INTRODUCTION

Various characteristics of plant organs and pollen contribute to biological diversity (Hwang and Masters, 2013) and data on their variability lay groundwork for the solution of many biological problems related to taxonomy, microevolutionary processes, hybridization, and gene pool protection (Polyakova and Gataulina, 2008; Warny, 2013). Pollen analysis or the study of pollen grain morphology (i.e., size, exine pattern, fertility, and viability) is a research method for measuring the reproductive potential of plants (Goodman et al., 2015). Pollen analysis deals with the estimation of normal and defective pollen fractions and with metabolism processes in pollen grains (Paushova, 1980; Riley et al., 2015). The problem of multipole anomalies in pollen grains has been a forefront in recent debates (Augenstein, 2016; Miroff, 2019). The quality of pollen grains is crucial to the reproductive biology of plants and their ability to generate full-fledged seeds (Lawrence, 2018; Laurence and Bryant, 2019). Normally, pollen in plants growing under normal conditions is of good quality and the amount of normal pollen grains is close to 100% (Bryant and Bryant, 2019). Increased pollution, on the contrary, can reduce their proportion (Ashikhmina, 2005).

Recent decades have saw a steady growth in various sectors of economy, which strengthened the role of anthropogenic factors, both biotic and abiotic (Legendre and Legendre, 2012). Cities with industrial zones functioning side by side with the green zones can serve a convenient model object in the judgment of pollution levels. Such cities can be found in many countries around the world, including China, Kazakhstan, Russia, and the United States. At the same time, the Western European countries have a strong experience in designing urban landscapes with ecological considerations in mind, something that developing countries, such as Kazakhstan, may need to consider. The widely used landscape plants are metal tolerant, mostly woody, such as the black poplar (Populus nigra L.).

The most solid and gas emissions concentrate in the air 15 to 20 m above the ground. This range of polluted air travel is the living zone for humans and most plant species (Ferguson et al., 2018). Therefore, research on the effective monitoring of pollutants in green zones is needed (Fauscon et al., 2017). This study chose plant pollen as a research object for its sensitivity to pollution and anthropogenic loads of different intensity. This kind of data is somewhat scarce, which defined the relevance of this study.

It is known that pollen formation takes place through many cell divisions, which vary among different plant species (He et al., 2019). This statement equally applies to pol-

The purpose of this work is to comparatively assess pollen viability of five plant species growing in different areas of the city of Aktobe, Kazakhstan (N 50°18’, W 57°10’). Pollen viability was assessed through iodine staining on the samples of pollen grains harvested between April and June 2017 from 100 plants of 5 species growing in the industrial zones, parks, and along highways: Ulmus laevis Pall., Ulmus pinnato-ramosa Dieck., Acer negundo L., Syringa vulgaris L., Populus tremula L. The minimum percentage of fertile pollen grains that was found in Ulmus laevis species has been detected in samples from the industrial zones, up to 68%. For Ulmus pinnato-ramosa, the minimum proportion of fertile pollen grains was 30%. For Acer negundo and Syringa vulgaris, similar trends were obtained. All plant species except Ulmus laevis (0.42) and Populus tremula (0.37) showed no relationship between the size of pollen grains and the intensity of their color change. Among all plant species under study, two (Ulmus laevis and Populus tremula) had the potential to act as model plant species and bioindicators of urban pollution. All five plant species demonstrated the highest proportion of defective pollen grains in the industrial zones, up 98%.

Keywords: fertility, pollen, sterility, viability, woody plant
other growing urban agglomerations that operate in similar high level of anthropogenic load. Hence, it may represent growing sterile, was calculated in ten different microscopic peroxidases decreases (Khlebova and Bychkova, 2016). In an urban environment, the variation in reproductive parameters of woody plants depends on the microecological conditions, tree age and health (Dmitrik et al., 2016). The morphology and quality of plant pollen are of significant interest in many fields and industries: taxonomy, phylogeny, palynology, forestry, seed production, beekeeping and medicine. Studies on breeding and biotechnology involve a significant amount of nitrogen metabolism and, subsequently, the composition of the synthesized amino acids (Meineke et al., 2019). The implication is the loss of pollen viability, expressed in the minimum rates of pollen germination. Moreover, these indicators will vary greatly in different types of plants, even if they grew in the same conditions. This study conducted a first comparative analysis of pollen fertility between different plant species growing in industrial and ecologically clean conditions.

Pollen viability is linked to weather and climatic characteristics in the formation phase (Horváth et al., 2019). The quality of pollen grains is influenced by the atmospheric pollution. In the conditions of an urban ecosystem, the ability of pollen grains to germinate decreases, the number of abnormal pollen tubes increases, and the frequency of cells containing active peroxidases decreases (Khlebova and Bychkova, 2016). In an urban environment, the variation in reproductive parameters of woody plants depends on the microecological conditions, tree age and health (Dmitrik et al., 2016). The morphology and quality of plant pollen are of significant interest in many fields and industries: taxonomy, phylogeny, palynology, forestry, seed production, beekeeping and medicine. Studies on breeding and biotechnology involve a significant amount of viable pollen. Data on the morphological characteristics of pollen grains are also widely used in the investigation of plant adaptability to dynamic and stressful living conditions (He et al., 2019).

Recently, ecological and palynological studies have been actively carried out in the urban cities (Lu et al., 2018) but no similar investigations have been yet conducted in the Aktobe region, Kazakhstan. The city of Aktobe is a center of urban agglomeration that has been emerging in the past two decades, with a total population of 1.3 million people, surrounded by several dozen neighboring settlements. Therefore, it experiences a rapid increase in the level of anthropogenic pressure. Aktobe is home to a variety of enterprises, including light manufacturing, food, and chemical enterprises, which add to the high level of anthropogenic load. Hence, it may represent other growing urban agglomerations that operate in similar lar, i.e., temperate continental, climatic conditions. The purpose of this study is to assess pollen viability of some woody plants growing in different areas of the city of Aktobe. The city of choice is a convenient model object because it combines zones with high and low anthropogenic loads.

### MATERIALS AND METHODS

The study was conducted on five species of woody plants: flittering elm (*Ulmus laevis* Pall.), Siberian elm (*Ulmus pinnato-ramosa* Dieck.), box elder (*Acer negundo* L.), common lilac (*Syringa vulgaris* L.), and common aspen (*Populus tremula* L.). These plant species were commonly found at all study sites across the city. Inflorescences were harvested from April to June 2017, packed in paper bags, and labeled with the date and place of harvest before being dried in the shade at room temperature. Specimen collection took place within different areas of the city of Aktobe (Table 1).

Pollen viability can be assessed by the germination of pollen grains on artificial liquid/solid media (in vitro germination tests) and by using stains that react with vital enzymes and other substances, i.e., iodine staining specifically (Isakov and Matsneva, 2015).

Normally, pollen viability and fertility are evaluated separately. According to Walden and Everett (1961), pollen viability may be defined as the ability of the male gametophyte to grow insuitable stylar tissue, and the fertilizing ability or zygotic potential of the pollen grain as the ability to complete fertilization. Sterile pollen is incapable of normal functioning (fertilization). It is formed as a result of irregular meiosis, an uneven distribution of chromosomes in the poles, most often at the reduction division (meiosis I). Sterile and fertile pollen grains are distinguished by using staining techniques. Fertile grains contain many starch granules in contrast to sterile grains that do not have any. For that, fertile pollen grains change color to dark purple, almost black, in cytological tests, and sterile pollen grains under the microscope look like golden yellow films, often folded.

After harvest, pollen was examined under an SXZ-207 light microscope (Ningbo Beilun Aofusen Instrument Co., Ltd., China). The total number of pollen grains, including sterile, was calculated in ten different microscopic fields. The results were presented in a tabular form. The per-

### Table 1 Plant sampling sites

| Site                          | Coordinates                              |
|-------------------------------|------------------------------------------|
| Ailya Maldagulova Avenue      | N 50.29255, W 57.167432                 |
| The First President's Park    | N 50.287350, W 57.18532                 |
| Retro Park                    | N 50.28092254795376, W 57.21757200377542 |
| Abalkhair Khan Avenue         | N 50.28933266149877, W 57.16737635405642 |
| Abai Avenue                   | N 50.3029155399453, W 57.162437324262385 |
| Pushkin Park                  | N 50.2864150479834, W 57.2285844767173 |
| Brothers Zhubanov’s Street    | N 50.29747456910552, W 57.145919897672194 |
| Sh. Ualikhanov Street         | N 50.281248125892205, W 57.2248525534222 |
percentage of fertile pollen was calculated by the following formula:

\[ PF = \frac{m}{M} \times 100\% \]

Where \( PF \) is the percentage of fertile pollen, \( m \) is the number of stained pollen grains, and \( M \) is the total number of pollen grains.

The pollen grain sizes were measured with a Phenom G2 pro desktop scanning electron microscope (SEM). Half of partially stained grains were classified as viable, the remaining portion as non-viable. The Spearman’s Rho test was conducted to determine if there is a relationship between grain size and color change. Differences were considered significant at \( P \leq 0.05 \).

RESULTS

The estimates of pollen viability showed high variation. The amount of starch granules in pollen grains varied depending on the harvest site (Table 2). For instance, the Ulmus laevis species growing in the industrial zones had the smallest fraction of fertile pollen grains, ranging from 66 to 68%. The amounts of fertile pollen in plants growing in the city parks and on the side of roads do not differ much, perhaps due to the near-road location of parks. The highest fertility of pollen grains, 92%, was demonstrated by samples collected in the First President’s Park. Similar results were displayed by pollen samples from the Acer negundo plant species. Lower viability values, 28%, for found in pollen harvested in the industrial zones and higher viability values, 88%, in pollen harvested in the First President’s Park and from trees growing along the Abay Avenue. Regards Acer negundo and Syringa vulgaris, more sterile grains were found in pollen samples from the industrial zones and from plants growing along the Brothers Zhubanov’s Street and one of the side of highways. Populus tremula plants showed a relatively low sterility percentage in pollen grains, 2 to 25%, with the greatest amount of non-fertile pollen in industrial zones and in plants growing on the sides of highways. In parks, the portion of sterile pollen grains ranges from 2 to 10% (Table 2).

The distribution of abnormal pollen across the city is uneven. The palynological study revealed a difference in the proportion of viable and teratomorphic pollen grains between parks, industrial zones, and busy highway roadsides. As in the case of fertility, the results showed a variation depending on harvest location (Table 3).

For instance, there was a clear variation in the size of pollen grains from Acer negundo. The smallest grains were collected in the industrial areas and along the Aliya Moldagulova Avenue. By the ratio of fertile-to-sterile pollen grains, one can distinguish several ecologically safe zones in the city where a small amount of abnormal pollen was found: the First President’s Park, the Retro Park, the Aliya Moldagulova and the Abulkhair Khan Avenues. A higher portion of teratomorphic pollen grains was found in trees growing along the Abai Avenue, in the Pushkin Park, along the Brothers Zhubanov’s Street and the Sh. Ulikhanov Street. The smallest fractions of fertile pollen were found alongside the highest amount of teratomorphic grains in the industrial zones of the city. The remaining sites under study experience less anthropogenic load, which manifested in higher pollen fertility.

For reliability assessment, a correlation between grain size and sterility percentage was calculated using the Spearman’s Rho test. The correlation showed a strong relationship, with a correlation coefficient of \( \rho = -0.78 \). This indicates that the pollen grains of Acer negundo are significantly smaller than those of Ulmus laevis and Syringa vulgaris.

| Table 2 | Percentage of fertile grains in some plant species growing in the city of Aktobe |
|---------|----------------------------------------------------------------------------------|
| No.     | Fertile fraction, % Ulmus laevis, Ulmus pinnato-ramosa, Acer negundo, Syringa vulgaris, Populus tremula |
| 1       | 89.8, 77.4, 84.0, 89.1, 86.4 |
| 2       | 92.0, 80.0, 94.0, 95.0, 75.0 |
| 3       | 89.0, 87.0, 98.0, 95.0, 86.0 |
| 4       | 87.0, 86.0, 97.0, 95.0, 86.0 |
| 5       | 68.0, 71.3, 69.8, 82.0, 75.0 |
| 6       | 66.0, 72.0, 61.0, 62.3, 84.9 |
| 7       | 91.0, 88.0, 98.0, 95.0, 98.0 |
| 8       | 83.0, 74.0, 94.0, 96.0, 90.0 |
| 9       | 85.0, 88.0, 81.0, 95.0, 98.0 |
| 10      | 87.0, 83.0, 97.0, 97.0, 95.0 |

| Table 3 | Dimensions of pollen grains in some plant species growing in the city of Aktobe |
|---------|----------------------------------------------------------------------------------|
| Plant species | Pollen grain size, length – width, μm | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 |
| ---------- | ----------------------------------- | -- | -- | -- | -- | -- | -- | -- | -- | -- | -- |
| Ulmus laevis | 29.8×23.2 | 25.9×24.3 | 25.9×24.3 | 32.5×27.6 | 27.6×20.7 | 27.3×22.3 | 30.1×25.7 | 28.9×22.4 | 27.5×23.4 | 25.6×22.7 |
| Ulmus pinnato-ramosa | 26.5×18.9 | 29.0×21.6 | 26.4×21.3 | 26.1×21.5 | 25.8×23.3 | 25.6×23.5 | 26.9×24.5 | 23.2×18.5 | 28.2×19.5 | 25.1×22.7 |
| Acer negundo | 32.5×21.8 | 39.4×17.5 | 22.0×17.5 | 34.3×17.9 | 23.2×20.0 | 21.4×13.1 | 36.1×16.0 | 34.9×16.0 | 35.7×19.7 | 37.3×18.1 |
| Syringa vulgaris | 29.8×18.8 | 28.2×19.7 | 28.2×27.6 | 35.6×25.6 | 36.0×24.6 | 28.0×16.6 | 31.4×18.8 | 25.2×21.8 | 33.6×21.9 | 31.4×19.1 |
| Populus tremula | 28.9×26.6 | 22.6×19.9 | 20.7×19.6 | 23.7×19.7 | 26.5×21.6 | 26.7×22.6 | 23.4×21.4 | 30.1×27.0 | 30.2×27.3 | 28.9×26.1 |

Vol. 59, No. 3 (2021) (31) 137
size and fertility was found. Since the intensity of color change in response to starch is a measure different from the numerical value of the pollen grain size, formulas to calculate the correlation coefficient must be applied in directly. Pollen grains have a normal size distribution, and deviations from the mean increase the likelihood of defective pollen. Although a simple staining method reveals fertile pollen grains, it may theoretically detect defective (non-fertile) ones that are larger or smaller than a typical fertile pollen grain. Therefore, if such variables as grain size and pollen fertility are independent of each other, then their mean values will not correlate. The Spearman’s Rho test showed no relationship between pollen size and color change after staining in all plant species under study except Ulmus laevis (0.42) and Populus tremula (0.37). The latter species demonstrated a positive correlation, which suggests that a fluttering elm and an aspen may act as bioindicators of urban pollution (Table 4).

The analysis showed that species Ulmus laevis and Acer negundo were sensitive to urban pollution and can be recommended for application in urban pollution monitoring. As it was found, the quality of pollen was influenced by the plant growing conditions and the size of pollen grains can vary in response to different anthropogenic loads. Therefore, pollen analysis may be used to assess the level of chemical pollution in the area, considering the senility of pollen to environmental factors.

DISCUSSION

The role of green zones in is to counter the industrial pollution (Liao et al., 2018). Green spaces have a structural effect on the urban landscape and provide aesthetic pleasure. These natural settings are effectively used to reduce the number of dust particles, pathogens and gas emissions per unit volume of air (Tulik et al., 2018). Green spaces can significantly reduce noise pollution, which is inherent to large cities with many motor vehicles and industrial facilities. Tree stands also add to humidity and air temperature around as well as to the prevailing wind direction and radiation regime (Mao et al., 2019).

The microclimatic conditions of large cities are fundamentally different from those of the natural habitats. Urban trees are exposed to extreme stress caused by the influence of adverse factors (Čehulíč et al., 2019). This manifests in the quantitative and qualitative changes of pollen. The research population of four plants consisted of one invasive species (Acer negundo) but no markedly different results were found for it. In fact, Acer negundo species growing in the industrial zones demonstrated higher sensitivity to anthropogenic impact and had a significant amount of teratomorphic pollen grains. On the other hand, it was hardly affected by insects because of its foreign origin. The same picture was observed in Kiev (Stukalyuk et al., 2020). Namely, the local insects (aphids) were reported to avoid populating the introduced box elders. Therefore, Acer negundo may be a suitable option for urban landscaping in conditions of strong anthropogenic load. With constantly increasing anthropogenic emissions from industrial enterprises and motor vehicles, plants are forced to deal with stronger stress (Dornelas et al., 2019). Non-fertile and thus non-viable pollen grains are increasingly detected in various plant species. According to present findings and those of other investigators, a constant large-scale plant health monitoring is required (McCullen et al., 2019). Thus, this study found two plant species showing the opposite responses to the anthropogenic load. These findings may be used in the comparative studies with other regions involved. The convenience of pollen as a model object lies in the fact that it clearly displays the impact of anthropogenic load by annually providing materials for the analysis. In other words, pollen analysis enables the comparison of plant health statuses by years without causing a significant harm to its viability.

Among all plant species under study, two (Ulmus laevis and Populus tremula) had the potential to act as model plant species and bioindicators of urban pollution. These species were the only species to show a positive correlation between pollen grain dimensions and fertility. The fertility of pollen samples harvested from Populus tremula was positively correlated with growing in polluted areas, whereas the pollution factor had a minimal effect on the Ulmus laevis plant species, which preserved two thirds of pollen grains viable. Trends for all five plant species were differently detected in various plant species. According to preliminary findings and those of other investigators, a constant large-scale plant health monitoring is required (McCullen et al., 2019). Thus, this study found two plant species showing the opposite responses to the anthropogenic load. These findings may be used in the comparative studies with other regions involved. The convenience of pollen as a model object lies in the fact that it clearly displays the impact of anthropogenic load by annually providing materials for the analysis. In other words, pollen analysis enables the comparison of plant health statuses by years without causing a significant harm to its viability.
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