

# Microbial Diversity in Rainwater with Correspondence to Particulate Matter and Environmental Factors

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**Abstract:** Microbial composition in rainwater has emerged as a trending research topic due to the increased use of rainwater harvesting systems (RWHS) worldwide. Studies have been conducted to analyze potentially pathogenic microorganisms in RWHS. However, the core focus in most papers has been on the rainwater collected in the storage tanks rather than the composition of microorganisms in fresh rainwater. Due to the microbial influence on air quality, this review dissects various studies that have identified the types of microorganisms in rainwater and their correspondence with atmospheric microbiota to understand airborne microbial movement and its effect. Current methods for rainwater analysis and technologies such as air mass models, that map the distribution of airborne microorganisms throughout the atmosphere, were evaluated to define factors that allow these organisms to move through different levels of the atmosphere. Additionally, the correlation between organisms found in rainwater and particulate matter was analyzed. This review discusses the importance of analyzing rainwater directly without limiting it to RWHS, like roof-harvested rainwater, in microbial source tracking. The paper also presents an overview of ecological contributions by microorganisms at a tropospheric level. Research gaps were noted in identification techniques, the type of microorganisms studied, and their ecological purposes, including paucity in viral detection. The uniqueness of this review is that potential new techniques were explored for prospective studies to improve and further analyze the microbial communities in rainwater. The more focus we give to microbial communities in fresh rainwater in the future, the easier it will be to predict the levels of pathogenicity and disease transmission related to airborne microbiota.

**Keywords:** Cloud formation, Microbial community composition, Particulate matter, Rainwater

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## 1. Introduction

Rain has been a source of drinking water since ancient times. With diminishing water resources, people are moving towards alternative sources for domestic use. One of these alternate sources is roof-harvested rainwater (Zhang et al., 2020). Rainwater harvesting equipment collects and stores rainwater (RW) through a conveyance system for domestic use. Despite rainwater harvesting systems (RWHS) gaining traction all over the globe, there is very little literature on the bacterial composition in pure RW compared to roof-harvested RW. Although RW was considered pure during ancient times, it contains several elements and microbial species (Reddy et al., 2011). Due to the abundance of unknown species in pure rainwater,

some parts of the world have begun to study, identify, and map these communities.

Information on RW-based microorganism circulation, especially bacteria, is studied through air mass history. Numerous factors that seemed to shape the bacterial communities, such as weather patterns, were at play. Weather patterns and relative source location can significantly influence the bacterial load. The total bacterial load is hypothesized to be determined by wind speed due to the arrival and the uplifting of organisms, mainly when some of the catchment surfaces were studied in roof run-offs (Hu et al., 2017). The research on the microbial consortia in fresh rainwater pales compared to the studies done on microorganisms inside the RWHS storage tanks. Some research has been conducted to map the microbial community present in RW. However, very

few correlate the microorganisms in RW and particulate matter. During the process of water droplets falling from the clouds to the Earth, various organisms are collected in a single droplet. Since RW contains many microorganisms from the troposphere to Earth, parallels can be drawn between similar microbial species in RW and air.

One study on RWHS found that twenty-eight tanks (53%) tested positive at least once for total coliforms and *Escherichia coli* (*E. coli*) out of the fifty-three tanks surveyed. Even in tanks with filtration systems, the concentration of *E. coli* was lower but not completely removed (Chubaka et al., 2018). Another finding compared the water in RWHS to waterworks; several pathogens, such as *Pseudomonas aeruginosa*, were observed in 12 of the 27 analyzed toilet water samples (Albrechtsen, 2002). These pathogens were not found in toilets flushed with water from waterworks, which means they must have come from RW. This concludes that RW introduced microorganisms into the harvesting tanks, which ultimately ended up in the household water systems. Testing RW for the presence of pathogens is exceptionally crucial as pathogens inflicting gastroenteritis infections, such as *Aeromonas*, were found to be emerging (Preston & Huddleston, 2016). In India, the association between rainfall events and diseases such as diarrhoea was stronger when heavy rain followed a 60-day dry period (Mertens et al., 2019). It was discovered that mechanisms whereby rain flushes accumulated environmental contaminants into groundwater and standpipe distribution systems, reached human contact which led to this. A study from the United States adds to this information. According to health officials, these outbreaks were often associated with rainfall as demonstrated that any rain four days prior was significantly associated with at least an 11% increase in visits to the hospital for acute gastrointestinal illness (Drayna et al., 2010). Studies on traffic congestion and airborne bacteria have shown more opportunistic pathogens in congested regions (Amarasekara et al., 2021). The current findings on pathogenic airborne bacteria are still linked to rainwater harvesting tanks, although evidence indicates some pathogenic strains did not originate from the tanks. Since very little investigation is carried out on the microbial influence of air quality through rain, it can be challenging to map the origins of emerging pathogens. Rain droplets carry surrounding microorganisms from high levels of the atmosphere to the ground as they fall. Therefore, it is vital to compare research on particulate matter and rain to understand the microorganisms present, especially potentially pathogenic ones, and their movement through the rain. Since most reviews related to RW microbiota are based on RWHS, this study dives into the findings so far to map microbial composition in fresh rainwater and discuss the environmental factors at play for their diversity which is the main aim of this review.

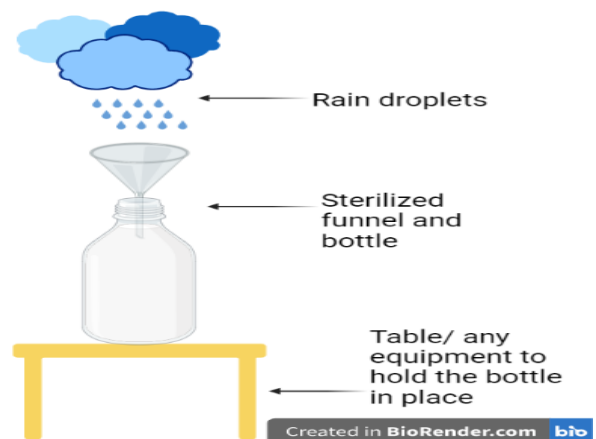
## 2. Materials and methods

This review article is mainly based on secondary articles on airborne microorganisms to dissect various studies that have identified the types of microorganisms in rainwater and their correspondence with atmospheric microbiota. Articles from search engines and databases such as SciVerse, Scopus, Google Scholar, and PubMed were selected for the current study. Relevant articles were searched using keywords akin to microbes in rainwater, airborne microbial community, particulate matter, and troposphere. As an understudied field, the main microorganisms discussed were bacteria, since most organisms discovered were bacterial. This paper dives into the contributing factors to their diversity in rainwater and the possible future directions to map entire microbial communities.

## 3. Results and discussion

### 3.1. Evolution of rainwater collection methods

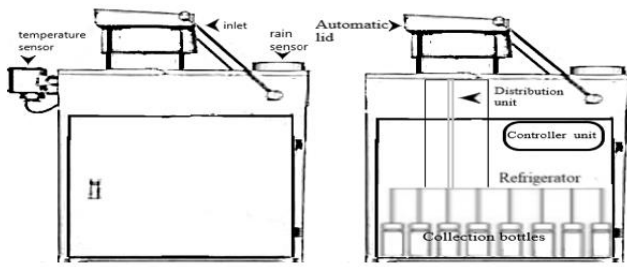
Although the main focus of microbes in RW has been in RWHS, the techniques used to collect and study fresh RW are an ever-evolving matter. One of the oldest yet widely used techniques is the bottle and funnel method. After sterilization of the instruments, the funnel is placed on top of the bottle (Figure 1) in an open space, usually a rooftop, during a rainfall event (Ahern, 2007). There have been modifications to this technique, such as Laurier Poissant's laboratory (1994), which created a sampler that consisted of a polyethylene funnel with an opening area of 0.32 m<sup>2</sup>. They measured the sample volume by creating a tipping rain gauge linked to a microprocessor. The microprocessor automatically controlled all the functions (detecting rainfall, opening, and closing of the cover) (Poissant & Béron, 1994).



**Figure 1:** A schematic diagram of the manual rainwater collection method (created in *BioRender.com*).

In recent studies, automated refrigerated rainwater samplers have become the go-to instrument for collection to avoid contamination (Kaushik & Balasubramanian, 2012). The samplers have built-in rainfall sensors that

open the lid automatically during the entire course of a rainfall event and collects the RW into the sterile storage vessel/s inside the refrigerator, as seen in Figure 2.



**Figure 2:** (i) An image of an automatic rainwater sampler and (ii) an overview of the inside of the sampler.

### 3.2. Identification methods

The methods for identification and viability of microbes are either culture molecular-based, or both in most studies. Primarily the microorganisms are identified by their morphology. Most studies initially grow the culturable bacteria in an agar medium and use the physical characteristics, such as colony morphology or cellular structure, to determine the organism. Some studies performed biochemical tests for further analysis before diving into molecular-based methods. If the experiment is done to identify a specific organism in rainwater, selective media were used, such as m-coli media to detect coliforms (Evans et al., 2005). The selective media enumerates the desired bacterial pathogens (Kaushik & Balasubramanian, 2012). A study on the virome in roof-harvested rainwater used the flocculation technique, where pre-flocculated skimmed milk proteins were used for virus adsorption (Prado T. et al., 2021). However, the culture-based method underestimates the level of contamination in RW caused by potential pathogens compared to the molecular techniques. In the study by Kaushik & Balasubramanian (2012), when culture-based and molecular methods were compared in 15 samples, only three samples showed the presence of *E. coli* for the culture-based method. Molecular diagnostic techniques revealed the presence of *E. coli* in four other samples in the same study.

As culture-based methods were proven to underestimate the presence of microorganisms, Polymerase Chain Reaction (PCR) is presently the most prominently used method for identifying isolated organisms in rainwater studies. The PCR-based approach is popular for rainwater studies as it is rapid and can detect non-viable bacteria in environmental samples. In most cases, real-time PCR is commonly utilized as a fast identification technique (Kaushik & Balasubramanian, 2012). Gene sequencing (Hu, Murata, Toyonaga, et al., 2017) and pyrosequencing (Cho & Jang, 2014) of amplicons of the 16S rRNA genes are mainly utilized for analyzing bacterial community structures. Phylogenetic trees can be constructed from clone libraries of DNA samples of fresh rainwater microorganisms. (Kaushik, R., et al., 2014). The pathogenicity of bacterial strains in rainwater was determined in a few studies by testing for the presence of

the Type III Secretion System, a critical virulence factor (Monteil, Bardin & Morris, 2014). The issue with molecular-based identification is that it only detects the type of organism, even unculturable ones, on a genetic level. No information is provided on the physical characteristics, such as morphology. Therefore, new fluorescence techniques are being introduced for better identification, although untested in rainwater studies.

### 3.3. Identified microbes in rainwater

Rainwater was discovered to have various microorganisms, from bacteria to fungi. Studies done by Hu, Murata, Toyonaga, et al. (2017) displayed that the most abundant bacterial phylum was Proteobacteria (approx. 37%) which consisted of Alpha (29.2%), Beta (2.3%), Gamma- (2.5%), Delta-(3.0%) and Epsilon-(0.01%) subclasses. RW also contained Bacteroidetes, Cyanobacteria, Actinobacteria, Acidobacteria, and Firmicutes. The total number of bacteria seemed to vary depending on rainfall events and temperature. In cases where a single temperature was employed, bacterial strains belonged to alpha-proteobacteria, beta-Proteobacteria, gamma-Proteobacteria, Firmicutes, and Actinobacteria (Cho & Jang, 2014). Fungal clones such as *Taphrina* and *Cryptococcus* in RW were also noted (Lu et al., 2016). Algae and fungal spores transported in RW appeared to be the cause of coloured rain, like the red rain that occurred in Kerala, India, in 2001 (Louis & Kumar, 2006). Furthermore, the rainfall microbiome also appears to depend on the precipitation type. For example, in acid rain, the presence of the fungus *Botrytis cinerea* (*B. cinerea*) was discovered to be promoted due to below-cloud scrubbing (Monteil et al., 2014).

### 3.4. Abundant species

Due to weather patterns and currents, most airborne microorganisms can travel to different regions. Therefore, some microbes in bacterial communities can be commonly found in many parts of the world. The levels of four major opportunistic waterborne pathogens, namely, *Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, and *Aeromonas hydrophila*, in RW samples were quantified by using a robust and sensitive quantitative PCR (qPCR) method in Singapore (Kaushik & Balasubramanian, 2012). *E.coli* was the most prevalent among the four pathogens tested in their RW samples (Kaushik et al., 2012). In the Asian regions, besides *E. coli*, some of the abundant microbes were of the genus *Burkholderia*, followed by *Massilia* and *Methylobacterium* (Lu et al., 2016)

Additionally, the abundance of organisms depends on rainfall events. In Amato's findings (2005), the relative abundance of Actinobacteria consistently increased in their aerosol samples collected after rainfall events. However, it tended to decrease in pre-rain air samples over time. It was hypothesized that variations in humidity and size and the number of raindrops during rainfall events likely provide environmental cues triggering the

release of diverse bacteria. In some instances, the rain was discovered to drastically impact the microbiota in inland water bodies, such as cyanobacterial blooms, as reviewed in-depth by Reichwaldt (2012). The abundance of RW microorganisms also changed once they entered RWHS. In Evans's (2009) case, Microbacteriaceae, Propionibacteriaceae, Proteobacteria, and Firmicutes accounted for the most identified bacteria in roof-harvested rainwater (RHRW). The remainders were members of the Actinobacteria and Bacteroidetes phyla. The other microorganisms identified under Firmicutes belonged to the Bacilli class, while the Proteobacteria comprised eleven separate orders from the  $\alpha$ ,  $\beta$ , and  $\gamma$  sub-classes.

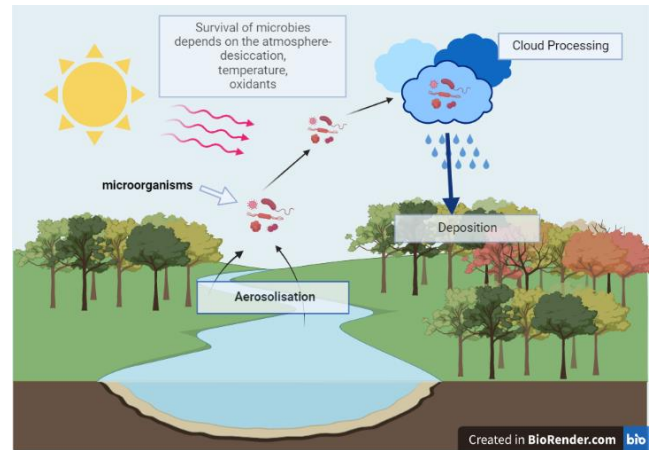
### 3.5. Environmental factors that affect microbial diversity

In conjunction with other factors, weather patterns can significantly influence microbial communities. Findings on roof-harvested RW demonstrated significant differences in microbial communities stored at different temperatures. There were increased variations among storage bottles noted at higher temperatures and storage time (Zhang et al., 2020). Some findings made comparisons of the distribution of Gram-negative vs Gram-positive bacteria during the seasons. Higher light exposure appeared to favor the presence of Gram-negative strains, which are usually more resistant to UV damage. When isolated, the Gram-negative strains appeared more during the summer as they are generally associated with vegetation. These parameters lead to a higher proportion of retrieved microorganisms related to Gram-negative strains during the summer (Amato et al., 2007).

The movement of microorganisms in RW, arriving at the sampling sites, was mapped in some findings on the day of the precipitation event by backward trajectories. The HYSPLIT (Hybrid Single-Particle Lagrangian Integrated Trajectory) models utilized backward trajectories to investigate the long-range transport journey of air masses from the precipitation event (Sarmiento-Vizcaíno et al., 2018). As displayed in figure 3, the movement of microorganisms passed through aerosolization from the ground to air and rain can be mapped through backward trajectory models.

Variations in reaching heights and geographical locations have been investigated to determine these air masses' associated climatic conditions through backward trajectories (Monteil et al., 2014). These findings investigated favorable conditions for disseminating bacteria like *Pseudomonas syringae* (*P. syringae*) in RW, by estimating the associated air masses' physical properties and backward trajectories in precipitation events. To better understand the populations, the microbial populations were also characterized through precipitation chemistry as well as genetic and phenotypic structures. The same study ran parallel research with the fungus *Botrytis cinerea* for comparison and uncovered the relationship of *P. syringae* to precipitation as a dissemination vector. When snowfall and rainfall were

compared using the trajectory of the air mass associated with the precipitation, and specific physical conditions of the air mass (i.e., distance traveled, temperature), *P. syringae* in rain was linked to electrical conductivity and pH of RW. In contrast, these predictions differed for *B. cinerea* (Monteil et al., 2014).



**Figure 3:** A schematic representation of the movement of microorganisms in the atmosphere. A diagram of the cyclic journey of airborne microbes from the ground to the atmosphere to accumulation in cloud systems and back to Earth by deposition (Created in *BioRender.com*).

### 3.6. Differences in the microbiome based on the geographical locations

The main focus of the new RW microbiota studies has been bacterial, and the current findings only provide insight into the bacterial load. The composition of the microbial community is different based on the location of the collected samples. The wind's direction at the site contributes significantly to the total bacterial load, while winds from other directions contribute a divergent mix of organisms (Evans et al., 2006). The magnitude of the rainfall seemed to cause changes in airborne bacterial composition.

Research on fresh rainwater organisms is sparse. Furthermore, depending on the environment and pollution of the collection site, the microorganism species appeared to vary. In one instance, a sample collected in a suburban area demonstrated that a rise in the relative abundance of non-spore-forming Actinobacteria decreased the relative abundance of Firmicutes in air columns (Jang et al., 2018). Currently, rainwater collected in RWHS can aid in determining the impact of raindrops on various surfaces that cause emissions of surface-associated bacteria using air columns (Jang et al., 2018). Additionally, the microbiome in RHRW may also give insight into mechanisms that cause changes in airborne bacterial community composition, resulting in health risks during long-term storage (Amato et al., 2005).

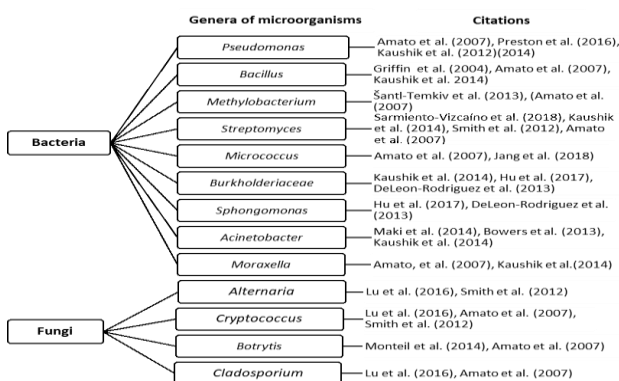
Furthermore, the consequences of natural selection are another factor for which the microorganisms can vary from the altitude and area of collection. In the upper layers of the Earth's atmosphere, the predominant proportion of

microorganisms is thought to be pigmented to adapt to UV radiation resistance (Imshenetsky et al., 1979). Dust rains are an effective means of transporting and delivering microorganisms to various locations. Rainclouds can protect organisms, especially bacteria, against desiccation and solar radiation; this transport promotes species' survival from soil and aquatic bodies (Itani & Smith, 2016). These results are pertinent to understanding microbial survival, emission sources, and atmospheric processes.

### 3.7. Microbial movement in the troposphere

Most of the cells in the mid/upper troposphere appear bacterial. They constitute an essential component of the total super  $\mu\text{m}$ -sized particles and support ice nuclei (IN) activity at high altitudes. Microbes embody a higher fraction of the total particles than observed near the surface of the Earth. Moreover, the faster growth of abiotic particles as cloud condensation nuclei (CCN) and IN from precipitation scavenging displayed responsibility for the microbial patterns observed (DeLeon-Rodriguez et al., 2013). Like the transfer of elements from the lower atmosphere to the tropospheric rain-forming layers, the microbial movement too is facilitated by winds and air pressure. These bacteria seem more competitive in non-crowded, nutrient-poor environments; cloud droplets provide an appropriate environmental niche for their activity and growth.

Numerous microorganisms were found to be abundant in the troposphere and fresh RW, as represented in figure 4. Furthermore, it was hypothesized that the upper atmosphere is a medium for disseminating viable airborne microbes, such as methanotrophs, from sources with dense and active populations to more remote or newly established environments. Also, it appears to act as an environment where significant methane oxidation could occur (Šantl-Temkiv et al., 2013).



**Figure 4:** Genera of microorganisms found in both the troposphere and rainwater. A corroborated compilation of studies in which bacteria and fungi of the same genera were present in both the troposphere and rain.

At high altitudes, the diverse bacterial population appeared to be dominated by gram-negative fractions, e.g., *Acinetobacter*, *Massilia*, *Serratia*, *Acidovorax*, *Sphingomonas*, and *Pseudomonas*. These organisms can

participate in nucleation by acting as CCN and IN in cloud systems. The bacterial community had shown a marked daily tendency between daytime and nighttime, where environmental factors, for instance, wind patterns, O<sub>3</sub> and SO<sub>2</sub>, and participation of some RW bacteria in IN, critically contributed to the variation of bacterial community structure (Xu et al. 2017).

### 3.8. Cloud formation

Most organisms in the troposphere, especially in clouds, help scavenge water and nutrients and utilize the cloud as a mechanism for their widespread dispersal, aiding ubiquity (Ahern et al., 2007). Bacteria are the primary organisms considered sources that activate atmospheric aerosols into CCN. Pseudomonads, having a higher level of surfactant production, make the bacterial genus the most active species for ice nucleation (INA). However, some RW studies only accounted for 2% of the bacterial clones. Further, neither the genus *Erwinia* nor *Xanthomonas*, also known as IN bacteria, was found in any samples (Lu. et al. 2016). These results suggest that the bacterial IN were dispersed in the atmosphere and clouds. Still, their overall contribution to heterogeneous ice nucleation in mixed-phase clouds may not be significant locally. It should be studied more broadly to understand their cloud formation participation.

### 3.9. Cloud water

There is an intrinsic difference in bacterial composition between clouds and rain. Ahern (2007) recognized that each rain sample distinctly reflected aerosols' spatial and temporal variability in different air masses captured when scrubbed by the rain. One study simulated cloud water conditions in an "artificial cloud water" medium to test the effect of major chemicals dissolved in cloud water on methane oxidation (Delort et al., 2010). This medium mimicked the complex chemical composition of natural cloud water. Thus, it was employed to evaluate the influence of organic compounds (acetate, formate, succinate, and oxalate) on methane oxidation. It was reported for the first time that viable methanotrophic bacteria were present in the atmosphere's dry and wet phases. However, natural cloud droplets offered an appropriate environment for methanotrophic activity, although only sparsely populated by bacteria (Šantl-Temkiv et al., 2013).

Comparisons of cloud water and snow with unpolluted groundwater demonstrated that the concentrations of bacteria were in the same range. Bauer et al. (2002) tried to determine the total number of concentrations of fungal spores and the contribution of microorganisms to organic carbon (OC) in atmospheric samples. Aerosol samples displayed that bacterial and fungal spores represent an average of 5.8% of the organic carbon in the coarse mode. In cloud water and precipitation samples, bacterial and fungal carbon contributed to an average of 1.7% of OC and 1.8% of total carbon. Although bacteria were generally more abundant in number, their contribution to

OC appeared to be lower than that of fungal spores because of their smaller size (Bauer et al., 2002).

### 3.10. Rainwater and particulate matter microbiome

Anthropogenic pollutants and terrestrial emissions result in high concentrations and low viability of bacteria in RW (Hu et al., 2017b). However, more significant fractions of bacterial species, presumably involved in in-cloud processes, signify a higher contribution of bacterial communities from continental emissions (Hu et al., 2017b). The viability of bacteria in RW was comparable to or higher than that in the air, significantly relatively higher than that in the dusty air or air parcels. Rainwater protects airborne bacterial cells against desiccation and contains organic compounds and elements that can act as nutrients (e.g., phosphorus, iron, copper, and magnesium) and sustain metabolism for bacteria. Some findings revealed negative correlations between bacterial viability and the ionic species  $\text{H}_3\text{O}^+$ ,  $\text{NO}_3^-$ , etc., in the RW samples signifying that rising pollutants and terrestrial emissions might inhibit the liveness of bacteria in RW.

Figure 4 demonstrates that some of the microbial genera listed, such as *Pseudomonas* and *Cryptococcus*, consist of pathogenic strains. Although the viability of microorganisms, such as bacteria, is low in RW, the pathogenicity is vastly under-researched. High concentrations of ionic species appear to potentially result in lower viability of bacteria (Hu et al., 2017a). Interestingly, a study on the urban atmosphere of Singapore during the Southwest monsoon displayed that the prevalence patterns of four pathogens in RW and particulate matter were quite similar (Kaushik et al., 2012). However, this is not always the case due to wind currents. Preston & Huddleston (2016) found different species collected on the same day, like *Novosphingobium/Sphingomonas*, in the wind but not RW.

The correlation between particulate matter and RW can be significantly seen during dust storms and haze events. Studies conducted on haze events in Singapore observed that the microbial quality of RW in portability was in the acceptable range in the non-haze period, with no gene copies per 100 ml. In comparison, the average numbers of microbial quality were approximately  $2 \times 10^3$  gene copies/100 ml during rain events under biomass burning (Kaushik et al., 2012). Similar conditions were reported in Sri Lanka during a haze event, which proved to impact the airborne bacterial load that increases with the intensity of the haze condition. At least twelve culturable bacterial species were identified through this study. The lowest bacterial load of  $1.12 \times 10^5$  cells/ $\mu\text{L}$  was observed during a non-hazy day. Meanwhile, the bacterial load was approximately 40% higher during haze events than on non-hazy days (Thilakarathne et al., 2021).

Immune-compromised people face the highest health risk due to pathogenic microbes in breathing air. Amarasekara's (2021) findings on the effect of traffic congestion and vegetation on airborne bacteria identified

28 bacterial types. Among them, 22 species were recognized as opportunistic pathogens. *Kocuria* spp., one of the Actinobacteria identified in that study, was known to survive even under solid oxidants such as  $\text{H}_2\text{O}_2$ , commonly used as an antimicrobial agent that can destroy bacterial membranes. These types of bacteria are even capable of multiplying under stress conditions. Since the existence of pathogenic bacteria is high in most of these studies, this is a cause for concern regarding people's well-being. As most airborne microbiome is bacterial, a focal point in RW research should be the pathogenicity of these microorganisms.

### 3.11. Possible future directions

The scarcity of research on a particular topic usually results in more significant gaps related to the identification and purpose of certain organisms. Such is the case for microbial studies in rainwater. Investigations on the pathogenicity of the identified organisms and resistance profiles are scarce (Thilakarathne et al., 2021). Furthermore, molecular-based methods do not provide physical characteristics such as the morphology of unculturable microorganisms; therefore, the fluorescence in situ hybridization (FISH) technique can benefit the preliminary phases for further studies. FISH uncovers not only culturable microorganisms but also unculturable organisms. This is done by detecting nucleic acid sequences using a fluorescently labelled probe that hybridizes to its complementary target sequence within the intact cell, which can help understand complex microbial communities (Batani et al., 2019).

Moreover, the viral community in rainwater is the most narrowly studied segment in the rainwater microbiome. Recently, findings on the virome in RHRW have been conducted to identify pathogenic viruses that may prevent RHRW from human consumption (Prado et al., 2021). Some of the viruses detected in this study through metagenomic sequencing were genera *Cyclovirus*, *Salivirus*, *Enterovirus*, and *Hepatovirus*. Unfortunately, research on viruses in fresh rainwater is still sparse. Hopefully, more experiments can be conducted to monitor emerging viruses and their impact on rainwater quality.

Regarding utilizing rainwater as a source for household purposes, a significant risk is the pathogens present in RW itself and contamination from faecal matter that precludes the use of rainwater for drinking. There are processes to disinfect rainwater, such as membrane filtration systems, chlorination, and solar panels (thermal inactivation of bacteria) (Zdeb et al., 2016). More research is needed to determine the efficiency of these filtration systems in eliminating pathogens. Some studies indicated that rooftop material and coating could impact metal contamination in harvested rainwater (Ahmed et al., 2018). Further studies are needed to see how different catchment surfaces connected to RWHS can affect water quality and the survival of pathogens. It has been demonstrated that approximately 40% of more particles were added during the catchment process (Kim, R.H. et al., 2005). Another study on *Legionella*'s survival in rainwater showed that



some strains, such as *Legionella pneumophila* survived better in rainwater with a complex bacterial flora than in others (Potočnjak et al., 2012).

In addition, the exact effects of biological IN on cloud droplets and their involvement in precipitation formation are under-researched (Lu et al., 2016). More research is vital to determine the microorganisms behind ice nucleation accurately, and their abundance globally since their contribution to heterogeneous ice nucleation is not significant at a regional scale.

## 4. Conclusion

In summary, although research on rainwater is starting to upsurge, microbial literature on fresh RW is still limited compared to roof-harvested rainwater. Current literature gives partial insight into the microbial diversity in rainwater. As mentioned above, many unknown facts about how they partake in cloud formation and other atmospheric chemical processes exist. With climate change and increasing pollution levels, the RW microbiome is a critical aspect that should be looked upon, as these droplets may contain pathogenic organisms. This is crucial as, during prolonged rainwater storage, the collected water can cause infections if the environment overpowers opportunistic pathogenic bacteria. Notably, with technological advances, the knowledge gap is shrinking on how microbial communities can be mapped and why they differ in each location. Since airborne microorganisms also seem to have more significant ecological functions, further research on this topic will aid in understanding their impact on human health and climate change.

## References

- Ahern, H.E., Walsh, K.A., Hill, T.C.J., & Moffett, B.F. (2007). Fluorescent pseudomonads isolated from Hebridean clouds and rainwater produce biosurfactants but do not cause ice nucleation. *Biogeosciences*, 4(1), 115–124. <https://doi.org/10.5194/bg-4-115-2007>
- Ahmed, M.U., Majumder, A.K., Hossain, M.S., & Nayeem, A.A. (2019). Assessment of the microbiological quality of rainwater: A case study of selected water scarce area in Bangladesh. *Stamford Journal of Microbiology*, 8(1), 24–26. <https://doi.org/10.3329/sjm.v8i1.42434>
- Albrechtsen, H.J. (2002). Microbiological investigations of rainwater and graywater collected for toilet flushing. *Water Science and Technology*, 46(6-7), 311–316. <https://doi.org/10.2166/wst.2002.0694>
- Amarasekara, R. W.K., Vithanage, M., Samaraweera, P., Goonetilleke, A., & Magana-Arachchi, D.N. (2021). Effect of traffic congestion and vegetation on airborne bacteria in a city of a developing country. *Air Quality, Atmosphere & Health*, 14(7), 1103–1116. <https://doi.org/10.1007/s11869-021-01001-1>
- Amato, P., Ménager, M., Sancelme, M., Laj, P., Mailhot, G., & Delort, A.-M. (2005). Microbial population in cloud water at the Puy de Dôme: Implications for the chemistry of clouds. *Atmospheric Environment*, 39(22), 4143–4153. <https://doi.org/10.1016/j.atmosenv.2005.04.002>
- Amato, P., Parazols, M., Sancelme, M., Laj, P., Mailhot, G., & Delort, A.-M. (2007). Microorganisms isolated from the water phase of tropospheric clouds at the Puy de Dôme: major groups and growth abilities at low temperatures. *FEMS Microbiology Ecology*, 59(2), 242–254. <https://doi.org/10.1111/j.1574-6941.2006.00199.x>
- Batani, G., Bayer, K., Böge, J., Hentschel, U., & Thomas, T. (2019). Fluorescence in situ hybridization (FISH) and cell sorting of living bacteria. *Scientific Reports*, 9(1). <https://doi.org/10.1038/s41598-019-55049-2>
- Bauer, H., Kasper-Giebl, A., Löflund, M., Giebl, H., Hitzemberger, R., Zibuschka, F., & Puxbaum, H. (2002). The contribution of bacteria and fungal spores to the organic carbon content of cloud water, precipitation, and aerosols. *Atmospheric Research*, 64(1-4), 109–119. [https://doi.org/10.1016/s0169-8095\(02\)00084-4](https://doi.org/10.1016/s0169-8095(02)00084-4)
- Bowers, R.M., Clements, N., Emerson, J.B., Wiedinmyer, C., Hannigan, M. P., & Fierer, N. (2013). Seasonal Variability in Bacterial and Fungal Diversity of the Near-Surface Atmosphere. *Environmental Science & Technology*, 47(21), 12097–12106. <https://doi.org/10.1021/es402970s>
- Cho, B.C., & Jang, G.I. (2014). Active and diverse rainwater bacteria were collected at an inland site in the spring and summer of 2011. *Atmospheric Environment*, 94, 409–416. <https://doi.org/10.1016/j.atmosenv.2014.05.048>
- Chubaka, C., Whiley, H., Edwards, J., & Ross, K. (2018). Microbiological Values of Rainwater Harvested in Adelaide. *Pathogens*, 7(1), 21. <https://doi.org/10.3390/pathogens7010021>
- DeLeon-Rodriguez, N., Latham, T.L., Rodriguez-R, L. M., Barazesh, J.M., Anderson, B.E., Beyersdorf, A.J., Ziemba, L.D., Bergin, M., Nenes, A., & Konstantinidis, K.T. (2013). The microbiome of the upper troposphere: Species composition and prevalence, effects of tropical storms, and atmospheric implications. *Proceedings of the National Academy of Sciences*, 110(7), 2575–2580. <https://doi.org/10.1073/pnas.1212089110>
- Delort, A.-M., Väitilingom, M., Amato, P., Sancelme, M., Parazols, M., Mailhot, G., Laj, P., & Deguillaume, L. (2010). A short overview of the microbial population in clouds: Potential roles in atmospheric chemistry and nucleation processes. *Atmospheric Research*, 98(2), 249–260. <https://doi.org/10.1016/j.atmosres.2010.07.004>
- Drayna, P., McLellan, S.L., Simpson, P., Li, S.-H., & Gorelick, M.H. (2010). Association between Rainfall and Pediatric Emergency Department Visits for Acute Gastrointestinal Illness. *Environmental Health*

- Perspectives*, 118(10), 1439–1443. <https://doi.org/10.1289/ehp.0901671>
- Evans, C.A., Coombes, P.J., & Dunstan, R.H. (2006). Wind, rain, and bacteria: The effect of weather on the microbial composition of roof-harvested rainwater. *Water Research*, 40(1), 37–44. <https://doi.org/10.1016/j.watres.2005.10.034>
- Evans, C.A., Coombes, P.J., Dunstan, R.H., & Harrison, T. (2009). Extensive bacterial diversity indicates the potential operation of a dynamic micro-ecology within domestic rainwater storage systems. *Science of the Total Environment*, 407(19), 5206–5215. <https://doi.org/10.1016/j.scitotenv.2009.06.009>
- Griffin, D.W. (2004). Terrestrial Microorganisms at an Altitude of 20,000 m in Earth's Atmosphere. *Aerobiologia*, 20(2), 135–140. <https://doi.org/10.1023/b:aero.0000032948.84077.12>
- Hu, W., Murata, K., Horikawa, Y., Naganuma, A., & Zhang, D. (2017a). Bacterial community composition in rainwater is associated with synoptic weather in an area downwind of the Asian continent. *Science of the Total Environment*, 601-602, 1775–1784. <https://doi.org/10.1016/j.scitotenv.2017.06.052>
- Hu, W., Murata, K., Toyonaga, S., & Zhang, D. (2017b). Bacterial abundance and viability in rainwater associated with cyclones, stationary fronts, and typhoons in southwestern Japan. *Atmospheric Environment*, 167, 104–115. <https://doi.org/10.1016/j.atmosenv.2017.08.013>
- Imshenetsky, A.A., Lysenko, S.V., & Lach, S.P. (1979). Microorganisms of the upper layer of the atmosphere and the protective role of their cell pigments. *Life Sciences and Space Research*, 105–110. <https://doi.org/10.1016/b978-0-08-023416-8.50017-9>
- Itani, G.N., & Smith, C.A. (2016). Dust Rains Deliver Diverse Assemblages of Microorganisms to the Eastern Mediterranean. *Scientific Reports*, 6(1). <https://doi.org/10.1038/srep22657>
- Jang, G.I., Hwang, C.Y., & Cho, B.C. (2017). Effects of heavy rainfall on the composition of airborne bacterial communities. *Frontiers of Environmental Science & Engineering*, 12(2). <https://doi.org/10.1007/s11783-018-1008-0>
- Kaushik, R., & Balasubramanian, R. (2012). Assessment of bacterial pathogens in fresh rainwater and airborne particulate matter using Real-Time PCR. *Atmospheric Environment*, 46, 131–139. <https://doi.org/10.1016/j.atmosenv.2011.10.013>
- Kaushik, R., Balasubramanian, R., & de la Cruz, A.A. (2012). Influence of Air Quality on the Composition of Microbial Pathogens in Fresh Rainwater. *Applied and Environmental Microbiology*, 78(8), 2813–2818. <https://doi.org/10.1128/aem.07695-11>
- Kaushik, R., Balasubramanian, R., & Dunstan, H. (2014). Microbial Quality and Phylogenetic Diversity of Fresh Rainwater and Tropical Freshwater Reservoir. *PLoS ONE*, 9(6), e100737. <https://doi.org/10.1371/journal.pone.0100737>
- Kim, R-H., Lee, S., Kim, Y-M., Lee, J-H., Kim, S.-K. , & Kim, S-G. (2005). Pollutants in Rainwater Runoff in Korea: Their Impacts on Rainwater Utilization. *Environmental Technology*, 26(4), 411–420. <https://doi.org/10.1080/09593332608618546>
- Louis, G., & Kumar, A.S. (2006). The Red Rain Phenomenon of Kerala and its Possible Extraterrestrial Origin. *Astrophysics and Space Science*, 302(1-4), 175–187. <https://doi.org/10.1007/s10509-005-9025-4>
- Lu, Z., Du, P., Du, R., Liang, Z., Qin, S., Li, Z., & Wang, Y. (2016). The Diversity and Role of Bacterial Ice Nuclei in Rainwater from Mountain Sites in China. *Aerosol and Air Quality Research*, 16(3), 640–652. <https://doi.org/10.4209/aaqr.2015.05.0315>
- Maki, T., Puspitasari, F., Hara, K., Yamada, M., Kobayashi, F., Hasegawa, H., & Iwasaka, Y. (2014). Variations in the structure of airborne bacterial communities in a downwind area during an Asian dust (Kosa) event. *Science of the Total Environment*, 488-489, 75–84. <https://doi.org/10.1016/j.scitotenv.2014.04.044>
- Mertens, A., Balakrishnan, K., Ramaswamy, P., Rajkumar, P., Ramaprabha, P., Durairaj, N., Hubbard, A. E., Khush, R., Colford, J. M., & Arnold, B. F. (2019). Associations between High Temperature, Heavy Rainfall, and Diarrhea among Young Children in Rural Tamil Nadu, India: A Prospective Cohort Study. *Environmental Health Perspectives*, 127(4), 47004. <https://doi.org/10.1289/EHP3711>
- Monteil, C.L., Bardin, M., & Morris, C.E. (2014). Features of air masses associated with the deposition of *Pseudomonas syringae* and *Botrytis cinerea* by rain and snowfall. *The ISME Journal*, 8(11), 2290–2304. <https://doi.org/10.1038/ismej.2014.55>
- Poissant, L., & Béron, P. (1994). Parameterized rainwater quality model in urban environment. *Atmospheric Environment*, 28(2), 305–310. [https://doi.org/10.1016/1352-2310\(94\)90105-8](https://doi.org/10.1016/1352-2310(94)90105-8)
- Potočnjak, M., Široka, M., Rebić, D., & Gobin, I. (2012). The survival of *Legionella* in rainwater. *Sanitarno Inženirstvo*, 6(1), 31–36. <https://journal.institut-isi.si/the-survival-of-legionella-in-rainwater/>
- Prado, T., Shubo, T., Freitas, L., Leomil, L., Maranhão, A. G., & Miagostovich, M.P. (2022). Virome in roof-harvested rainwater of a densely urbanized low-income region. *Science of the Total Environment*, 807, 150778. <https://doi.org/10.1016/j.scitotenv.2021.150778>
- Preston, K.E., & Huddleston, J. (2016). Gram-Negative, Oxidase-Positive Bacteria in Rainwater and Wind Samples. *Fine Focus*, 2(2), 92–103. <https://doi.org/10.33043/ff.2.2.92-103>
- Reddy, Y.K., Maddirala, P., Vamshigoud, R., Reddy, S.N., Krishna, S., & Mamatha, M. (2011). Analytical study and microorganisms present in rainwater of different areas. *International Journal of Environmental Sciences*, 2(1), 194-200.
- Reichwaldt, E.S., & Ghadouani, A. (2012). Effects of rainfall patterns on toxic cyanobacterial blooms in a changing climate: Between simplistic scenarios and



- complex dynamics. *Water Research*, 46(5), 1372–1393. <https://doi.org/10.1016/j.watres.2011.11.052>
- Šantl-Temkiv, T., Finster, K., Hansen, B. M., Pašić, L., & Karlson, U. G. (2013). Viable methanotrophic bacteria enriched from air and rain can oxidize methane under cloud-like conditions. *Aerobiologia*, 29(3), 373–384. <https://doi.org/10.1007/s10453-013-9287-1>
- Sarmiento-Vizcaíno, A., Espadas, J., Martín, J., Braña, A. F., Reyes, F., García, L.A., & Blanco, G. (2018). Atmospheric Precipitations, Hailstone and Rainwater, as a Novel Source of Streptomyces Producing Bioactive Natural Products. *Frontiers in Microbiology*, 9. <https://doi.org/10.3389/fmicb.2018.00773>
- Smith, D.J., Jaffe, D.A., Birmele, M.N., Griffin, D.W., Schuergel, A. C., Hee, J., & Roberts, M. S. (2012). Free Tropospheric Transport of Microorganisms from Asia to North America. *Microbial Ecology*, 64(4), 973–985. <https://doi.org/10.1007/s00248-012-0088-9>
- Thilakarathne, S.M.N.K., Ekanayake, A., Madamarandawala, P.S., Weerarathne, W.B.C.P., Thotawatthage, C.A., & Magana-Arachchi, D.N. (2021). Impact of haze events on airborne bacterial consortia—a case study. *SN Applied Sciences*, 3(1). <https://doi.org/10.1007/s42452-020-04022-0>
- Xu, C., Wei, M., Chen, J., Sui, X., Zhu, C., Li, J., Zheng, L., Sui, G., Li, W., Wang, W., Zhang, Q., & Mellouki, A. (2017). Investigation of diverse bacteria in cloud water at Mt. Tai, China. *Science of the Total Environment*, 580, 258–265. <https://doi.org/10.1016/j.scitotenv.2016.12.081>
- Zdeb, M., Zamorska, J., & Papciak, D. (2016). Studying microbiology of rainwater for their use in the economy. *Journal of Ecological Engineering*, 17(3), 203–208. <https://doi.org/10.12911/22998993/63314>
- Zhang, X., Xia, S., Zhao, R., & Wang, H. (2020). Effect of temperature on opportunistic pathogen gene markers and microbial communities in long-term stored roof-harvested rainwater. *Environmental Research*, 181, 108917. <https://doi.org/10.1016/j.envres.2019.108917>



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