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Using of *Taraxacum officinale* (L.) pollens for the urban park bioindication

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Palinoindication of 6 parks of the urban ecosystem of Kiev using *Taraxacum officinale* (L.) Weber ex F.H. Wigg. population, was carried out. There is a general trend towards an increase in the amount of nonviable pollen with an increase in anthropogenic load. In the example of T. officinale, this is manifested in a gradual increase to 27% of non-viable pollen on the pollution gradient. With the deterioration of the ecological situation in the studied test polygons, an increase in the number of teratomorphic pollen grains up to 41.10% in Babin Yar tract was recorded. According to the level of palynotoxic action (PE, %), only 2 gradations were revealed in the parks under study: initial and effective. The sublethal effect (highly toxic) has not been established, since the level of pollen sterility in our studies did not exceed 45,2%. The largest number of pollen grains with developmental pathologies (teratomorphic) – 41.10% – was recorded in the Babin Yar tract, the state of which, according to the anthropogenic load index (Jal), is characterized as poor. At the same time, the percentage of teratomorphic pollen grains in T. officinale plants in more favorable environmental conditions (control) does not exceed 16.50%. For the bioindication assessment of the park ecosystems state in the megalopolis, the most indicative were the index of sterility, palynotoxic effect and the content of teratomorphic pollen grains, which increases with the gradient of anthropogenic load.
Keywords: Anthropogenic load; Pollen sterility; Fertility; Palynotoxic effect; Teratomorphic pollen; Urbanization

Introduction

Anthropogenic pressure on urban ecosystems is currently accompanied by environmental pollution with xenobiotics. The negative human impact is manifested in the destruction of biotic groups, changes in functioning, degradation of natural biotopes, global changes in landscapes and climate (Cuinica et al., 2014, Azzazy, 2016, Leghari et al., 2018). In cities, air pollution is one of the main problems that affect the biological systems and the quality of life of residents (Khannanova, Arkanova, 2017, Miroshnik, Teslenko, 2018). Industry and motor transport are the main contributors to air pollution. Kyiv is the largest city, capital, industrial, scientific and cultural center of Ukraine; located in the center of Eastern Europe, in the north of Ukraine, on the border of Polissya and Forest-Steppe on both sides of the Dnieper River. The area of the city is 836 km^2, including the green zone (460 km^2 or 55%), water areas (62 km^2, 7%), artificial urban ecosystems (314 km^2, 38%), built-up lands of the city – 364.0 km^2 (43.5%) (The main indicators..., 2017). The territory of Kyiv is characterized by a complex relief, in the conditions of which air masses with a high concentration of pollutants are formed. The city has a powerful system of green spaces and objects of the natural reserve fund.

The high level of air pollution in Kyiv as one of the consequences of urbanization poses a threat to public health and the natural environment. Airborne industrial pollution of the city is caused by emissions from stationary (industrial enterprises, CHP) and mobile sources (river and motor transport, aviation equipment). Air pollutant emissions from stationary and mobile sources indicate an annual increase in harmful outflow into the atmosphere (Miroshnyk&Teslenko, 2018). Environmental monitoring is carried out primarily to receiving the information and control anthropogenic impact on the biosphere (Cuinica et al., 2014, Grishko & Komarova, 2016, Morozova et al., 2019). The most sensitive to environmental changes are bioindicator plants, which are characterized by a pronounced adaptability to certain environmental conditions; with their help one can assess the environmental health and the consequences for nature and humans (Dzyuba, 2006, Ibragimova, 2015). Many scientists today are conducting research on the state of biota in metropolitan areas at different levels of the organization. Systematic studies of the green plantations state in the conditions of urbanization at the cellular level with the help of pollen sensitivity, can be carried out with the use of long-term dynamics or with the correlation of urbanization level parameters and data on the integrated state of plantations.

One of the most promising and available approaches to determining the state of the environment at the cell level is a change of the reproductive plant structures (pollen), which exhibit significant sensitivity to pollutants (Komarova, 2019). Palinoindication is used as

a unit of an environmental monitoring system and a quantitative indicator of the negative impact of xenobiotics (Cuinica et al., 2014, Leghari et al., 2018).
The study of the urbanization influence on the reproductive capacity of plants, as well as on the assessment of the quality of the environment using palinoindication, is characterized in the articles (Dzyuba, 2006, Grishko, Komarova, 2016, Hannanova, Arkanova, S. 2017, Komarova, 2019, Morozova et al., 2019, Soldatova, Samsonova, 2019). The analysis of the variability of pollen traits in different plant species growing in the city and the possibilities of its use for assessing the quality of the environment was carried out by (Dzyuba, 2006, Matyashuk, 2014, Ibragimova, 2015, Cuinica et al., 2014, Grigoruk & Optasyuk, 2018, Leghari et al., 2018, Grishko & Komarova, 2016, Komarova, 2019, Morozova et al., 2019, Soldatova & Samsonova, 2019). Researchers note a decrease in fertility, an increase in the number of teratomorphic and sterile pollen grains, a change in morphology, a decrease in viability, and other changes in the pollen quality.
Using the example of several cities in the North-Western region of the European part of Russia, O. Dzyuba demonstrated that the nature of pollen grains pathologies in 36 species of angiosperms is a consequence of pollution (Dzyuba, 2006). The possibility of using flower crops (Hemerocallis L., Canna L.) to determine the effect of pollutants on their generative organs was investigated (Chipilyak, 2014, Matyashuk, 2014). When studying the generative sphere of woody species *Salix babylonica* L*., Aesculus hippocastanum* L., *Tilia cordata* Mill., *Pinus sylvestris* L., *Larix sibirica* Ledeb. and others, a direct relationship between the level of environmental pollution and the sterility of pollen was demonstrated. It has been shown that the amount of abortive pollen increases in plants growing in zones of industrial enterprises and near roads with heavy traffic, therefore they can be used as bioindicators for assessing technogenic territories (Kaigorodov et al., 2010, Cuinica et al., 2014, Ibragimova, 2015, Grigoruk & Optasyuk, 2018, Leghari et al., 2018, Soldatova & Samsonova, 2019). The relationship between the quality and quantity of the *T. officinale* male gametophyte with the growing conditions, characteristics of the habitat and pollution was studied by (Kaigorodov et al., 2010, Hannanova & Arkanova, 2017, Komarova, 2019, Morozova et al., 2019). However, a comprehensive study of the relationship between the quality of *T. officinale* pollen and anthropogenic impact within the metropolis has not been carried out. We have begun bioindication assessment of the park ecosystems state in Kyiv, depending on the conditions of the urban environment, using the pollen sensitivity indicators of *T. officinale*.

Materials and Methods

The object of the research is *T. officinale*, a perennial herb of the family *Asteraceae*, which spontaneously grow in almost all biotopes of Kyiv and are convenient objects of research. Earlier, we assessed the ecological situation in the administrative districts of Kyiv by calculating the integral index of anthropogenic load (*Jal*). Then the ecological zoning of the city districts was carried out according to the degree of ecological dysfunction (Miroshnyk & Teslenko, 2018) (Table 1). For research in 2019, test polygons were taken in five administrative districts of Kyiv, which differed from each other in terms of the air pollution level, water objects, saturation of the city's territory with a street and road network, etc. (Miroshnyk & Teslenko, 2018). Goloseevsky district is located on the outskirts of the city and borders on the "green belt" of forest parks, the anthropogenic load index (*Jal*) is 1.66 (Figs 1 and 2). Therefore, the ecological situation here is characterized as very good. In the park, the monument of landscape gardening art "Feofania" (hereinafter MLGA "Feofania") is a control, test polygon No 1 was selected, which is located about 1000 meters from the road. This park is surrounded by the remains of a forest, so the index *Jal* situation is also very good. A good ecological situation in terms of the numerical values of the index (*Jal* is 3.64) is also in the Peremoha Park (Darnytsia region), which is located on the left bank of the Dnieper River. There is a test polygon No 2; the distance from the road is 80 meters. The most unfavorable are the central districts of the city, including Shevchenkivsky. It houses 71 enterprises, ranks first in terms of the amount of municipal solid waste and is densely populated (The main indicators..., 2017). Indicators of anthropogenic load are the highest in Kyiv: *Jal* = 12.82 and the ecological situation is bad. In the Babin Yar tract, test polygon No 3 was selected, which is located 6 meters from the intensive traffic central road (Figure 1). Mariinsky Park in the Pechersk district (central district of the city), the smallest in area, but densely populated, with a high percentage of high-rise buildings and roads, ranks third in terms of solid waste emissions, has a satisfactory environmental situation (*Jal* = 7.37). The distance to the road of the experimental landfill is about 226 m. In the Obolon district, the ecological situation of which is characterized as very good, the anthropogenic load index is 1.99, in the Pushcha Voditsa Park there is a test site No 6, the distance from the road is 238 m (Fig. 1).
For the study, *T. officinale* pollen was taken at the stage of mass flowering. Considering that fertile and sterile cells of plant pollen differ in starch content, the quality of pollen was determined by the Iodine method (Gram staining) (Pausheva, 1980). All pollen grains without typical signs of morphological structure (that is, everything except normally developed ones) were considered teratomorphic. Laboratory studies were carried out using a Nikon ECLIPSE microscope and a Canon DS 126291 camera.

Pollen sterility (*PS*) was calculated using Formula 1:

$$PS = \frac{G}{N} \times 100\%, \quad (1)$$

where *G* is the amount of sterile pollen (pieces), *N* is the amount of pollen being examined (pieces).

Pollen fertility (*PF*) was calculated using Formula 2:

$$PF = \frac{P}{N} \times 100\%, \quad (2)$$

where *P* is the amount of fertile pollen (pieces), *N* is the amount of pollen studied (pieces).

The sterility coefficient (*Cstp*), which determines how many times the frequency of the induced level of sterility exceeds the level of spontaneous sterility in the control, was calculated using the Formula 3:

$$Cstp = \frac{Ssa}{Sc}, \quad (3)$$

where Ssa is the number of sterile pollen grains in the study area, pieces; Sc – number of sterile pollen grains in the control area, pieces.

The pollen sensitivity coefficient (*Cps*) of *T. officinale* pollen was determined by the Formula 4:

$$Cps = \frac{F}{S}, \quad (4)$$

where *F* is the number of fertile pollen grains at the test site, pieces; *S* is the number of sterile pollen grains at the test site, pieces.

Palynotoxicity of environmental pollutants using the reproductive system of plants was determined by the Formula 5:

$$PE = \frac{Fc - Ft}{Fc} \times 100\%, \quad (5)$$

where *PE* – palynotoxic effect, *Fc* – indicator of the value of plants pollen fertility in the control zone, pieces; *Ft* – indicator of the value of plants pollen fertility growing on the test polygon, pieces (Balichieva et al., 2006).

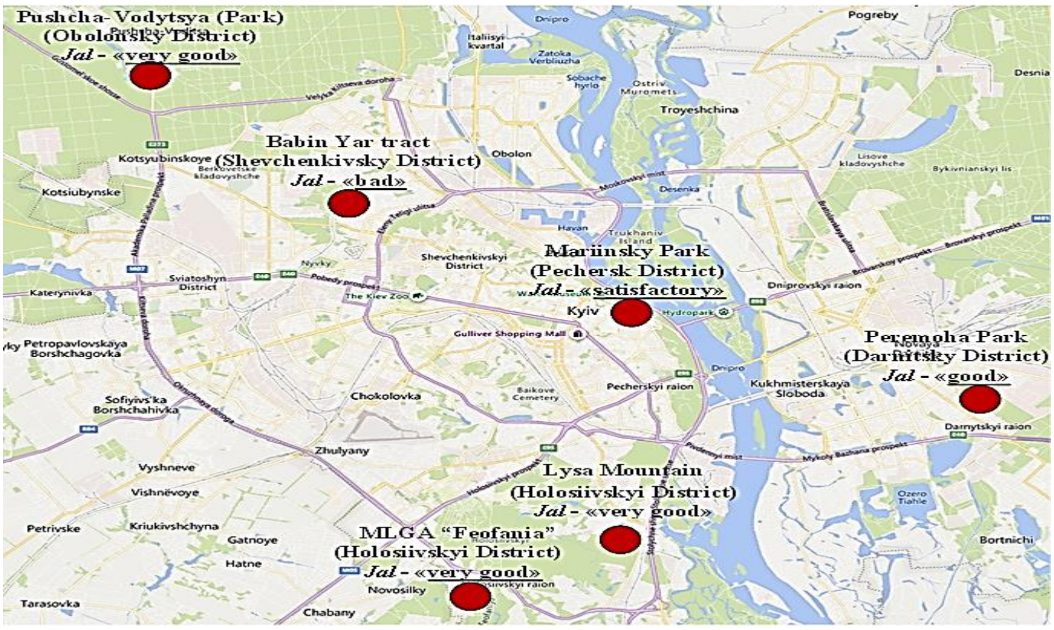

Fig. 1. Test polygons in Kyiv.

The test polygons were ranked according to the environment effect – EE_{10-90} classification: initial (slightly toxic), effective (medium toxic) and sublethal (highly toxic), the content of toxic concentrations of pollutants in the studied areas – respectively EE_{10}, EE_{50}, EE_{90}, at which inhibition of the fertile pollen production in the studied plants was observed as 10, 50 and 90% compared to control. Based on the obtained results, a conclusion about the degree of park ecosystems pollution in Kyiv was made (Balichieva et al., 2006). Statistical analysis of the data was carried out using the Microsoft Office Excel 2013 program. The mean value of the trait (M), variance (D) and coefficient of variation (V) were calculated. Variation of the trait was considered weak at V <11%, average at V = 11–25%, and strong at V> 25% (Lakin, 1990).

Results and Discussion

Differences in the sterile pollen production were found between the populations of *T. officinale* growing in areas with different levels of pollution. Sterility of pollen in *T. officinale* plants at the test site of Pushcha Voditsa Park was 13%, which does not exceed this indicator in the control. This is due to the very good ecological situation of the Obolon district and the park remoteness from the center of the metropolis. So, in the Babin Yar is the highest level of pollen grains abortion was recorded – 26.86%, this is 2.3 times more than in the control. In the Peremoha Park, Darnitsky region, which has a good ecological situation, the percentage of starch-free pollen grains exceeded the control by 1.7 times (Figure 2). Thus, with the increase of Kiev's park ecosystems pollution there is an increase in production of abortive pollen of *T. officinale*.

Calculation of the sterility coefficient of pollen grains (*Cstp*) of *T. officinale* revealed a difference in the production of sterile male gametes between test polygons No. 3 and No. 4. In Shevchenko district, where the level of anthropogenic load is characterized as poor, the pollen sterility indicator is 1.64 (test polygon No. 3). The test site No 4 is located near high-traffic roads. The highest indicators of pollen sterility coefficient 1.64 and 1.54 were recorded at test sites in Babin Yar and Lysa Hora, respectively. At the test site No 6 in the Pushcha Voditsa park, this coefficient is equal to 1, i.e. the ecological situation is equated to control (Figure 2).

Since plants that alter the production of abortive pollen with an increase in environmental pollution are sensitive, they can be recommended as phytoindicators of green spaces in megalopolicies. In different test sites, we observed a different ratio of fertile and sterile pollen grains (*Cps*) of *T. officinale*, depending on the level of anthropogenic load. The highest sensitivity coefficient 7.66 was noted in ecologically favorable conditions in the MLGA "Feofania", Goloseevsky district (control) and in Pushcha Voditsa park – 6.56. At other test sites there was a tendency to reduce this indicator, so in the Babin Yar this coefficient decreased by 2.7, and in Lysa Hora by 2.4 times. In the most polluted Babin Yar tract of Shevchenkivsky district, this indicator decreased 2.7 times compared to the control. The trend of 2.1 times decrease in *Cstp* compared to the control was also observed in the Darnytsky district in Peremoha Park. Between the experimental test polygons, the Csp indicators differ insignificantly; in the Babin Yar tract this indicator is 1.2 times lower than in Peremoha Park.

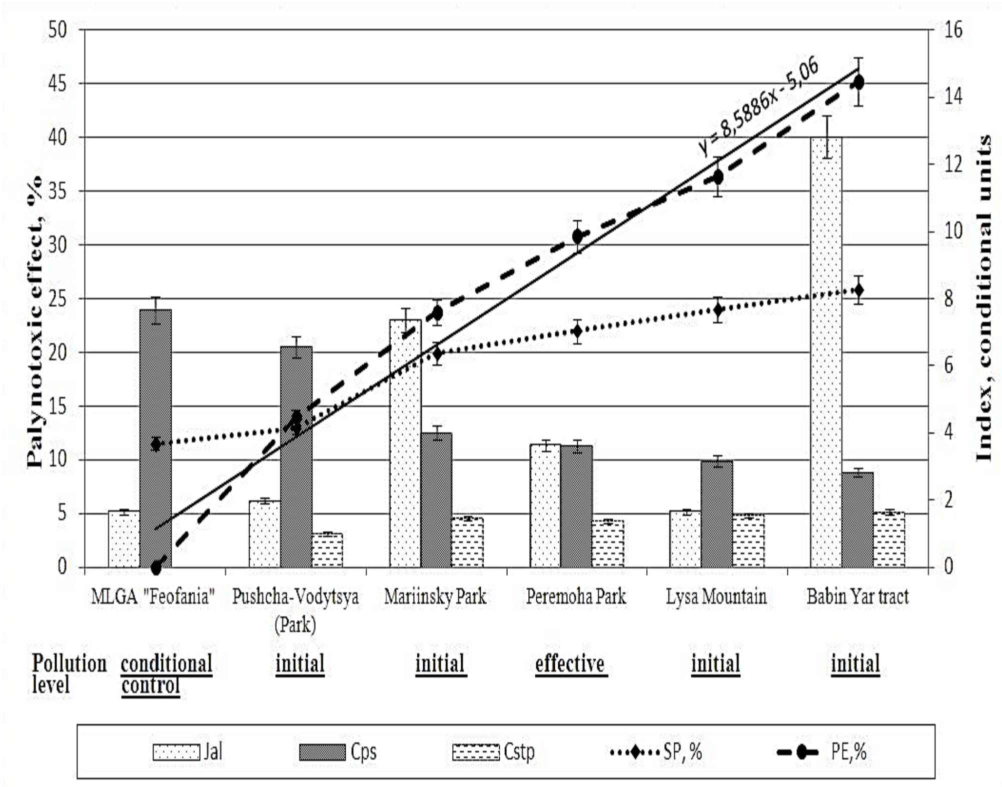

Fig. 2. Indicators of *T. officinale* pollen sensitivity in park ecosystems of Kyiv. SP, % – sterile pollen grains, *Cps* – pollen sensitivity coefficient, *Cstp* – pollen sterility coefficient, PE, % – palynotoxic effect.

When analyzing our data, the studied districts of Kiev were ranked according to the EE10-90 classification. It was found that at the test site No 2, 4, 5, 6 toxicants showed a slightly toxic effect on gametogenesis, where the PE index did not exceed 36.4%. In the Babin Yar tract of Shevchenkivsky district, pollutants effected (moderately toxic) on the male reproductive organs; the index PE was 45.2%. Therefore, in regions with an increased anthropogenic load, pollutants affect the production of pollen from *T. officinale* plants. According to the level of palynotoxic action (PE, %), only 2 gradations were revealed in the parks under study: initial and effective. The sublethal effect (highly toxic) has not been established, since the level of pollen sterility in our studies did not exceed 45.2% (Figure 2). Thus, the *T. officinale* male generative system is sensitive to high levels of pollution. The production of abortive pollen increases with the growth in pollution of park ecosystems in Kiev. Similar changes, namely, an increase in the sterility of *T. officinale* pollen on the gradient of industrial air pollution, were noted by other researchers (Azzazy, 2016, Grishko, Komarova, 2016, Hannanova, Arkanova, 2017, Morozova et al., 2019). Researchers (Grishko, Komarova, 2016) noted that indicators of sterility and palynotoxic effect are more informative for bioindication of an industrial city using *T. officinale*. Our research confirms that under conditions of different levels of anthropogenic impact on park ecosystems in Kiev, indicators of infertility and palynotoxic action are effective. At the same time the sensitivity coefficient does not always show a clear difference with the level of anthropogenic load.

An additional indicator of the gametocidal effect of pollution is the amount of morphologically altered (teratomorphic) pollen. The worse the ecological situation is, the higher the percentage of pathologically developed pollen is and vice versa (Dzyuba, 2006). In our study, in addition to the typical pollen grains of *T. officinale* (symmetrical, rounded), teratomorphic grains with the following anomalies were revealed: nanism (pollen dwarfism), pollen grains with a diameter less than 18 microns – 29.7–34.6%; gigantism of pollen grains, pollen diameter exceeds 48 μm – 31.5–38.6% of teratomorphic pollen; deformation of the shell (crumpled or torn), this kind of anomalies is in 32.8–40.3% pollen grains of *T. officinale* (Table 1).

Table 1. Characteristics of *T. officinale* pollen in the park ecosystems of Kiev.

Test polygon	Sterile				Fertile				Teratomorphic			
	d, M ± m	D	V	W	M ± m	D	V	W	Number	D	V	W
MLGA “Feofania”	24.29±0.51	18.26	18.00	11.55	41.40±0.65	33.43	14.13	88.45	336	2.55	31.23	16.50
Lysa Mountain	26.34±0,40	12.09	13.20	23.97	38.08±0.60	45.89	18.00	76.03	606	9.62	32.00	40.24
Pushcha-Vodytsya (Park)	26.58±0.38	13.25	14.27	13.00	43.40±0.50	24.64	11.00	87.00	351	2.60	22.50	19.70
Mariinsky Park	26.06±0.41	22.52	18.40	19.92	34.91±0.61	76.47	25.31	80.08	582	3.16	19.08	30.79
Peremoha Park	26.35±0.50	18.19	16.21	19.60	40.81±0.54	29.11	13.00	80.40	546	2.64	18.00	33.90
Babin Yar tract	29.31±0.46	15.96	13.63	26.86	40.84±0.32	14.48	13.63	73.14	585	2.19	20.37	41.10

d – pollen siameter, μm, D – variance, V – variation, %, W – share of pollen grains, %.

The largest number of pollen grains with pathologies in development – 41.10% – was recorded in the Babin Yar tract, the state of which, according to the anthropogenic load index (*Jal*), is characterized as poor. High rate of teratomorphism – 40.24% of pollen grains in *T. officinale* plants was also noted at the test site No 4 in the tract of Lysa Hora, which is located in the center of a metropolis with developed traffic. At the test site No 6 (Pushcha Voditsa Park), the percentage of anomalies in the development of pollen in *T. officinale* plants was 19.7%. This is due to the remoteness of the park from the city center. At the same time, the percentage of teratomorphic pollen grains in *T. officinale* plants in more favorable environmental conditions of MLGA "Feofania" does not exceed 16.50%. Nevertheless, there is the greatest variation of this feature (V = 31.23%) (Table 1).

Conclusion

Palinoindication of 6 parks of the urban ecosystem in Kiev using *T. officinale* was carried out. There is a general trend towards an increase in the amount of nonviable pollen with an increase in anthropogenic load. In the example of *T. officinale*, this is manifested in a gradual increase to 27% of non-viable pollen on the pollution gradient. With the deterioration of the ecological situation in the studied test polygons, an increase in the number of teratomorphic pollen grains up to 41.10% in Babin Yar tract was recorded. According to the level of palynotoxic action (PE, %), only 2 gradations were revealed in the parks under study: initial and effective. The sublethal effect (highly toxic) has not been established, since the level of pollen sterility in our studies did not exceed 45.2%. The most indicative of all the proposed sensitivity indices of the *T. officinale* male gametophyte under pollution conditions of park ecosystems in Kiev are the content of teratomorphic pollen grains, the indices of sterility, palynotoxic effect. The indicator of production of T. *officinale* abortive and teratomorphic pollen can be used in the system of ecological monitoring of environmental objects experiencing various degrees of anthropogenic pressure. In the future, we are going to study the relationship of morphological structure pollen and anthropogenic load.

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