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Review

Pollution of the Environment and Pollen: A Review

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Abstract: Bioindication of the environment is one of the actively developing directions of ecology. Information about pollutants and the level of environmental pollution can be obtained as a result of studying the biological reaction of plants to pollution. Ecological palynology is a new direction, when pollen of various woody and herbaceous species is used for bioindication of the level of environmental pollution and the presence of mutagens. The review considers the morphological variability of pollen, its fertility and viability under the influence of pollutants, the possibility of its use as a bioindicator of pollution of urban areas by emissions of vehicle transport and industry.

Keywords: environment; pollution; pollen; bioindication

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1. Introduction

Ecological palynology is an actively developing direction of environmental monitoring. Traditionally, results of palynological studies have been applied to paleogeographic and paleoecological reconstructions. Recently, palynomorphology has been used also for assessment of quality of environment, both past and present [1]. Fresh pollen can provide valuable information about plant phenology, ecophysiology, population dynamics and gene flow [2]. Monitoring of the airborne pollen of urban areas is of great interest for ecological studies and environmental health [3]. The number of plants species involved in palinology monitoring is very large and varies from herbaceous plants to different species of trees, the pollen of which may or may not be allergenic [4]. A large amount of research is focused on studying morphology and physiology of pollen grains of plants, like allergenic *Betula*, *Ambrosia* and other species, growing under polluted conditions [5–10]. Information about pollutants and the level of environmental pollution can be obtained as a result of studying the biological reaction of plants to pollution [11,12]. Researchers propose to use pollen as a biological indicator of the quality of atmospheric air [13–20]. The actual direction is bioindication of atmospheric air quality under the traffic load [21–26], and impacts of industrial emissions [27–31]. Palynoecological studies of urban areas are carried out for the purpose of using of pollen as biomonitors for capture heavy metals and other pollutants [32], for the study of anomalies of the development and viability of pollen in conditions of environmental pollution [1,15,33–35], and its allergenicity [36–38]. It is known that pollen of urban areas and polluted industrial regions has a higher number of allergens [39]. Pollen grains can change their surface and biochemical properties, which can exacerbate allergic symptoms [35]. The aim of the review is to summarize the results of the research carried out so far on the key points about the relationships between pollen quality and environmental pollution. This review considers correlations between: pollen and pollution of urban environment, pollen morphology, its viability and contamination by vehicle and industrial emissions.

2. Pollen as Indicator of the Urban Environment

2.1. Monitoring of Pollen and Air Pollution: Correlations with Abiotic Environmental Factors

In modern cities the monitoring of concentration of particulate matter (PM 10 and PM 2.5) in the air is carried out in most countries of the world. Studies have shown that

PMs can bind with airborne pollen and fungal spores, changing their morphology and making pollen allergens more accessible [40]. PMs 2.5 are the most dangerous for human health. T. Womiloju [41] showed that plant pollen and fungi cell materials can make up 4–11% of the mass of PM 2.5. There are some studies of the correlation of pollutants (including PM), the amount of pollen and abiotic factors in urban areas [42–46]. In the city of Cincinnati (OR, USA), the study of the relationship between airborne pollen and fungi of various genera, particulate matter, ozone, carbon, some trace elements (cadmium, zinc, copper and lead), temperature and relative humidity was conducted [42]. It was found that concentrations of fungal spores and pollen of some genera of plants, ozone and particulate matter are positively correlated with air temperature. Inverse correlations were obtained between bioaerosols and toxic heavy metals, but in most cases, they were not statistically significant [42]. The similar study of the impact of atmospheric air pollution (ozone, sulfur dioxide, carbon dioxide, PM 10, nitrogen dioxide) and meteorological factors (daily level of precipitation, wind speed, relative humidity and air temperature) on the concentration of pollen of some taxa was carried out in the city of Szczecin (Poland) [43]. The authors obtained a statistical correlation between the concentration of pollen of various genera of plants and pollutants of the atmospheric air, which is a consequence of the relationship between the latter and meteorological conditions. Ozone and PM had the greatest impact on variability of pollen taxa composition. When PM concentrations were high, production of *Carpinus* pollen was very large, and at low PM values, there were the highest concentrations of pollen of *Secale*, *Chenopodiaceae*, *Urtica*, *Artemisia*, *Tilia*, *Rumex*, *Poa*, *Plantago* and *Pinus*. With a high content of NO_2 in the air, was observed the increasing of the amount of pollen of *Salix*, *Populus*, *Ulmus*, *Corylus*, *Alnus*, *Cupressus*, *Fraxinus*, *Rosa*, *Platanus* and *Quercus*. High ozone concentrations correlated with an increase in the pollen production of *Salix*, *Morus*, *Platanus*, *Quercus*, *Aesculus*, *Rosa*, *Carpinus*, *Pinus* and *Secale.* The authors come to the conclusion that the correlations between the pollen production and air pollutants are in consequence with the influence of weather factors [43]. In the city of Poznan (Poland), was conducted the 12-year monitoring of chemical (PM 10, ground-level ozone), biological (pollen grains and fungal spores) pollutants and the dynamics of temperature of the urban environment [46]. As the object of palynological study were used the pollen of birch (*Betula* sp.), alder (*Alnus* sp.), grasses (*Poaceae*), sagebrush (*Artemisia* sp.), as well as spores of *Alternaria* sp. and *Cladosporium*. It was found that the highest concentration of pollutants was in summer (June–August), during the dusting season of allergenic cereals and *Artemisia* sp., which correlated with an increase in the content of fungal spores and ozone in the urban air. Increased concentrations of ozone cause an increase in pollen allergens of various grass species [45]. It was revealed that high temperatures prior to mass dusting of grasses and *Artemisia* sp. significantly reduce the level of concentration of pollen at the beginning and end of the vegetative season. Perhaps this is due to drought, which leads to a slowdown of reproductive development and reduces pollen production [47]. At higher temperatures the concentration of PM 10 decreases. The impact of pollutants on the pollen morphology of 24 plant species was studied in the city of Hyderabad (India) [44]. In conditions of high levels of PM particles, SO_2 and nitrogen oxides in the atmospheric air from industrial and transport sources, it was found the shrinkage of exine of pollen of many species (*Cyperus rotundus*, *Syzygium cumini*, *Hyptis suaveolens*, *Cocos nucifera*, *Acacia nilotica*, *Eucalyptus tereticornis*, *Azadirachta indica* and *Zea mays*). The authors come to the conclusion that pollutants can affect by two ways: among the tapetal fluids of pollen or by the attachment of particulate matter to the surface of the exine of pollen after dehiscence of anthers and cause developmental abnormalities. Aerobiological monitoring of cypress pollen was carried out in the city of Evora (Portugal) [48]. Pollen with exine ruptures, dilatation of the intine wall and release of the protoplast to the environment, was detected. The authors consider that these morphological changes can promote the release of pollen allergens and possibly enhance their allergenic activity. These free allergens, most likely in the form of small particles, are easily inhaled and can enter the lower respiratory system, causing aggravation of symptoms in sensitized individuals.

2.2. Pollen, Biotesting and Bioindication of the Urban Environment

Many species of plants are good biomonitors and bioindicators of genotoxicity of polluted air due to the accumulation of pollutants [49,50]. Ukrainian scientists conducted a study of pollen in the six parks of the city of Kyiv for the purpose of environmental bioindication [51]. The content of fertile, sterile pollen and the coefficient of sterility was determined. As an additional indicator of the gametocidal effect of pollutants, the content of teratomorphic pollen was studied: dwarf, giant, with exine disorders (with cracks or crumpled). With the purpose of studying the palynotoxic effect, the EE10-90 biotest was used, which determines the content of toxic substances in the parks areas, when the content of fertile pollen was 10, 50 and 90% less, than in the control [52]. It was found that with an increase in the anthropogenic load, associated with the intensity of vehicle traffic, the content of abortive and teratomorphic pollen increases. According to the level of palynotoxic effect, territories with initial (EE 10) and medium levels of pollution (EE 50) were identified.

Another genotoxicity biotest, based on the quantity of aborted pollen of *Tradescantia pallida*, was used for passive monitoring of vehicle pollution of the city of Rio Grande (Brazil) [53]. The biotest for the analysis of abortive pollen makes it possible to identify the physiological responses of gametes to short-term changes of concentrations of air pollutants. This genotoxicity biotest is especially applicable in cities with high levels of ozone. The results of the study shows, that genotoxicity of *Tradescantia* plants, which grow near the roads with intensive traffic, and ozone concentrations, were high. The authors point out that this species can be successfully used not only for mutagenic biotests for chromosomal aberrations (analysis of micronuclei), but also for pollen sterility. The effectiveness of this biotest is also noted by other researchers, which notes its high sensitivity, since lethal mutations are well determined in haploid microspores [54,55]. In biotesting, the ratio of fertile and sterile pollen is often used. The lower the proportion of fertile pollen, the higher the level of environmental pollution. The fertility of pollen of five species of trees and shrubs (*Ulmus laevis* Pall., *Ulmus pinnato-ramosa* Dieck., *Acer negundo* L., *Syringa vulgaris* L., *Populus tremula* L.) was studied in the city of Aktobe (Kazakhstan) [20]. In the industrial areas of the city, a significant decrease in pollen fertility was revealed: *Ulmus laevis*—66–68%, *Ulmus pinnato-ramosa*—72%, *Acer negundo*—61–70%, *Syringa vulgaris*—62.3%, *Populus tremula*—75%. The proportion of fertile pollen of these species in the city parks and along the roads was high, and differ not so much, possibly due to the roadside location of parks. Among the studied species of woody plants, the authors identified *Ulmus laevis* and *Acer negundo*, whose pollen is most sensitive to urban pollution and recommended them as biomonitors [20].

Recently, large attention of researchers is given to the possibility of use of pollen sizes in the bioindication of air pollution. Measurements of the size of pollen in Aktobe [20] showed its significant variation depending on the species and location of the experimental sites. The *Acer negundo* pollen has the highest variability, in industrial zones this species produces a lot of small pollen grains. The highest content of small pollen (90–98%) was found in the industrial areas and along highways, while in the parks its proportion ranged from 15 to 35%. Similar research of pollen sizes with the purpose of bioindication was carried out in the city of Belgrade [19]. Pollen morphology of 12 tree species was studied in the parks of the city: *Aesculus hippocastanum* L., *Betula alba* L., *Ginkgo biloba* L., *Paulownia tomentosa* Steud., *Platanus x acerifolia* Willd., *Quercus cerris* L., *Quercus robur* L., *Quercus rubra* L., *Tilia argentea* Desf., *Tilia x euchlora*, *Tilia grandifolia* Ehrh., and *Tilia parvifolia* Ehrh. The length of the polar and equatorial pollen axis was measured and determined the pollen grain shape index (the ratio of the polar axis to the equatorial, expressed in %). The authors revealed the decrease in the sizes of pollen grains of the tree species in the parks located in areas with high air pollution in the center of the city of Belgrad. Scientists come to the conclusion that pollen size can be used as an indicator of changes of pollen morphology under the influence of anthropogenic stress.

Pollen has morphological, ultrastructural, biochemical, and physiological changes under the influence of environmental pollution [15]. Physiological changes can be assessed by pollen viability using various tests. The most common are based on pollen germination and pollen tube growth. Unexpected results of such biotesting were obtained in the city of Barnaul (Russia), where the viability of *Pinus sylvestris* pollen was study [56]. Pollen grains, which tubes were longer than the diameter of the body of pollen grain with sacci, were considered as germinated and viable. It was found that on the 6th day of the experiment, the pollen germination was low and varied from 11 to 35% depending on the location of the experimental site. The minimum values were obtained along one of the main highways of the city. The low viability of the male gametophyte was due to the fact that the pollen grains swelled, there was a slight protrusion of the cytoplasm in the region of the aperture, the growth of the pollen tube was blocked, and its size never exceeded the diameter of the body of the grain. Pollen tubes were often "clogged" by callose plugs, which prevented moving of the nuclei of the vegetative and generative cells into the tube. The cytoplasm of the vegetative cell was located at the apical end of the tube, connecting with the contents of the pollen grain by a thin cytoplasmic cord. Such pollen tubes, as a rule, were subsequently lysed. The authors revealed a wide variety of forms of growth and branching of pollen tubes. Together with the dichotomous branching, which is characteristic to the pine, there were variations of multiple branching. The most common anomaly was the bifurcation of the pollen tube and its hypertrophied thickening. Beside this, there was pollen, which germinated on both sides of the body [56].

2.3. Palynoindication of the Urban Environment

Palynoindication, as one of the promising and new methods of the assessment of quality of the environment, is based on determination of the proportions of normal and abnormal pollen, when the quality of environment assessed by the share of normal pollen in the samples [1]. Pathologies of pollen, which indicate the presence of mutagens of various kinds, and a high level of pollution, are also under study. It is known that a large number of teratomorphic (with developmental anomalies) pollen grains—from 45 to 100%, are recorded in the areas with a high level of gas pollution from transport emissions, the impact areas of industrial enterprises, and in the territories, with excessive, maximum permissible concentrations of zinc, cadmium, and lead in the soils [57]. While the percentage of natural polymorphism of pollen of various species in favorable environmental conditions usually does not exceed 5–10% [1], plants under the influence of pollution produce a large amount of abnormal pollen, and the higher level of environmental stress, the more developmental anomalies [1,28,29,58]. Studies of morphological features of pollen grains allow establishing of the presence of gametopathogenic compounds in the environment and also giving a comparative assessment of the pollution of various areas [59,60]. Research are conducted on the species of plants of different life forms—woody (*Pinus sylvestris*, *Picea abies*, *Tilia cordata*, *Syringa josikae*, etc.) and herbaceous (*Alopecurus pratensis*, *Dactylis glomerata*, *Plantago major*, *Chenopodium album*) [1]. Such studies are carried out in many cities of various regions of Russia: St. Petersburg [1,61], in Karelia [29,62], Murmansk region [58,63], Ural [64–67] and Siberia [60,68].

O. F. Dzyuba [1] studied the pollen of 40 species of trees and shrubs and find that all these species, under conditions of environmental pollution produce a large number of teratomorphic and sterile pollen grains. As a result of the palynomorphological study of *Tilia cordata* of St. Petersburg herbarium of 1892 year, it was found only 6.5% of teratomorphic pollen grains; in 1996 their content in herbarium samples was 44.80–64.0%. The author came to the conclusion that the ratio of normal and teratomorphic pollen grains is a good bioindicator of environmental pollution. In the city of Astrakhan, it was found that *Tilia cordata* produces 70–90% of teratomorphic pollen [69]. This was typical for trees growing in the park in the vicinity of the Aksarai gas condensate field. The low content of normal and large proportion of anomalous pollen indicates a high level of environmental pollution.

Studies of morphological variability of *Pinus sylvestris* L. pollen were carried out in the cities of Monchegorsk [58] and Murmansk [63], which located on the Far North of Russia. Palynoindication of the environment of the cities was carried out on the proportion of normally developed pine pollen in the samples, using the classification of N. A. Kalashnik [64]. According to this scale of ecological zoning of territories, pollen samples of conditionally clean territories contain more than 90% of normal pollen of Scots pine, moderately polluted—89.4–82.9%, heavily polluted—82.3–75.2%, critically polluted—68.6–62% [64]. The city of Monchegorsk is located in the impact area of "Severonickel" metallurgical Plant. The main pollutants of the Plant during the processing of copper-nickel raw materials are sulfur dioxide and dust containing heavy non-ferrous metals (nickel, copper, cobalt). In the parks and squares of Monchegorsk, 9 test sites were laid. It was found that the largest amount of normally developed pollen was contained in the samples of the control (90%) and Ecopark (86%). The content of teratomorphic pollen of *P. sylvestris* in the samples of the experimental plots varied from 14 to 55% (10% in the control). The most often were small pollen without content or with a reduced body, a significant amount of pollen were with anomalies of the sacci (with single sacci and with two various sacci), less often with three and four saccus. Almost all samples contained giant pollen [58]. According to the proportion of normal pollen in the samples, moderately, heavily and critically polluted areas of Monchegorsk were identified.

A similar study was carried out in the Arctic Murmansk, where 5 experimental sites were laid in the different districts of the city [63]. In all tested samples, the ratio of normal pollen of *Pinus sylvestris* was low and varies from 29.4 to 45.8%, which was 2–3 times less than in the control (84.4%). The proportion of teratomorphic Scots pine pollen in Murmansk varies from 54.2 to 70.6% (15.5% in the control). These pollen grains differ from the normal ones by pathologies of exine or sacci, size and shape. Eleven morphological anomalies of pollen have been identified: without protoplast (not colored, sterile), with plasmolysed protoplast (unevenly stained), without sacci, with reduced sacci, with a single saccus, with two dissimilar sacci, hypertrophied with three sacci, with exine rupture, dwarf, giant and dwarf with outgrowths (Figure 1). The most common developmental anomaly was sterile uncolored pine pollen without a protoplast (10.8–28%), in the control 3.4%), with complete degradation of the nucleus and cytoplasm (Figure 1b). The samples often contain unevenly colored pollen with a plasmolysed protoplast (9–18.6%) (Figure 1c). N. A. Kalashnik [67] indicates that pollen grains of coniferous with such developmental anomaly are most often encountered under conditions of strong technogenic pollution. A significant amount of *P. sylvestris* pollen with disorders of development of sacci was detected (Figure 1): with two dissimilar sacci, with a single saccus, with reduced sacci, without sacci and hypertrophied with three saccus. The most common pathology was pollen with reduced sacci (Figure 1e), it's considered as one of the most frequent for the male gametophyte of Scots pine. Such pollen has very low volatility, since the reduced sacci have a small volume and are practically not filled by air. There were significant quantities of the pine pollen with two dissimilar sacci (Figure 1h), without sacci (Figure 1f), that excludes the possibility of its participation in pollination. The proportion of pollen grains with a single saccus (Figure 1g) was significantly lower. The hypertrophied pollen with three sacci (Figure 1d) was found in the samples of four experimental sites. Such pollen with three, four and more sacci are produced by species of the genera *Pinus* and *Picea* only under extreme environmental conditions [70]. In the city of Murmansk, the teratogenic pollen with various pathologies of sacci most often occurs in the vicinity of the shipyard (25%) and the waste incineration plant (21.4%). The pine pollen with exine ruptures (Figure 1k) were often, its maximum was in the impact zones of industrial facilities. Exine ruptures are observed under the high level of environmental pollution [61]. All tested samples contained giant pollen, 1.5 or more times larger than typical, presumably diploid (Figure 1i). The large size and rounded shape of the pollen grain is a feature of polyploidy [71]. Dwarf pollen (Figure 1j) was more common, its share was especially high in the vicinity of the waste incineration plant. The change in the size of pollen is associated with a violation of the process of growth and cell division during the formation of primary cells of archesporium and tetrads of microspores. G. M. Levkovskaya [72] notes that under geobotanical stresses, the general trend of teratomorphosis is the nanism of pollen. Perhaps, this is a reaction to low air humidity, which is characteristic of the technogenic environment.

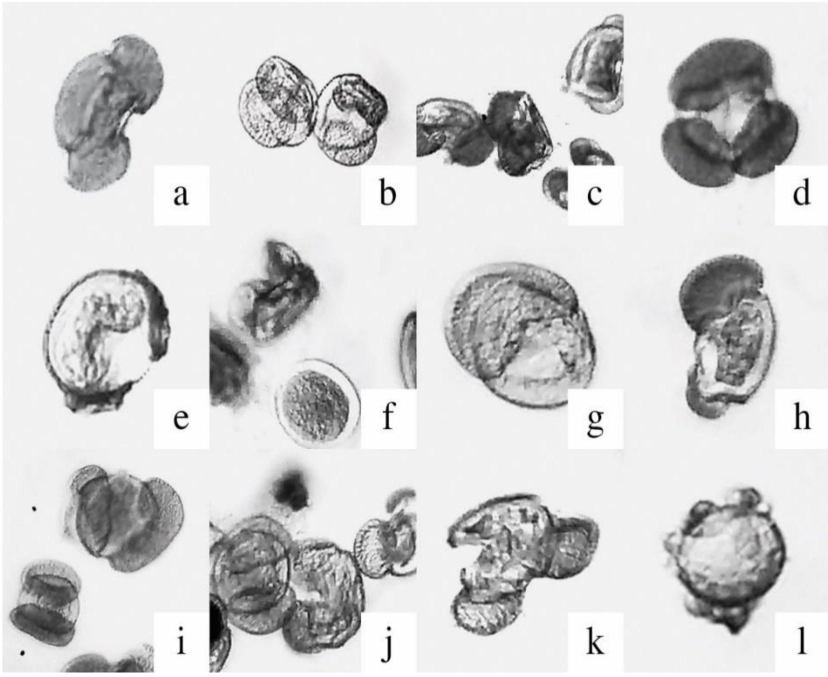

Figure 1. Pollen grains of *Pinus sylvestris* L. in Murmansk: (**a**)—normal, (**b**)—without protoplast (sterile), (**c**)—with plasmolysed protoplast, (**d**)—hypertrophied with three sacci, (**e**)—with reduced sacci, (**f**)—without sacci, (**g**)—with a single saccus, (**h**)—two dissimilar sacci, (**i**)—giant, (**j**)—dwarf, (**k**)—with exine rupture, (**l**)—dwarf with outgrowths. (Vasilevskaya, Domakhina, 2016 [63]).

According to the palynological scale of ecological zoning of territories by N. A. Kalashnik [64], the territory of Murmansk can be determined as critically polluted (the content of normal pollen in the samples 29.4–45.8%). In the city of Kostomuksha (Republic of Karelia, Russia), located in the impact zone of the "Karelsky Okatysh"—Plant which produces the iron ore raw materials, the proportion of normal pine pollen averaged 57% [29,62]. The content of teratomorphic pollen in the city of Kostomuksha was 4 times higher than in the vicinity of the mining and processing plant [29]. The same study of Scots pine pollen was carried out in the industrial city of Krasnoyarsk (Siberia, Russia) and its vicinity with different levels of geochemical air pollution [28]. The samples revealed a high percentage of anomalous pollen: horseshoe, lenticular, collar-shaped and pollen grains with one, three and four sacci. Most of the pollen grains were small, immature and degenerated. Pollen germination on cultured medium showed that *P. sylvestris* pollen in all samples was sterile. Studies of the morphological variability of pollen and the nature of its teratologies under the influence of environmental pollution require further research, since they are promising for revealing the gametocidal effects of pollutants.

3. Pollen Morphology and Viability under the Vehicle Pollution

Vehicle transport is one of the main sources of anthropogenic impact in the cities, polluting the air by carbon, nitrogen, sulfur oxides, ozone, PM 10 particles, heavy metals and polyaromatic hydrocarbons. Studies have shown that emissions of urban transport affect the shape, size, viability and sculpture of pollen exine [4,16,17,34,35]. Heavy metals, fluorides are absorbed on the surface of pollen grains, causing morphological changes, which makes them sensitive bioindicators of atmospheric pollution [73]. It has been shown that an increasing in temperature and CO_2 concentration stimulate the production and growth of pollen [74,75], as well as its allergenicity [76]. Pollutants of exhaust gases interact directly with pollen grains, affecting the structure of the cell wall, resulting in increased release of allergens or sub-pollen particles containing allergens [77]. The pollen allergenicity of trees in urban environment is significantly modified by atmospheric pollutants that cause an increase in amounts of allergenic proteins or changes of lipid composition [78,79].

Other research have shown that exposure of air pollutants leads to the formation of less amount of pollen, which has a smaller size and deformed shape compared with pollen of the same species growing in less polluted areas [35]. In the study of pollen grains of *Chenopodium album* of the urban territory with heavy traffic (Porto, Portugal) by the method of scanning electron microscopy received, that surface of the exine have modified structure, presenting by thin film which covering the exine, and deformed opercula's [35]. The analysis of carbon-containing particles of urban air showed that their spectrum is comparable to those particles contained in the exhaust gases of diesel engines. They accumulate on the pollen surface and can cause its modification. The pollen catches not only bioaerosols, but also other pollutants. Pollen grains can accumulate heavy metals, such as lead, cadmium, and mercury, as well as sulfur compounds [34]. Japanese scientists, studying the particles of pollutants on the surface of pollen grains of *Cryptomeria japonica* growing in the urban area with intensive vehicle traffic, found that particles of heavy metals (Al, Fe and Ti) can deposit on pollen perigonium [34]. The granular particles were less than 1 μm in diameter and aggregated. On the surface of the pollen, crystalline particles were also found, apparently of secondary origin, as ammonium salts [34]. Pollen has the ability to adsorb particulate matter that can lead to thinning and rupture of exine [80], facilitating release of allergens into the environment [77]. Pollen from plants growing along the roads with intensive traffic has higher pollen allergenicity [7]. Main air pollutants from vehicles (such as fine atmospheric particles, sulfates and gases) can be absorbed on the surface of the pollen and enhance its allergenic properties [81]. For example, nitrogen dioxide, a major traffic-related pollutant, increases the allergenicity of birch pollen [7]. Studies of the influence of components of spent motor oil on the morphology of pollen of *Atriplex canescens* and *Tamarix nilotica* were carried out near the highway El Katameya-El Ain Sukhna in Egypt [82]. For the study of the surface of exine of pollen grains, scanning electron microscopy was used. Simultaneously, the chemical analysis of the air and soil samples was carried out. It was found, that soil polyaromatic hydrocarbons and air pollutants (SO_2, NO_2, CO, CO_2) cause the deformation of the exine sculpture of *Atriplex canescens* pollen, change the pollen shape and damage the apertures of *Tamarix nilotica* [82].

Experiments on the effects of polluting gases on pollen were conducted in Shanghai: pollen of *Platanus orientalis* was treated by mixture of exhaust gases NO_2, SO_2 and NH_3 [81]. Studies have shown that after the exposure, the pollen swelled, and accumulations of polluting particles appeared on the surface of exine. This increases the production of allergenic proteins. Morphological changes of pollen under the influence of pollutants can increase its allergenicity [83]. Polish scientists conducted a study of the impact of vehicle transport on the morphology and chemical composition of pollen of *Artemisia vulgaris* L. in the city of Rzeszow [84]. The use of scanning electron microscopy (SEM) has shown that there were no changes in pollen morphology and structure of exine, but with more intensive traffic, the pollen grains became smaller. Chemical analysis by infrared spectroscopy (FTIR) has detected changes in biochemical composition. The biggest differences were found in the protein part of the spectra. Along the roads with intensive traffic, the secondary structure of pollen proteins changed. That may be the result of mutations of the genetic material caused by pollutants, which can increase the risk of allergies [84]. It has been shown that diesel emissions led to increasing in production by pollen the proteins associated with pathogenesis [85].

The amount of sterile (abortive) pollen increases in plants growing near roads with heavy traffic and it can be used as bioindicators for the assessment of technogenic territories [68]. Ibragimova [26] applied the sterility test of male gametophyte, using the pollen of *Aesculus hippocastanum* L. for screening the palynotoxicity of emissions of vehicle transport.

The number of fertile and sterile pollen, the percentage of cells with anomalies calculated from the total number of gametes, and sterility index (SI) were studied. Palynotoxic effect of vehicle exhausts—PE (%) was calculated based on the pollen fertility of experimental plants. It was found that the sterility of *A. hippocastanum* pollen growing along highways increases several times. Under the vehicle pollution, a lot of small, giant and narrow rod-shaped pollen grains were formed. On medium pollution, a statistically significant increase in small pollen was revealed. Such pollen is formed at the early stages of microsporogenesis and then eliminated. On heavy vehicle traffic, the proportion of giant pollen increases. The shape and size of pollen grains are genetically determined, and their violation may indicate mutational changes due to the genotoxic effect of pollutants. The obtained data were ranked according to biotest EC10-90 [86]: the initial (EC10), effective (EC50) and sublethal (EC90) concentrations of toxic pollutants, when the content of fertile pollen of the studied plants is 10, 50 and 90% less than in the control. It was found that vehicle emissions have effective (medium) toxicity (EC50). The quality and degree of pollen damage can be used as a biotest for the mutagenic impact of environmental factors [26]. Similar results were obtained in the study of pollen of *Thuja occidentalis* [87]. It was found that with the increasing in toxic emissions of vehicle transport, the production of sterile pollen grains increases linearly.

Studies in the Trentino region, Italy [15] have shown that the most toxic for plant reproduction is not ozone, but nitrogen oxides, which reach high concentrations during this period. The pollen fertility of *Corylus avellana* and *Pinus nigra* have been researched under conditions of high and low traffic. The viability of both species was low under heavy traffic. The pollen of *P. nigra* proved to be most sensitive bioindicator, because the germination of pollen and the length of pollen tubes were very low on the sites near the roads with heavy vehicle traffic. In subsequent studies the scientists randomized samples of *P. nigrum* according to the height of location of the sites, trees age, and their vitality [88]. It was found that the emission of nitrogen dioxide statistically significantly affects the pollen viability. Pollen viability was higher at low NO_2 levels (in rural areas) than at high levels (in the vicinity of the city with high traffic intensity) [88]. In the city of Ufa (Russia) the fertility and morphology of *Picea obovata* pollen were studied along the highways [66]. Under the pollution 6 teratomorphs were identified. The most common were sterile, hypertrophied, and small pollen grains, as well as pollen with one saccus or without saccus [66]. As a result of pairwise comparison using the x^2 criterion of indicators of abnormality and fertility of pollen grains of spruce from the conditions of technogenic pollution and control, the reliability of the differences between these indicators was of high significance. The research of the influence of heavy metals on the viability of pollen of the 8 herbaceous and woody plant species (*Convolvulus sepium* L., *Cynodon dactylon* (L.) Pers., *Dactylis glomerata* L., *Parietaria diffusa* (M.&K.), *Daucus carota* L., *Quercus ilex* L., *Tilia cordata* Mill., *Hedera helix* L.) was carried out in the city of Perugia (Italy) [23]. Pollen was collected from plants growing on the sites, which differ by the intensity of traffic. Scientists used the TTC test, which shows the enzymatic viability of pollen. TTC is a specific biologic stain, which is often used in the studies of airborne heavy metals. The results for different species were opposite. Among woody species, *Tilia cordata* has the lowest viability under the high and medium vehicle traffic (12 and 14%). The pollen of *Quercus ilex* has high resistance (84 and 89%), as the shrub *Hedera helix* (84 and 85%). Among the herbaceous species, the lowest viability has *Convolvulus sepium* (25%), *Daucus carota* (39%), and *Dactylis glomerata* (61%). *Cynodon dactylon* was the most resistant species (91 and 88%). The phenomenon was revealed on ruderal *Parietaria diffusa*, which pollen vitality rises as the traffic load increases from 8% (without transport) to 80% (intensive traffic). The authors suggest that this species has a specific metabolism to control air pollutants. Several parameters of *Prunus avium* pollen were studied along the road with an intensive traffic in Quetta (Pakistan): productivity, viability, size, germination rate and length of pollen tubes [18]. The influence of exhaust gases led to inhibition of viability, pollen sizes, its germination, pollen tube growth. In this regard, fertilization and the ability to reproduce were declined. Scientists come to the conclusion, that air pollution leads to anomalies of the development of anthers, decreases the number of pollen grains, and causes male infertility [18]. Similar results were obtained by Bharadwaj, Chauhan [22], Rezanejad [89,90] in the study of the pollen of *Spartium junceum* L. The research of the impact of heavy metals (Cu, Zn and Pb) and abiotic environmental factors on the viability of high allergic *Parietaria judaica* pollen was conducted in the city of Thessaloniki (Greece) [91]. Studies have shown that the viability of *Parietaria judaica* pollen varied significantly depending on the intensity of traffic and environmental pollution, but its values did not differ during the season. High positive correlation coefficients of pollen viability were found with concentration of copper and lead in the shoots, but not with zinc. The pollen of plants growing near the roads with heavy traffic and a high level of environmental pollution by heavy metals has the highest viability. This indicates a stimulating effect of copper and lead on the fertility of *P. judaica* pollen. Similar results for this species were obtained by Iannotti [23]. The authors concluded that *Parietaria judaica* is well adapted to warm and polluted urban environment. In this way the pollen of some plant species is resistant to pollution and cannot be used for environmental bioindication [15].

4. Impact of Industrial Emissions on Pollen

The assessment of the quality of atmospheric air and soils of industrial areas is carried out using the pollen of herbaceous and woody species [30,31,58,65,67,80,92]. Fertility, viability and anomalies of the development of pollen grains are in the study.

Fertility and pollen viability of two morphological forms of *Taraxacum officinale* Wigg. s.l., which grows on industrial dumps were studied in the Middle Urals (Russia) [30]. The proportion of fertile pollen was significantly higher on agrozems (65%) than on technozems (55%), which contain high concentrations of heavy metals (Co, Cu, Fe, Zn). The percentage of fertile grains of *f.dahlstedtii* differs insignificantly within the studied soils from 57 up 54%, respectively. The proportion of fertile pollen grains of the forms of dandelion on agrozems was higher than on technozems. The authors come to the conclusion that the revealed differentiation of morphological forms of *Taraxacum officinale* on the basis of the fertility of pollen grains suggests that edaphic and coenotic stresses lead to increasing in differences on apomictic and amphimictic properties. The studied forms of dandelion do not differ in the proportion of viable pollen grains [30]. Similar results were obtained in the study of pollen fertility of *Sorbus gorodkovii* Pojark. in the city of Zapolyarny (Murmansk region, Russia), located in the vicinity of processing plant of the Kola Mining and Metallurgical Company [93]. The soil of the city contains high concentrations of heavy metals (Cu, Ni, Cd, Zn, Pb). However, the proportion of fertile pollen grains of *S. gorodkovii* varied insignificantly among the experimental plots, from 71.2 to 77.7% (in the control– 76.6%). At the same time the study of fertility of pollen of *Sorbus gorodkovii* in the vicinity of the Incineration plant and Heating Plants of the city of Murmansk (Russia), shows the other results [94]. In the vicinity of the environmentally hazardous industrial facilities the ratio of fertile pollen was 35–52% (in control –72%). The minimum of fertile pollen was in the samples of the South Heating Plant (35%), Central Heating Plant (41%) working on the fuel oil, and the coal-fired boiler house "Roslyakovo Yuzhnoye" (45%). The share of sterile pollen near these enterprises was very high (55–65%), in the control—28%. This indicates the high toxicity and mutagenicity of the emissions of boiler houses in the city of Murmansk. It is known that environmental stress can cause asynchronous processes during the development of the male gametophyte, affecting the amount of fertile pollen [95]. In the haploid gamets lethal mutations occur during the formation of pollen grains [96]. Developmental disorders, its asynchrony is often considered as meiotic instability, including under the influence of environmental stress, which can cause male sterility [95]. Pollen sterility is a sign of disturbance of reproductive process [97]. Sterile pollen grains are characterized by deformation and degeneration of nuclei, cytoplasm, or whole cells. The formation of such pollen in plants, as a rule, is associated with disorders during meiosis and microsporogenesis [98].

In recent years, studies of fertility, viability and teratogenes of pollen of coniferous affected by industrial pollution became very actual due to their high sensitivity [27–29,31,61,66,67,99–103]. For toxicological expertise of harmful compounds are often applied biotest of pollen tubes growth [104]. Experiments on the effect of various concentrations of lead on the germination of *Picea wilsonii* pollen showed that there was a strong inhibition of germination and elongation of pollen tubes [101]. The shape of pollen tubes changed, their diameter expands, and occurs the vacuolization of cell protoplasts. The authors found that lead affects the thickness of the cell wall, acid pectin and cellulose are deposited on it unevenly, and their quantity decreases towards the apical and subapical parts of the tubes. Confocal microscopy studies showed that lead stress disrupts the structure of the cytoskeleton by change of the structure and arrangement of cell wall microfilaments. As the concentration of lead increases, they become shorter, have another form, their number decreases, especially near the apex. Similar experiments were carried out by Wang [102], who studied the effects of As, Hg, Cd, Cr and Cu on the germination of pollen and elongation of pollen tubes of *Picea wilsonii in vitro*. Microscopic studies have shown that heavy metals inhibited the growth of *P. wilsonii* pollen tubes. The most toxic were cadmium and mercury. Under the influence of these metals, the pollen tubes have strong vacuolization of the cytoplasm, uneven increase in diameter and swelling of tube tips. Chromium and copper showed less toxicity and did not cause such structural changes of pollen tubes [102]. Cadmium and mercury are the most toxic for pollen germination and pollen tube growth [105]. Such pollen with a high concentration of heavy metals becomes more allergic. Studies of the quality of pollen of *Pinus sylvestris* L. were carried out in the vicinity of the Karabash copper smelter (Urals, Russia). Fertility, developmental anomalies, and pollen viability were studied [103]. Pollen fertility in all cenosis was high and varied in the range of 87.0–93.7%, there were no significant differences between the experimental sites. Sterile pollen with different developmental teratologies was separated and divided into three groups:

(1) With morphological disorders (small, large; with anomalies of the sacci, body shape and exine);
(2) With cytological disorders (plasmolysis, pycnosis, chromatolysis);
(3) With both types of disorders (degenerated).

It was found that most of the sterile pine pollen was small and underdeveloped; pollen with cytological disorders and anomalies of sacci was less common. The germination of pine pollen grains *in vitro* showed that the pollen tubes were characterized by a significantly higher frequency of swelling and branching. Frequency of pollen tubes with such anomalies of development in the impact zone of the copper smelter was higher than in the control. At the same time, pollen from polluted sites contained more starch, as a result of which pollen tubes were longer than in the control [103].

The cytogenetic anomalies of coniferous pollen (*Picea obovata*, *Pinus sylvestris*, *Larix sukaczewii*, *Abies sibirica*) were study under conditions of long-term critical and severe environmental pollution in the areas of copper smelting (Karabash), mining (Satka, Uchaly), and metallurgy industries (Zlatoust) in Russia [67]. The maximum allowable concentrations of pollutants in the air, water and soil were exceeded by tens and even hundreds of times. Sterile, small, hypertrophied, with abnormal sacci pollen grains were revealed in the samples. The significance of differences of detected anomalies between the studied tests plots of coniferous were determined by the χ^2 criterion. The results of the study of chromosome disorders in the generative tissue of coniferous showed that the average level of disturbances per stage of meiosis under conditions of pollution was 0.7–9.88%, in the control and background conditions only 0.5–1.8%. As a result of pairwise comparison of the quantity of chromosomal disorders detected at the stages of antelophase I and antelophase II of meiosis of coniferous under the anthropogenic pollution and in the control, using the χ^2 criterion, it was mainly established reliability of differences between these indicators. Comparison of the level of chromosomal disorders detected at the stage of metaphase I of meiosis, the reliability of differences only between control, critical and severe pollution was installed.

In addition to conifers, research of the use of pollen for bioindication of pollution of industrial areas is carried out on other species of different living forms, as *Taraxacum officinale*, *Chenopodium botrys*, *Lilium longiflorum*, *Nicotiana tabacum*, *Alyssum* L., *Eucalyptus globulus*, and others [11,30,73,80,104,106,107]. There are some studies showing the inhibitory effect of heavy metals on pollen of plants of natural populations [73,104,108]. In the Mediterranean, many species of the *Brassicaceae* family are known to be nickel hyperaccumulators [106]. Nickel affects plant reproduction by causing abnormal gamete development, disruption of embryogenesis and seed formation [109]. In several Mediterranean countries, the samples of stamens and pollen of several species of genera *Alyssum* L. were collected in natural populations, growing on ultrabasic soils with high concentrations of Mg, Ni, and Fe [107]. Pollen was studied by light transmission and scanning electron microscopy. The presence of Ni was found in the stamens, rarely in the pollen grains of all species. The authors suggest that there are some special protective physiological mechanisms and nickel accumulation in pollen is less than in anthers and its filaments [107]. The shape of pollen grains differed between *Alyssum* sp. species and their populations. In populations of *A. murale subsp. mural*, a large variety of shape and size of pollen was found, which, as the authors suggest, may be due to the effect of nickel on the process of microsporogenesis. The great variation of pollen shape may be the result of polyploidy. Most *Allisum* sp. species have a high proportion of sterile pollen. This may be the result of the negative impact of heavy metals contained in the soil, which is consistent with the data of other researchers [109,110]. At the same time the study of nickel accumulation in pollen of *Streptanthus polygaloides* and *Noccaea fendleri* ssp. *glauca* (*Brassicaceae*), which are considered hyperaccumulators of heavy metals, showed that the concentration of nickel in pollen of both species was 100 times higher than in the control [111].

The metal accumulation in pollen can affect plant reproduction by reducing pollinator visits to flowers and pollen germination [109]. Researchers have found that heavy metals have an inhibitory effect on pollen germination, especially of dicotyledonous plants [109,112]. Experiments on the influence of cadmium, copper, lead and mercury on pollen germination *in vitro* were conducted on 10 cherry cultivars [113]. The proportion of germinated pollen (%) and the length of pollen tubes (μm) were determined. It was found that with an increase in the concentrations of Cd, Cu, Pb and Hg from 50 to 250 ppm, there were decreases of the proportion of germinated pollen and the length of pollen tubes of all 10 sweet cherry cultivars. At the highest concentration of heavy metals (250 ppm), the studied parameters were close to zero. Cadmium showed the greatest, and lead the least toxicity, what may be due to the genetic characteristics of the resistance of sweet cherry to various heavy metals [113]. This is confirmed by the results of experiments of Gür and Topdemir [114], which treated plum and quince pollen by the salts of heavy metals (Cd, Cu, Hg and Pb). Cadmium was the most toxic for pollen germination and growth of plum pollen tubes, and mercury was the most toxic for quince. The authors [114] shown, that cadmium inhibits the extensibility of the cell walls of growing pollen tubes. Indian scientists experimentally studied the bioaccumulation of heavy metals (As, Pb, Ni, Cd, Fe, Al and Cu) by pollen of *Cyperus rotundus*, *Cassia siamea* and *Kigelia pinnata* [32]. It was found that aluminum and iron were the most accumulated. The remaining elements were found in trace amounts. Even at very low concentrations, heavy metals strongly inhibit pollen germination and pollen tube growth [104,108].

Pollen viability of *Cercis siliquastrum* L., *Medicago sativa* L., *Robinia pseudoacacia* L., *Melilotus officinalis* (L.) Lam., *Trifolium repens* L., and *Sophora alopecuroides* L. were determined in the vicinity of aluminum smelter with high levels of fluoride pollution [11]. Using staining by acetocarmine, it was found that all studied species produce a lot of abnormal, sterile pollen on the sites contaminated by fluorides, in contrast to the control. The pollutants changed the shape of the pollen [11]. The authors conclude that pollen provides important information about the biological effects of pollutants, which can be used for biomonitoring of air and soil pollution. Studies of pollen fertility were conducted on ten species of the family *Brassicaceae* in the vicinity of natural gas Power plant [108].

It was found that the proportion of anomalous and sterile pollen grains increased in the impact zone of the Power plant. However, the results varied significantly between species. *Isatis kotschyana* has the largest amount of teratomorphic (50.8%) and sterile pollen (45.5%). For all other species, these indicators were lower. The authors come to the conclusion, that pollen viability can be used as a bioindicator of air quality. It is known that phenolic compounds are indicators of environmental stress. An increased content of flavonoids was found in the pollen [108]. Similar results were obtained by Rezanejad [115] under the high levels of traffic and elevated concentrations of SO_2, NO_2, CO and hydro carbonates. In the pollen of *Spartium junceum* L., *Lagerstroemia indica* L., *Thuja orientalis* L. were obtained a significant increase in flavonoids compared to the control. There is an assumption that the increase in the content of flavonoids may be associated with their protective role under environmental stress [116].

5. Conclusions

Modern ecologists are interested in the research of reactions of living organisms to environmental pollution. Methodological approaches to bioindication of the environment are being developed with the help of various morpho-physiological reactions of plants and animals. Ecological palynology in this way seems to be a very promising direction. Pollen has morphological, ultrastructural, biochemical, and physiological changes under the influence of environmental pollution. Morphological variability and formation of teratomorphs, as studies have shown, can be used for palynoindication of the environment of urbanized and industrial areas. Physiological changes can be assessed by pollen viability using various tests. The biotest for the analysis of abortive pollen makes it possible to identify the physiological responses of gametes to changes of concentrations of air pollutants and the presence of mutagens. Widely used for toxicological studies tests based on pollen germination and pollen tube growth. Studies of ultrastructural changes in pollen grains and their biochemical reactions to environmental pollution make it possible to understand the mechanisms of increasing in pollen allergenicity under the influence of an anthropogenic factor.

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