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Research Article


Variation of *Pseudomonas aeruginosa* in Rainwater Harvesting Systems: Effects of Seasons, Catchments and Storage Conditions

The effects of different catchment types and storage conditions on *Pseudomonas aeruginosa* in harvested rainwater under wet and dry seasons were investigated. Both horizontal (roof intercepted to outlet point) and vertical (surface to bottom) quality variation inside storage tanks of different rainwater harvesting (RWH) systems was also monitored. The numbers of *P. aeruginosa* varied from 30 to 400 colony forming units (CFU)/100 mL during the dry season and 200 to 1800 CFU/100 mL during the wet season. A relatively good quality of harvested rainwater was observed in dry season. The horizontal and vertical quality variation of *P. aeruginosa* revealed best quality at the supply point. The number of *P. aeruginosa* was the highest (about 1800 and 1000 CFU/100 mL during wet and dry seasons, respectively) in rainwater harvested from mountain catchment while the lowest (about 30 and 1000 CFU/100 mL during dry and wet seasons, respectively) numbers was seen from concrete roof catchment. Dark, covered storage conditions resulting low rainwater temperatures showed better microbial quality of rainwater than uncovered and open storage conditions exposed to light. The study suggests that the improvement in the quality of harvested rainwater is possible when appropriate tank designs, maintenance of catchment surfaces, and proper storage conditions are considered in RWH systems.

Keywords: Catchment type; Heterotrophic bacteria; RWH systems; Water quality; Water shortage

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1 Introduction

Water shortage is the major problem of this Era. Changing climate conditions and increasing demand of growing population reduced the water resources. According to the latest available information by the joint study of UNICEF and WHO, still 783 million people do not have access to safe water resources [1]. Rainwater harvesting (RWH) in this regard is reviving as a new paradigm. An efficient RWH system consists of catchment area, storage tank, supply facility, pipes and a treatment facility [2–4]. The harvested rainwater has not only been used for non-potable purposes [5, 6] but also receiving increased attention worldwide as an alternative potable water source [3, 7–10].

The poor microbial quality of harvested rainwater makes it unsafe for human use especially for potable purposes. The microbial quality of the harvested rainwater depends upon the characteristics of the harvested area, such as topography, weather conditions, and proximity to pollution sources [11, 12], the type of the catchment

area [13, 14], the type of storage [13, 15], dry periods, and the handling and management of the water [11, 16].

Thus, the catchment types and storage conditions are the important factors for deciding the quality of harvested rainwater in any RWH system. The catchment surfaces can significantly deteriorate the microbial quality of rainwater especially after long dry season. Once the wet season starts, rainwater upon contact with the catchment surfaces wash many types of bacteria, algae, dust, leaves, bird droppings, and other contaminants into the water tank which in return deteriorate the microbial quality of harvested rainwater [17, 18]. This shows that along with catchment, weather conditions also affect the microbial quality of harvested rainwater. On the other hand, better storage conditions/storage tanks can be regarded as means of treatment as they offer a range of beneficial and natural treatment processes to improve the quality of harvested rainwater [19, 20].

The main focus of this study is to investigate the effects of different seasons (dry and wet), catchments (concrete roof, concrete + green roof + terrace and mountain), and storage conditions on *Pseudomonas aeruginosa* in order to lay down the proper design and maintenance guidelines for RWH systems. *P. aeruginosa* is an opportunistic heterotrophic bacterium which not only affects the physical characteristics such as taste, odor, and turbidity but also is

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Abbreviations: CFU, colony forming unit; FF, first flush; PoS, point of supply; RWH, rainwater harvesting

responsible for many human diseases [21] and nosocomial infection in immune-compromised people [22]. Moreover, this microorganism was also used to indicate the presence of nutrients in water and biofilms on surfaces [23]. Its presence is unacceptable because it has been implicated in waterborne and food borne diseases and now considered to be a primary infectious agent [24].

2 Materials and methods

2.1 Experimental site description

This study was carried out at Seoul National University (SNU), South Korea where the RWH systems were installed at three locations. The components of RWH systems are described in Tab. 1 and the schematic diagrams showing the tanks, catchment types, and collection area are presented in Fig. 1, except for RWH system 3.

The rainwater enters into the storage tanks of RWH systems 1 and 2 through calm inlet (shown in Fig. 2) while in RWH system 3; water is delivered from settling tank (T_3) to the supply tank (T_3') through pipe (Supporting Information Figs. S1 and S2). These sites are all at urban locations and the harvested rainwater is used for toilet flushing, gardening, infiltration, and groundwater recharge.

In RWH system 2, the rainwater harvested from concrete + green roof and terrace is stored in auxiliary tank (T_2') at first stage and then flows into the main tank (T_2) while T_2' also collects water directly from concrete roof. The storage tanks in RWH systems 1 and 2 are divided into two halves and water moves through a baffle from inlet to outlet section. None of the RWH systems has any device to separately treat the first flush (FF) and FF also enters directly into the storage tanks.

2.2 Sampling points

Rainwater samples were collected from three RWH systems, with different catchment sources, installed at Seoul National University,

South Korea. Both horizontal and vertical quality variation in *P. aeruginosa* was investigated in the main storage tanks of RWH systems 1 and 2. For this purpose, three samples (S_{11} , S_{12} , and S_{13} in Fig. 2a and S_{21} , S_{22} , and S_{23} in Fig. 2b) were collected from the each storage tank (T_1) of RWH system 1 and T_2' of RWH system 2, representing the horizontal quality variation from the roof intercepted to the outlet point. The samples from filters (roof intercepted) were directly collected in 0.5 L polyethylene containers while 1 L acrylics water sampler was used to collect the samples from inlet and outlet of the storage tanks. The water sampler includes a brass messenger for activation and a lead collar for rapid descent and minimal drift caused by water currents.

Comparison of three vertical rainwater samples (S_{14} , S_{17} , and S_{18} in Fig. 2a and S_{24} , S_{27} , and S_{28} in Fig. 2b) represent the vertical quality variation from surface to the bottom in T_1 and T_2' at the outlet section. The point of supply (PoS) was situated at a height of about 0.5 m in RWH system 1 and 1.35 m above the base of the storage tanks in RWH system 2. Different samples (S_{21} , S_{22} ... S_{28} in Fig. 2c) were also collected from T_2' to check the horizontal and vertical variation of *P. aeruginosa* in the auxiliary tank of RWH system 2. But the major emphasis was on the variation of *P. aeruginosa* in T_2' as the rainwater was supplied for various purposes from the main tank in the RWH system 2.

Additional samples (S_{15} , S_{16} in Fig. 2a and S_{25} , S_{26} in Fig. 2b and S_{25} , S_{26} in Fig. 2c) at the outlet sections of T_1 , T_2 , and T_2' were collected for the catchment effects. The rainwater samples (S_{34} to S_{38}) from T_3 of RWH system 3 were also collected to exhibit the catchment effects on the variation of *P. aeruginosa*. The variation in the number of *P. aeruginosa* in all cases is shown with error bars representing the minimum and maximum values in Figs. 3–6. All samples were collected for more than, at least, five times during one year of the analysis separately for dry and wet season. The samples were collected in sterile 0.5 L screw cap containers, immediately chilled, and analyzed within 24 h. Sampling from RWH system 3 is only included to compare the effects of different

Table 1. Description of three RWH Systems

Description	Catchment type (area)	Tank capacity	Filter type	Use of harvested rainwater	In operation
RWH system 1 (dormitory)	Concrete roof (2098 m ²)	200 m ³	VF6	Toilet flushing and gardening	Since 2003
RWH system 2 (engineering building)	Concrete + green roof (2828 m ²)	250 m ³	WFF 100	Toilet flushing	Since 2006
RWH system 3 (buddle-gol)	Terrace (824 m ²) Mountain	27 m ³ 3 tanks 24 m ³ each	AFS 200 N/A	Infiltration and groundwater recharge	Since 2008

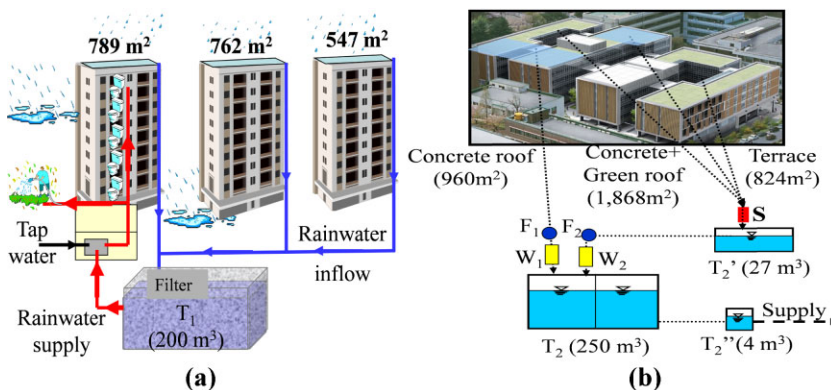


Figure 1. Schematic diagram of RWH systems: (a) system 1, (b) system 2.

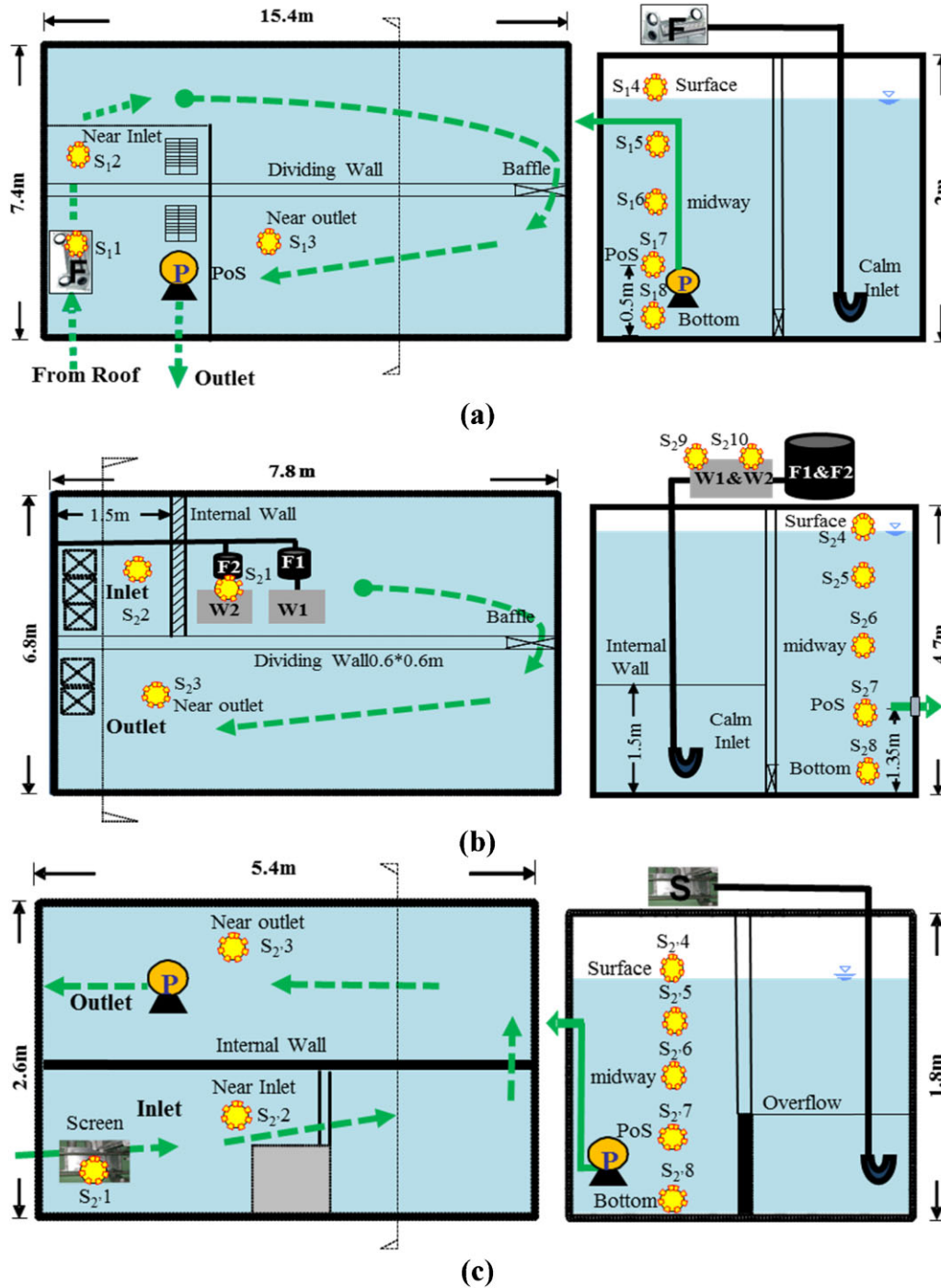


Figure 2. Plan and section views of different storage tanks with sampling points; (a) 'T₁' of RWH system 1, (b) 'T₂' of RWH system 2, and (c) 'T₂' of RWH system 2.

catchment and storage on *P. aeruginosa*. Rainwater samples were diluted in some cases, where the *P. aeruginosa* concentration expected to be high, prior to analysis by most probable number method.

2.3 Microbial analysis

The microbial water quality analysis was carried out by standard methods in the Soil and Water Quality Laboratory, SNU, at room

temperature 25°C. The detection of *P. aeruginosa* was made by multiple tube method. Asparagine broth (Titan Biotech) for the presumptive stage of *P. aeruginosa* was used in a series of fifteen test tubes for three dilutions (10, 1, and 0.1 mL) and thus, five tubes per dilution. The tubes were then incubated at 35°C for 24 h. To check the number of *P. aeruginosa*, the tubes were examined under black light after 24 and then after 48 h. The tubes with green fluorescent pigments were selected and then 0.1 ml of culture was further

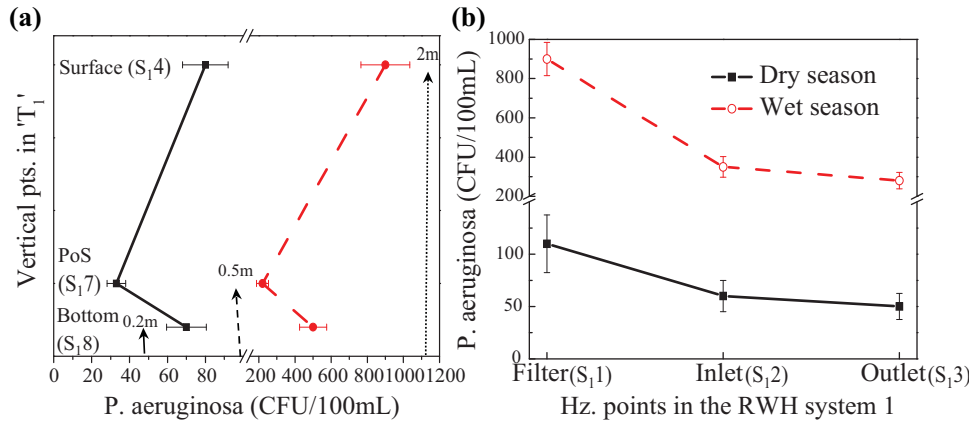


Figure 3. (a) Vertical, and (b) horizontal *P. aeruginosa* variation under dry and wet seasons in 'T₁'.

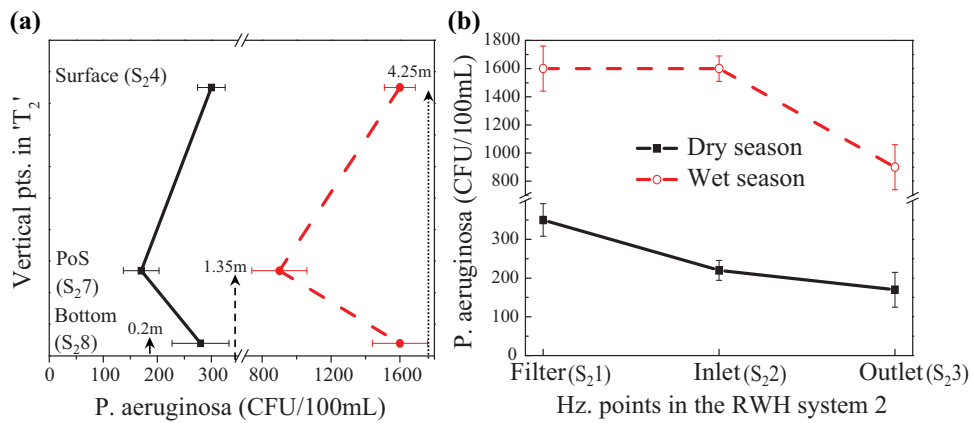


Figure 4. (a) Vertical, and (b) horizontal *P. aeruginosa* variation under dry and wet seasons in 'T₂'.

incubated at 35°C into Acetamide broth (Titan Biotech) to complete the confirmation stage [17, 25].

3 Results and discussion

Simple physicochemical and microbial parameters of the harvested rainwater in 'T₁' and 'T₂' were analyzed on bimonthly basis and the average minimum and the maximum range of the values are mentioned in Tab. 2. The physicochemical parameters, however, were not discussed in detail and the focus of the study was to investigate

the variation in number of *P. aeruginosa* from mountain catchment and roof intercepted rainwater to the supply point under dry and wet seasons with different storage conditions. Reference values of bacteria (total coliform, fecal coliform, *Escherichia coli* and heterotrophic plate count) are included in Tab. 2 from a previous study, which was conducted for the similar RWH systems [3].

As shown in Tab. 2, the difference between the minimum and maximum value of both physicochemical and microbial parameters is considerably significant. Usually the higher values of the parameters were obtained in wet weather except pH, which shifted

Table 2. Reference values of parent rainwater samples

Physicochemical parameter		Microbial parameter (CFU/100 mL)	
Turbidity (NTU)	2–5	<i>P. aeruginosa</i> ^{b)}	Dry season 30–400 Wet season 200–1800
Temperature (°C)	17–26	Total coliform ^{a)}	880–1100 ^{a)}
pH	6–8.5	Fecal coliform ^{a)}	400–450 ^{a)}
EC (μS/cm)	150–450	<i>Escherichia coli</i> ^{a)}	200–250 ^{a)}
DO (mg/L)	6–9	Heterotrophic plate count ^{a)}	1500–2000 ^{a)}

^{a)} Reference values of bacteria [3].

^{b)} Current study.

from slightly acidic to basic in dry weather. One possible reason for this could be the longer storage period of harvested rainwater in concrete storage tanks.

3.1 Vertical and horizontal variation of *P. aeruginosa* in 'T₁' under wet & dry seasons

Figure 3a and b represents the horizontal (S₁1 to S₁3) and the vertical (S₁4, S₁7 to S₁8) *P. aeruginosa* variation, from roof intercepted to the outlet section, in 'T₁' of RWH system 1. Low concentration of *P. aeruginosa* at PoS (S₁7) was observed than at surface/bottom (S₁4/S₁8) during dry season (October to February), as shown in Fig. 3a. One of the possible reasons for the lesser number of *P. aeruginosa* at PoS may be due to the formation of biofilm at the surface and walls of the tanks as *P. aeruginosa* is well known for making the biofilms [26]. This fact is also supported by the earlier research establishing the fact that many bacteria conglomerate in a macro-layer on the water surface, whereas other contaminants precipitate out of the water column and settle at the bottom of the tank [20]. The higher number at the bottom than at the supply point of the storage tank supports the assumption that contaminants settle at the bottom of tanks. This also may be due to the natural treatment processes including flocculation, settlement and bio-reaction, which appear to operate during storage of rainwater in tanks [19].

The water quality improved horizontally in the system from the roof intercepted to the supply point (S₁1 to S₁3) as shown in Fig. 3b. The worst quality inside the filter assembly ('F' in Fig. 2a) as compared with the inlet (S₁2) or outlet (S₁3) samples was probably due to presence of FF water (S₁1) in addition to the absence of natural treatment processes as inside storage tank. The higher number of *P. aeruginosa* at all sampling points during wet season (July to September) may be due to the larger amounts of FF and lesser storage time inside the tanks due to the frequent rainfall events. The longer dry period before the beginning of wet season allows the deposition of dust particles and feces of birds and rodents on roof top which directly entered to storage tank with rainwater runoff as no separate device for FF removal was installed. Another reason of high number of *P. aeruginosa* in wet season could be due to the presence of enough nutrients in FF required for the rapid growth of *P. aeruginosa*. The microbial analysis of 5–6 samples each, drawn from different sampling points inside 'T₁' (Fig. 3) of RWH system 1, during one year, revealed that the average values for *P. aeruginosa* were well in excess of the guidelines (0 CFU/100 mL) when the water is used for potable purposes [27].

3.2 Vertical and horizontal variation of *P. aeruginosa* in 'T₂' under wet & dry seasons

Figure 4 represents the horizontal and the vertical variation of *P. aeruginosa* in 'T₂' wherein the samples (S₂1, S₂2, S₂3, S₂4, S₂7, and S₂8 in Fig. 2b) were collected from the filter to the outlet point. As shown in Fig. 4a, better microbial quality was observed at the PoS (S₂7) than at the surface (S₂4) or at the bottom (S₂8) of the tank. The phenomena of biofilm formation and natural treatment processes inside the tank could be the possible reasons for the reduction of *P. aeruginosa* at PoS in 'T₂'. As it was the case in RWH system 1, a similar trend was observed for horizontal variation of *P. aeruginosa* from the filter (S₂1) to the outlet (S₂3) point in the RWH system 2. The division of 'T₂' into two halves, inlet and outlet sections, allows more sludge accumulation near the inlet point which could also be the reason for lesser

number of *P. aeruginosa* at the outlet point inside tank. The provision of baffles, through which the water flows from inlet to outlet section, further enhances the sedimentation in order to give the better quality water at the outlet section.

A slight higher values at the respective points of RWH system 2 than at the RWH system 1 (comparing Fig. 3a with Fig. 4a and Fig. 3b with Fig. 4b) could be due to the addition of rainwater from the green roof+ terrace catchment in addition to the relatively clean concrete roof. The highest concentration of *P. aeruginosa* in 'T₂' (results not shown) also reflected the effects of green roof+ terrace catchment on deteriorating the microbial quality of the harvested rainwater in RWH system 2. The sample collected from 'T₂' was comparatively dirty in appearance and was alkaline in nature with very high turbidity value (Supporting Information Fig. S11). Better quality in 'T₂' as compared to 'T₂' was due to the dilution factor as the rainwater in 'T₂' was also collected directly from concrete roof in addition to the flow of stored rainwater from 'T₂' to 'T₂' (Fig. 1b).

3.3 Effects of different catchments on *P. aeruginosa* variation under wet and dry seasons

The effects of the different catchments were observed by comparing the concentration of *P. aeruginosa* at the outlet sections in 'T₁', only concrete roof catchment, 'T₂', concrete + green + terrace catchment, 'T₂', rainwater from 'T₂' further mixed/diluted from concrete roof only and 'T₃', mountain catchment. The average of five samples (S₁4 to S₁8 for 'T₁' in Fig. 2a), (S₂4 to S₂8 for 'T₂' in Fig. 2b), (S₂4 to S₂8 for 'T₂' in Fig. 2c) and (S₃4 to S₃8 for 'T₃') revealed the highest numbers of *P. aeruginosa* in 'T₃' with 'T₁' being the lowest, as shown in Fig. 5.

This variation in number of *P. aeruginosa* appears to reflect the difference in the catchment materials in all three RWH systems. At first, the difference in number of *P. aeruginosa* in 'T₁', 'T₂', and 'T₂' may reflect the effects of the cleaning/management which were more easy and frequent for concrete surfaces which was the only catchment source for harvesting rainwater into 'T₁' of RWH system 1.

The number of *P. aeruginosa* in 'T₃', rainwater harvested from mountain, was the highest. The possible reason could be the carriage of many nutrients, helpful for *P. aeruginosa* growth, from mountain catchment surface. The human activity involved in terms of hiking, recreational purposes etc. could be another factor for highest

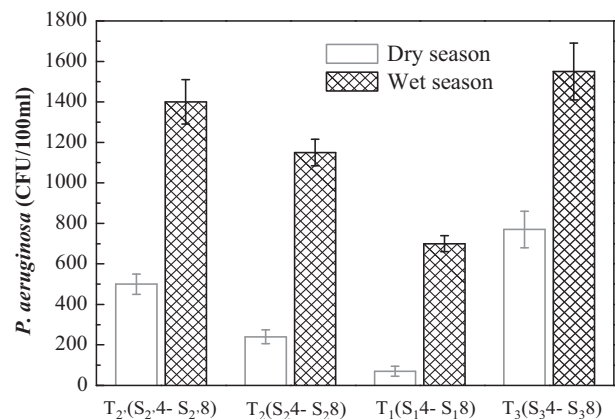


Figure 5. Effects of different catchment sources on *P. aeruginosa* under wet and dry season in 'T₁', 'T₂', 'T₂', and 'T₃'.

concentration of *P. aeruginosa*. The lower concentration of *P. aeruginosa* in 'T₂' than 'T₂' may be due to the dilution factor as 'T₂' also collects rainwater directly from the concrete roof catchment. In the case of the terrace catchment ('T₂' and 'T₂' of RWH system 2), much of the pollution could be associated with human activities including walking and smoking on the terrace surface (personal observations). The quality of runoff may be ameliorated or become worse depending on the kinds of materials used for the catchment surface [28, 29]. A suitable catchment surface and regular cleaning/maintenance, especially during long dry periods, can be a solution for the quality management of rainwater when a FF removal device is unavailable. Bird or rodents activity like rats, observed in this study, could be the primary contributor to the presence of *P. aeruginosa* in the tanks which resulted mostly from the terrace catchment, in addition to the human activities. The effects of different seasons were also reported by earlier researchers [30, 31] as reflected in Fig. 5. In dry seasons, the concentration of *P. aeruginosa* was very low in all three storage facilities due to the lesser transport of the catchment contaminations into the storage tanks than in the wet seasons.

3.4 Effects of storage conditions on *P. aeruginosa* variation under wet and dry seasons

One important factor, which influences the *P. aeruginosa* variations in RWH systems is the storage condition. In this study, the covered and dark storage conditions of 'T₂' and 'T₂' in RWH system 2 and supply tank (T₃) of RWH system 3 were compared with the uncovered storage of harvested rainwater in light. For this purpose, the concentration of *P. aeruginosa* in rainwater samples collected from 'T₂' (S₂7), 'T₂' (S₂7) and 'T₃' (S₃3) was compared with samples collected from 'W₁' (S₂9), 'W₂' (S₂10) and 'T₃' (S₃6) of RWH system 3 as shown in Fig. 6.

The rainwater in 'W₁' was harvested only from concrete roof catchment and was filtered through 'F₁' while in 'T₂', along with the addition of rainwater from 'W₁', the rainwater was also added from the green roof and terrace catchment. The concentration of *P. aeruginosa* in 'W₁', harvesting rainwater only from concrete roof catchment, should be better than that in 'T₂' connected to multiple catchment including concrete and green roof and terrace surfaces. The number of *P. aeruginosa*, however, was almost 50% less in 'T₂' than 'W₁' during dry season and almost 10–15% during wet season. Similarly, there was a difference in number of *P. aeruginosa* between

the rainwater samples collected from 'T₂' (S₂7) and 'W₂' (S₂10). The catchment source in case of both (T₂ and W₂) was concrete+ green roof and terrace. The rainwater in 'T₂' was only screened while it was both screened and filtered in 'W₂', so the concentration of *P. aeruginosa* in 'W₂' should be lower than that of the rainwater in 'T₂' but this was not the case, as shown in Fig. 6. The number of *P. aeruginosa* was almost 30% less in 'T₂' than 'W₂' during dry as well as during wet season.

In case of RWH system 3, which has the mountain catchment, the higher concentration of *P. aeruginosa* was observed in 'T₃' (S₃6) as compared to 'T₃' (S₃3) due to the different storage conditions. The difference in numbers of *P. aeruginosa* was >90%, being lesser in 'T₃' as compared to 'T₃' during dry season while almost 70% reduction was observed during wet season.

Possible reasons of less numbers of *P. aeruginosa* inside the concrete storage tanks ('T₂', 'T₂' and 'T₃') could be due to the naturally occurring set of treatment processes including the processes of sedimentation and the action of biofilms at the tank-water interfaces in addition to the favorable dark and covered storage conditions. 'W₁' and 'W₂' were exposed to ordinary light and other associated factors like presence of flies, mosquitoes, and insects, etc. However, in 'T₃', which was open to atmosphere, the number of factors including the dropping of birds, foliage, and easy access to the rodents and animals could be considered the possible reasons for high microbial contamination.

Finally, the temperature difference of about 7–8°C between the tank water and rainwater taken from the open weirs and settling tank (results not shown) could be another factor as a result of different storage conditions. Higher temperatures in open weirs and settling tank than in storage tanks may have an impact on the presence of *P. aeruginosa* by increasing their growth. This finding is also supported by [16, 32]. Hence, the appropriate rainwater storage conditions can be considered one of the important parameters for the design of the RWH systems. Regular cleaning of storage tanks and appropriate length of storage time for arising natural cleaning processes can further improve the microbial quality of harvested rainwater [33]. The physical appearance of the harvested rainwater samples separately stored in plastic tanks under light and dark conditions is also depicted in Fig. 7, highlighting the effects under different storage conditions. The sampling from the plastic tanks was done and the high number of *P. aeruginosa* was found in the samples stored in light (results not shown). As mentioned earlier, in addition to the natural processes inside rainwater storage tanks, dark and covered storage condition is also an important factor in reducing the number of *P. aeruginosa* in RWH systems.

4 Concluding remarks

The effects of different catchment types and storage conditions on *P. aeruginosa* in harvested rainwater under wet and dry seasons were investigated in order to lay the proper design and maintenance guidelines for RWH systems. The lesser number of *P. aeruginosa* in harvested rainwater was observed in dry seasons than in wet seasons. The horizontal quality improvement from the inlet to the outlets points concludes that longer flow path and the provisions of the baffles can be the important design parameters for storage tanks. Due to the naturally occurring processes and biofilm formation inside the tanks, relatively better water quality was observed at the supply point. The worst microbial quality at the bottom of the tank indicates that the supply point at height of about 0.5–1 m from the base of the

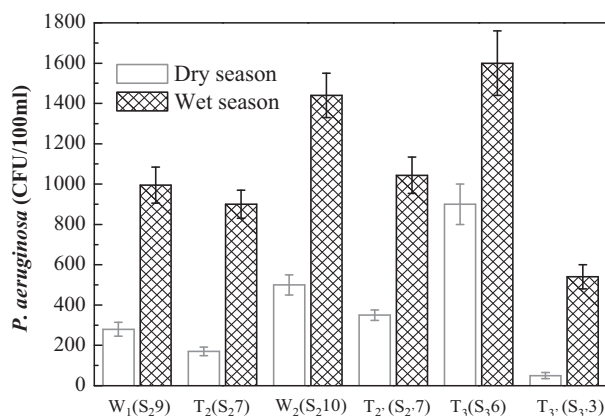


Figure 6. Effects of different storage conditions on *P. aeruginosa* in RWH system 2 and RWH system 3 under wet and dry seasons.

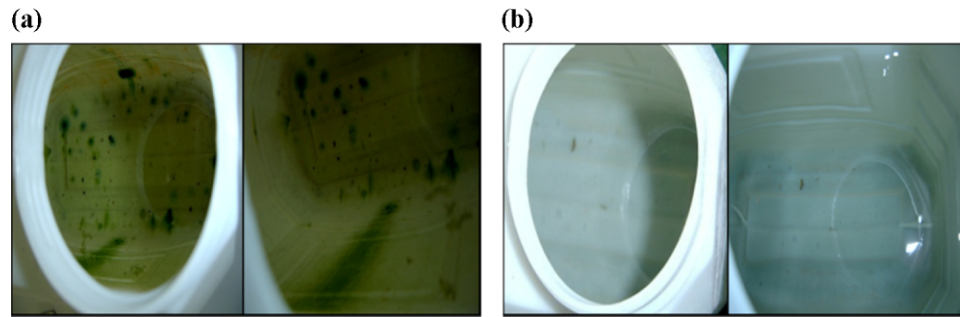


Figure 7. Effects of different storage conditions on *P. aeruginosa* (samples stored in plastic tanks); (a) under light and (b) under dark conditions.

tank may deliver better quality water. The addition of separate facility for FF treatment, in RWH systems, may also help to improve the microbial quality of harvested rainwater. The water harvested only from concrete roof was better in quality than that collected from concrete + green roof + terrace mainly. The selection of appropriate roof materials and regular cleaning of the catchment surfaces to remove dust, leaves, and bird droppings are the important factors to ensure the better microbial quality of the harvested rainwater. The better microbial quality in the covered storage tanks having low temperature and dark conditions than in the uncovered open weirs and settling tank signifies the importance of proper storage conditions.

The authors have declared no conflict of interest.

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