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Bacterial Characteristics of Dust Particle Saltation in Gobi Dust Sites, Mongolia

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Abstract: The Gobi Desert is a major source of Asian dust events, and the resulting health hazards have increased significantly in recent years. We reported that a variety of live bacteria were distributed in the Gobi Desert in relation to land use. Bacterial distribution was confirmed in the environment and on the land used by animals; however, bacterial saltation due to dust events has not been investigated in detail. In this study, to understand the distribution of surface bacteria in the atmosphere by dust saltation, live bacteria in four dust-generating areas in the Gobi area were monitored using an artificial dust generating device. The live bacteria were detected by experimental saltation at a wind speed of 6.5–8 m/s in all areas. A certain number of live bacteria are constantly saltated by dust events, and these bacteria depend on land use. Moreover, the bacterial saltation strain depended on land use and diversity, indicating that live bacteria are lifted into the environment by dust events. These findings indicate that dust events saltate environmental bacteria on the ground, suggest the risk of animal-derived bacterial saltation affected by land use, and present cross-border public health challenges to be considered in the future.

Keywords: bacteria; Asian dust storm; dust saltation; Gobi Desert

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1. Introduction

Recent developments in socio-economic activity have led to changes in atmospheric circulation due to climate change [1]. Cross-border infectious diseases are caused by viruses and bacteria that can threaten human and animal health, and zoonotic diseases are becoming increasingly problematic [2–8]. Asian dust events occur in arid and semi-arid areas, such as desert areas and the yellow soil plateau of East Asia, where substantial dust is blown by strong winds caused by low pressure [9–14]. This dust is carried by westerly winds to Northeast Asia and the western Pacific Ocean [15–21].

The threshold friction velocity for saltation is a critical parameter for describing the initiation of mobilization of sand particles from the ground surface into the atmosphere. The airborne sand particles fall due to gravity and hit the ground, dislodging dust particles that are then emitted and transported regionally and globally [22]. Knowing how much dust is emitted into the atmosphere is very important for understanding the global climate, environmental changes, and resulting health issues. However, critical wind speed has not been observed precisely in the field due to instrumental limitations and complicated ground surface conditions, such as sand particle size distribution, soil moisture and snow cover. For these reasons, few papers by dust modelers have presented practical values of critical wind speed [23]. The findings of Greeley et al. [24–27] for wind "threshold" curves derived from laboratory experiments showed: "wind has the potential for directly eroding material and redistributing it to other areas. Wind transports sediment via: suspension (mostly silt and clay particles, i.e., ≤60 μm), saltation (mostly sand size particles, 60 to 2000 μm in diameter), and surface creep (particles ≥ 2000 μm in diameter)". Additionally,

they define the minimum wind friction speed [28] to initiate the movement of particles in different planetary environments [29,30]. High winds above 8 m/s blow away dust from sand particles around Beijing [31]. In addition to wind speed, soil moisture and vegetation on the ground surface (surface roughness) affect the saltation of sand particles. Aeolian processes are capable of redistributing enormous quantities of sediment over planetary surfaces, resulting in the formation of various landforms and deposition of windblown sediments that can be hundreds of meters thick [32,33]. Therefore, it is expected that environmental microorganisms bound to sand will be scattered in the atmosphere together with dust. However, there are still unknowns about the information that was actually verified.

Our previous studies have revealed that seven predominant bacterial strains survived environmental stress on the surface of the dust-producing areas of the Gobi Desert [34]. Their distribution was characterized by the environmental factors of the land use; however, it is unclear whether the bacterial characteristics of the surface saltated by strong winds affect the characteristics of land use.

Bioaerosols are particulate matter derived from living organisms, including microorganisms such as bacteria, viruses, pollen, and organic dust [35]. During dust events, bacteria can attach to dust particles and travel long distances, causing environmental cross-border pollution [36]. In fact, bacteria attached to sand particles in the Gobi area are saltated by dust [37]. Previous findings indicate that various bioaerosols, including microorganisms, are alive [38,39]. Dust-borne microorganisms may have a direct impact on human health through etiology, exposure to allergens, promotion of immune-mediated disease, and increased susceptibility to asthma due to long-term exposure [40–42]. Previous reports indicate that dust saltation is concentrated in April–June and is related to the cyclone [43,44]. It has been reported that the dust generated in the Gobi Desert contains a large number of microorganisms; however, the characteristics of dust particles and the species of bacteria by saltation are poorly understood [37]. If dust-borne microorganisms are zoonotic disease pathogens or risk factors for human health, they can also affect livestock or humans directly or indirectly through animals. There is no detailed quantitative verification of actual saltated bacteria, and the consideration of their environmental risks is insufficient. Therefore, we developed our own saltation experimental device and conducted experimental verification to clarify the particle size of dust saltation and the characteristics of the strain in topographical change.

2. Materials and Methods

2.1. Sample Collection Sites

Four different locations were used as the study areas, classified according to their land use: a dry lake bed, a wadi, a well, and a desert steppe (Figure 1). The site for this survey was at the same location where surface microorganisms were surveyed in April 2018 [22]. (1) Dry lake bed (DL): Dry lake floors without vegetation are a source of sandstorms and a place where water has previously flowed. Topographically, dry lakes are called inland depressions (playas) and are shallow plains during the rainy season; however, when evaporated, they become salt pans with clay, salt, and gypsum deposits [43]. (2) Wadi (WA): wadis are dry waterways formed in the lowlands and seasonal rivers that appear temporarily during the rainy season, where small particles accumulate on the ground. (3) Well (WE): Wells are distributed in the Gobi Desert every 20–30 km. The area around the well is used by livestock and wildlife. (4) Desert steppe (DS): Desert grasslands possess the most widely distributed land vegetation for livestock. The areas are home to annual and perennial shrubs, which are involved in the control of dust storms. This research project was carried out by inheriting the following project: "International Observation of Yellow Sand and Environmental Regime Shift in Source Area", Grant Scientific Research (A: 16H02703). The analysis of the survey sample was carried out with the permission of the Agriculture, Forestry, and Fisheries Plant Protection Station (29Y2490).

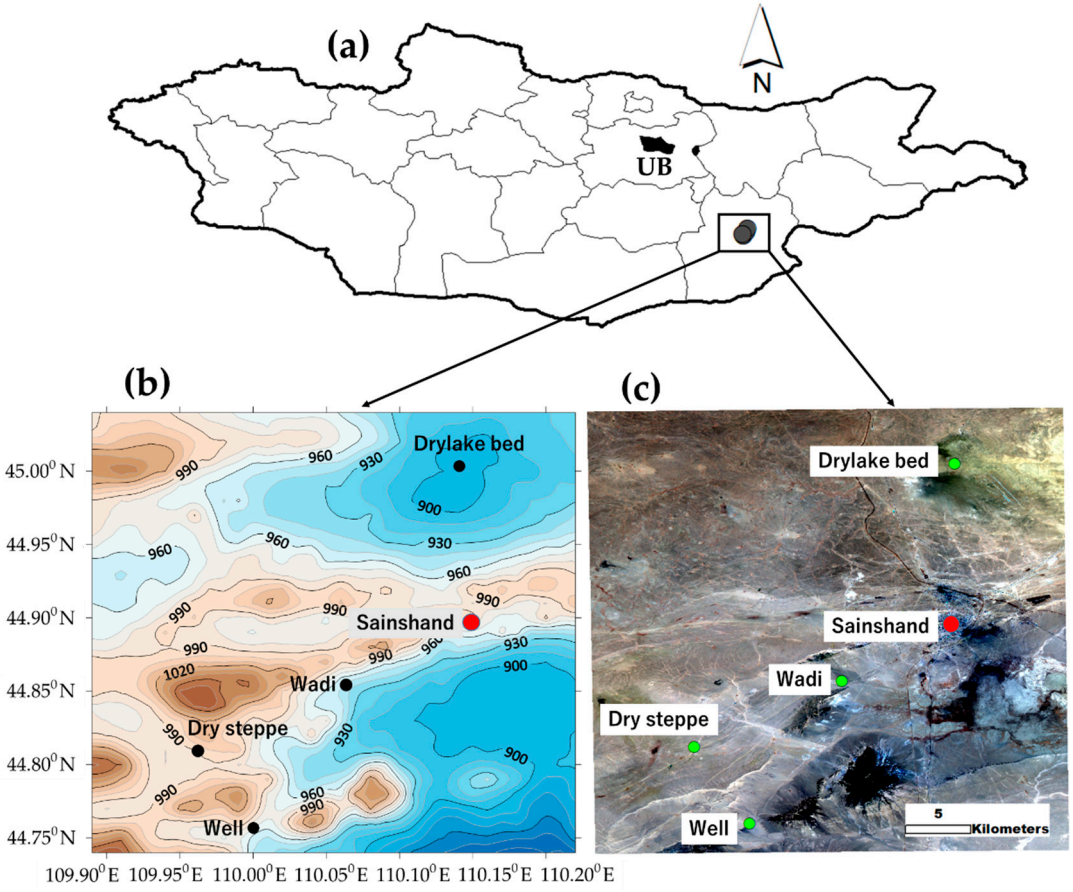


Figure 1. The study points are shown on the map, where (**a**) indicates the location in Mongolia, and (**b**) indicates the four research survey points. The vertical axis shows latitude, the horizontal axis shows longitude, and the numbers show the altitude; (**c**) the study points in Sentinel 2 image.

2.2. Dust Particle Saltation Experiments

Dust particle saltation experiments were conducted at four sites in the desert area, DL, and dry lake area. The water content and temperature of the soil collected at the sample points were measured using dedicated measuring instruments: the HH2 Moisture Meter© (Delta-T Devices Ltd., Cambridge, UK) and a radiation thermometer (Shinwa Rules Co., Ltd., Niigata, Japan), respectively. The conditions of the field environment are listed in Table 1.

Table 1. Environmental information at the observation point.

Location	Ground Surface Temperature (°C)	Soil Moisture (%)	Temperature (°C)	Humidity (%)
Well	40.2	3.0	26	14
Wadi	37.8	6.8	19.7	19
Dry lake bed	41.4	6.5	22.5	20
Desert Steppe	37.8	6.8	19.7	19

Data show the average value of five measurements.

The dust concentration was measured for two particle sizes: 1 μm < 3 μm (3 μm) and 3 μm < 5 μm (5 μm). To measure the number of dust saltations, a handheld particle counter Model 3886 (KANOMAX Co., Ltd., Osaka, Japan) was used. The dimensions of the measuring equipment and photographs of the saltation experiments are shown in Figure 2. To minimize the influence of the outside air, the air inlet of the blower was installed on the windward side, and the blower and the measurement point were sealed, except for the air inlet and outlet. In the measurement method, an artificially generated wind was blown

onto the ground surface stepwise by a blower, and the concentration of the dust particle saltation was measured. Here, stepwise means five stages of rotation speeds of the blower: 1200, 1400, 1600, 1800, and 1900 rpm (revolution per minute, corresponding to 5, 6.5, 8, 9, and 11 m/s at 1 cm from the land base). The wind speed was calculated from the number of revolutions using the following formula [45,46].

Estimation for 10 m wind speed:

$$U_{10} = U_s \left(\frac{ln \frac{h_{10}}{z_0}}{ln \frac{h_s}{z_0}} \right) \quad (1)$$

U_{10}: wind speed at 10 m.

(The standard exposure height of wind instruments over level; open terrain is 10 m above the ground).

U_s: wind speed at 0.01 m.

(Height of wind speed measurement by fan).

h_{10}: 10 m height; h_s: 0.01 m height; z_0: roughness length (flat desert).

In the saltation experiment, we carried out spray disinfection with 70% ethanol for each test to inactivate the environmental bacteria in the saltation area in the tent. Air was blown for one minute at each stage, and the dust particle saltation at each stage was measured. Furthermore, the wind speed for each rotation speed of the blower was measured. In this way, we examined the difference in the number of dust saltations for each wind speed in the research area. The dust particle data were analyzed using kernel density (Spatial Analyst, (c) Systat software version 13.2., Systat Software, Inc., Chicago, IL, USA) for each particle system. Furthermore, the relationship between topography and saltated particles was analyzed and drawn using GS + Geostatistics (Gamma Design Software, LLC., Plainwell, MI, USA).

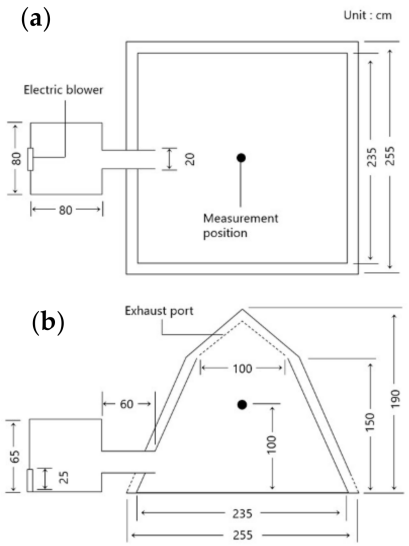


Figure 2. Outline of the saltation experiment. The inlet and the fan installation box are connected to one side of the tent, and the air is blown by the fan. For saltated dust, a particle counter probe is installed on the opposite side of the tent. A sterilized Petri dish that captures dust particles is placed in the center (dot mark point). (**a**) top view, and (**b**) side view shown the mobile dust chamber for the measurement of particle concentrations.

2.3. Bacterial Isolation

Saltated particles were collected in a sterile Petri dish installed in the tent (mark point in Figure 2). The obtained particles were suspended in 10% sterile phosphate-buffered saline (PBS), and bacterial culture was carried out as follows. The suspension was diluted to 10^{-1}–10^{-5} in PBS and aerobically cultured in LB agar media (BD Difco™, East Rutherford,

NJ, USA) at 37 °C for 24 h. The generated colonies were first counted as the total number of bacteria and classified by morphology based on Bergey's Manual [47]. DNA was subsequently extracted from three colonies of the same morphology from each sample location using the MagExtractor™-Genome-kit (Toyobo, Osaka, Japan) according to the manufacturer's instructions.

Amplification of 16s rDNA and Determination of Gene Sequence

Amplification of 16s rDNA was performed using the extracted DNA as a template, and primers 27F (5′-AGAGTTTGATCMTGGCTCAG-3′) and 1492R (5′-CGGTTACCTTGTTACG ACTT-3′). Gene amplification was carried out by polymerase chain reaction (PCR) using TaKaRa Ex Taq® (Takara Bio Inc., Shiga, Japan) as follows: an initial denaturation at 95 °C for 2 min, followed by thermocycling (35 cycles) at 98 °C for 10 s, 55 °C for 30 s, and 72 °C for 1 min, and a final extension at 68 °C for 5 min. DNA amplicons were resolved on a 1.0% agarose gel via electrophoresis, and an amplified band of 1466 bp in length was confirmed. Amplified DNA was purified using the Fast Gene Gel/PCR Extraction Kit (Genetics, Tokyo, Japan) to prepare samples for direct sequencing. Sequence analysis was performed using the 27F sequence primer. The resulting nucleotide sequence was aligned using BioEdit (version 7.0.9) [48]. The 16s rDNA variable regions 1–4 were subjected to BLAST analysis using the GenBank database (NCBI) to identify bacteria based on their previously determined classification.

2.4. Sample Data Analysis

The water content and temperature of the soil collected at the sample points were measured using dedicated measuring instruments: the HH2 Moisture Meter© (Delta-T Devices Ltd., Camberwell, UK) and a radiation thermometer (Shinwa Rules Co., Ltd., Sanjō-shi, Japan), respectively. Longitude–latitude interactive simulation was analyzed by GS + Geostatistics software (version 10) to compare with the same 2018 season of the scattering dust event. Sequence analysis was performed using MEGA-X (version 10) [49].

3. Results

In the Gobi Desert, four locations were selected based on the characteristics of the topography, and saltation experiments were conducted in each area. During the study period, the weather was fine, and no abnormal weather conditions, such as sandstorms, were observed. The outside air temperature was maintained in the range of 13.4–26.0 °C, and the wind speed was 2.5–4.4 m/s. These weather conditions did not affect the installed test tents.

In the artificial saltation experiment, a particle counter and a sterile dish were installed to monitor particle scattering and microorganisms. For saltated particles, the values were divided into 3 μm and 5 μm, and the changes were analyzed by kernel density (Spatial Analyst, (c) Systat software version 13.2).

As a result of the scattering test, it was clarified that the scattering tendency of the particles differed depending on the change in wind speed, and that the scattering amount increased most between the wind speeds of 6.5 and 11.0 m/s (Figure 3A–D). In the DL, 8–11 m/s showed the most saltated particles. In other places, the wind speed of 6.5–8 m/s showed the largest saltated particles. Furthermore, it was shown that the relationship between the saltated particles and the topography was characteristic. The longitude–latitude interactive simulation was analyzed for dust size (3 μm and 5 μm) in the research area. From the field experiment, we found that the critical wind speed for dust generation was 9 m/s, and the dust particles were saltated more easily from the WA in different landscapes. Next, we analyzed the saltation from the DL. This is because many dust particles are saltated on the ground surface of these places. Dust is unlikely to occur in desert steppes where dry grass (non-photosynthetic vegetation) is distributed (Figure 3A–D).

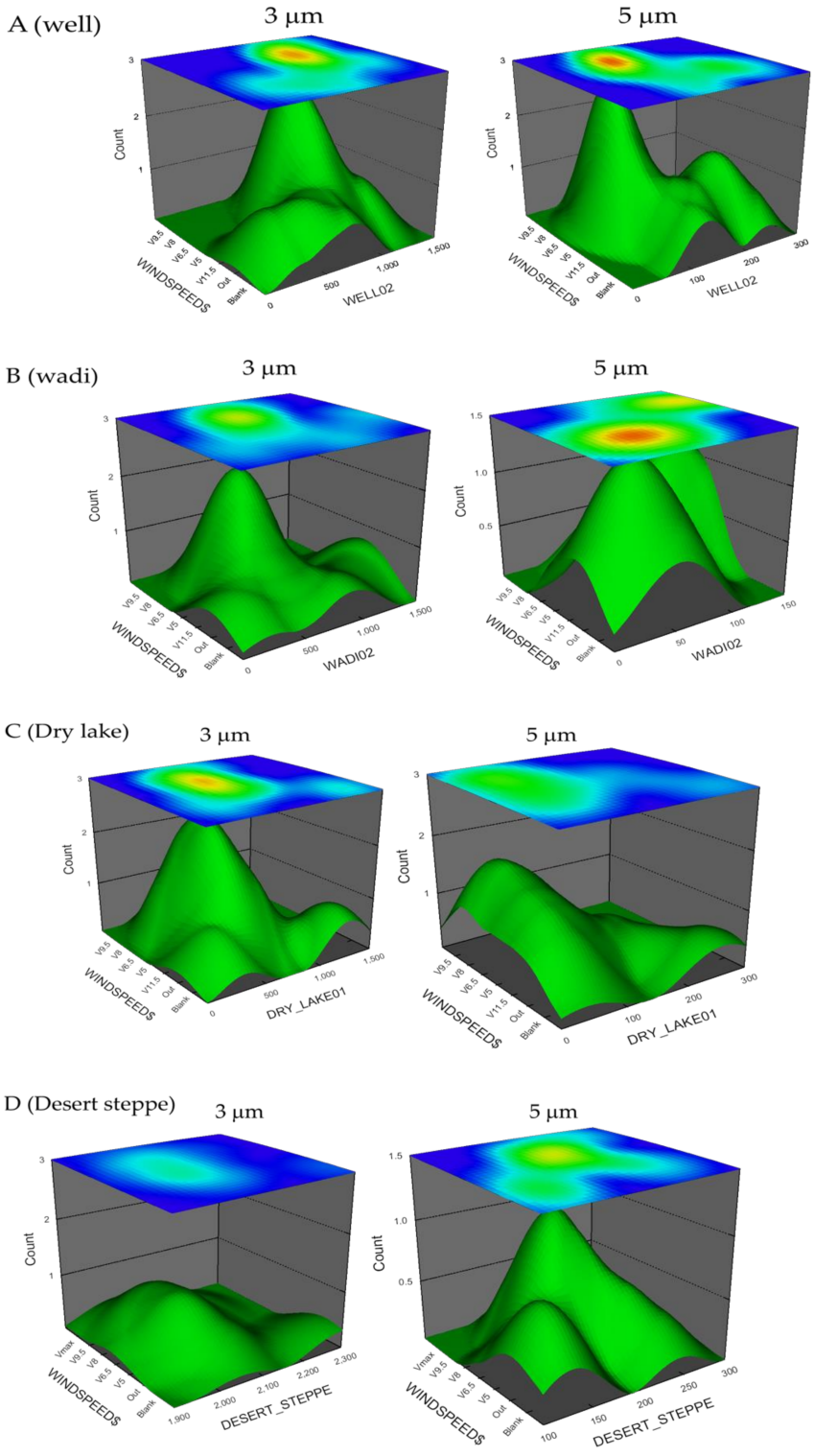
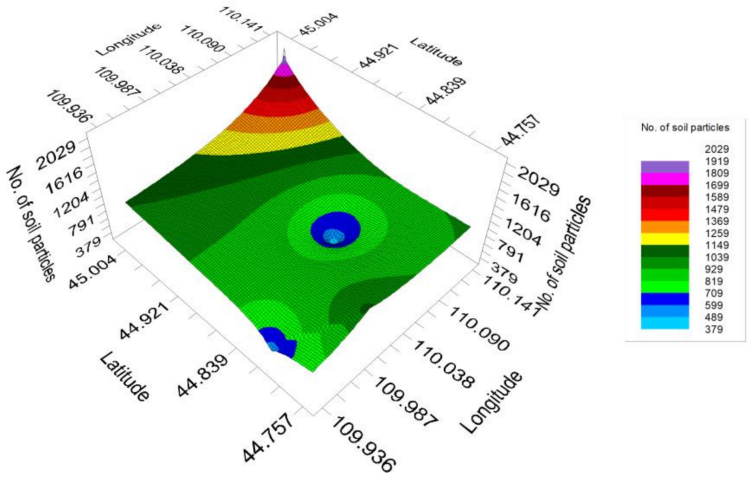


Figure 3. The particle data are analyzed by kernel density by saltation experiment. The figure shows the pattern of dust particle saltation at study sites according to the wind speed. Dust particles are more likely to saltate near the well due to overgrazing, and it can be seen that the wadi is easy to saltate in the wind because the size of the sand particles is considerably small. The measurement results at each location are shown in the particle size (3 μm, 5 μm), (**A**) well, (**B**) wadi, (**C**) dry lake, and (**D**) desert steppe.

The relationship between topography and saltated particles was analyzed and drawn using GS + Geostatistics. Figure 4 shows the dust scattering concentration at a critical wind

speed of 9 m/s along the latitude and longitude. The figure shows the number of saltated particles by the diameter together with latitude and longitude. The amount of saltated dust varies depending on the terrain. As shown in Figure 1, a large dry lake is distributed in the northeastern (high-latitude and high-longitude) part of this area, and a large amount of dust is present on the ground surface in the high-latitude and high-longitude directions.

(**a**)



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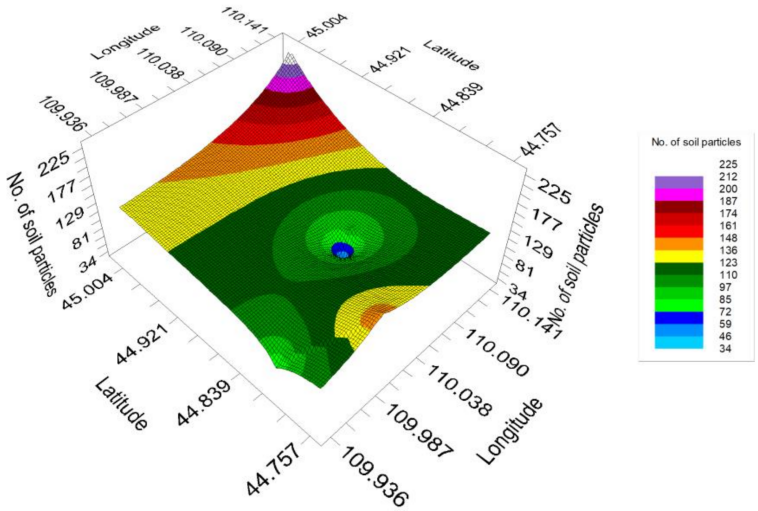

Figure 4. The relationship between topography and saltated particles was analyzed and drawn by GS + Geostatistics. The figure shows the dust scattering concentration at a critical wind speed of 9 m/s with latitude and longitude. (**a**) is 3 μm particles, and (**b**) is 5 μm particles.

The number of bacteria on the ground surface at the four locations was detected at the DL ($10^{7.0}$ CFU/g), the WA ($10^{6.5}$ CFU/g), the WE ($10^{7.3}$ CFU/g), and the DS ($10^{6.4}$ CFU/g), and the saltation experiment revealed that approximately 1/1000 of the number of live bacteria was saltated. Next, the total number of bacteria obtained from the culture of saltated bacteria in a container installed in the experiment tent was examined. In the examination of the number of saltated bacteria at four locations, 10^{4}–$10^{4.9}$ CFU/g was detected for 10 min blown by the fan. The WE had the largest number of bacteria ($10^{7.3}$ CFU/g) detected from the ground surface, and the number of saltated bacteria was $10^{4.9}$ CFU/g (Table 2).

Table 2. Comparison between the number of bacteria on the ground surface and saltation bacteria.

Location	Surface Bacteria	Saltation Bacteria		
	Total (log CFU/g)	Total (log CFU/g)	Family	log CFU/g
Dry lake bed	7.0	4.1	Bacillaceae Planococcaceae Micrococcaceae Cellulomonadaceae	3.7 3.7 3.2 3.2
Wadi	6.5	4.5	Staphylococcaceae Streptomycetaceae	4.4 3.5
Well	7.3	4.9	Bacillaceae Micrococcaceae	4.7 4.4
Desert Steppe	6.4	4.0	Micrococcaceae Planococcaceae Streptomycetaceae	3.7 3.5 3.2

The bacteria detected in the saltation experiment belonged to six families at four sites. Supplementary Table S1 provides detailed information on the bacterial family determined by the 16s rDNA analysis detected at each location. It also shows the homology rate (%) with the sequences listed in the gene bank. In each region, the DL had the largest number of four families, followed by three families in the DS, and two families in the other two locations. The saltated bacterial families had similar results to those of bacterial species on previous surfaces. *Bacillaceae* was identified in the DL and WE, and *Planococcaceae* was identified in the DL and DS. *Staphylococcaceae*, generally detected in animals, was detected in the WA ($10^{4.6}$ CFU/g), suggesting that land use is related to animals. In the DL and WE, bacteria on the ground surface were easily saltated, and in the WA, animal-derived *Staphylococcaceae* were saltated. *Micrococcaceae* was detected at the DS and WE, and it is closely related to *Arthrobacter* through genetic analysis and is widely present in soil, suggesting that it may have been widely distributed in the layer directly below the ground surface and spread with scattering. In the WA, the dominant bacterial species, *Staphylococcus*, was detected following the saltation experiment (Figure 5).

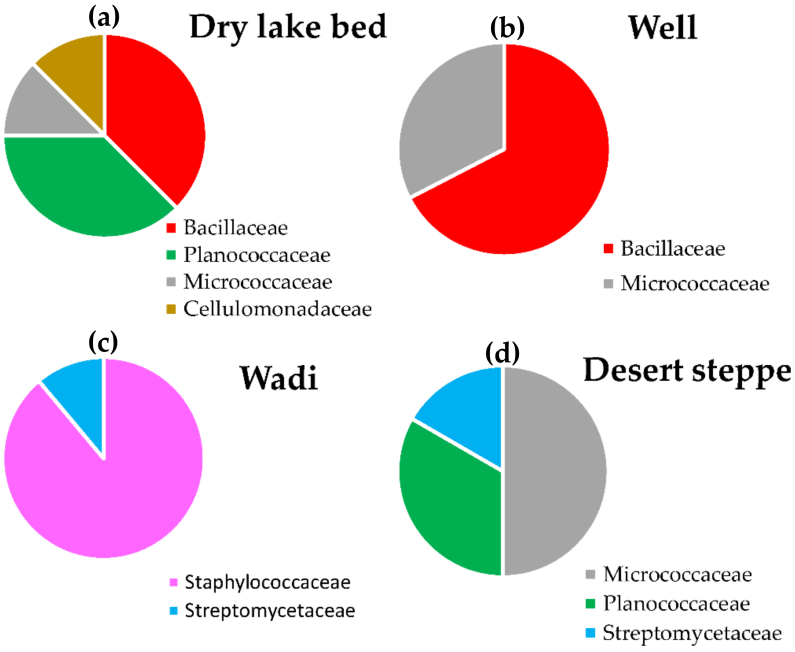

Figure 5. The bacterial species isolated from the dust saltation particles are shown in a pie chart. Each color chart shows the detection rate of each bacterial family at each research area, (**a**) dry lake bed, (**b**) well, (**c**) wadi, and (**d**) desert steppe.

4. Discussion

In this study, an artificial saltation experiment was conducted in the dust-generating area, and the conditions of the dispersed particles and the characteristics of the bacteria attached to them were compared with those on the ground surface. In the dust saltation experiment, a wind speed of 9 m/s was confirmed as a condition for dust scattering, and the saltated wind speed was found to be slightly different depending on the land use; however, the findings were almost identical. Dust particles were more likely to saltate near the WE due to overgrazing, and it could be seen that the WA was easily saltated in the wind, because the sand particle size was considerably small. This study revealed that the number of saltated particles differs depending on the geographical influence of dust saltation, and that the bacteria on the ground surface differ depending on the land use [34].

Bacterial diversity on the ground surface was similar to that previously reported [34,50,51]. When comparing the number of bacteria on the ground surface with the number of saltated bacteria, the number of bacteria was high in the DL and WE on the ground surface. However, the number of bacterial saltations was almost identical in each region. From this result, it was found that the numbers of saltated bacteria from the dust on the ground surface were similar with the same wind speed. Notably, the bacterial species differed depending on land use. Genetic analysis of each bacterial strain revealed that animal-derived bacteria *Staphylococcaceae* were detected in the WE and DL, which originated from animals. The genus *Staphylococcus* is associated with important health risks due to zoonotic diseases, including methicillin-resistant strains [52–54]. This finding assisted in characterizing the bacteria contained in the dust and reaffirmed the requirement for detailed public health research on dust and health [40,41,55].

Moreover, from the saltation experiment results, we found that the bacteria on the ground surface as indigenous bacteria did not always scatter with dust saltation. The sizes, structures, and components of dust particles to which these bacteria adhere would be influenced by bacterial binding to dust saltation. Soil moisture affects the critical friction rate; therefore, it shows that the effect of soil moisture on dust saltation is significant, as it is an important element of dust events [56]. Shao et al. reported that the dust particle size distribution depends on a surrogate for surface shear stress and a descriptor for saltation-bombardment intensity, which in turn is dependent on atmospheric boundary-layer stability [57]. In the DL and WE (soil moisture 3.0% and 6.5%, respectively), bacteria on the ground surface were easily lifted by artificial wind, and the WA (soil moisture 6.8%) was a land where dust with animal-derived bacteria blew relatively frequently. This indicates that bacterial saltation is observed at a soil humidity of 3.0–6.8% and a wind speed of >6.5 m/s. The DS has been shown to spread *Micrococcaceae*, which is detected from bacteria on the ground surface [34,58]. In addition, since the bacteria are closely related to *Arthrobacter* through genetic analysis and are widely presented in soil, they may have been widely distributed in the lower layers of the ground surface. In the saltation experiment, bacterial saltation of species other than those detected on the surface layer was observed by artificial wind. From this result, it was inferred that the dust layer on the ground surface was sequentially saltated by wind power and time. To understand this phenomenon, it is necessary to analyze the structure of sand on the ground surface and conduct multifaceted structural analysis, such as evaluating the molecular ionic strength of the dust surface and the degree of bonding between dust particles [59]. The number of bacteria was $>10^4$ CFU/g in the saltation experiment for 10 min. In an actual dust event, it is estimated that 100–1000 times this amount or more is present in the atmosphere. Therefore, it is easily presumed that humans and animals are exposed, and this could have a considerable effect on health [40–42,60,61]. These results support the relationship between the risk of various diseases and dust events.

In conclusion, the dust saltation occurred with live bacteria from the ground surface being released into the environment, and the bacterial species depended on land use and exhibited diversity. These findings indicate that the spatial movement of microorganisms occurs with dust saltation and maintains the diversity of the microbial layer in the environ-

ment. Furthermore, animal-derived bacteria can move spatially depending on their land use and weather conditions, thus requiring cross-border public health attention.

5. Patents

There is no patent matter in this manuscript.

Supplementary Materials: The following are available online at https://www.mdpi.com/article/10.3390/atmos12111456/s1, Table S1: Identification of bacteria family at research area (captions: location shows dry lake bed (DL), wadi (WA), well (WE), and desert steppe (DS); the sequence region is a variable region of 16s rDNA (V1-4); the homology rate (%) is shown as compared to the Genebank listed sequences; accession refers to the gene sequence accession number).

Author Contributions: Conceptualization, K.H. and B.H.; methodology, K.H. and B.H.; software, T.M., B.H. and P.T.; validation, B.H., T.M. and K.H.; formal analysis, T.M., K.B. and P.T.; investigation, T.M., P.T., K.B., K.H. and B.H.; resources, B.H., K.B. and K.H.; data curation, T.M., B.H. and K.H.; writing—original draft preparation, K.H. and T.M.; writing—review and editing, K.H. and B.H.; visualization, B.H.; supervision, K.H. and B.H.; project administration, K.H. and B.H.; funding acquisition, K.H., K.B. and B.H. All authors contributed equally. All authors have read and agreed to the published version of the manuscript.

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Institutional Review Board Statement: The study was conducted according to the guidelines of the Declaration of Helsinki, and approved by the Agriculture, Forestry, and Fisheries Plant Protection Station (29Y2490).

Informed Consent Statement: Not applicable.

Data Availability Statement: All the data are shown in the manuscript.

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Conflicts of Interest: The authors declare no conflict of interest.

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