Review

A Critical Mini-Review on Atmospheric Ozone Mediated Alterations of Bioaerosols and Their Effects on Human Health

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Abstract: Bioaerosols are airborne particles that contain microorganisms and their derivatives, attracting much attention recently due to global epidemic of COVID-19. In fact, characteristics of bioaerosols can be significantly influenced by pollutants in air. As one of the most common ambient air pollutants, ozone (O_3) may influence the characteristics of bioaerosols and finally affects their health effects. However, the interaction association between the atmospheric ozone pollution and bioaerosols are poorly understood. In this critical mini-review, recent research about the influences of O_3 on biological components, physical characteristics, bio-activity, evolution of community structure as well as health risk of bioaerosols is reviewed. In addition, this mini-review also highlights that atmospheric O_3 may play a potential role to boost the spread of antibiotics resistance genes to some extent, which warns the public to properly control atmospheric O_3 and bioaerosol pollutions synchronously.

Keywords: bioaerosol; ozone pollution; alteration of characteristics; change of community structure; health risk

1. Introduction

Bioaerosols are airborne particles that contain microorganisms and their derivatives, such as bacteria, fungi, viruses, endotoxin, myctoxins, pollen and so on [1,2]. Bioaerosols exist outdoor and indoor with the bacterial and fungi concentration ranging from tens to thousands and several to hundreds CFU·m^{-3}, respectively, depending on seasons and locations [3,4]. The size distribution of bioaerosols shows large span from 10 nm to 100 μm, which size of airborne bacteria majorly distributes within 1.0–7.0 μm, while fungi within 1.0–11.0 μm [5,6]. Diversity of bioaerosol communities reaches thousands bacterial and fungal genera with tens or hundreds dominant genera, usually including pathogenic microorganisms [7]. Exposure to these airborne bio-particles may pose potential health risks to humans and animals, which include respiratory infections, allergies, and other infectious diseases [8].

Besides the geographical factors, contemporary research indicated that bioaerosols are also influenced by air quality [9], including humidity, ozone (O_3), sulfur dioxide (SO_2) and other atmospheric pollutants. Nowadays, O_3 is one of the important air pollutants, exhibits the capability to oxidize all types of organic and inorganic compounds [10]. Thus, O_3 can directly and indirectly interact with bioaerosols due to its ability to capture electrons from other molecules and release them to promote further chemical reactions [11,12]. Direct impacts include the absorption of O_3 by target molecules on bioaerosols, while indirect effects focus on ozonation of water to produce reactive species [13]. At atmospheric O_3 pollution concentrations of tens to hundreds of μg·m^{-3}, O_3 has demonstrated remarkable effectiveness in reacting with various types of airborne pathogens, particularly in high humidity conditions (RH > 80%) [14]. Thus, the characteristics of bioaerosols would be significantly influenced by O_3.

Recently, associations of air pollutions with their health risks have been intensively investigated [5,15]. However, the specific studies on relationships of reactive O_3 with pathogenic bioaerosols have not comprehensively summarized. In this critical mini review, we have highlighted recent research progresses on the mechanisms of atmospheric O_3 mediated characteristics change of bioaerosols, including the influences of O_3 on biological components, bio-activity, size distributions and community structures (concentrations) of bioaerosols, as well as their effects on human health risk in modern scientific cognition, which may alert the public to properly control O_3 and bioaerosol pollutions simultaneously.

2. High Concentration Atmospheric O_3 Affects the Size Distribution and Community Structure of Bioaerosols

The World Health Organization (WHO) has established a guideline value of 100 μg·m^{-3} as the maximum 8 h average concentration for O_3 (The Grade II standards of the Chinese NAAQS is 160 μg·m^{-3} for O_3), which can induce the mucosal irritation of the human nose and throat [16]. At high concentration of O_3 pollution episodes with >100 μg·m^{-3}, O_3 can destroy bioaerosols leading to microorganisms' inactivation, as it is a powerful oxidant [17].

O_3 exhibits the capability to oxidize essential enzymes involved in glucose decomposition and also directly react with and dismantle the organelles, DNA, and RNA of bacteria and fungi, disrupting their metabolic processes and resulting in their demise [18]. O_3 may also exert an inhibitory effect on microorganisms due to its strong oxidizing effect, like oxidizing bacterial cell wall and cytoplasmic membrane, leading to cell lysis and death through damaging the membrane components such as glycoproteins, glycolipids, or amino acids of the nucleic acids [19]. Moreover, O_3 possesses the ability to penetrate cell membranes and target lipoproteins in the outer membrane and lipopolysaccharides in the inner membrane, causing bacterial cell permeability to distort, dissolve, and ultimately perish [16]. On the other hand, O_3 also reacts with other air pollutants including organisms, volatile organic compounds (VOCs), NO_x, particulate matter (PM) and H_2O, generating reactive oxygen species (ROSs) like •OH, •O_2^-, •OOR and so on [19]. These ROSs are also able to inactivate bioaerosols by damaging their proteins or cell wall, resulting in nucleic acid leakage and further degradation [20,21]. As shown in Figure 1, these resulting ROSs can strongly destroy various bacterial cellular tissues and virus [14,22], resulting in reducing bio-activity or death of microorganisms on bioaerosols.

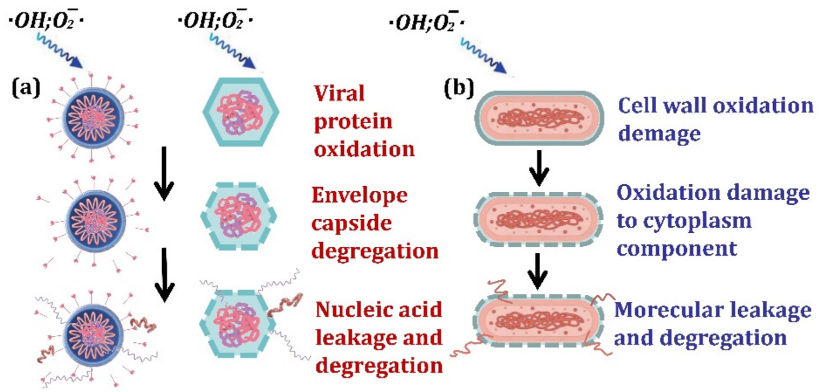

Figure 1. Inactivation mechanisms of virus (**a**) and bacterium (**b**) with ozone. Reactive oxygen species (ROSs) such as hydroxyl radicals (•OH) and superoxide anion-radicals (•O^{2-}) are produced via O_3 interactions with H_2O. Reproduced with permission. Copyright 2023, Springer Nature.

Due to the bactericidal property of O_3, it is easy to consider that exposing to high concentration of O_3 (>100 μg·m^{-3}) may lead to reducing concentration of bioaerosols [23]. Actually, as different types of microorganisms on bioaerosols show various tolerance to O_3 (some still alive, while others are inactivated), leading to evolution of community structure, microorganism diversity and altering of size distribution of bioaerosols when exposed to high concentration of O_3. For fugal aerosols, at high concentration of O_3 pollution, concentration of fugal aerosol decreased from ~950 to ~650 CFU·m^{-3} at normal days (14.2 ± 13.7 μg·m^{-3}) and O_3 pollution days (102.3 ± 66.2 μg·m^{-3}), respectively, and for bacterial aerosol decreased from ~350 to ~210 CFU·m^{-3} at normal days and O_3 pollution days, respectively [24]. The bacteria attached to dust on bioaerosols are mainly gram-positive bacteria, which have a stronger tolerance to high O_3 concentration environments than gram-negative bacteria [24]. What should be noted that antibiotic-resistant bacteria (ARB) on bioaerosols are found to be more resistant to O_3 than antibiotic-susceptible bacteria (ASB), indicating that O_3 may increase the proportions of ARB on bioaerosols. Berrington and Pedler found that in contrast to methicillin-sensitive *Staphylococcus aureus* (MSSA), methicillin-resistant *Staphylococcus aureus* (MRSA) strain showed better resistance to high O_3 concentration (100–120 μg·m^{-3}) [14]. The number of average survivors of MSSA and MRSA after exposure to 100–120 μg·m^{-3} O_3 for 4 h was 3.9 and

31.7, respectively, showing methicillin-resistant MRSA shows better tolerance to O_3. The results indicate that ARBs on bioaerosols remain alive when expose to high concentration of O_3.

Even for virus aerosols, Alimohammadi and Naderi stated that enveloped viruses such as SARS-CoV-2 are less persistent against O_3 molecules (disinfection concentration of O_3 500 μg·m^{-3}), compared to non-enveloped viruses [14]. Zuo et al. used redundancy analysis (RDA) and partial RDA (pRDA) to assess the contribution of environmental factors (air pollutants and meteorological conditions) to variation of airborne microorganisms. They found that air pollutants including O_3 played more important role in bacterial community and variations of airborne microorganisms than meteorological conditions [25,26].

After persistent and severe oxidations by high concentration of O_3, the sensitive bioaerosols eventually die, leading to change of size distribution/diversity and variation of community structure of themselves in some areas [25]. Yang et al. [24] found that high concentration of O_3 (102.3 ± 66.2 μg·m^{-3}) showed a trend of gradual decrease coarse to fine particle size of bioaerosols, with peak values of 3.3–4.7 μm. It can be seen from Figure 2, fine culturable bacteria and fugal aerosols (<2.1 μm) in O_3 polluted days are similar with normal days at around 20% and 40%, respectively. (HOs is high concentration of O_3 days >100 μg·m^{-3}, NDs is normal days). However, fine aerosols of total airborne microbe in HOs were ~52.2% among all microorganisms, which was much higher than that in NDs (~29.6%), indicating that 20% increase in fine particles and most microorganisms can exist in fine particles under high concentration of O_3 [24]. Similarly, Hao et al. [27] also found that a relatively larger decrease in viability over time for bio-particles within the larger size of 0.5–1.0 μm under ozone and UV exposure conditions, while no such a trend obtained for the smallest bio-particle size range of 0.3–0.5 μm on bioaerosols. These results give certain evidences that O_3 may promote the reduction of size distribution of bioaerosols, as the viability was higher for smaller particle sizes than for the larger ones.

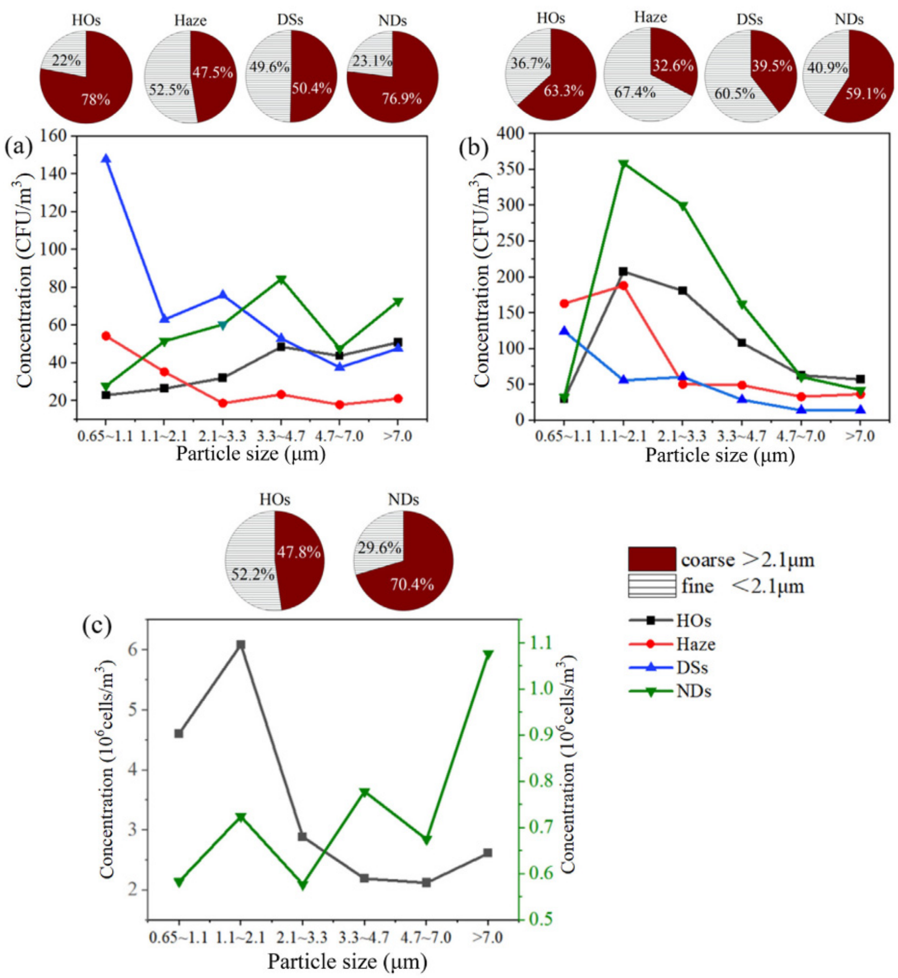

Figure 2. The size distribution of bioaerosols under different types of pollution (HO is high concentration of O_3 days >100 μg·m^{-3}, NDs is normal days): (**a**) airborne bacteria; (**b**) airborne fungi; (**c**) total airborne microbe. Reproduced with permission. Copyright 2024, IOP Publishing [24].

As compared with in normal days (concentration of $O_3 < 20$ μg·m^{-3}), fungi, bacteria and virus on bioaerosols exposure to high concentration (>100 μg·m^{-3}) of O_3 in HOs days, so that the community structure (species O_3 tolerance increase), concentration (decrease), and size distribution (fine bioaerosols increase) of bioaerosols are really modified due to strong oxidization of O_3. These investigations alert the public that bioaerosols oxidized by high concentration of O_3 may significantly change the concentrations, sizes, categories and community structures of bioaerosols.

3. Low Concentration Atmospheric O_3 Affects the Biologic Components and Activity of Bioaerosols

At low concentration of atmospheric O_3 condition (20–100 μg·m^{-3}), O_3 can also target to membrane glycoproteins, glycolipids, and amino acids through modifying the basic chemical structures in nucleic acids and influencing the sulfhydryl groups of vital enzymes, disrupting normal cellular activity. Although the morphology changes of airborne microorganisms caused by O_3 was not so much obvious, there were some researchers claimed that the cell membrane of microbes could be damaged when exposed to high RH and low concentration of O_3 [22].

Actually, the relationship between concentration of microorganisms on bioaerosols and low concentration of O_3 (20−100 μg·m^{-3}) is still under debate. Some researchers reported that O_3 (79.7–87.6 μg·m^{-3}) was negatively correlated with many microorganisms, possibly because the toxicity of O_3 inhibits the growth of microorganisms in atmosphere [28], while two works claimed that the total number of Operational taxonomic units (OTUs) was positively correlated with atmospheric O_3 concentration (10–90 μg·m^{-3}) [29,30].

For bacterial aerosols, at low concentration of O_3 ranging from 10−90 μg·m^{-3}, the total number of bacterial OTUs was positively correlated with O_3 concentration ($R^2 = 0.15$) (from 5200 to 5600 CFU·m^{-3}, 8% increase), indicating that O_3 may promote bacteria growth to some extent, while the relative abundance of pathogenic bacteria was negatively correlated with O_3 ($R^2 = 0.10$) concentration (decreasing from 6% to 4%) [29].

For fungi aerosols, some studies stated that negative relationship was obtained between O_3 and fungal levels [31], which indicating inactivation of fungi on bioaerosols by O_3 even at low concentration of O_3 (<100 μg·m^{-3}). At very low concentration of O_3 (5–6 μg·m^{-3}), there are some studies found that microorganisms belonging to *phylum Ascomycota* are not especially sensitive to O_3 [32], illustrating that the concentration of specific fungi on bioaerosols didn't being inactivated with increase of O_3. Actually, different types of fungi show variant tolerance to low concentration of O_3. Recently, some studies found that specific species were negatively correlated with *Penicillium* but positively correlated with *Alternaria* [32] for harmful organisms being oxidized by O_3, leaving bioaerosols' growth.

As weak interaction of low concentration O_3 with microorganisms, a slight variation of chemical compositions of microorganisms on bioaerosols is not easily detected. Recently, the scientists have developed Raman spectroscopy strategy for detecting surface chemical composition of a single microorganism before and after O_3 exposure [33]. They investigated Raman spectra of seven different single-trapped fungal aerosol (like *Penicillium camembertii*) in lab, and found that specified several individual chemical function groups of bioaerosols such as lipids and proteins that undergo chemical reactions with O_3. By comparing Raman spectra of bioaerosols, some new specific characteristic peaks and the change of the ratio of lipid–protein in seven fungus materials can be found after interaction with O_3 for 40 min, indicating that the change of biological compositions of microorganisms on bioaerosols. Similarly, Pan et al. [34,35] reported that both the absorption and emission spectra of aromatic amino acids (tryptophan, tyrosine, and phenylalanine) of bioaerosols were changed by O_3 during oxidation process. Tryptophan, tyrosine, and phenylalanine are main UV fluorescent molecules in bioaerosols, and their fluorescence dramatically decreased at UV but reversely increased at visible range. These results indicate that the biological components (lipids, proteins, DNA and amino acids) of microorganisms on bioaerosols are changed after exposure to O_3, leading to altering biological activity (metabolism, respiration, mobile performance, reproductive vitality) of microorganisms on bioaerosols.

However, even at low concentration of O_3 pollution, with high relative humidity (RH), the oxidizability of O_3 can be promoted notably. Griffiths et al. claimed that no viability decrease of *P. expansum spores* was found with low concentration of O_3 at low RH, while significant decline can be seen with low concentration of O_3 at high RH, indicating that the reaction of moisture and O_3 seems to enhance the inactivation effects of microorganisms on bioaerosols. For examples, MS2 is found to be more sensitive to O_3 than BtAH (10 μg·m^{-3} with RH of 81%) [36]. The reasons are the reactions of O_3 with H_2O and some low molecular weight hydrocarbons producing large amounts of ROSs and intermediates which maybe toxic to microorganisms on bioaerosols.

In one word, although low concentration of O_3 may not completely inactivate bioaerosols as high concentration of O_3, owning to its weak oxidation and small possibility of contact with microorganisms, some minor changes of bioaerosols were also really observed. Newly appearing groups and modified lipid-protein ratios can be found from various microorganisms on bioaerosols when exposure to low concentration of O_3, which may also influence the biological activity of bioaerosols.

4. Human Health Risks of Ozone-Mediated Bioaerosols

As bioaerosols contains pathogenic microorganisms, which may pose human health risks for example allergy, asthma, infectious diseases and so on. The potential health risks caused to humans mainly depend on the pathogenicity of bioaerosols or potential immunity of human beings, which can be affected by community structures, size distributions, biological compositions and biological activity of bioaerosols. However, these characteristics of bioaerosols can be affected by atmospheric O_3 as discussed above, leading to changes of health effects of them to humans.

At high concentration of O_3 (>100 $\mu g \cdot m^{-3}$), some researchers believed that health risks of bioaerosols reduced due to decrease of concentration of bioaerosols, while others claimed that the health risks of bioaerosols increase because producing more harmful components from bioaerosols when exposed to high concentration of O_3. Some specific microorganisms on bioaerosols are easily inactivated by O_3, resulting in the total concentration of bioaerosols shows even an order of magnitude decrease. As the pathogenicity of the specific microorganisms and the compounds produced on bioaerosols are reduced, inactivation of these microorganisms by high concentration of O_3 may potentially reduce the health risks of bioaerosols. On the other hand, some studies have shown that O_3 at appropriate concentrations (5–10%) may chemically react with other harmful substances presented on PM, thereby reducing the harm to microorganisms on bioaerosols as well [36]. Bioaerosols containing pathogenic microorganisms are not inactivated, which may increase health risk of them to humans. Moreover, the size distribution of bioaerosols can be decreased at high concentration of O_3, which may increase health risk of bioaerosols because that the finer size of bioaerosols is able to deposit on the deeper position of human respiratory system (The size of bioaerosol also affects the efficiency of being inhaled and deposited within human respiratory system) [32]. It is because majority of fine-particle bioaerosols can easily enter human body through the respiratory system and can cause a range of diseases, resulting in higher health risk. Both views make sense, as after interaction with high concentration of O_3, the community structure and size distribution of bioaerosols are really changed, leading to different targeted cells or organs that they attacked or deposited. Therefore, the health risk of bioaerosols at high concentration of O_3 should be further investigated.

At low concentration of O_3 (<100 $\mu g \cdot m^{-3}$), the biological compositions and bio-activity of bioaerosols are also changed to some extent. The dominant opinion is that health risks of bioaerosols increases when being exposed to low concentration of O_3, as they believe that the low concentration of O_3 cannot inactivate bioaerosols, while the biological composition changes of bioaerosol possibly produce more allergenic and toxic contents of pathogenic microorganisms to humans. Since pollen and fungal spores are able to interact with O_3 pollutants, researchers found that they linked to the climate-change-driven (O_3 pollution) worsening respiratory health effects. The exposure to several contaminants (including bioaerosols and O_3) in urban settings is linked to severe episodes of asthma attacks and/or exacerbations [37]. More precisely, Tiedemann and Firsching [38] reported that the pathogenicity of rust fungi on bioaerosols could be increased after exposure to low concentration of O_3 (20–70 $\mu g \cdot m^{-3}$). Similarly, Zoran et al. considered that low concentration of O_3 (20–50 $\mu g \cdot m^{-3}$) acts as a COVID-19 virus incubator, being positively correlated with COVID-19 infections and fatalities [39]. As strong oxidant, low concentration of ozone induces oxidative stress of bioaerosol, which also cause antimicrobial resistances [40]. In addition, it has been verified that ozone causes conjugative transfer and transformation of antibiotic resistance genes, which may lead severe health risks of ozone mediated bioaerosols [41].

Actually, at really atmospheric environments, both bioaerosols and O_3 at low concentration pose health risks to humans simultaneously [42]. It is known that WHO standard value is 100 $\mu g \cdot m^{-3}$, however, the total mortality associated with levels greater than 70 $\mu g \cdot m^{-3}$ account for 0.26% of the deaths, and it is worth noting that even low levels of O_3 (50 $\mu g \cdot m^{-3}$) may contribute to mortality [43]. High concentrations of O_3 exposure stimulate the human respiratory system, damage lung cells, central nervous, cardiovascular systems and aggravate other chronic lung diseases, therefore posing a great threat to human health [44]. O_3 not only affects the characteristics of bioaerosols, but can also be inhaled by humans, indicating that both bioaerosols and O_3 pose health risks to humans. Therefore, the co-exposure to bioaerosols and O_3 pollution, the synergistic effects of O_3 and bioaerosols may significantly increase health risks of humans, which should be systematically investigated in the future.

5. Summary and Prospects

With increased safety concerns of bioaerosols and air pollutants, health risks of O_3 mediated bioaerosols and O_3-bioaerosols co-exposure are under investigated. In heavy O_3 pollution (>100 $\mu g \cdot m^{-3}$) with strong interaction between bioaerosols and O_3 molecular, some microorganisms on bioaerosols are inactivated, leading to concentration and size reduction with change of community structure. The total concentration of microorganisms shows 30% decrease and fine particle size of bioaerosols increase 20% among total bioaerosols due to partially being inactivated by O_3. In addition, community structures and diversity of bioaerosols change due to different types of microorganisms have varied tolerance to O_3. Health risks of O_3-mediated bioaerosols are complicated. To a certain extent, pathogenic microorganisms on bioaerosols can be inactivated by O_3, thus reducing health risks of bioaerosols. However, the size distribution of bioaerosols decreases as well, improving the potential for a deeper deposition onto respiratory system, which may also increase health risk to humans.

At relatively low concentration of O_3 pollution (20–100 $\mu g \cdot m^{-3}$), debating opinions believe that concentration of bioaerosols may increase slightly (8% increase) due to clearance of other harmful organisms that toxic to bioaerosols, as well as decrease (2% in pathogenic microorganisms) because of different tolerances to oxidization by O_3. Even though, O_3 may not able to effectively react with bioaerosols due to long distance and limited oxidizability, biological compositions including ratio of proteins, lipids, and amino acids of bioaerosols are changed, as well as bio-activity of bioaerosols are decreased. Although microbial vitality of bioaerosols decreases, researchers believe that health risk of O_3-mediated bioaerosols may rise due to usually more allergenic and toxic contents of pathogenic microorganisms on bioaerosols are exposed.

From what discussed in this review, we know that the characteristics of bioaerosols can be changed when interacting with different concentrations of O_3. However, the investigation of interactions between bioaerosols and O_3 is insufficient due to complex compositions of bioaerosols and lack of proper detection strategy. We summarized following research gaps that should be noticed in future study.

(1) **Ignoring of assessing viable but non-culturable bioaerosols.** Most of existing researches only focused on O_3 affecting specific or culturable microorganisms on bioaerosols, remaining a lot of unknown and/or viable but non-culturable microorganisms.

(2) **Influences of O_3 on bioaerosols have not been systematically and deeply investigated.** Most underlying mechanisms of interaction between O_3 and bioaerosols including dose-response relationship, species abundance, metabolic toxicity and so on have not been investigated.

(3) **Other components on bioaerosols like pollen, endotoxin, and so on have not been considered.** Complicated components on bioaerosols can also be affected by O_3, causing complex health risks to human beings.

(4) **Lack of reliable health risks assessment of O_3-mediated bioaerosols.** The only concentration of total number of culturable microorganisms on bioaerosols has been considered, which is not reasonable.

Most importantly, as both bioaerosols and O_3 are air pollutions with relatively low concentration that cause health risk to humans and animals, there is a lack of researches to summarize the general rules for accurately evaluating health risk of co-exposure to O_3 and bioaerosols.

As there are a lot of unclears remaining, more efforts should be put on investigating interactions and health risks of O_3 and bioaerosols. First of all, detection and sampling strategy of living bioaerosols should be improved, thus viable but not culturable microorganisms can be considered on bioaerosols. Metagenomes sequencing and other methods can be used to reveal the change of community structure, abundance and other components of O_3-mediated bioaerosols. Secondly, more laboratory investigations to simulate interactions between O_3 and bioaerosols even other air pollutants are needed to reveal underlying mechanisms. Finally, for health risks, as antibiotic resistance gene on bioaerosols poses health risk to human beings, what should be considered in future study is that O_3-mediated bioaerosols may promote the horizontal transform of genes. A methodology for assessing health risks of O_3-mediated bioaerosols and co-exposure of bioaerosols and O_3 also should be urgently built. We believe comprehensive investigations on the interactions of O_3 with bioaerosols to accurately reveal deep mechanisms through advanced technologies are still on the way. This critical mini review may alert the public to reveal the health risks of exposure to bioaerosols and O_3, as well as properly control O_3 and bioaerosol pollutions.

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