the normal operation of the division’s spindle. The increase in quantity of cells in telophase, especially in Northeast regions of the city and industrial suburb, to all appearances, is a consequence of disturbance in the phragmoplast formation.

A sterility level of pollen characterises not only general toxicity of environment, but mediated reflects intensity of mutagenic pressure [3] because appearance of sterile pollens is the consequence of disturbance, that took place in meiosis.

On the basis of the mutagenic background evaluation [3], it is possible to say that the state of the investigated terrains can be described as: satisfactory – in the Southeast and South districts of the Ivano-Frankivsk, alarming – in the Northeast district, critical – in the North district and dangerous – in the Central district and industrial suburb.

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Була проведена диференційна оцінка мутагенного фону урбосистеми Івано-Франківська. Для цього ми використали однорідні (за віком, статтю і санітарним станом) дерева *Populus simonii* Carr. Забраний матеріал (вегетативні бруньки) був досліджений на вміст мікроелементів: Fe, Mn, Cu, Al, Pb, Zn, Cr, Cd, Ni (метод енергодисперсного рентгенівського іміджингу), а також для вивчення мітотичної активності та рівня аберацій хромосом (анафазний метод) у меристематичній тканині зачаткових листів. У деревних рослин вивчали також стерильність пилкових зерен. Концентрація більшості важких металів у рослинних тканинах збільшувалась із зростанням техногенної навантаження. В окремих районах спостерігалося значне зростання кількості хромосомних аберацій, мітотична інгібіція і підвищення рівня стерильності пилку. За результатами досліджень дана оцінка стану районів міста за мутагенним фоном.

Стаття надійшла до редколегії 12.11.2002
Прийнята до друку 20.01.2002