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Pollen Quality in Woody Plants in the City Parks of Dnipro, Ukraine

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Abstract. This research was conducted to study pollen quality in woody plants in the city parks of Dnipro, which are located at various distances from the sources of anthropogenic pollution. It has been revealed that sterile pollen in its greatest abundance was present in plant species in the Park of Memory and Reconciliation and Molodyozhny park, as both are situated within the range of emission transfer from the Western Industrial Complex, while the least abundant sterile pollen was detected in the park named after Yu. Gagarin and that named after V. Dubinin. According to sterility indices, the pollen in *Betula pendula* Roth. and that in *Acer platanoides* L. were most sensitive to environmental pollution. In the city parks, the amounts of shrunk and abnormally shaped pollen grains increases; giant forms with grain diameters exceeding standard, as well as dwarf forms are not rare in occurrence. The highest percentage of pollen grains of atypical shape and size was observed in the same parks where the pollen sterility index had its highest values.

Introduction

In Ukraine, the city of Dnipro is one of the most heavily industrialized centers exposed to considerable anthropogenic loads attributable to the facilities of the metallurgical, machine-building, construction, chemical and other branches of industries operating in the region. This cumulative effect of the harmful air pollutants results in high levels of air pollution per unit of the urban area [1]. It is known that urban environment, in fact, is generally prone to accumulate sizable dust concentrations and abundances of hazardous air and soil pollutants, a substantial proportion of them being mutagens.

The concept of the comprehensive ecological monitoring [2] developed for the qualitative assessment of the environment involves the use of bioindicators as an integral part of the procedure. It is evident that this technique of evaluation of the environmental status is not intended to be a substitute or an alternative to the conventional physical and chemical methods of environmental research. Nevertheless, the application of this biomonitoring technique allows of substantial upgrading the accuracy of forecasting the environment status and plausible environmental changes resulting from human activities. Testing the pollen sterility, as a mediate indicator of mutagenic effects of chemical compounds, has been used for detection of mutagens in industrial effluents [3], for assessment of pollen characteristics in plants exposed to mutagenic impacts [4, 5], and also for estimation of phytotoxicity levels and mutagenicity of polluted environment [6–8].

Certain constituents in industrial and automotive emissions have negative impacts on pollen viability. Pollen exhibits a high sensitivity to ozone [9], sulfur dioxide [10] and nitrogen dioxide, hydrogen sulfide, and mercury and lead nitrates [11]. The concurrent influence of phytotoxicants can exacerbate their detrimental effects. Many studies report that the atmospheric pollutants at concentrations typical of urban levels resulted in a decline in pollen viability along with a strongly pronounced synergistic interrelation between various contents of sulfur dioxide, ozone and aldehyds [12].

The analyses of pollen collected from the trees of *Pinus silvestris* L. in polluted areas showed a decrease in the amounts of viable pollen grains and as much as 85 % of them were sterile and devoid of starch [13]. According to the data presented by E.A. Valetova and G.I. Yegorkina [14], the pollen from the background area (10 km from the source of pollution) had a higher percentage of pollen fertility and accumulated greater amounts of nutrients and physiologically active compounds than the pollen at the distance of 1 km from the urban industrial zone. Depression of the pollen grain viability potential in arborous and herbaceous species in the anthropogenic environmental conditions has also been revealed in a number of other investigations [15–18].

Recently, a series of studies have been undertaken with the view to assess the environmental situation in various urban industrial areas, on the basis of intensity of the gametocidal action on plant species [19–22].

Taking into account the fact that an anthropogenic stress has a pronounced negative impact on the qualitative characteristics of pollen in plant species, pollen sterility may be used as an indicative parameter of pollution levels in the urban ecosystems. A number of authors, in particular N.V. Vasilevskaya and N.V. Petrova [23] (the town of Monchegorsk, Russia), V.P. Bessonova et al. [21] (the city of Dnipro, Ukraine), I.I. Kosinova et al. [24] (the town of Petrozavodsk, Russia), E.E. Ibragimova and V.D. Balichiyeva [25] (the town of Simferopol, Ukraine), I.N. Tretyakova and N.E. Noskova [26] (the city of Krasnoyarsk, Russia), L.M. Kavelenova [27] (the city of Samara, Russia) and others have carried out zoning of the city territories according to their pollution levels based on the plant pollen characteristics.

The purpose of the present study was to use phytoindication as a method of environmental quality assessment based on pollen sterility parameters in woody plants as mediate bioindicators of the ecological status of park phytocoenoses under various conditions of aerotechnogenic origin.

Materials and Methods

The investigations have been carried out in the city parks of Dnipro that are exposed to varying levels of the aerogenic influence, including, the park named after Yu. Gagarin and that named after V. Dubinin, the Park of Memory and Reconciliation, the park named after B. Khmelnitsky and that named after L. Globa, Sevastopolsky park, Molodyozhny park, and the park named after T.G. Shevchenko. It is important to note that the latter four parks are reserved in Ukraine as the historical and cultural legacy of landscape-and-gardening art. The locations of the parks under study are depicted in Figure 1.

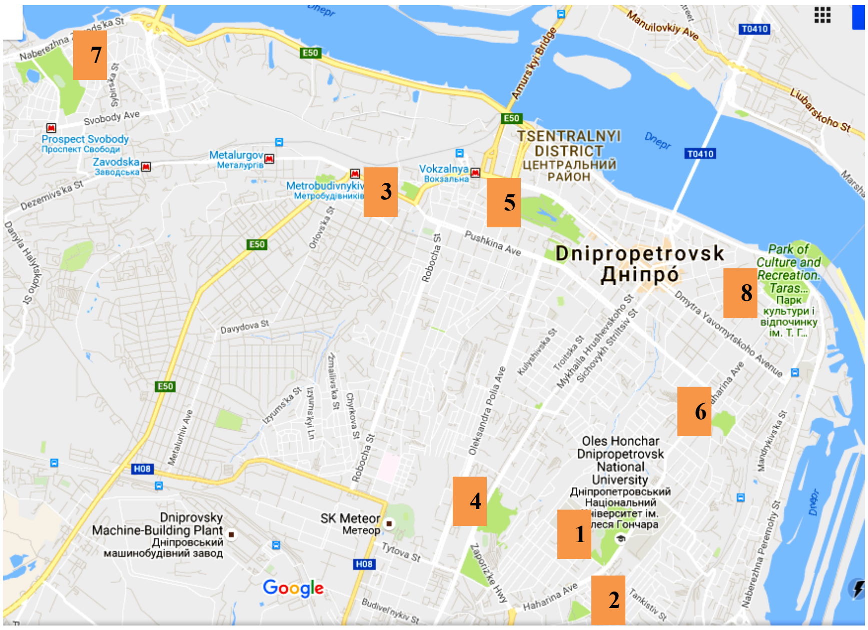

Figure 1: Park locations on the sketch map of the city of Dnipro: 1 – the park named after Yu. Gagarin; 2 – the park named after V. Dubinin; 3 – the Park of Memory and Reconciliation; 4 – the park named after B. Khmelnitsky; 5 – the park named after L. Globa; 6 – Sevastopolsky park; 7 – Molodyozhny park; 8 – the park named after T.G. Shevchenko

The Park of Memory and Reconciliation and Molodyozhny park are situated within the range of prevailing industrial emission transfer from the Western Industrial Complex. In the immediate vicinity of the above mentioned parks, there are motor roads with heavy traffic (about 4,000 vph). The park named after L. Globa and that named after B. Khmelnitsky are also contiguous with the streets with heavy traffic (4,000 vph and 3,000 vph, respectively). The plants in the park named after T. G. Shevchenko are affected by emissions from the facilities of the Eastern industrial group and those of the Northeastern industrial groups. These enterprises produce a less severe effect on the plants in Sevastopolsky park due to its location at a secluded site behind the buildings of a residential area. The park named after Yu. Gagarin is located in the area remote at a distance of about 10 km away from the intensively industrialized zones. Pollen samples for analyses were collected from the following woody species: pendent birch (*Betula pendula* Roth.), Norway maple (*Acer platanoides* L.), big-leaf linden (*Tilia platyphyllos* Scop.), black locust (*Robinia pseudoacacia* L.). The present research was carried out in 2015 at the beginning of the flowering period typical of each woody species being studied. The flower buds in the first two species mentioned above are set one year prior to flowering, whereas in the two latter species the flower buds are set in the flowering year. The samples were collected from the lateral branches of the similar order of branching on the southeast side of each tree crown and in the similar storeys of the tree canopies.

Pollen viability was determined by application of the iodine and acetocarmine methods [28] and using a microscope of Biomed-4 model. Photos of pollen grains were taken with the Digital Camera for Microscope DCM130. The test results obtained in the experiment were treated statistically. An individual sample of each plant species subjected to analysis consisted of minimal counting 1,000 pollen grains. An arithmetic mean value and a standard error value (SE) were calculated for each specific characteristic. The data were checked for normality of distribution by calculations of the parameters of asymmetry and excess. The Student's t-test criterion was applied to determine the significance of differences between the samples of the similar plant species in different parks and those in the control site.

Results

All the plants used as the test-objects in the present research are diploid species, which generate haploid pollen grains, and therefore they have offered a way of identifying lethal mutations. The plant pollen samples were of adequate quality and the pollen sterility in the control site did not exceed 5 %, by that was meant that the samples fully satisfied the quality requirements for a parameter or characteristic to be used for monitoring phytotoxicity and mutagenicity of the environmental pollution [6].

The pollen sterility in woody plants in the city parks was higher than that in the plants at the control site (Table 1). The most significant abundances of sterile pollen in all the investigated species were revealed in the plants growing in the Park of Memory and Reconciliation and Molodyozhny park, i.e. in the parks situated within the potential range of emission transfer from the Western industrial complex, while the smallest amounts of sterile pollen were found in the park named after Yu. Gagarin and the park named after V. Dubinin (Figure 2).

Since the contents of sterile pollen in all the plant species being tested differed from those contents in their plant counterparts in the control site, the pollen sterility index was used to ensure an unbiased comparison of the data. The pollen sterility index for each plant species was calculated as the ratio between the pollen sterility content in a test sample and the similar parameter in its counterpart sample in the control site [15]. Comparison of these characteristics in the investigated species of woody plants showed that the pollens in *Betula pendula* and *Acer platanoides* were actually most sensitive to the environmental pollution (Table 2). It should, however, be noted that in some instances the pollen sterility indices in *Tilia platyphyllos* and *Robinia pseudoacacia* had the closest values to the first two plant species mentioned above; and in the Park of Memory and Reconciliation, *Robinia pseudoacacia* exhibited even higher values of the pollen sterility indices then the first two plant species previously mentioned.

Table 1: Percentage of sterile pollen in woody plants in the city parks of Dnipro (n>1000)

Sampled locations	Sterile pollen contents [%]			
	Betula pendula	*Acer platanoides*	*Tilia platyphyllos*	*Robinia pseudoacacia*
Control site	2.41±0.09	3.10±0.10	2.25±0.09	1.96±0.07
Park named after Yu. Gagarin	6.60±0.13	8.03±0.25	4.95±0.15	3.96±0.14
Park named after L. Globa	17.30±0.56	23.21±0.95	14.74±0.53	12.09±0.45
Park named after V. Dubinin	7.70±0.18	10.91±0.44	6.52±0.21	6.08±0.23
Park of Memory and Reconciliation	25.48±0.89	30.44±0.96	20.47±0.83	31.20±0.98
Molodyozhny park	29.05±0.78	25.43±1.03	19.26±0.66	16.21±0.75
Sevastopolsky park	11.35±0.46	13.05±0.46	8.12±0.20	6.58±0.23
Park named after T.G. Shevchenko	14.70±0.53	16.42±0.68	8.82±0.22	10.99±0.47
Park named after B. Khmelnitsky	10.58±0.33	14.30±0.56	8.26±0.18	7.07±0.20

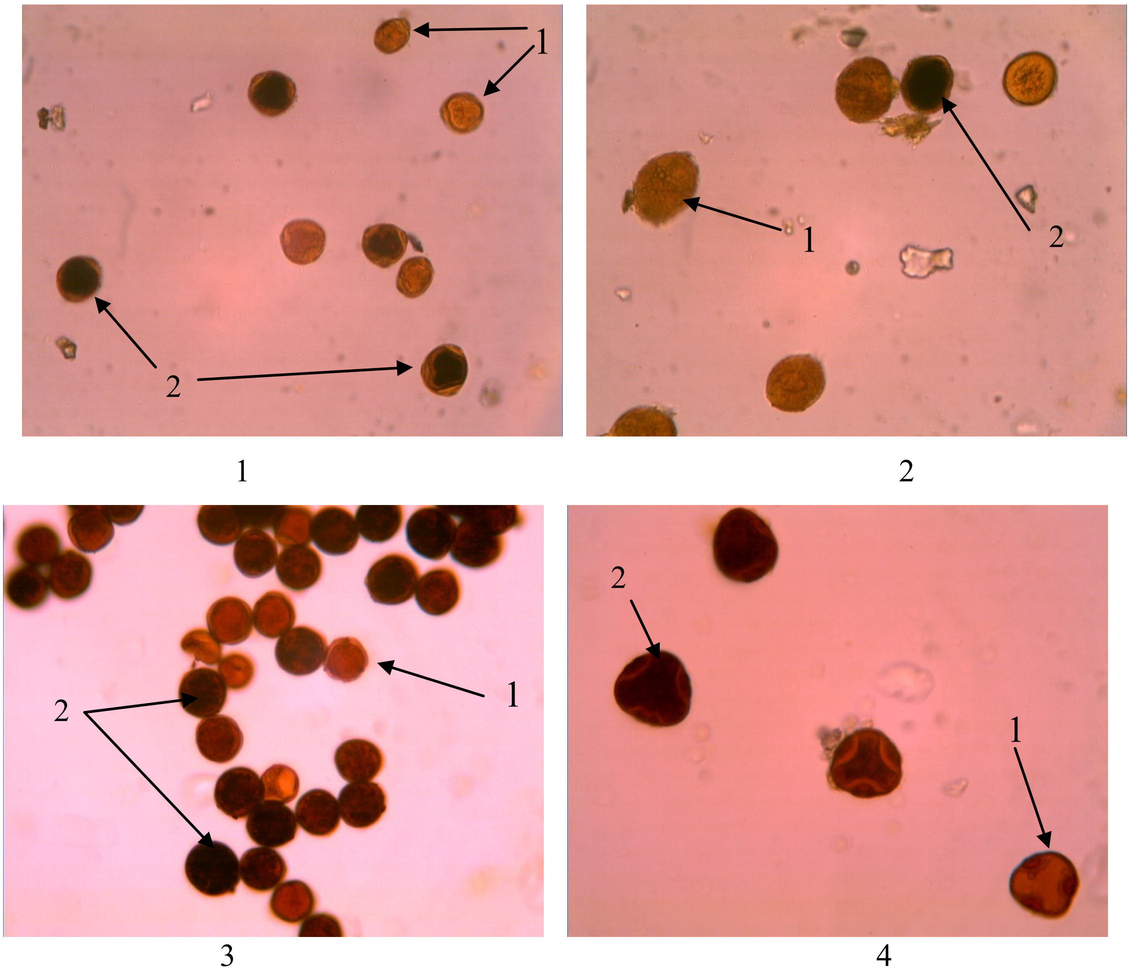

Figure 2: Pollen quality in woody plants in the city parks of Dnipro: 1 – *Betula pendula* (the park named after L. Globa); 2 – *Acer platanoides* (the Park of Memory and Reconciliation); 3 – *Robinia pseudoacacia* (the park named after T.G. Shevchenko); 4 – *Tilia platyphyllos* (the park named after Yu. Gagarin); 1 – sterile, 2 – fertile

Table 2: Pollen sterility indices in woody plants in the city parks of Dnipro

Sampled locations	*Betula pendula*	*Acer platanoides*	*Tilia platyphyllos*	*Robinia pseudoacacia*	Average index
Park named after Yu. Gagarin	2.73±0.12	2.59±0.11	2.20±0.10	2.02±0.09	2.38±0.09
Park named after L. Globa	7.18±0.25	7.48±0.27	6.55±0.21	6.17±0.20	6.89±0.21
Park named after V. Dubinin	3.19±0.13	3.52±0.14	2.89±0.10	3.10±0.13	3.17±0.10
Park of Memory and Reconciliation	10.57±0.33	9.82±0.39	9.09±0.33	15.91±0.25	11.15±0.12
Molodyozhny park	12.05±0.40	8.20±0.21	8.56±0.20	8.27±0.20	9.26±0.22
Sevastopolsky park	4.71±0.13	4.21±0.10	3.61±0.12	3.36±0.12	3.97±0.14
Park named after T.G. Shevchenko	6.09±0.21	5.29±0.16	3.92±0.15	5.61±0.11	5.24±0.12
Park named after B. Khmelnitsky	4.39±0.11	4.61±0.19	3.67±0.14	3.61±0.11	4.06±0.10

According to the data obtained, the highest values of pollen sterility indices were found in the plants of the Park of Memory and Reconciliation and Molodyozhny park, both located within the urban industrial area. Somewhat lesser, but still rather high values of pollen sterility indices were recorded in the plant species in the park named after L. Globa. The value of the pollen sterility index in the plant species in the park named after B. Khmelnitsky and that in the plants in Sevastopolsky parks were very close to each other. As represented in Table 2, depending on a particular plant species accepted as a test-object for monitoring, the values of the pollen sterility indices, for example, in the park named after T.G. Shevchenko were either higher than those in Sevastopolsky park and those in the park named after B. Khmelnitsky or did not differ much from one another, being almost equal, as it was the case with the big leaf linden.

Plant pollen in the control site was characterized by moderate polymorphism. Polymorphic pollen grains in greater abundances were detected in the trees of the parks, therefore the city parks were ranked in the order of increasing abundance of polymorphic pollen grains: the park named after Yu. Gagarin < the park named after V. Dubinin ≤ Sevastopolsky park = the park named after B. Khmelnitsky < the park named after T.G. Shevchenko < Molodyozhny park < the park named after L. Globa < the Park of Memory and Reconciliation. In the city parks, the amounts of crumpled pollen grains and those of abnormal shape and size have increased; gigantic pollen grains, with diameters many times greater than standard, as well as dwarf pollen grains were not rare in occurrence (Table 3, Figure 3). The most noticeable increase in the abundances of defective pollen grains in the plants was observed in the same parks, where the plants had the highest values of pollen sterility indices, and that interrelation was further evidence of unfavourable environmental conditions.

Discussion

The highest levels of pollen sterility were recorded in the woody plants growing in the parks located either in the city industrial areas (the Park of Memory and Reconciliation, Molodyozhny park) or in close proximity to these areas, within less than 4 km off production facilities (the park named after T.G. Shevchenko and the park named after L. Globa), as these sites are exposed to the most hazardous anthropogenic air pollutants, in particular SO_2, NO_x, NH_3, phenol compounds, heavy metals, and other contaminants. The lowest pollen sterility in the plants was revealed in the parks located farther away from the pollution sources (the park named after Yu. Gagarin and the park named after V. Dubinin). Present findings are in agreement with the data obtained by other researchers who reported that pollen sterility levels were highly correlated to environmental pollution concentrations [21, 22, 29]. It has been found that the values of pollen sterility indices increase when the plants are exposed to the impacts of polluting substances of industrial emissions, i.e. heavy metals [15, 30], gaseous air pollutants [31, 32], and motor vehicle emissions [7, 33]. For the purpose of bioindication of the environmental status in different functional areas of the city of Dnipro, a number of researchers have undertaken investigations into pollen viability in the plants growing in the territories of industrial enterprises, in the sanitary zones [34, 35] and in some streets [36] in the city of Dnipro. These surveys indicate that the levels of pollen grains sterility at the examined sites were greater than those in the control site. However, a complicating factor for the comparison of the results obtained by other researchers and our findings is experimental conditions used. In most cases, the observations were carried out in different localities and there were only few instances, if any, when the same plant species were selected as test objects for analyses.

Table 3: Percentage of dwarf, gigantic and abnormal forms of pollen grains, [%], (n>1000)

Sampled locations	*Betula pendula*	*Acer platanoides*	*Tilia platyphyllos*	*Robinia pseudoacacia*	Average index
Control site	0.64±0.02	0.76±0.02	1.21±0.05	0.92±0.03	0.88±0.03
Park named after Yu. Gagarin	3.56±0.14	3.21±0.14	2.10±0.09	2.71±0.11	2.65±0.11
Park named after L. Globa	6.67±0.23	10.12±0.33	8.95±0.34	7.29±0.26	8.25±0.21
Park named after V. Dubinin	3.15±0.13	3.11±0.14	4.35±0.19	2.75±0.12	3.34±0.12
Park of Memory and Reconciliation	10.29±0.41	12.32±0.46	8.24±0.32	9.44±0.32	10.07±0.32
Molodyozhny park	8.17±0.29	9.20±0.31	6.31±0.23	5.90±0.25	7.40±0.21
Sevastopolsky park	3.75±0.15	4.97±0.22	4.29±0.20	5.13±0.20	4.53±0.20
Park named after T.G. Shevchenko	4.91±0.20	5.10±0.21	3.22±0.14	4.71±0.21	4.48±0.19
Park named after B. Khmelnitsky	5.17±0.19	4.86±0.20	2.94±0.12	4.02±0.18	2.24±0.09

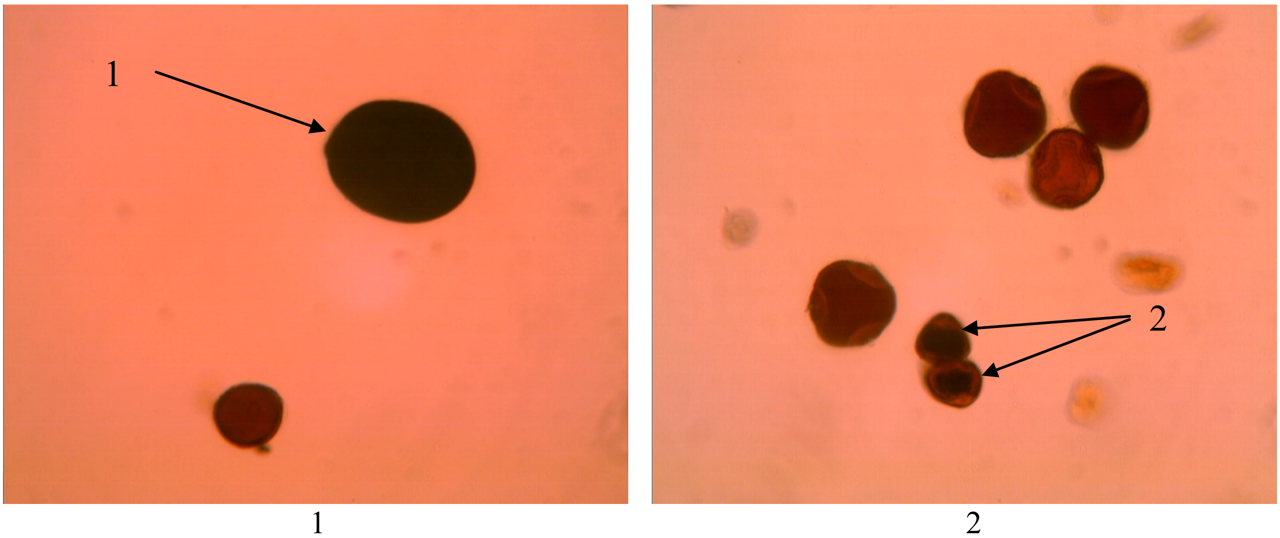

Figure 3: Morphological alterations (polymorphism) of pollen grains in woody plants in the city parks of Dnipro: 1 – a gigantic pollen grain in *Robinia pseudoacacia* (the Park of Memory and Reconciliation); 2 – dwarf pollen grains in *Tilia platyphyllos* (Molodyozhny park)

It is known that pollen grains in various plant species differ in starch contents, enzyme activity, proline contents and in other specific characteristics, as well as in pollen viability and longevity [35]. Reasoning from this, we calculated an average value of pollen sterility index for each of four investigated plant species (Table 2). On the basis of a given index, used as a mediate indicator of mutagenicity and intensity of the gametocidal action on the plants in the technogenic environment, the park territories were grouped in the following order of increasing risk: conventionally clean parks (1–3.5), areas at low-level risk (3.6–7.0), areas at moderate-level risk (7.1–10.5), areas at high-level risk (10.6–14.0). The park named after Yu. Gagarin and the park named after V. Dubinin belong to the first group; the territory of Sevastopolsky park, those of the park named after B. Khmelnitsky and the park named after T.G. Shevchenko are considered to be the areas at low-level risk; Molodyozhny park belongs to the area at moderate-level risk; and the Park of Memory and Reconciliation was at the bottom of the order as the area at high-level risk.

As it has already been stated above, the most pronounced increase in the abundances of pollen with abnormal shape and size was observed in woody plants growing in the same parks where the pollen of the plant species demonstrated the highest sterility indices. Despite the fact that numerous experiment data show that most of dwarf and gigantic pollen grains give reaction of starch and acetocarmine reaction, the opinion persists that dwarf and gigantic pollen grains are infertile. The proportion of dwarf pollen grains is commonly much larger than that of gigantic grains. Differences in the quality of pollen are attributed to disturbances in microsporogenesis, therefore, not only tetrads but also monads, dyads and other cell clusters are formed [16].

The frequency of occurrence of morphological alterations in pollen quality can serve as a bioindicative hallmark of an aerogenic pollution level in the park territories, because there is a significant positive correlation between increasing abundance of poor quality pollen grains and the proximity of the park location to the source of technogenic pollution. For instance, L.M. Kavelenova [27] used the results of testing the amounts of defective pollen in *Taraxacum officinale* L. for zoning the territory of the city of Samara (Russia) into different areas according to the levels of their pollution. This author recognized pollen as defective when it was abnormal in shape and size or sterile. However, since the pollen of abnormal shape and size is quite rare in occurrence, the pollen sterility index, in the author’s opinion, is more appropriate for comprehensive assessment of pollen status.

Thus, in the present research, the pollen sterility index, as a mediate bioindicator of mutagenicity of the environment, has been used as a means for testing the environment status in the city parks. According to the data obtained, it was reasonable to rank the city parks in Dnipro in the following order of increasing phytotoxicity of their environments: the park named after Yu. Gagarin < the park named after V. Dubinin ≤ Sevastopolsky park = the park named after B. Khmelnitsky < the park named after T.G. Shevchenko < Molodyozhny park < the park named after L. Globa < the park of Memory and Reconciliation.

Conclusion

Our research shows that:

1. The assessment of pollen quality in woody plants, such as *Betula pendula*, *Acer platanoides*, *Tilia platyphyllos* and *Robinia pseudoacacia,* has provided evidence of degradation in the plant generative potential and deterioration in the environmental quality in the parks located in different functional urban areas and at various distances from the sources of harmful pollutants.

2. The most unfavorable ecological situation on account of gametocidal action has evolved in the Park of Memory and Reconciliation and Molodyozhny park. According to the quality of environment in other parks, their sites have been assessed as either conventionally clean areas or areas at low-level risk.

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