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Brochosomes and Other Bioaerosols in the Surface Layer of the Atmosphere of Moscow Metropolis

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Abstract: The paper presents the results of the morphological study of aerosol particles in the urban air of Moscow (Russia) in 2019–2022 by scanning electron microscopy (SEM). Our monitoring revealed mineral and anthropogenic particles, and also primary bioaerosols (PBA), such as pollen, spores, plant fibers, etc., typical for the urban environment. Moreover, in July 2021, brochosomes, lipid secretions of semi-hard-winged insects Cicadellidae (or leafhopper), were found in several aerosol samples. They are quasi-spherical hollow porous semi-regular polyhedra (truncated icosahedra) of 0.2–0.7 microns in size, consisting mainly of carbon and oxygen. Despite the prevalence and diversity of leafhoppers, identification of their secretions in atmospheric aerosols in situ is rather rare: single articles from South Korea, Spain, the Himalayas, and the United States. In this sense, the results obtained are interesting and novel. PBA particles cover a wide size range and have a complex and diverse shape, which determines the distance and efficiency of their atmospheric transport. Pollen and fungal spores have a high allergenic potential and can have harmful effects on human health. Any new information about PBA can be useful for studying the development and dynamics of ecosystems.

Keywords: atmosphere; Moscow; urban aerosol; primary biological aerosol; brochosomes; morphology; meteorology

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1. Introduction

The processes of interaction between land, ocean, and atmosphere are largely determined by aerosol particles [1,2]. A significant part of atmospheric aerosol is represented by a subgroup of particles of biogenic origin—primary biological aerosol (PBA). These particles include living and dead organisms (algae, archaea, bacteria, etc.), dispersion units (fungal spores, plant pollen), and various fragments or secretions of organisms directly released into the atmosphere [3–9].

PBA can have different particle shapes and structures, and their size vary over a wide range (from nanometers to hundreds of micrometers) [4]. These particles make a significant contribution to the total aerosol emission on the Earth. On a global scale, the emission of primary biogenic particles is about 1000 Tg per year, whereas global sea salt emissions and mineral dust ones are about 3300 Tg/yr and 2000 Tg/yr, respectively [6].

Potentially, PBAs should influence climate-forming processes [3–9] because of their involvement in global cycles of carbon, nitrogen, sulfur and phosphorus, in cloud and precipitation formation processes, through heterogeneous and multiphase physico-chemical atmospheric reactions. The specificity of PBA effect on atmospheric processes, radiation, and optical characteristics of the atmosphere is determined by the high reactivity of such particles. They are fundamentally different from inorganic aerosols due to their more complex shape, variety of sizes, and their nature of often having a large surface area. On their surface, bioaerosols carry various functional groups that react differently to changes in physical conditions (humidity, temperature) and in chemical activities of small gas components in the atmosphere [6]. Moreover, PBA activates the clouds/ice formation in the upper atmosphere more efficiently and easily than other particles, and large pollen grains or fungal spores can themselves be giant condensation nuclei [3–7].

Some biogenic particles (microbial pathogens, allergens, for example, endotoxins, mycotoxins and glucans) can have infectious, allergic, or toxic effects on biota and humans on a local, regional, and global scale. Many pathogens of plants, animals, and humans are able to rise beyond the tropospheric mixing layer and are transported by air masses over long distances, spreading diseases across and between continents [4]. The role of atmospheric PBAs in shaping the state of the biosphere is increasing in large industrial megacities. Here, local anthropogenic sources of aerosols and special air flow regimes formed by dense buildings, microclimate, and relief of the city create specific conditions for the existence of aerosols in the near-surface atmosphere. In particular, some biogenic particles (microbes, viruses and other microorganisms) spread in urban air together with anthropogenic ultrafine particles penetrating into human lungs [10,11]. A variety of anthropogenic activities, accompanied by air pollution with dangerous bioaerosols, leads to high risks to public health [12–14].

Since the reactivity of PBA (and other aerosol particles) in atmospheric heterogeneous processes is related to their morphological structure [6–8], much attention in the world is currently focused on the study of the morphology of various aerosol particle types in the atmosphere. Particularly, intensive studies of morphological types and the composition of microparticles in the atmosphere are carried out in large cities of East and Central Asia [9,15–24]. In these publications, the shape, size and elemental composition of aerosol particles of anthropogenic and natural origin in different seasons are studied; their main sources in cities are identified. The information important for modeling the climatic effects of aerosols was obtained on the morphology and composition of mineral dust [25,26] and carbon-containing particles formed in various combustion processes (fly ash, soot, organic carbon, resin balls, etc.) [9,27–32].

In recent years, thanks to the development of analytical methods and instruments, qualitatively new studies of the characteristics of bioaerosols, including their morphological structure, have appeared in the world scientific community [7–9,33,34]. However, there are few Russian publications on the research of bioaerosols in the atmosphere (for example, [35–40]), and there are practically no articles on the morphology of atmospheric PBA. This work partially fills this gap.

The purpose of this study was to identify the primary biological aerosols in near-surface atmospheric aerosol in Moscow metropolis and to study their morphological characteristics. The article discusses the results of the morphological analysis of PBA particles found in the near-surface aerosol of Moscow metropolis. In particular, the main types of identified biogenic particles and the details of their morphological structure are described. Special attention is paid to the brochosomes, lipid secretions of semi-hard-winged insects Cicadellidae (or leafhopper), first discovered in the air of Moscow. We analyze the meteorological features of the warm season of 2021 in Moscow, which could contribute to the identification of brochosomes in the city aerosol. The results of the work can be useful for a broader studying and modeling of the processes of bioaerosol propagation in the atmosphere.

2. Materials and Methods

Aerosol sampling for morphological analysis was carried out in 2019–2022 as part of a continuous experiment to study the variability of physical and chemical characteristics of near-surface aerosol in Moscow metropolis [41,42]. The observation point was located in the courtyard of A.M. Obukhov Institute of Atmospheric Physics (IAP) of Russian Academy of Sciences, placed in the center of the city, in a zone of dense administrative and residential development, at a distance from major highways and railways. The underlying surface in this district is mainly urban soils with planted vegetation. Significant areas are sealed by road surface and buildings, unsealed areas make up 3–5%. Several old trees grow in the vicinity of the sampling point, the yard of the IAP RAS is sealed with asphalt pavement.

In this work, the objects of research were individual particles of atmospheric aerosol contained in aerosol samples. Over the years, a total of 189 atmospheric aerosol samples were collected on hydrophobic membrane filters from polytetrafluoroethylene or Petryanov's cloth; the area of each filter is 20 cm^2. Samples are taken at a height of 2 m from the underlying surface using a low-volume sampler. The sampling time may be from 12 to 24 h depending on the season, the synoptic situation, and meteorological conditions.

Urban aerosol samples were taken at different levels of atmospheric pollution. A high increase in the concentration of near-surface aerosol was observed during the regional or long-range atmospheric transport of fire aerosols [41] or dust [42–44], as well as during the operation of a close intense local source [42]. Sampling did not stop during the lockdown caused by the COVID-19 pandemic, when the anthropogenic load on urban ecosystems was reduced. Various meteorological and synoptic conditions could contribute both to the accumulation (anticyclone, calm) of pollutants in the air and to a significant cleansing (dominance of the arctic air masses, heavy precipitation, etc.) of the urban atmosphere from aerosol particles [40,43].

We interpreted our morphological data using open Internet resources [45] and meteorological data for the closest weather station "Balchug" (at a distance of about 850 m from the sampling point) in Moscow [46–48].

The properties of individual aerosol particles were studied by electron sonde methods, which are widely used for such tasks [2,9,15,17,18,23,24,49]. We worked with the high-resolution scanning electron microscope (SEM) JSM 7500F from JEOL, Japan with an auto-emission cathode. The images were obtained in the mode of low-energy secondary electrons, which provides the best spatial resolution (up to 1.5 nm under primary beam energy of 15 keV).

Only pieces (about 5 mm^2 in size) of a dry filter with aerosol sample, cut out randomly while taking into account the filling of the filter surface with aerosol, were used for SEM analysis. A thin platinum film (with a thickness near 5 nm) was sprayed on such pieces by magnetron sputtering to prevent the dielectric surface from electrification under the action of electronic sonde. Three or more images of each piece were analyzed at various multiplicity of magnification from 1000$\times$to 45,000$\times$.

The method of X-ray spectral microanalysis makes it possible to determine the local elemental composition of individual particles by their energy dispersion spectra (EDS) under electron beam scanning [50]. The analytical nozzle INCA Penta FETx3 (OXFORD INSTRUMENTS, Oxford, UK) was used for the scanning electron microscope (SEM+EDS). The silicon-lithium detector with liquid nitrogen cooling gives a resolution (by carbon) of 129 eV. Its calibration was carried out using a cobalt reference metal by $Co_{K\alpha}$ line.

Our morphological analysis allowed us to identify the main types of particles of natural and anthropogenic origin in Moscow metropolis: aluminosilicates and salts, often enriched with heavy metals, agglomerates of soot particles, etc. This work is devoted to aerosol particles of biological origin (bioaerosols), including brochosomes, rarely found in such studies and, possibly, firstly identified in Moscow air.

3. Results and Discussion

The study and classification of aerosol particles by morphological structure and composition allowed us to determine their nature. Along with mineral particles, as well as anthropogenic particles containing metals, sulfur and carbon, biogenic particles of various types were detected in samples of near-surface atmospheric aerosol in Moscow, mainly in the warm season (April–October). They belong to the group of organic aerosols and consist mostly of carbon and oxygen. There were mineral elements (Na, Mg, Ca, K, Al, Fe, Si) and elements of predominantly anthropogenic origin (S, P, Pb and Cu) in trace amounts on some particles of PBA [41,42].

3.1. Spores and Conidia of Fungi

Fungal spores (Figure 1) and conidia (Figure 2) are the most common types of PBA particles detected in different years in summer samples during the main activity of their reproduction in Moscow region. Spores and conidia are ejected by fungi in liquid jets or droplets (effects of osmotic pressure and surface tension), but it is possible to separate dry spores by wind or other external forces [5,34,51]. The concentration of spores released by the dry method, as a rule, increases in warm, dry weather, while the number of spores released by the wet method increases in humid conditions, at night and in the early morning hours. Therefore, there may be a relationship between the processes of spore emission/dispersion and various meteorological parameters [5].

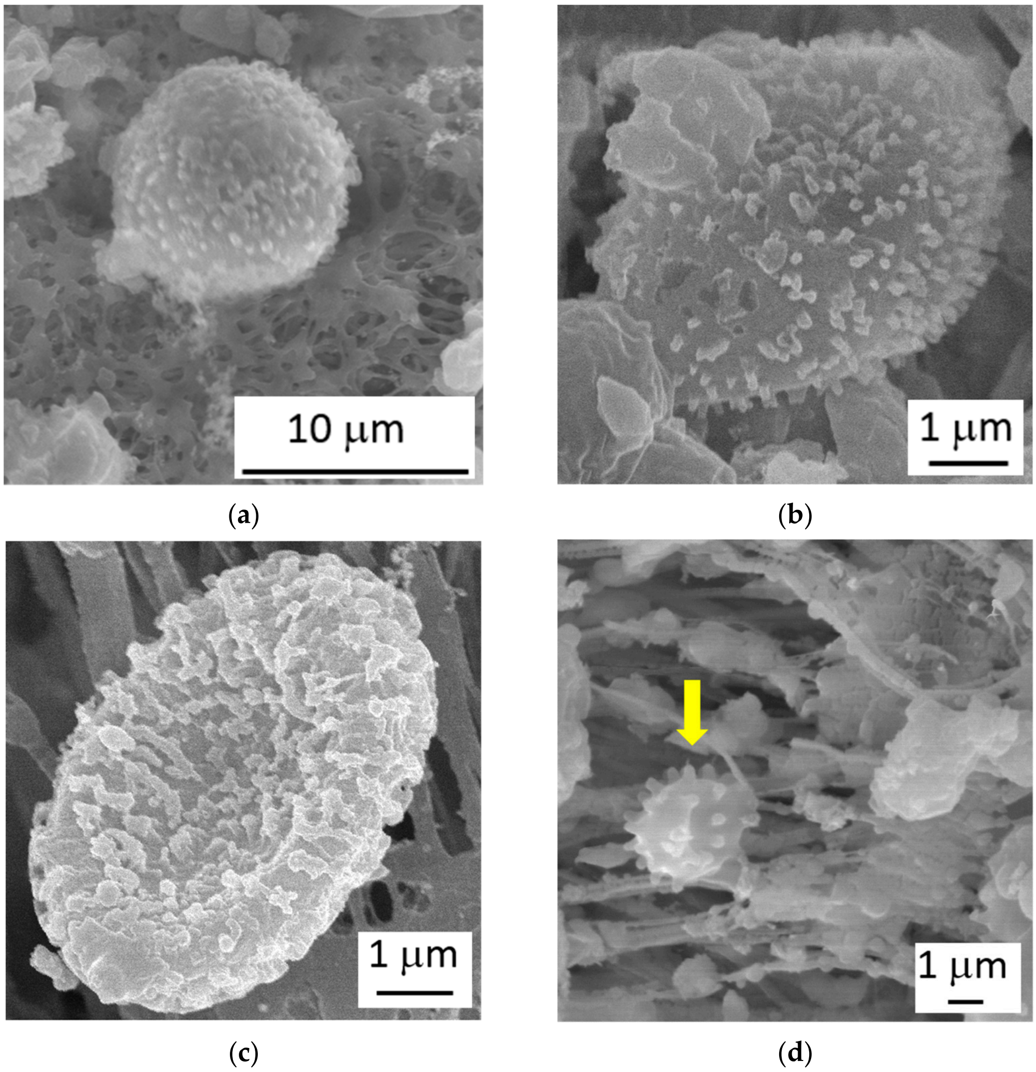


Figure 1. SEM images of various types of fungal spores found in aerosol samples in summer, Moscow, in different years: (**a**)—2 July 2019; (**b**)—28 June 2021; (**c**)—14 July 2021; (**d**)—14 July 2022, Yellow arrow indicates a fungal spore.

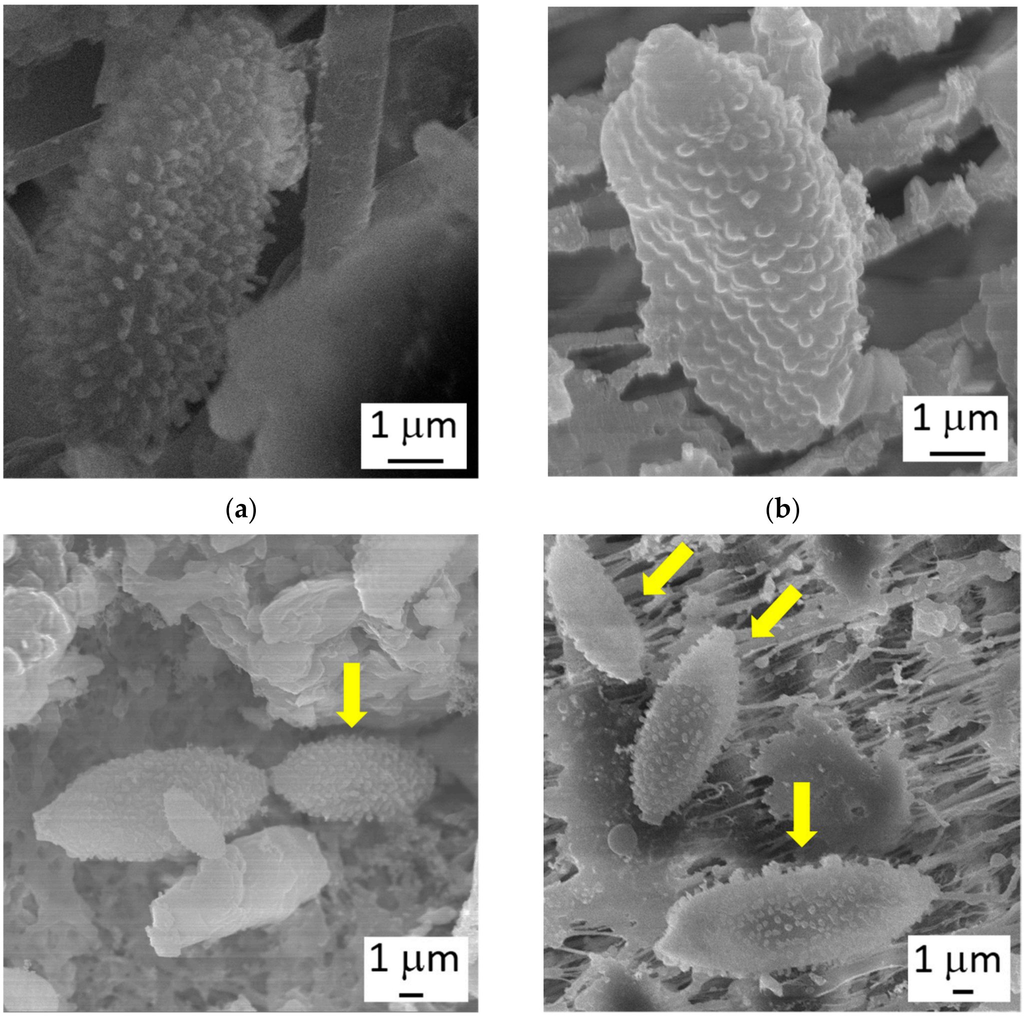


Figure 2. SEM images of fungi conidia found in aerosol samples in summer, Moscow, in different years: (**a**)—7 July 2020; (**b**)—27 June 2021; (**c**)—2 July 2019; (**d**)—28 July 2022. Yellow arrows indicates fungi conidia in the figures (c) and (d).

The diameter of fungal spores varies in the range of 1–50 μm depending on the biological species, age, and environmental conditions [4,5]. The spores and conidia of fungi identified in samples of near-surface aerosol in Moscow were characterized by an average size of 4–7 μm. Moreover, conidia combined in pairs were found (Figure 2c) in several samples. The ability of fungal spores to combine into long chains determines their aerodynamic diameter. This parameter determines the lifetime of these biogenic particles in the atmosphere and the ability of their deposition in the respiratory tract and in the lungs of animals and humans [5].

The number and mass concentrations of fungal spores in the air of the atmospheric continental boundary layer are estimated, respectively, to be about 104 m^{-3} and 1 $\mu g/m^3$. Up to 10% of organic carbon is contained in fungal spores and conidia, which are found on about 5% of PM_{10} aerosol particles in cities and suburbs [5].

3.2. Pollen

Pollen grains are the second most common type of PBA and are one of the largest in size (up to 100 μm). In the near-surface atmosphere in Moscow, the largest number of pollen grains was found in spring samples (Figure 3), which is associated with the specifics of vegetation growing season. Pollen grains can have different shapes and hard shells; they exist in aerosols as whole units or fragments. At high humidity, they are capable of break up into fragments, ranging in size from 30 nm to 5 μm [5]. The dispersion of pollen grains in the atmosphere depends on meteorological conditions (humidity, temperature, wind, precipitation). The presence of pollen in the air clearly corresponds to the seasonal cycle associated with the activity of plant flowering sources. Furthermore, as for fungal spores, a certain daily course is typical for pollen. Pollen grains, such as large-sized PBAs, usually have a short life time in the atmosphere. However, under certain conditions, they are able to rise to great heights with concentrations sufficient for their participation in ice crystal formation processes at the upper levels of the troposphere [5].

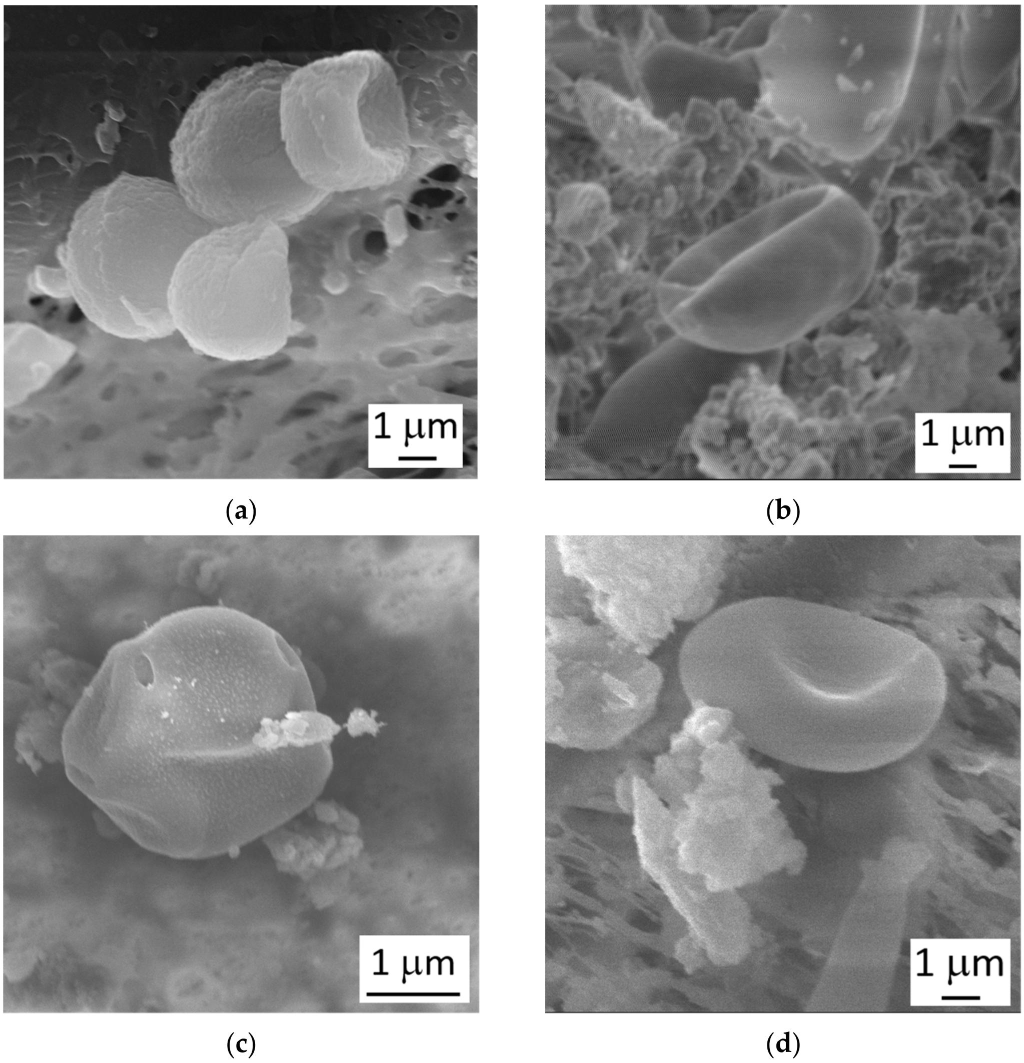
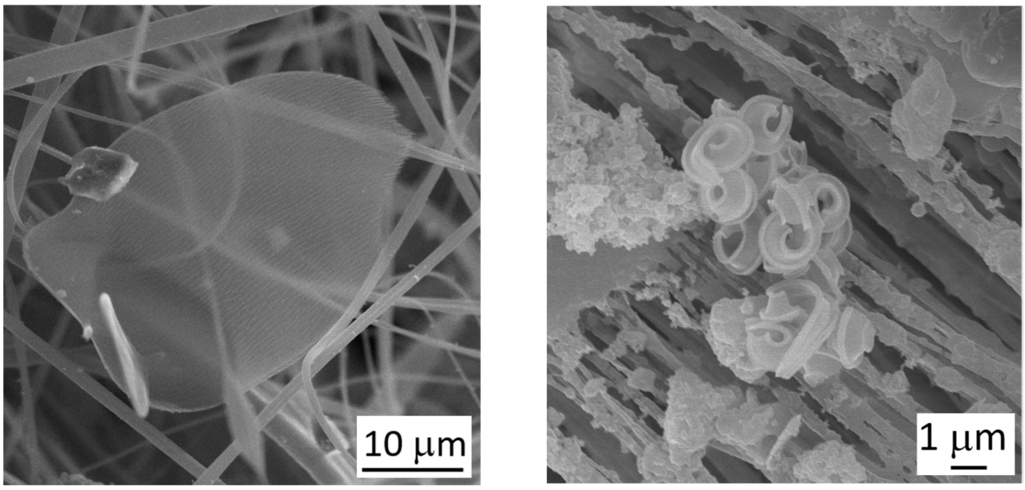


Figure 3. SEM images of various forms of pollen grains found in aerosol samples in Moscow in different years: (**a**)—20 June 2019; (**b**)—23 October 2020; (**c**)—12 April 2021; (**d**)—3 October 2022.

3.3. Fragments and Secretions of Living Organisms

In warm season, other types of PBA were also found in the samples of near-surface aerosol in Moscow. The largest of them in size (20–40 µm) were flat insect scales (Figure 4a) detected in the spring of 2021. Insect scales are quite common primary biogenic particles in the atmosphere of cities [34]. In the summer of 2021, a rather rare type of PBA was identified—epicuticular wax of the plant (Figure 4b). This is a wax coating on the outer surface of plant cuticle forming super hydrophobic and self-cleaning surface. Epicuticular wax mainly consists of aliphatic hydrocarbons containing various functional groups, forming two- or three-dimensional structures. The most common morphological types of epicuticular wax are thin films and several three-dimensional structures: massive crusts, granules, plates, threads, rods, and hollow tubes. The sizes of these morphological structures usually vary in the range of 0.2–100 µm [52]. Within the framework of this work, epicuticular wax in a form of resembling pasta was identified (Figure 4b). The same morphological structures were observed by the authors [51].



(**a**) (**b**)

Figure 4. SEM images of other types of PBA: (**a**)—flat insect scale, (**b**)—epicuticular wax of a plant.

Certainly, there are many other biogenic particles in the biosphere. In particular, plant fragments make one of the largest contributions to the total mass PBA concentration. Often, it is difficult to reliably determine the nature of such particles and other organic substances because plant materials can eventually be split into humic-like substances by oxidation and degradation of biopolymers [52].

3.4. Brochosomes

The secretions of living organisms also include brochosomes. This is the most remarkable and rare species of PBA, found for the first time in near-surface aerosol in Moscow. That is why, in this paper, we consider them in a separate paragraph.

3.4.1. Identification and Morphology of Brochosomes

Brochosomes are quasi-spherical hollow porous formations with a size of 200–700 nm, extracted from proteins and fats by individuals of the cicadas family Cicadellidae (or leafhoppers) [52–56]. Similar to epicuticular wax produced by plants for the protective purpose, super hydrophobic brochosomes serve to protect the surfaces of the wings, body and laid eggs of insects from water and pollution, and also weaken the reflection of light from these surfaces to mask from predators.

A distinctive feature of brochosomes is their shape, which was the feature that attracted our attention and subsequently caused the long process of their identification. Previously, such particles were not detected in the experiments of the authors and other Russian research groups studying the physical and chemical properties of near-surface aerosol. The search for particles resembling the identified objects in shape first led to the microcapsules of sporopollenin of dandelion pollen grains [57], which are geometrically similar to the shape of brochosomes. However, the difference in outward appearance and the larger sizes of dandelion grains, compared with the particles we found, stimulated further steps to search for the sources of the latter.

It was found that a major part of the brochosomes found in Moscow have a shape similar to the structure of the fullerene C_{60} molecule [58–60], which is a semi-regular polyhedron (truncated icosahedron) (Figure 5). That is why, in the literature, brochosomes are often called "biological fullerenes" [61]. However, the sizes of geometrically similar structures of brochosomes and fullerene C_{60} differ significantly. The brochosomes found in aerosol samples in Moscow in the summer of 2021 (Figure 6) are characterized by an external size of about 200–400 nm, which is significantly (by several orders of magnitude) larger than the size of the fullerene C_{60} molecule. According to experimental and calculated data [59], the length of the intramolecular double bond in the carbon frame of the C_{60} molecule is 0.139 nm, and the length of the single one is 0.145 nm (Figure 5).

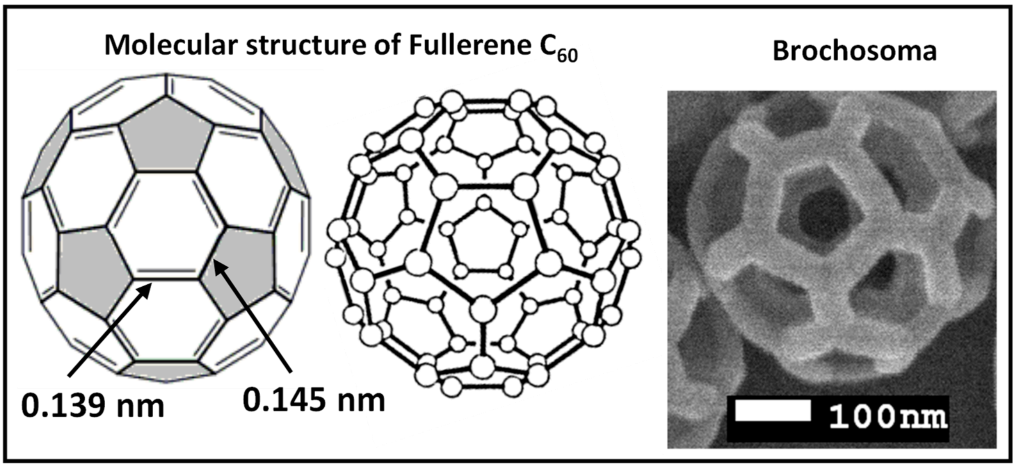


Figure 5. Comparison of brochosome shape with the molecular structure of fullerene C_{60} [59,60].

A specific feature of the brochosomes identified in the real city air should be noted: they are found in the form of large agglomerates (intricate chains and friable aggregates such as fractal clusters of soot particles), which are either pressed to the surface or in direct contact with larger mineral particles or their clusters, as can be seen from Figure 7. In addition, the size of a brochosome is 10 or more times smaller than other PBA particles detected in aerosol samples in Moscow (Figure 8).

3.4.2. Meteorological Conditions

In the warm season, there should be a huge amount of insect-secreted brochosomes and their agglomerates in the near-surface atmosphere of the middle latitudes, since hundreds of different species of cicadas live on shrubs and trees (lilac, rose, linden, birch, poplar, etc.) both in cities and in suburbs. However, in the publications of scientists experimentally studying the morphology of near-surface aerosols, information about brochosomes is scarce (for example, in [9,34,51]), particularly, there is, at present, an absence of this topic in Russian works. In this sense, this study is a pioneer.

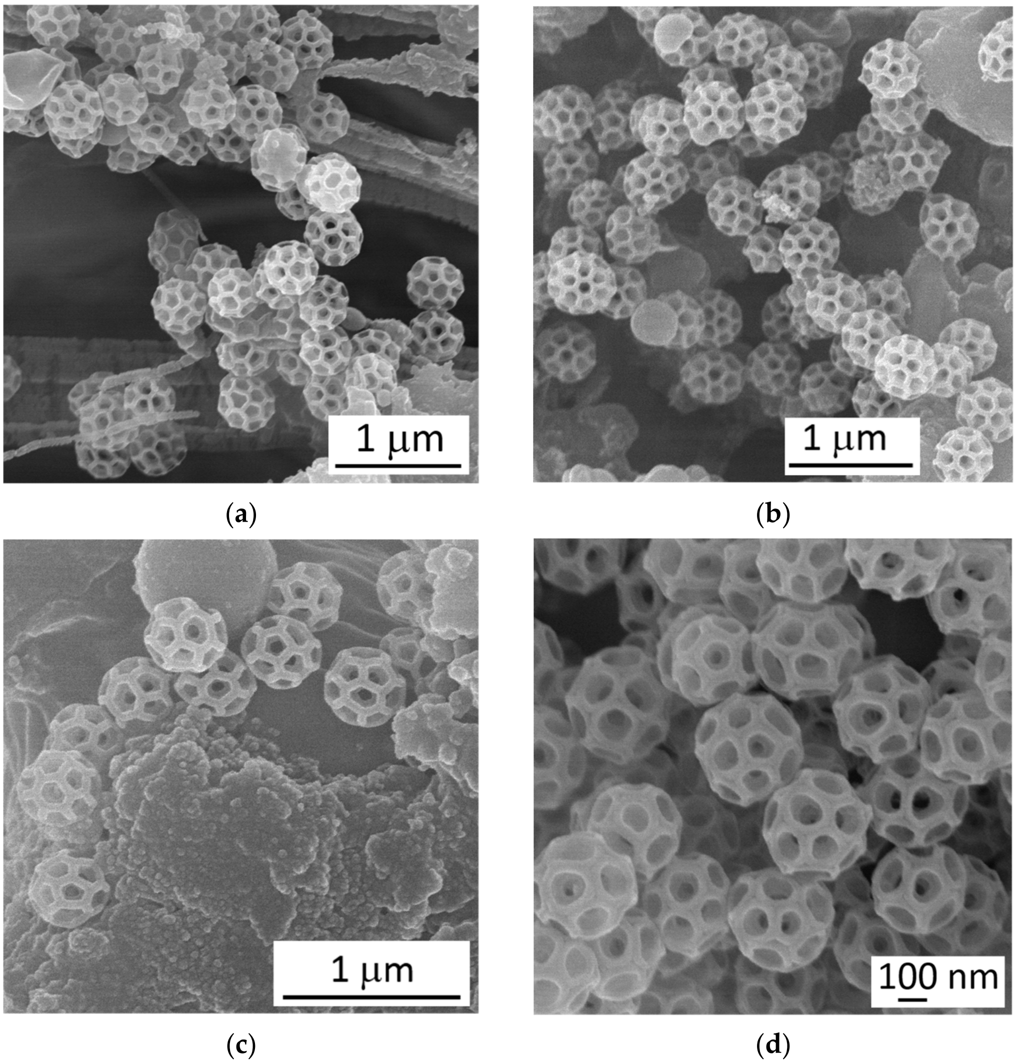


Figure 6. SEM images of brochosome agglomerates found in four aerosol samples in Moscow during summer 2021: (**a**)—29 June.; (**b**)—14 July.; (**c**)—28 July; (**d**)—31 July.

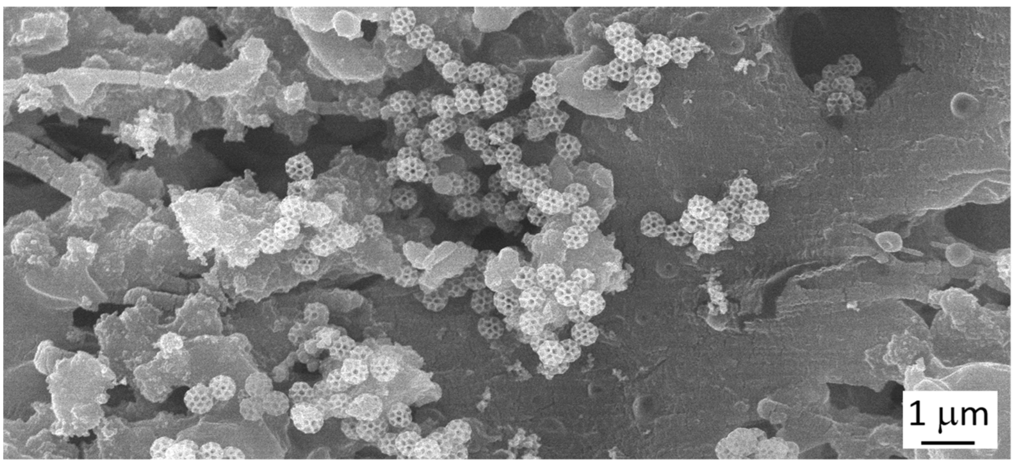


Figure 7. An example of the location of brochosome agglomerates in the aerosol sample.

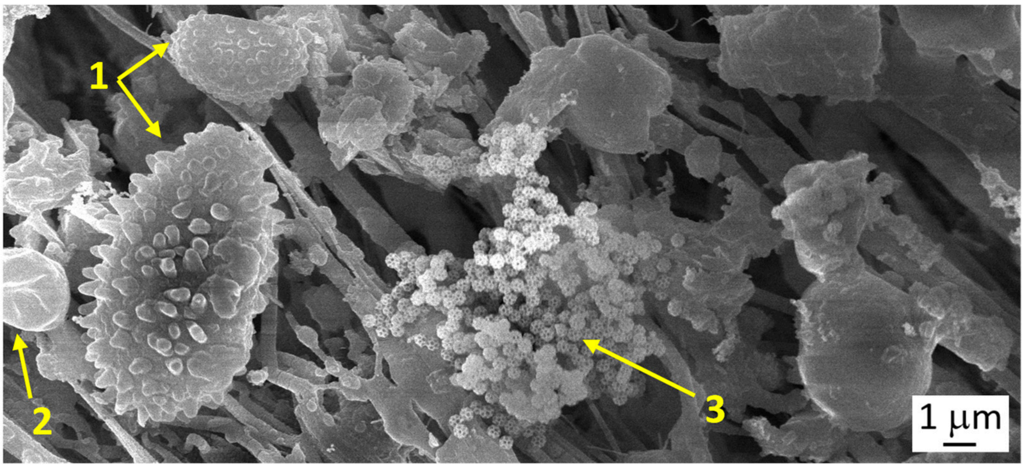


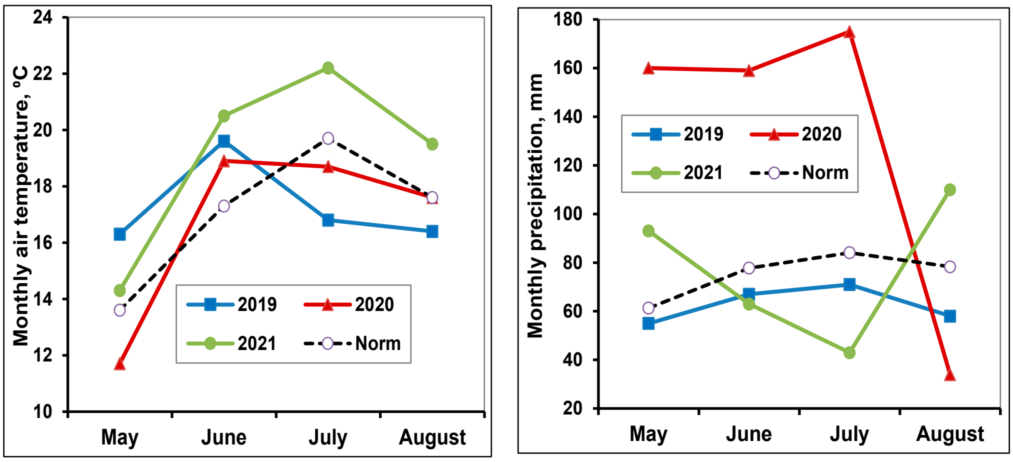
Figure 8. Comparison of shapes and sizes of different types of PBA: (**1**)—spores of fungi, (**2**)—pollen grain, (**3**)—agglomerate of brochosomes.

We registered brochosomes in Moscow in four aerosol samples (out of 21) collected in summer 2021: on 29 June and on 14, 28, and 31 July. Since this was the first detection of brochosomes during three years of near-surface aerosol observations (including the morphology of aerosol particles) in Moscow, the question arose as to why we did not observe these particles in other months and years. Of course, one of the explanations is trivial, consisting in the fact that brochosomes on the aerosol filters simply did not fall into the areas of our SEM analysis. However, another explanation is also possible.

In the middle latitudes, the development cycle of cicadas Cicadellidae covers the warm period from April to September. The output of the first generation of larvae from overwintered ovipositors occurs in the spring. The development cycle of the next generation larvae is about 20 days during the period from the second half of June to mid-September. During the development and laying of eggs, Cicadellidae cicadas secrete brochosomes to protect organs and eggs from pollution and viruses.

The weather changes from year to year, and it can be assumed that in a year when spring and early summer are wetter and at the same time warmer, the release of brochosomes may occur earlier, and under the opposite meteorological conditions, later. Our observations in summer usually began on 22–26 June and ended on 3–5 August.

Let us compare the meteorological conditions of the warm months of 2021 in Moscow with the previous two years based on meteorological data archives [46,47]. It can be seen from Figure 9 that May and the whole summer of 2021 were abnormally hot: the average monthly air temperature exceeded the long-term average values by 2–3 °C. In the second half of June 2021, Moscow meteorological stations even recorded air temperature of 34.7 °C during daytime hours, which is the absolute maximum for the last 160 years of observations. Besides this, the total amount of precipitation in May 2021 significantly exceeded the normal value. Such conditions could contribute to the abnormally early development of cicadas and their emission of brochosomes as early as in May. July, on the contrary, was very hot and dry; the amount of precipitation was half the norm. From about mid-June to the end of July, hot days with weak winds (up to 1 m/s) prevailed in Moscow, and occasionally there were short torrential rains with thunderstorms and a short-term increase in wind speed. Under such conditions, the brochosomes could dry out and come off by wind or raindrops, arriving into the aerosol composition in near-surface air. Since in 2019–2020 the meteorological conditions of the warm months were closer to normal (Figure 9), it can be assumed that the development of cicadas, accompanied by the production of brochosomes, occurred at the standard time for mid-latitudes, and during our experiments, we might not have had the time to register brochosomes in aerosol composition, since our observations stopped in early August.



(**a**) (**b**)

Figure 9. Meteorological conditions in the warm months of 2019–2021 in Moscow in comparison with the norms [48]: (**a**)—the monthly average air temperature in near-surface air; (**b**)—the total monthly precipitation.

It can be assumed that in order for brochosomes to enter the atmospheric aerosol, it is not only their presence on vegetation and insects themselves which are necessary, but also the presence of suitable meteorological conditions for their separation and removal into the atmosphere. Long-term trends of changes in meteorological parameters in Moscow show that over the past 50 years, the air temperature in the summer months has increased (Figure 10) at a rate of 0.4–0.8 °C (for different months) over 10 years in accordance with [48]. It is quite possible that brochosomes are common in the surface air of Moscow region on certain days or weeks of each summer. Thus, climatic changes in weather conditions, time shift and/or warming of the summer season could increase or decrease the probability of detecting brochosomes at stable dates of the observation period.

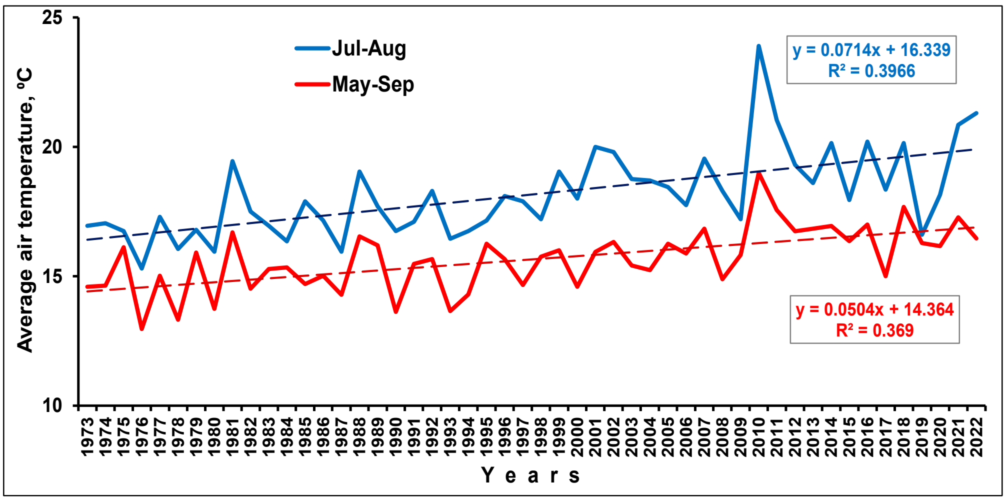


Figure 10. Average air temperature through July–August and May–September over the past 50 years from 1973 to 2022 with linear trends and their parameters.

4. Conclusions

The paper presents the results of studying the morphology of primary biological aerosol (PBA) contained in the near-surface atmosphere of Moscow metropolis. The main types of PBAs, their shapes, and sizes were identified by the SEM+EDS method according to long-term observations (2019–2022) in the central district of Moscow. In the spring period, pollen grains of plants and their fragments (ranging in size from 30 nm to 5 microns) predominate among the particles of biological aerosols in the city, and in the summer period, the same is true for the spores and conidia of fungi (an average size of 4–7 μm). In addition, fragments and secretions of living organisms, such as insect scales (20–40 μm), epicuticular wax, and plant fragments were identified in urban aerosol samples during the warm season.

The composition of PBAs is characterized by seasonal and diurnal variability. The specifics of their emission, transport, and transformation largely depend on meteorological parameters. Pollen and fungal spores can have a negative impact on human health and vital activity. In particular, their high allergenic potential can be further enhanced by participating in heterogeneous reactions with gas impurities in the urban atmosphere.

A new interesting result is the detection and identification of brochosomes ("biological fullerenes") in the composition of a surface aerosol in Moscow in the summer of 2021. This is a rare (on a global scale) result obtained for the first time during field observations of atmospheric composition in Russia. Brochosomes, lipid secretions of semi-hard-winged insects Cicadellidae (or leafhopper), were found in several aerosol samples only. They are quasi-spherical hollow porous semi-regular polyhedra (truncated icosahedra) of 0.2–0.7 μm in size, mainly consisting carbon and oxygen.

Brochosomes in aerosol composition in Moscow were registered only in four samples collected in the summer of 2021 (out of three summer seasons of daily aerosol sampling for morphology from the end of June to the first days of August 2019–2021). The analysis of meteorological conditions showed that this year was distinguished by abnormally hot and dry summer with abnormally wet spring preceding it. Activity in the development of leafhoppers, as is known, has its own pattern of seasonal changes. Perhaps, namely this weather anomaly in 2021 contributed to the appearance of brochosomes in Moscow aerosol only during the period of sampling aerosol material for morphological analysis. Further observations and a set of representative aerosol samples are needed to test this hypothesis.

PBA particles cover an extremely wide range of sizes, are very complex, and diverse in morphological structure; information about these features in the literature is still insufficient. However, it is the size and shape of an aerosol particle that determines its lifetime and range of transport in the atmosphere, as well as the ability of its deposition in respiratory tracts and lungs of animals and humans. The results obtained in this work provide new information, partially filling this gap, and can be used in studying and modeling the role of PBA in the development, evolution, and dynamics of ecosystems.

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