공학석사 학위논문

가시광선이 플라스틱 빗물저장탱크 내부의 미생물 수질 및 바이오필름 형성에 미치는 영향

Effects of visible light on microbiological water quality and biofilm formation in plastic rainwater storage tanks

2021 년 8 월

서울대학교 대학원

건설환경공학부

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이 논문을 공학석사 학위논문으로 제출함

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By

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Abstract

Effects of visible light on microbiological water quality and biofilm formation in plastic rainwater storage tanks

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Insufficient water supply systems and population growth lead to water shortages, and rainwater collection is in the spotlight as a potential solution. However, because storage facilities are expensive, developing countries use a simple makeshift rainwater collection system. Each household uses a small recycled translucent plastic material instead of an opaque thick rainwater reservoir. The plastic reservoir reflects, transmits, and absorbs visible light under sunlight. These properties of plastic reservoirs can affect bacterial growth and microorganisms in stored water.

The black plastic reservoir absorbs visible light and converts it into heat, increasing the water temperature, which in turn promotes the proliferation of

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Suspended Bacteria (SB). The thin plastic reservoir lets the visible light wavelength to pass through according to color. The red reservoir can transmit a wavelength of 630 nm, which has a strong sterilization effect. The blue reservoir (425nm and 470 nm) produces reactive oxygen specifications (ROS) which harms cells, inhibiting or killing the growth of floating bacteria. On the other hand, the white reservoir penetrates the entire wavelength of visible light.

In this study, the effects of visible light on microbiological water quality in typical household plastic tanks were studied, and the microbiological water quality and biofilm formation due to the coloration of reservoirs were studied.

First, a small acrylic reservoir was used to test the effect of visible light on the growth of suspended and surface-attached bacteria (SAB), and experiments were conducted for three months one exposed to the sun (TES) and the other not to the sun (TNES). The number of SB decreased faster when not exposed than when exposed. This represents a better microbiological water quality without exposure to white light. SAB stabilized when exposed to visible light, but decreased in dark conditions.

Second, five cylindrical reservoirs (1L) were painted white, red, yellow, blue, and black to assess the effect of the color of the storage on microbiological water quality and biofilm formation. Each reservoir was filled with rainwater and installed under a small blue fluorescent lamp for six weeks. The concentration of suspended and attached bacteria was measured by HPC (Heterotrophic Plate Count) method, and the total amount of biomass in the bacterial structure attached to the surface was measured by crystal violet analysis.

Red light has been shown to inhibit bacterial growth. However, white, yellow, blue, and black light stimulated the growth rate of SB. The bacteria decreased after three weeks, and at the end of the experiment, the number of

bacteria in the red storage was the lowest. Furthermore, the spectrum of light transmitted also affected the development of biofilms on the surface. Red and blue light caused stress-induced proteins in extracellular polymer substances. Since protein-rich EPS is a protective layer, the number of bacteria attached to the surface remains longer. In the white and yellow reservoirs, the EPS-integrated heterotrophic bacteria decreased, but photosynthetic organisms increased. Biofilm formation was low in black storage. In conclusion, the color priorities of the plastic reservoir are red, black, blue, yellow, and white, with the worst color being white.

The study found that the presence of visible light in plastic rainwater reservoirs affects microbiological water quality and biofilm formation. Furthermore, the color of the reservoir has a significant impact on maintaining the quality of stored rainwater, which should be considered carefully in color selection.

Keyword: visible light, suspended bacteria, biofilm, color of storage, extracellular polymeric substance, rainwater, heterotrophic bacteria

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Chapter 1. Introduction

1.1. General background

Water is critical for the survival of human beings. It is a precious resource for human life and development (Zabidi *et al.*, 2020). The availability of water around the world is variable, and it has decreased more and more. The constant population growth and climate change increase the pressure on water resources and water consumption (Water, 2006; UN, 2020).

At present, globally, millions of people live in areas with extreme water vulnerability and do not have access to clean water (Water, 2006) and around 3.1 billion individuals depend on unreliable, non-piped water supplies that are located off-premises (Thomas *et al.*, 2020). Furthermore, water demand has been predicted to rise (Yannopoulos, Giannopoulou and Kaiafa-Saropoulou, 2019), while, by 2050, more than half of the global population will live in area that suffer water scarcity at least one month a year (World Water Assessment Programme, 2018).

Serious actions to tackle this threat should then be implemented through the improvement of water management, increase the efforts of water conservation, and adoption of nature-based solutions (World Water Assessment Programme, 2018). It has been reported that rainwater harvesting (RWH) is a natural-based solution that can promote significant water saving in residents in different countries (Abdulla *et al.*, 2009; Bernard and Joyfred, 2020).

1.2. Motivation

RWH technology consists of collecting rainwater from a roof or other catchment area and directing it into a storage tank (Yannopoulos, Giannopoulou and Kaiafa-Saropoulou, 2019). This storage tank ranges from a sample rainwater barrel to a more complex multiple tank system (Innovative

Water Solutions, no date). Despite the fact that rainwater is the most effective access to water sources (Sun *et al.*, 2016), RWH few households have adopted the strategy in many areas (Bernard and Joyfred, 2020). In developing countries, the challenge that limits households from acquiring and using this tool include the excess cost of storage tanks, the uncertainty of water quality, and poor installation or maintenance (Mwenge Kahinda and Taigbenu, 2011; Thomas, 2014; Yannopoulos, Giannopoulou and Kaiafa-Saropoulou, 2019).

Consequently, makeshift household roof catchment systems emerged and became more and more popular. Many of the systems make use of the recycled or scavenged storage plastics barrels, jerrycans, and plastic drum, that allow the penetration of visible light or often used instead of the more idea; opaque tanks (UN-Habitat, 2016a; Bernard and Joyfred, 2020). Such system helps the households to address water shortage for both potable and non-potable purposes (Yannopoulos, Giannopoulou and Kaiafa-Saropoulou, 2019)

A common issue with RWH, nonetheless, is the quality of rainwater harvested. Because of the presence of the dry and wet particle that settle on the roof catchment, such as dust and bird droppings, many researchers have observed poor quality of the harvested water (Lee, Bak and Han, 2012; Alim *et al.*, 2020), which may contain pathogens that are harmful to human health (Al-Batsh *et al.*, 2019; Hamilton *et al.*, 2019). Few studies, which utilized opaque and large storage tanks. Have investigated the factors affecting microbial growth in RWH (Coombes, 2006; Kim and Han, 2016). However, to the knowledge of the authors, no research has been done on the quality of rainwater using storage items that households in the developing countries generally use, which are cheaper, more accessible, and allows penetration of visible light (UN-Habitat, 2016a).

1.3. Aim and objectives of the study

Thus the purpose of this thesis is to fill the current knowledge gap on microbiological water quality of rainwater kept in storage of small capacity allowing light penetration. Water quality of harvested rainwater is influenced by several physiochemical, spatial, temporal, and microbiological factors (Evison and Sunna, 2001; Evans *et al.*, 2009). The color of the storage is related to the temperature of the water inside the storage tanks. A high temperature of water can stimulate bacterial proliferation (Evison and Sunna, 2001). On the other hand, baseline low concentration of total organic carbon in rainwater has the potential to inhibit the bacterial growth of suspended bacteria (SB) (Kim and Han, 2011). The bacterial concentration also differs depending on the water depth and overtime for both SB and surface-attached bacteria (SAB) (Spinks AT., 2007; Kim and Han, 2011; Amin *et al.*, 2013). Some researches support that there is a positive relation between SAB development in the storage tank and the quality of the water from the outlet (Evans *et al.*, 2007). The penetrance of the visible light inside the tank also has an impact on SB and SAB. Visible light can stimulate or inhibit the bacteria growth of SB (Ruiz-González *et al.*, 2013). Furthermore, the SAB exposed to visible light, despite its stable counts over time, were significantly higher in number compared to the non-exposed condition (Schmidt *et al.*, 2018). These findings however, are yet to be seen in the studies on harvested rainwater.

In sum, studies that analyzed harvested rainwater quality have not taken into account the effect of visible light, while research that examined the effects of visible light on the quality of harvested rainwater are yet to be done. Thus, this study aims to determine the microbiological quality of harvested rainwater in storage that allows penetration of visible light. Specifically, this paper attempts to investigate the effects of visible light on both surface-attached and suspended heterotrophic bacteria present in rainwater storage,

also in relation to water depth and duration of storage. The effect of visible light on the physicochemical properties of harvested rainwater was also explored.

Chapter 2. Literature review

2.1. Water supply and human health

Water supply has an impact on the health of the community or a household. Having access to a safe and adequate quantity of water is primordial for good health and economic development. Waterborne diseases, such as cholera, typhoid fever, amoebic dysentery remain a cause of human morbidity and mortality worldwide (Griffiths, 2017). According to the water barometer, 297,000 children under the age of 5 die every year from diarrhea due to consumption of unsafe water (Goldberg *et al.*, 2021).

The diseases linked with water are divided into non-infectious diseases and infectious diseases. The chemical properties of water can be responsible for non-infectious diseases, while infectious diseases are associated with the consumption of water-containing pathogens. The presence of fecal contaminants in the water is an indicator of potential health risk for individuals exposed to that water (Ogbozige, Ibrahim and Adie, 2018). Fecal matter carries numerous pathogenic microorganisms (parasites, bacteria, and viruses) that cause a wide range of waterborne diseases.

2.2. Water quality parameters

Despite the RWH being ancient technology, it has regained attention in recent years, to provide water for domestically use (Alim *et al.*, 2020). The Organization for Economic Co-operation and Development also suggested utilizing RWH as an alternative source in terms of water supply (OECD, 2009). Rainwater is considered the safest source of clean water; however, the catchment area might introduce contaminants such as sediments, pathogens, and organic matters (Alim *et al.*, 2020). Whether the systems provide the households with adequate safe water is still an unanswered question (Abdulla *et al.*, 2009).

Owing to the potential exposures to microbiological and chemical contaminants several studies have investigated the quality of RHW (Hamilton *et al.*, 2019). The concentration of these contaminants will fluctuate as physical, chemical, and biological processes continuously occur (Spinks AT., 2007). Consequently, water quality is a dynamic process, and researchers choose the value of bacterial concentration to represent water quality.

2.3. Physio-chemical water quality parameters

2.3.1.Physical water quality parameters

The physical parameters of water are observable by human senses, such as temperature, color, and taste. The temperature of the water is not related to water drinkability, but it is an essential factor in maintaining the microbiological quality of stored water. In terms of water quality, the water temperature has three main effects: change the concentration of dissolved oxygen, regulate the rate of biological activity, and influence the rate of change of gas transfer into water (Wilkerson *et al.*, 2011).

The temperature of water affects the growth of microorganisms. The ideal temperature for the bacteria replication depends on the type of microorganism. The limitation of substrate affinity in low temperatures of water engenders a reduction of bacterial growth and reproduction (Nedwell, 1999). The higher the temperature, the faster the rate of biological activities. The temperature of water ranging from 20 to 30°C stimulates the proliferation of bacteria (Majdi *et al.*, 2020), and higher temperatures induce a decline in some species of heterotrophic bacteria in marine and freshwater ecosystems (Phillips, Godwin and Cotner, 2017). Several factors affect the water temperature inside of a storage tank, including air temperature, ground temperature, and solar radiation. Solar radiation depends on the material, reflectiveness, and shading.

The color of the water is visible by naked eyes, and the presence of minerals and algae impacts it. The growth and decomposition of algae may impart a green, brown, or reddish to the water (Team clean water, no date). The general public is usually reluctant to consume colored water. This aesthetical aspect of the water is essential because people tend to choose clear and transparent water over greenish water.

2.3.2.Chemical water quality parameters

Chemical parameters of water comprise pH, dissolved oxygen, total dissolved solids, total organic carbon, etc. The pH represents the concentration of hydrogen ions in water; it regulates the bioavailability of nutrients through redox reactions and microbial growth and survival. The deviation of the environmental pH from the optimal growth decreases the replication of bacteria (Jin and Kirk, 2018). Furthermore, pH is not static but changes over time and can be affected by the biological process within the storage and the material (Ogbozige, Ibrahim and Adie, 2018).

DO is a crucial factor of the water's body because it represents the ability of the water to support aquatic life. Through photosynthesis and gas transfer, oxygen enters the water, and respiration of microorganisms, evaporation of water, and decomposition of organic matters are responsible for the depletion of oxygen in the water (Fondriest Environmental, no date).

Total dissolved solids (TDS) measures the sum of organic and inorganic substances in water. The TDS test provides a quantitative measure of the amount of minerals, salts, and organic matter that determine the general quality of the water. The TDS concentration is an aesthetical rather than a health hazard and may affect the taste of the water. An elevated TDS may suggest that toxic metal might be present, whereas a lower TDS concentration may be the sign of corrosive water (World Health Organization, 2003).

TOC determines the number of carbon atoms tied up in organic compounds in water, and it is non-specific water quality. Measuring TOC is imperative because a higher organic content leads to an increase in the growth of microorganisms. Furthermore, a low concentration of TDS and organic carbon in rainwater can potentially inhibit the growth of suspended bacteria (Kim and Han, 2011). Phytoplankton produces organic carbon through photosynthesis. Heterotrophic bacteria assure the remineralization of the dissolved organic carbon throughout the water column (Calleja, Al-Otaibi and Morán, 2019).

2.4. Microbiological water quality

Safety and quality are principal concerns when managing water for human consumption and household usage (WHO, 2002). Numerous approaches are taken to ensure microbial water quality. However, it is not feasible to monitor routinely the full range of pathogenic or opportunistically pathogenic bacteria. Assessing numerous specific bacteria is time-consuming and engenders high costs. Consequently, some indicators are employed to evaluate the potential health risk of water supplies (Spinks AT., 2007).

The water quality of harvesting rainwater depends on the physiochemical and microbiological factors (Evison and Sunna, 2001; Evans *et al.*, 2009). The measure of HPC, total coliform counts, thermotolerant coliform counts (specifically *Escherichia Coli*) assess the microbial water quality of stored rainwater (Hamilton *et al.*, 2019). The number of colonies formed units (CFU) in selective media can quantify these indicators.

However, the bacteria fecal index did not accurately indicate the presence of protozoan and virus pathogens, WHO (2003) proposed the *Clostridium perfringens*, anaerobic spore-forming organisms, as a new index for protozoan pathogens and viral contaminants. Nevertheless, many drinking water guidelines still operate with the assessment of E. coli.

HPC is a procedure for estimating the number of heterotrophic microorganisms in water. Heterotrophic bacteria uptake organic carbon from the environment to survive. Consequently, they are assumed to provide a general indication of the harvested rainwater storage conditions. The test can be carried out by several standardized techniques like pour plate, spread plate, or membrane filtration method (Bartram and World Health Organization., 2003).

The accurate interpretation of the water quality should take into account the limitation of the indicator organisms. A degree of uncertainty must be acknowledged because the HPC test does not distinguish between pathogenic and nonpathogenic microorganisms. Besides the assumption that the presence of fecal contamination equates to the presence of the pathogenic organisms. Bird's droppings or small mammal fecal matter does not contain the human pathogen. Consequently, the probability of the existence of harmful bacteria depends on the sources of fecal contamination. The truthfulness of the indicator diminishes with the appearance of false positives in the literature, but it is impractical to monitor every possible pathogen making the use of indicator a cost-effective alternative.

2.5. Water storage tanks

RWH can provide water in regions where water supplies and groundwater are scarce or unreliable. The interest in this technology both in developing and developed countries is growing. In high-income countries, the RWH is not restricted to a small-scale roof collection system but extended to the large and complex system serving a great number of people. In developing countries, RWH is on a small scale for households (Yannopoulos, Giannopoulou and Kaiafa-Saropoulou, 2019).

Even if rainwater is the cheapest water source, RWH needs high investment costs for the storage tank, particularly in middle and low-income

countries (Thomas, 2014). Consequently, casual and low-cost storage such as clay pots, jerrycans, plastic, or steel barrels, is placed under the roof in many rural areas (UN-Habitat, 2016a; Bernard and Joyfred, 2020). The different materials definitely will affect the quality of water stored inside in different ways. The most used type of tank in Africa is a galvanized steel tank or a plastic storage with small volumes and different colors (Eniola *et al.*, 2007; Ogbozige, Ibrahim and Adie, 2018).

2.5.1.Clay pot

Since the discovery of clay properties by prehistoric humans, it has been shaped like a pot to store food and water. The clay pot is cheap to purchase, easily breakable, and hard to repair. Farmers and people living in rural areas still employ clay jars to transport and stock rainwater (Bernard and Joyfred, 2020).

Figure 1. Clay pot used to harvest rainwater

2.5.2.Metallic barrel

Metallic barrels, a cylindrical container used for shipping petroleum oil, are recycled as a rainwater storage tank in developing countries. They can

hold 200 liters or more, but they are expensive at 60 $/500 liters (UN-Habitat, 2016a). Besides, there is a problem of corrosion associated with this storage.

Figure 2. Informal steel barrel rainwater in Uganda (Kisakye, Akurut and Van der Bruggen, 2018)

2.5.3.Jerrycans

A jerrycan is a square container made of high-density polyethylene (HDPE) or polypropylene (PP). The first purpose of this container is to store edible oil and the capacity varies from 20 to 30 liters. The thickness of a jerrycan range from 1.5 mm to 3 mm depending on the manufacturers and different colors are available (white, red, yellow, and green) but the yellow color is the most common. The family buys and recycles this jerrycan for storing harvested rainwater (Bernard and Joyfred, 2020).

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Figure 3. Plastic jerrycan of 20 liters with it dimension used as roof rainwater harvesting storage In Nigeria (Bernard and Joyfred, 2020)

2.5.4. Plastic drum storage

According to the UN-Habitat, more than 150 000 persons have found their water with RWH. The system was not technically sophisticated and made of scavenged materials such as plastic drums (UN-Habitat, 2016b).

A plastic drum or HDPE or PP blue drum is a cylindrical container used to transport chemical hazardous, lubricants, food, etc. The thickness of the plastic drum ranges from 1.5 to 2.2 mm and the capacity from 30 to 220 liters. People use plastic storage because they offer some advantages: easy to move and repair if they leak; HDPE is resistant to ultraviolet radiation.

Figure 4. Dimension of plastic drum of 30 L and a roof rainwater harvesting system with a plastic drum in Madagascar (SEED Madagascar).

2.6. Changes in the microbiological quality of stored rainwater

The process occurring within the storage time is complex and depends on several factors. However, numerous studies have shown that the microbiological quality of the water improves with storage.

2.6.1.Biofilms in a storage tank

Biofilms or surface-attached bacteria structures are a layer of bacteria that colonize then proliferate on a surface in contact with water (Coombes, 2006). The primary adhesion of planktonic bacteria is reversible and regulated by the physiochemical interaction between the substratum properties and the bacteria cell (Donlan, 2001). After the attachment of the bacteria to the surface, they replicate, secrete extracellular polymeric substances (EPS), and form a complex ecology of microorganisms. Different types of compounds made of polysaccharides, proteins, and deoxyribonucleic acid DNA compose the EPS (Gambino *et al.*, 2019).

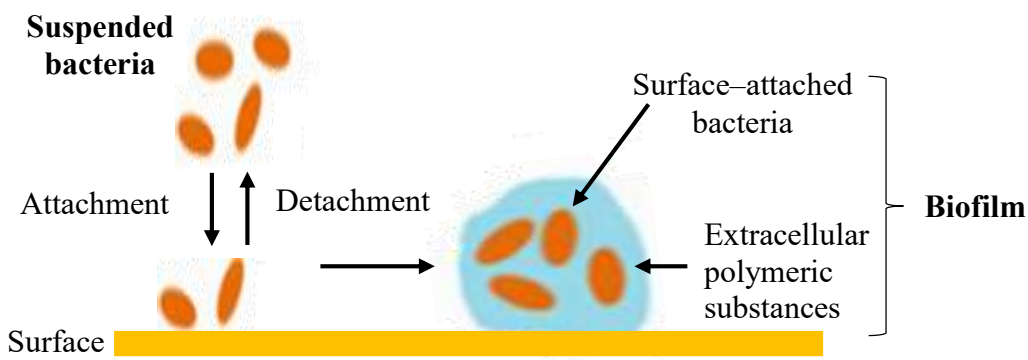

Figure 5. Interaction between suspended and surface-attached bacteria and the composition of a biofilm

The biofilm characteristic is known to significantly affect physiochemical environments like pH, temperature, availability of nutrients, and presence of light. High temperature and nutrient level stimulate bacterial

growth, whereas irradiation by UV, production of biofilm matrix slows down the bacteria replication. External stress like desiccation and exposure to specific light wavelengths elicits EPS production (Rao, 2010; Gambino *et al.*, 2019). SAB are known to be more resistant than SB, and the EPS plays a protective and structural role in this strong resilience (Costa, Raaijmakers and Kuramae, 2018).

Research on the effects of the surface-attached bacteria on the microbiological water quality yielded a conflicting result. SAB have been found to carry waterborne pathogens in drinking water distribution systems (Fu *et al.*, 2021). Abberton et al. (2016) reported that *Escherichia Coli*, fecal pathogens responsible for gastrointestinal illnesses, persisted in the SAB community (Abberton *et al.*, 2016). The advantages, however, were found only in investigations that involved stored rainwater not exposed to visible light.

A study carried by Kim and Han (2011) observed that after inoculating *Pseudomonas Aeruginosa* in rainwater storage with SAB, 99% of removal of the Pseudomonas after five days in full-scale tanks (Kim and Han, 2011). In addition, Coombes et al. (2006) stated that in underground rainwater tanks, SAB removed toxic metals and compounds from the water column by playing the role of a bioreactor (Coombes, 2006). Suspended heterotrophic bacteria can also ingest dissolved organic compounds, attach to the SAB, and die naturally due to starvation (Coombes, 2006; Kim and Han, 2016). Thus, in a RWH tank not exposed to visible light, SAB improves microbiological water quality (Spinks AT., 2007; Kim and Han, 2016). Although these studies highlight the potential benefits of SAB, studies on their growth and their effect on heterotrophic bacteria in stored rainwater exposed to visible light are lacking. Nevertheless, the influence of visible light on water quality has been investigated in other sources of water.

2.6.2.Sedimentation

Several water treatment plants use the sedimentation process to enhance the quality of the water. In Australia, Coombes et al. reported that an incidental train treatment improves the quality of stored rainwater over time. The settlement of particles, bacteria, and organic matter at the bottom of the tank decreases the bacterial concentration in the water column (Coombes, 2006). Similarly, Amin et al., after investigating the number of heterotrophic bacteria at different levels inside of the storage tank, found that bacterial counts at the bottom of the tank were higher than in the upper level (Amin *et al.*, 2013).

Eniola et al. (2007) and Ogbozige et al. (2018) observed a decline of bacterial concentrations in a typical household water container over the storage period. The reduction was attributed to the settlement of the total dissolved solids (Eniola *et al.*, 2007; Ogbozige, Ibrahim and Adie, 2018).

2.7. Visible light availability

The rainwater storage can be installed outdoors, exposed to sunlight, or indoors, kept in the dark. A conventional rainwater tank is opaque (Columbia, 2020); either steel, concrete, or brick material does not allow light to pass through, or plastic material is thick (4.5 mm or greater), so light cannot penetrate inside it (Team poly, 2018).

However, the typical storage used in developing countries is from recycled and scavenged materials. HDPE and PP, material for jerrycans and plastic drums, are food-grade plastic and ultraviolet treated. The manufacturers add carbon black or another ultraviolet stabilizer to prevent storage degradation by sunlight exposure (Anderson, 2020). Even if HDPE and PP have an ultraviolet barrier, it is transparent to the visible light with a

transmittance between 10 to 40 % depending on the plastic's color (Coltro and Borghetti, 2007).

Visible light is the small part of the electromagnetic spectrum having a wavelength from 400 nm to 800 nm that human eyes can see. Furthermore, different wavelengths correspond to a specific color.

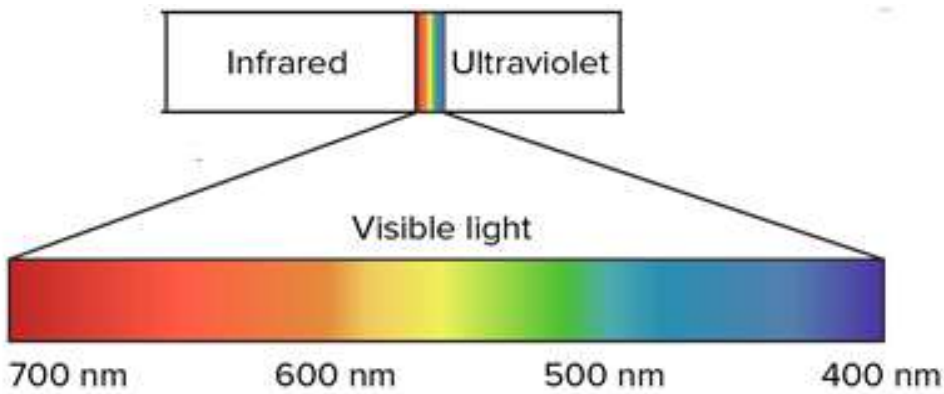

Figure 6. Visible light spectrum with the color corresponding to the different wavelengths

Some research on the effects of visible light on heterotrophic bacterial activity found out that visible light is not always beneficial. The spectrum of light, type of water, and water depth influence the response of the bacteria to light exposure. Published studies cover a wide array of the positive and negative impacts of visible light on bacterial growth.

The bacterial cell damage by visible light mainly results from a photodynamic process involving reactive oxygen species (ROS), which cause oxidative damage (Khaengraeng and Reed, 2005). The additional stress on the bacteria will hinder the bacteria replication or induce the death of the bacteria (Kamel, Saeed and Hassan, 2016).

However, a literature review conducted by Ruiz-Gonzalez et al. (2013) suggested that visible light induces photosynthesis; the organic compounds

passing through the microbial food chains are affected. The photochemical transformation of dissolved compounds increases the availability of food for heterotrophic bacteria (Ruiz-González *et al.*, 2013).

Within the literature, the effect of visible light on suspended bacteria in the aquatic ecosystem had attracted attention. However, these findings are yet to be seen in studies on harvested rainwater.

Chapter 3. Effect of Visible Light on Suspended and Surface-attached Heterotrophic Bacteria in a Typical Household Rainwater Harvesting Tank

3.1. Experimental design and methods

3.1.1.Experimental set-up

Two 34 L acrylic tanks, having a diameter of 25 cm and a height of 70 cm each, were fabricated with a tap at the bottom. As this study focused on the effect of visible light, a PVC coating (ORACAL 8300) that was purchased from Orafol, Germany was used to prevent the penetration of UV radiation into the tank. The two tanks were first washed with detergent and then rinsed with double-distilled water. Furthermore, they were sterilized with 70% ethyl alcohol.

Coupons were made (each with a surface area of 16 cm^2) using polyethylene terephthalate (PET), with each coupon having a dimension of 2 × 4 cm. After sterilizing with 70% ethyl alcohol, three of these coupons were attached to a single sterile fishing line at different heights that corresponded to the top water level (T) (at a depth of 15 cm from the water surface), middle water level (M) (at a depth of 35 cm from the water surface), and bottom water level (B) (at a depth of 55 cm from the water surface), as illustrated in Figure 7. T was set at 15 cm, as many researchers have shown that solar disinfection is ineffective at depths greater than 10 cm (Dessie *et al.*, 2014). Furthermore, 24 strings were tied vertically with three coupons each (T, M, B) to a cable tie, as shown in Figure 7.

Rainwater was collected from the RWH system with a first flush tank in building number 39 at Seoul National University, South Korea, and introduced into the acrylic tank until the water level reached 30 L. The physiochemical characteristics of the water source are as follow: pH and temperature were 7.83 and 22°, the concentration of DO was 5.28 mg. L^{-1},

and the level of TDS was 57 mg. L^{-1}. The total phosphorus and total nitrogen were very low <0.01 mg. L^{-1} and 1.64 mg. L^{-1}, and the TOC was 2.58 mg. L^{-1}.

This procedure was repeated for the other tank. Then, one tank was kept on the roof of building number 35 at Seoul National University and the other tank was kept inside a room with no openings to provide a dark environment. The experiment was conducted from July to September 2020, that is, during the summer. The average meteorological condition and the visible light intensity surrounding the study area are as follows: the temperature was 27.28 ± 1.34 °C, the humidity was 58 ± 21%, and the visible light intensity was 70.12 ± 9.20 kW/m^2 (James Diebel; Jacob Norda; Orna Kretchmer, no date).

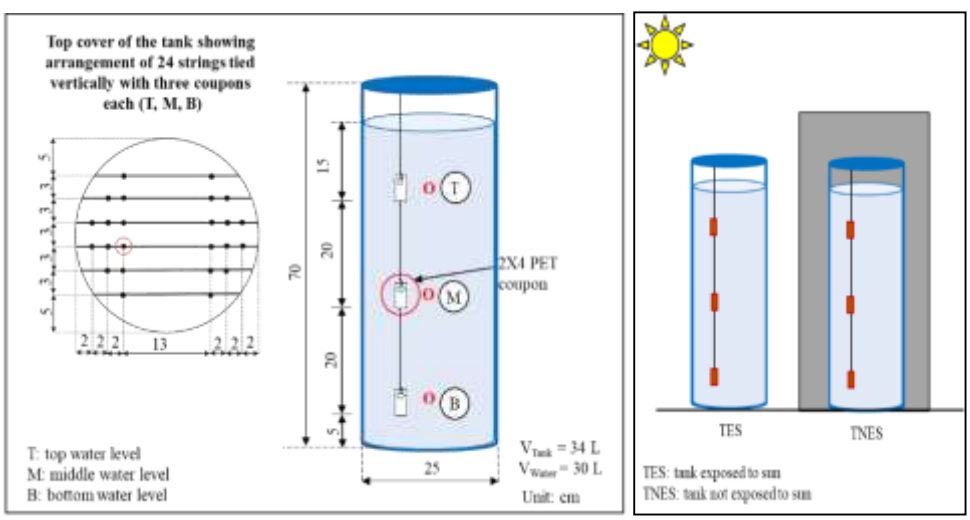

Figure 7. Experimental setup of sampling coupons in rainwater tanks (top and side view of the tank storage and the effect of sunlight)

3.1.2.Sampling and collection of coupon

Water samples (20 mL) were collected weekly at each level using a sterile 10 mL pipette, corresponding to the water level of the coupon. The collected water was then poured into a 50 mL sterile conical plastic tube. During the experimental period, the tanks were not refilled with water. The

volume of the water sample did not exceed 1 L, and water loss was minimized by placing a parafilm at the edge of the tanks. At the end of the experiment, less than 2 cm of the water was depleted.

Every week, two strings, located at the opposite ends of the tank, were removed and washed with 5 mL of sterile phosphate-buffered saline (PBS) to separate the non-attached bacteria. Then, three coupons (of T, M, and B) were placed inside the sterile conical plastic tubes filled with 20 mL of PBS to investigate the microbial properties. Another three coupons were placed in sterile Petri dishes until they were assessed for biomass.

3.1.3.Bacterial enumeration

The culturable suspended heterotrophic bacteria plate counts in the collected water samples were assessed using 3 M petrifilm Aqua Heterotrophic Count (AQHC) plates. The collected water samples were diluted using sterile distilled water to measure the plate counts (15–200 colonies). After dilution, 1 mL of the diluted solution was spiked onto the AQHC plates and evenly spread using a spreader that was provided with the AQHC plates. The spiked AQHC plates were then incubated at 37 °C for 48 h. After incubation, the number of colony-forming units (CFU) of each plate was counted. The plate counts were performed in triplicate, and the average CFUs were expressed in CFU/mL.

The bacteria had to be detached from the surface to measure the SAB. To detach them, this study used the method described by Kobayashi et al. (2009) (Kobayashi *et al.*, 2009). First, test tubes containing the coupons were shaken using a vortex shaker (SI-0246A Vortex-Genie-2, Scientific Industries Inc., USA) for 3 min, and then immersed in a sonication bath (SAE-HAN Ultrasonic cleaner SH-1025, SAE HAN Ultrasonic Co., Jongno-Gu, Seoul) for 15 min at 60 Hz. The optimum sonication time was determined during the preliminary study to maximize the detachment.

During the preliminary study, the sonication bath was on for time: 5, 10, 15, 20, and 25 minutes. After 5 minutes, 1 mL of the solution was spiked on the petrifilm in triplicate. We repeated the process at different times. The number of CFU did not increase from 15 to 20 min. Therefore, 15 min was retained as the optimum sonication time.

After the sonication bath, the number of CFUs in the water (detached from the coupon) was measured using the same method described for the SB plate counts. However, for the SAB, the number of colonies observed was multiplied by the volume of the PBS and divided by the surface area of the coupon to estimate the number of CFUs per unit area of the coupon. Therefore, all the SAB plate counts are expressed in CFU/cm^2 units. Every bacterial enumeration was made in triplicate.

3.1.4.Total biomass quantification

The total amount of biomass is the structure formed by SAB, dead cells, and EPS. The quantification of the total amount of biomass is an indirect way to assess the presence of bacteria attached to a surface (Wilson *et al.*, 2017). The biofilm formed by SAB was estimated using the crystal violet staining assay, following the method used by Stiefel et al. (2016) (Stiefel *et al.*, 2016). The coupon collected in the Petri dish was dried at 25 °C in the room. Then, 5 mL of 0.5% crystal violet (CV) was added to the Petri dish and the coupon was immersed for 30 min. A 0.5% CV solution was prepared following the Cold Spring Harbor Laboratory protocol (Cold Spring Harbor Protocols, 2016). Additionally, crystal violet powder (Daejung Chemicals & Metals, South Korea) was mixed with 20 mL of methanol (Daejung Chemicals & Metals, South Korea) and 80 mL of double-distilled water. The coupons were retrieved from the staining solution and washed thrice with 5 mL of sterile distilled water. After the coupons were dried at 25 °C in the room, 5 mL of 96% ethanol was added to dissolve the surface-attached bacteria-bound CV. The Petri dish was gently shaken after the introduction of ethanol. Alcohol

solution containing the stain was analyzed using a water analyzer and spectrophotometer (HS-3300, Humas, Daejeon, Korea). Absorbance corresponding to a wavelength of 595 nm was measured (with a standard error of 1% of absorbance), which was an indicator of the biomass.

3.1.5.Measurement of physiochemical parameters

The pH (with a standard error of ±0.02) and temperature (with a standard error of ±0.2) of rainwater were measured using a pH meter (HM 31 P, TOADKK, Japan). Furthermore, TDS (with a standard error of ±0.002) was measured using an HM Digital COM-300 EC TDS pH 4 in 1 Combo Meter (New York, NY, USA). DO (with a standard error of ± 0.1 mg/L) was measured using an optical dissolved oxygen meter (ProODO, YSI, Yellow Springs, OH, USA). The concentration of TOC in stored rainwater was measured with (Shimdazu total organic carbon analyzer ASI-V autosampler).

3.1.6.Statistical analysis

Data were first normalized by a log-transformation due to the assumption required in parametric statistical analysis methods such as t-test and ANOVA (Hammouri *et al.*, 2020; Lee, 2020). Then it was subjected to repeated measures ANOVA, and paired *t*-tests were performed using this software at a 95% confidence interval. Two fixed factors were considered: time with 12 levels (1–12 weeks) and water depths (top, middle, and bottom). Statistical analyses were performed on the data that were converted to a logarithmic scale using SPSS version 25 software. Non-linear regression with the exponential function was performed to assess the decay rate of the SAB, total amount of biomass, and SB.

3.2. Result and discussion

3.2.1.Effect of visible light on suspended bacteria

Figure 8 shows the comparison of SB between TES and TNES over time and different water depths. The decay rate of heterotrophic bacteria summarized in Table 1 (0.3 CFU day^{-1}) in the TNES was significantly higher than that in the TES (0.03 CFU day^{-1}; $p < 0.05$). According to Ruiz-Gonzalez et al. (2013) and Hameed et al. (2020), visible light triggers the uptake of DOM and cell division of heterotrophic bacteria (Ruiz-González *et al.*, 2013; Hameed, W. A. Lai, *et al.*, 2020). Therefore, a decrease in the bacterial count was alleviated by the production of new cells. In addition, with visible light exposure, phototrophs produce carbohydrates that sustain heterotrophic bacteria (Valverde *et al.*, 2015; Villa *et al.*, 2015). Furthermore, the oligotrophic condition of the rainwater tank not exposed to sunlight, with fewer bacteria due to lack of food, might have accentuated the difference in the plate counts of the two tanks (Kim and Han, 2015).

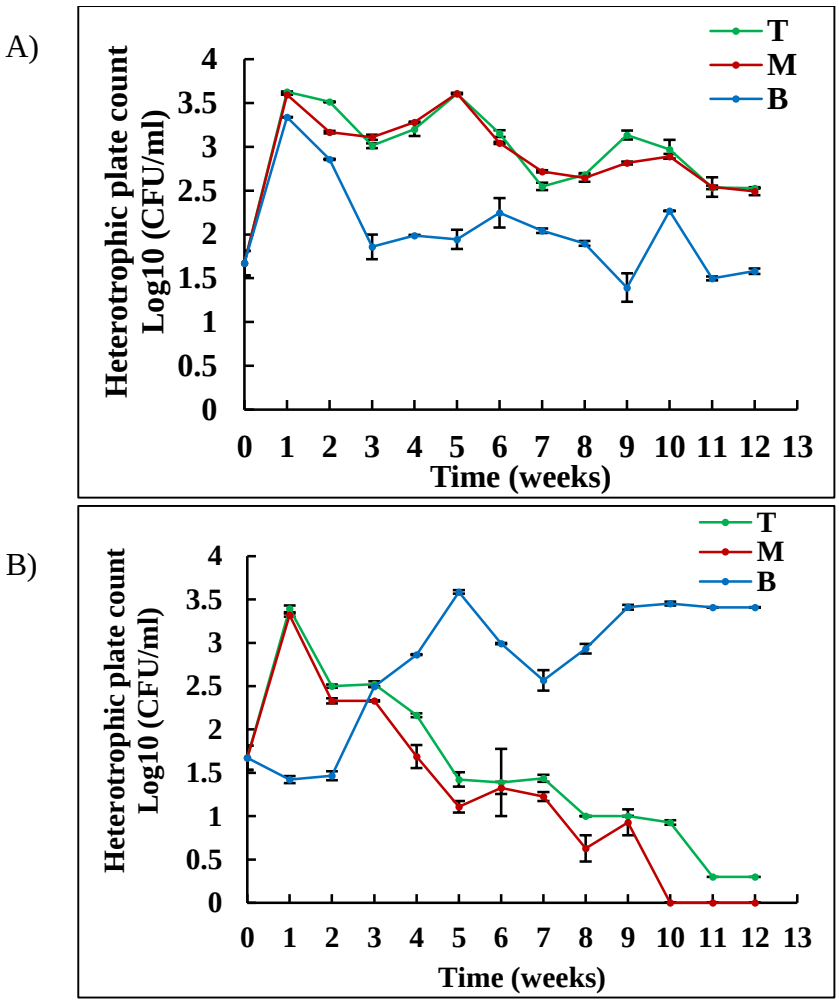

Figure 8. Variation in the suspended heterotrophic bacteria (A) in TES and (B) TNES at different water depths over time (T: top, M: middle, and B: bottom)

The effect of time on the number of SB was significant, with greenhouse Geisser F (1.33) = 130. 245, $p < 0.001$ for TES and F (1.33) = 90.250, $p < 0.001$ for TNES. Coombes et al. (2006) also reported that the bacterial counts in stored rainwater diminish over time due to sedimentation, natural death, and bacterial attachment to the surface. Besides, the main effect of water depth on the average SB plate counts across the time is also significant, with a Greenhouse Greisser F (3.33) = 17.839, $p < 0.001$, and F (3.33) = 130.871, $p < 0.001$ respectively in TES and TNES.

Changes in the amount of SB in TES and TNES showed different trends at varying depths (Figure 8). In the TES, suspended plate counts were higher at the top and middle levels, and their numbers decreased during the experimental period at all water depths. Furthermore, the pairwise comparison indicated a significant difference only between the top and bottom level ($p < 0.01$) as well as the middle and bottom level ($p < 0.01$).

This stratification in the number of suspended heterotrophic bacteria is similar to a previous study conducted by Spinks et al. (2007), which explained that a potential reason for these findings could be the occurrence of thermal gradient and difference of bacteria buoyancy (Spinks AT., 2007). These factors may disturb the settlement of bacteria and slower the decay rate of bacteria at the upper level. In the TNES, a gradual decrease in the number of bacteria at the top and middle levels was observed over time, with an opposite trend observed at the bottom level. Also, statistical differences were observed between all the water depths ($p < 0.05$) This phenomenon can be attributed to the sedimentation of particle-attached bacteria in water not exposed to visible light, which eventually increases the number of bacteria at the bottom (Amin *et al.*, 2013). Furthermore, natural sedimentation, biofilm formation, and bacterial death reduce bacterial counts in the water body (Coombes, 2006; Amin *et al.*, 2013).

Similarly, a non-linear regression with exponential function was used to estimate the decay rate of the SB. The result is summarized in Table 1.

Table 1. Comparison of the growth rate, coefficient of determination R^2, and 95% CI of SB in the TES and TNES at different water depths.

Water Depths	**Tank Exposed to Sun**			**Tank Not Exposed to Sun**		
	Rate (d^{-1})	**R^2**	**95% CI**	**Rate (d^{-1})**	**R^2**	**95% CI**
Top	−0.03	0.57	$[-0.039 - 0.019]$	−0.26	0.97	$[-0.305 - 0.215]$
Middle	−0.03	0.48	$[-0.037 - 0.014]$	−0.30	0.98	$[-0.340 - 0.256]$
Bottom	−0.17	0.97	$[-0.162 - 0.148]$	0.02	0.38	$[0.010 - 0.032]$

3.2.2.Effect of visible light on surface-attached bacteria

3.2.2.1. Effect of visible light on SAB at different water depths

All the coupons that were analyzed in this study contained heterotrophic bacteria on their surfaces. The surface-attached heterotrophic bacterial counts are shown in Figure 9.

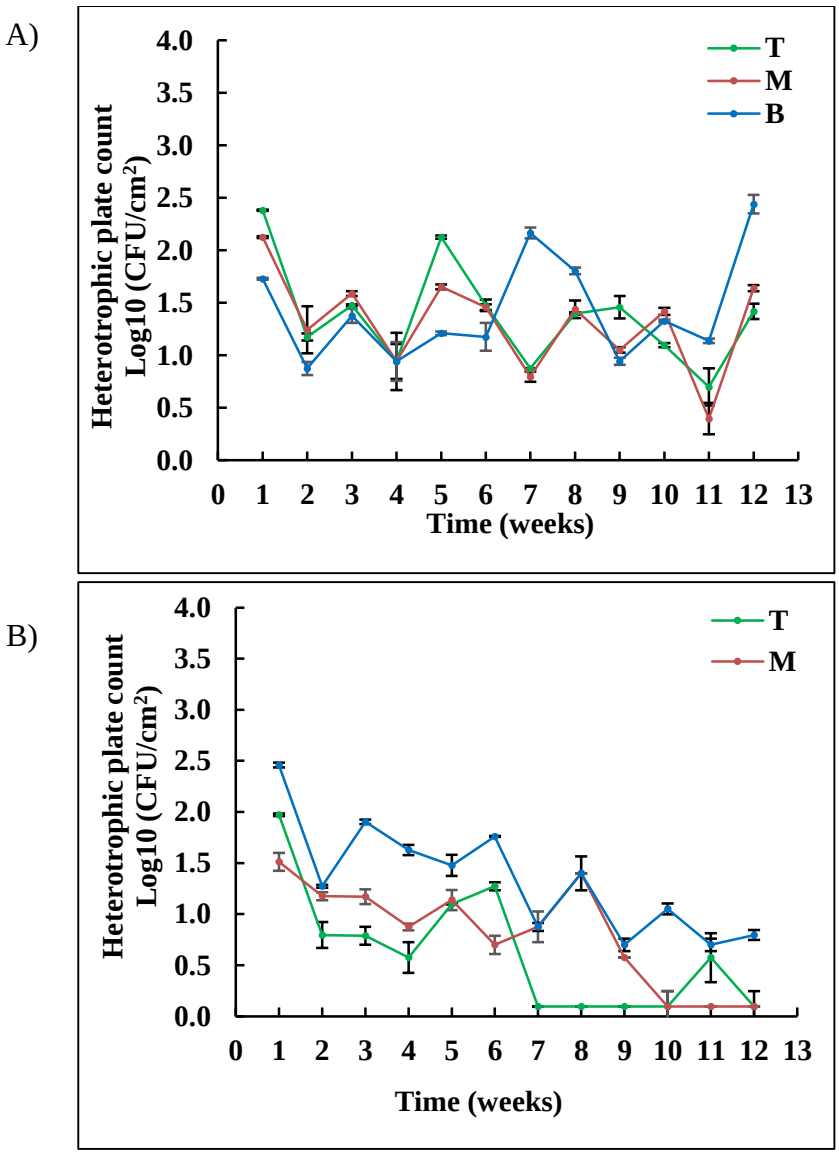

Figure 9. Variation of surface-attached heterotrophic bacteria (A) in the TES and (B) in the TNES at different water depths (T: top, M: middle, and B bottom).

Exposure to visible light had a significant effect on the amount of surface-attached bacteria in the tank ($p \leq 0.02$). While the number of heterotrophic bacteria was relatively stable in the TES, it decreased in the TNES. The stability of heterotrophic bacteria is probably caused by the higher availability of food produced by phototrophs during photosynthesis (Schmidt

et al., 2018). This hypothesis is corroborated by a significant difference in the concentration of total organic carbon in the two tanks, illustrated in Figure 10, (Student T-test $p < 0.05$). According to Schmidt et al. (2018), phototrophic bacteria in biofilms contribute to their stabilization and cultivation; additionally, low light intensity results in a significant reduction in biofilm development (Schmidt *et al.*, 2018).

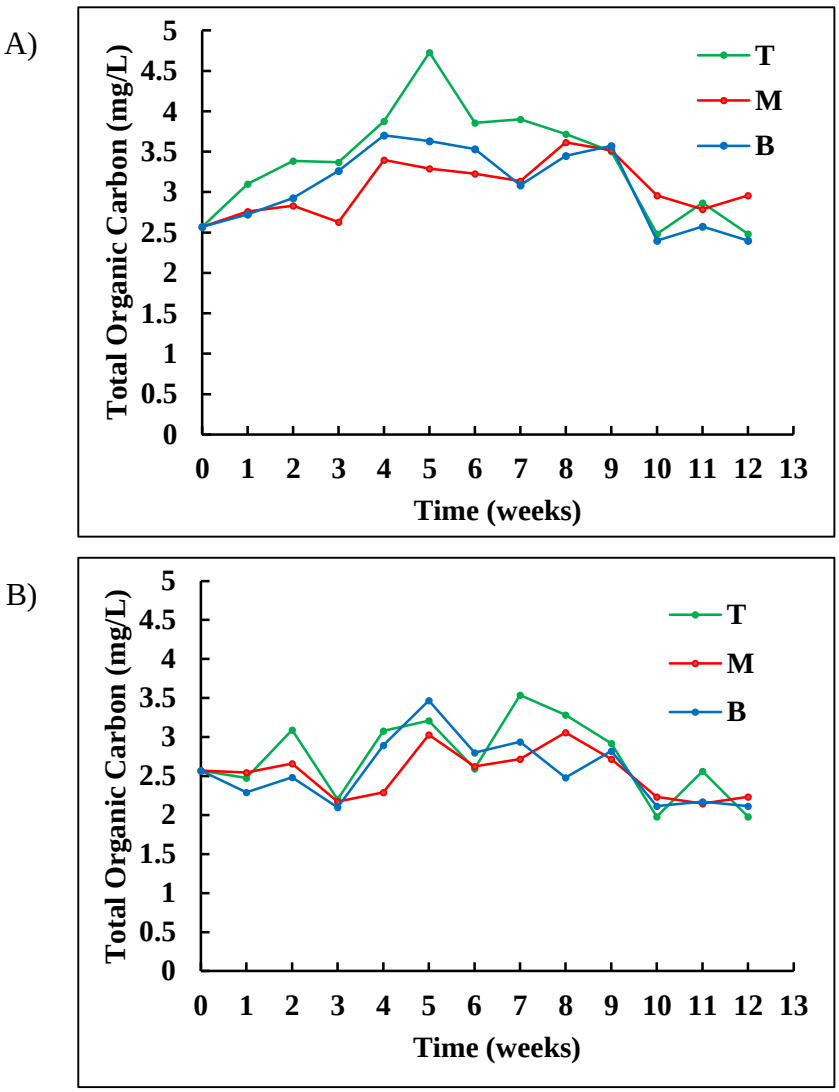

Figure 10. Variation of concentration of total organic carbon (A) in the TES and (B) in the TNES at different water depths (T: top, M: middle, and B bottom).

Figure 9 also shows the time trends in SAB plate count, with changes observed in two stages in both tanks: (1) from the 1st week until the 6th week, then (2) from the 6th week until the end of the experiment. This effect of time on the surface-attached plate counts is significant in the TES and TNES, Greenhouse-Geisser F (2.33) = 46.303, $p < 0.01$ and F (2.33) = 90.415, $p < 0.01$), respectively. In this regard, Kim et al. (2016) and Amauri et al. (2020) both reported that the accumulation of bacteria on the surface is a dynamic process involving the adhesion, growth, and maturation of surface-attached structures (Van Der Merwe, Duvenage and Korsten, 2013; Paula, Hwang and Koo, 2020).

The main effect of water depth on the average SAB plate counts across time is also significant. In the initial phase, SAB plate counts at the top (1.51 Log_{10} CFU/cm^2) and middle (1.46 Log_{10} CFU/cm^2) were higher compared to the bottom level (1.21 Log_{10} CFU/cm^2). Van der Merwe et al. (2013) reported a similar trend, with higher bacterial attachment at the top and middle levels (Van Der Merwe, Duvenage and Korsten, 2013). During the second phase, however, the number of SAB at the bottom level increased. Towards the end, the concentration of SAB at the bottom surpassed both the top and middle-level SAB plate counts. The pairwise comparisons indicated a significant difference only between the middle and bottom water levels ($p \leq 0.09$). Spinks et al. (2007) and Kim et al. (2016) observed that the sedimentation of particle-attached bacteria induced a better development of biofilm at the bottom level of an underground rainwater tank (Spinks AT., 2007; Kim and Han, 2015). Another possible explanation is the biota exchange between the sludge and SAB at the bottom resulting in increased count over time, as also argued by Tu et al. (2020) in their study on biofilm formation on microplastics (Tu *et al.*, 2020). This strongly supports the results of this study. A similar sedimentation process took place in TNES, which is responsible for statistical variation between the different water depths ($p < 0.05$). Some authors also argued that the bacterial count differences between levels are due to the water

temperature gradient (Spinks AT., 2007), but no significant differences in temperature were observed in the current study. However, the temperature was taken only once weekly, which might not measure precise temperature gradient.

Non-linear regression with an exponential function was calculated to determine the decay rate of SAB at the top, middle and bottom levels as seen in Table 2. The regression equation was $y = A \times e^{(b.t)}$, b represents the decrease of the bacteria per day. The coefficient of determination R^2 and the 95% confidence interval (CI) for the decay rate.

The extremely slow growth rates, as shown in Table 2 confirmed again the effect of visible light on the SAB. Without visible light exposure, it had a similar pattern as that of the first phase, except that the number of SAB declined after the 6th week, with a higher decay rate being observed at the top and middle levels.

Table 2. Growth and decay rate, coefficient of determination R^2, and 95% CI of SAB in TES and TNES at different water depths.

Water Depths	**Tank Exposed to Sun**			**Tank Not Exposed to Sun**		
	Rate (d^{-1})	**R^2**	**95% CI**	**Rate (d^{-1})**	**R^2**	**95% CI**
Top	−0.34	0.60	$[-0.676 - 0.010]$	−0.33	0.91	$[-0.462 - 0.192]$
Middle	−0.17	0.41	$[-0.279 - 0.072]$	−0.03	0.53	$[-0.050 - 0.020]$
Bottom	0.26	0.66	$[0.093 - 0.417]$	−0.25	0.80	$[-0.367 - 0.137]$

3.2.2.2. Effect of visible light on total biomass of SAB at different water depths

Figure 11 shows that the amount of total biomass that was exposed to visible light was significantly larger than that of the non-exposed tank. Visible light induces the production of organic compounds necessary for the metabolism of heterotrophic bacteria as seen in Figure 10. Augusti et al. (2020) and Music et al. (2019) have emphasized that light is a primary energy source for autotrophic organisms, linking photosynthesis directly to the growth of biomass and uptake of nutrients (De Tender *et al.*, 2017; Agustí *et al.*, 2020). In addition, the secretion of the extracellular substance by SAB might also contribute to the disparity between the amount of total biomass in TES and TNES. Schmidt et al. (2018), found a higher amount of extracellular polymeric substances with light exposure (Schmidt *et al.*, 2018).

Storage time also had a significant impact on the variation of the amount of total biomass of surface-attached in TES (Figure 11). Until the 6^{th} week, the augmentation of total biomass was slow, and then it showed a rapid increase. These temporal dynamics of the biomass pattern on the PET surface were in accordance with those of a previous study done by Tender et al. (2017) (De Tender *et al.*, 2017). The first stage was argued to be due to bacterial colonization and early biofilm formation. Over time, bacterial proliferation intensified, and biofilms grew in planar expansion and thickened (De Tender *et al.*, 2017). Moreover, the faster growth at the bottom level during the early phase can be explained by the integration of dead cells, a source of DOM for heterotrophic bacteria that contribute to total biomass (Ruiz-González *et al.*, 2013), into the biofilm due to sedimentation (Spinks AT., 2007). Indeed, this study has added to the literature that highlights the effect of time on biomass accumulation (Tu *et al.*, 2020).

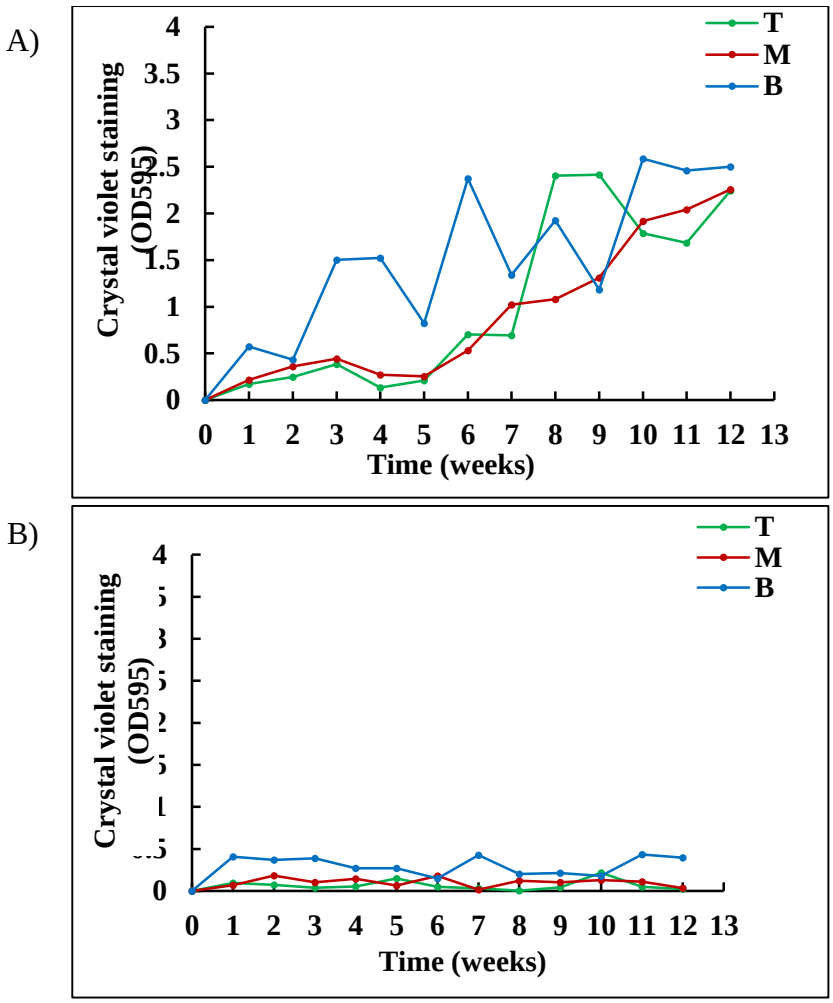

Figure 11. Variation of the total SAB biomass (A) in the TES and (B) in the TNES at different water depths over time (T: top, M: middle, and B bottom).

In the TNES, the variation of the amount of biomass over time was almost constant, but there were significant differences in biomass count in water depth as shown in Figure 11 and in Table 3. The total biomass at the bottom level was significantly higher than that at the upper level Stiefel et al. (2016) stated that the total amount of biofilm is formed by live cells, dead cells, and extracellular substances (Stiefel *et al.*, 2016). Dead cells settle and accumulate at the bottom of the tank. Furthermore, the placement of the coupon near the bottom of the tank might have enhanced biota exchange (Tu *et al.*, 2020). The growth rate of the amount of biomass was estimated by non-

linear regression for TES. The statistical analysis result was summarized in Table 3. The regression analysis of the total amount of biomass revealed that there is no correlation between time and the total amount of biomass.

Table 3. Comparison of the growth rate, coefficient of determination R^2, and 95% CI in the TES at different water depths.

Water Depths	**Tank Exposed to Sun**		
	Rate (d^{-1})	**R^2**	**95% CI**
Top	0.03	0.67	[0.009 0.043]
Middle	0.03	0.94	[0.023 0.004]
Bottom	0.01	0.59	[0.005 0.023]

3.2.3.Effect of visible light on physiochemical parameters

The pH, TDS, and DO concentration were not affected by different water depths, as shown in Table 4. The difference in temperature between the top and bottom levels was around 1 °C for more than half of the experimental period and the temperature ranged from 20.7 to 28.9 °C. Heterotrophic bacteria grow rapidly when the temperature ranges between 20 and 30 °C; that is higher the temperature, the faster the replication (Valverde *et al.*, 2015; Villa *et al.*, 2015). The difference in temperature between the TES and TNES was approximately 1.5 °C, as depicted in Table 4, and was not statically significant. A noticeable difference in the physiochemical characteristics of water between the tanks was the slightly higher concentration of dissolved oxygen in the TES. This difference was possibly due to photosynthesis by phototrophs in the presence of visible light

Table 4. Variation in the physiochemical parameters of rainwater: pH, temperature, total dissolved solids (TDS), and dissolved oxygen concentration.

Physiochemical Parameters		**Tank exposed to sun**		**Tank not exposed to sun**		**Average difference between TES and TNES**
		Min	**Max**	**Min**	**Max**	
pH	Top	5.42	8.29	5.97	8.4	0.48 ± 0.4
	Middle	4.9	8.29	5.72	7.95	0.46 ± 0.4
	Bottom	4.09	7.83	6.07	7.83	0.49 ± 0.4
Temperature (°C)	Top	21.9	28.9	20.3	27	1.51 ± 1.0
	Middle	20.2	28.9	20.6	27	1.34 ± 0.9
	Bottom	20.7	28.6	20	26.9	1.39 ± 1.0
Dissolved oxygen (mg/L)	Top	5.28	8.94	5.28	7.54	1.26 ± 0.4
	Middle	5.28	8.94	5.28	7.56	1.24 ± 0.4
	Bottom	5.28	8.7	5.28	7.44	1.31 ± 0.5
Total dissolved solid (mg/L)	Top	41	57	37	57	4.33 ± 3.8
	Middle	38	57	35	60	4.58 ± 4.4
	Bottom	31	57	39	57	3.08 ± 2.7

3.3. Limitation of the research and further study

This study has some limitations. Firstly, the microbial species that compose the SAB and SB at both TES and TNES conditions were not identified, so it is not possible to explain the exact reasons why such phenomena occurred. Secondly, although microbial activity is a function of various factors, such as color of the tank, TDS of the water in the tank, the surface to volume (S/V ratio), dosage of disinfectant, and temperature, this study focused only on the effect of visible light.

Therefore, we recommend conducting further studies characterizing the types of organisms and microbial species in SAB and SB by using PCR tests. Furthermore, other important factors that influence the microbial quality in rainwater tanks.

Chapter 4. Effects of the color of plastic rainwater storage tanks on microbiological water quality and biofilm formation

4.1. Experimental design and methods

4.1.1.Experiment setup

Ten food containers (1 liter) of transparent polypropylene, having a diameter of 15.5 cm and a height of 8.7 cm each, were used. The container outer surface was uniformly painted with white (code: N010), red (code: 100), yellow (code: N200), blue (code: 319), or black (code: N001) spray paint (Nabachem LPC77, made in South Korea). The painted storage tank was sterilized with 70% ethanol and filled with 900 mL rainwater obtained from the RWH at Seoul National University. During the 6 weeks of the experiment, storage tanks were installed inside an acrylic box with a white surface (50 cm × 40 cm × 20 cm) and under simulated daylight (12-h-light/ 12-h-dark) emitted by three blue compact fluorescent lamps, (100W E39, model ELC100EX-D with white color, manufactured in HANGZHOULI-TECH, China) (Figure 1). The colored storage tanks were rotated every 2 hours in different positions so that all tanks receive the same amount of intensity from the light source and also to avoid overexposure to light.

Sterile forceps were used to gently put twelve coupons, made of polyethylene terephthalate (4 cm × 2 cm) and sterilized with 70% ethanol, at the bottom of each storage tank (Figure 1). Every week, two coupons and approximately 3 mL of water sample were removed from each colored storage tank for microbial analyses. The tanks were refilled every day with 1 mL of sterile distilled water to compensate the evaporation of water.

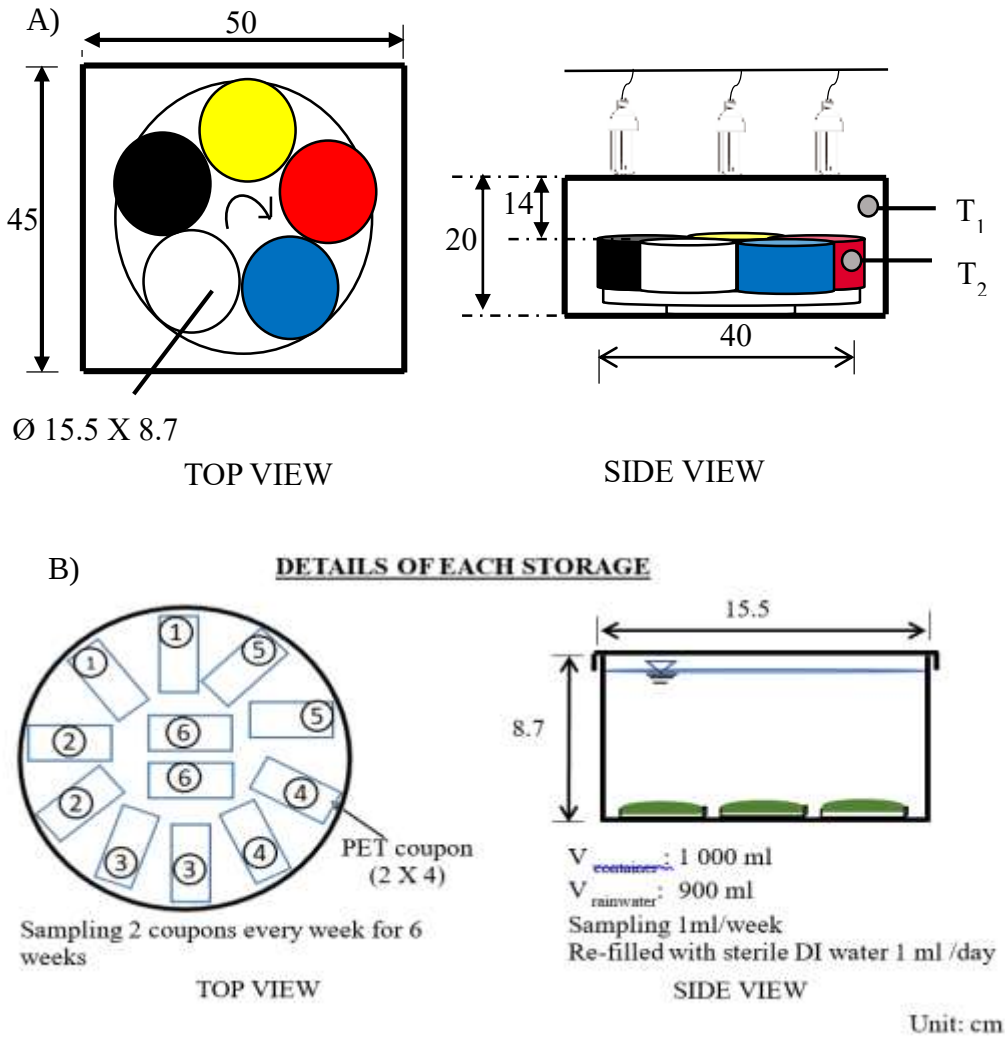

Figure 12. Experimental setup to investigate the effect of the color of storage tanks. (A) one set of five storages on a turntable under light bulbs (B) twelve coupons immersed in rainwater at each colored storage

The quality (spectral composition) of the light sources and transmitted by each storage cover was recorded via a Stellar Net Blue-Wave spectrometer (Tampa, Florida, USA). The spectral composition of the light ranging from 300 to 800 nm of the light source was represented in figure 2. The used light source showed a multimodal spectrum with several peaks at 404 nm, 436 nm,

487 nm, 545 nm, 579 nm, and 612 nm. The peaks at the blue (436 nm), green (545 nm), and red (612 nm) wavelengths were significant.

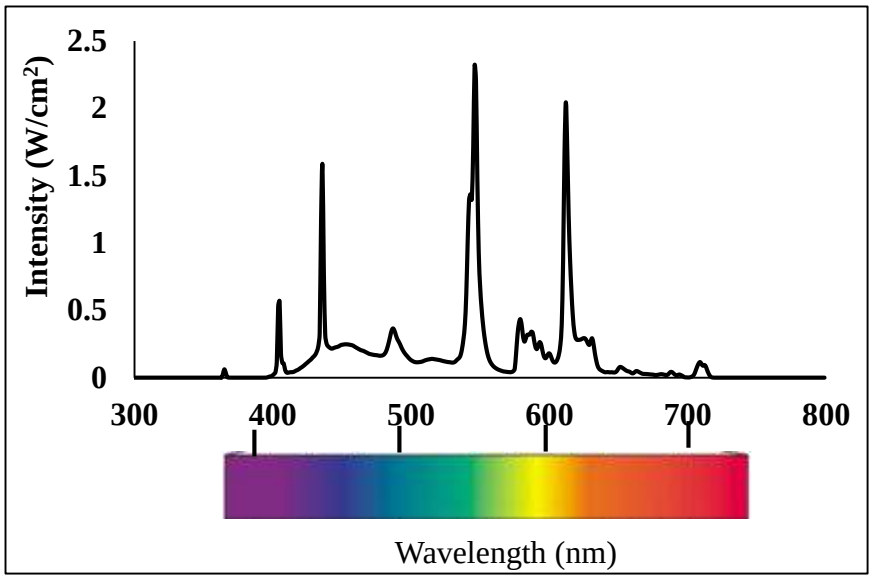

Figure 13. Light quality in the range of 300- 800 nm (visible light) of the light source

The transmitted light quality from the white cover showed all the wavelengths but at a different intensity. The transmitted light quality from the white cover showed all the wavelengths (from 413 to 799 nm) but at a different intensity. The red cover conveyed band of wavelength from 588 nm to 799 nm. The yellow cover transferred a large spectrum from 526 to 799 nm, whereas the blue cover transmitted tight wavelengths from 438 nm to 534 nm with a peak at 474 nm. The black cover did not diffuse any visible spectrum (Figure 14).

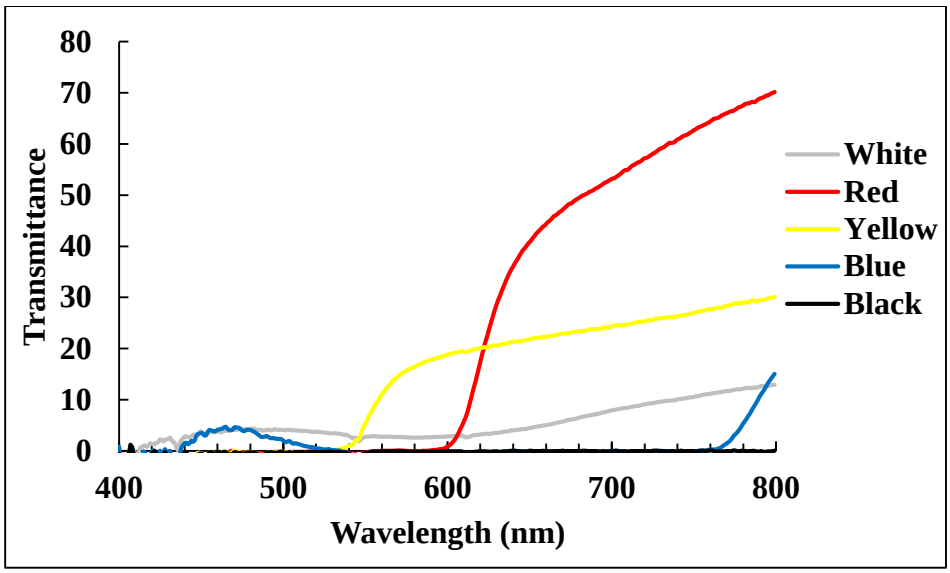

Figure 14. The quality (spectral composition) in the range of 400- 800 nm (VIS light) of the light the transmitted light

The quantity (intensity of radiation) of the light source, reflected, and transmitted light by each storage cover were measured by a solar power meter (with standard error ± 10 W/m^2) (CEM DT-1307, Shanghai, China). The transmittance, reflectance, and absorbance of the painted storage cover were calculated with the following equations (Angelo V. Arecchi, 2007):

$$Transmittance = \frac{I_T}{I} * 100$$

$$Reflectance = \frac{I_R}{I}$$

$$Absorbance = \frac{I - I_T - I_R}{I}$$

Where

- I: incident ray (emitted from light sources)
- I_T: transmitted ray
- I_R: reflected ray

The quantity of transmitted, reflected, and absorbed light were measured and shown in figure 15. It is worth noting that the white and yellow cover reflected more light, and the black cover absorbed more than 90% of the light emitted by the source.

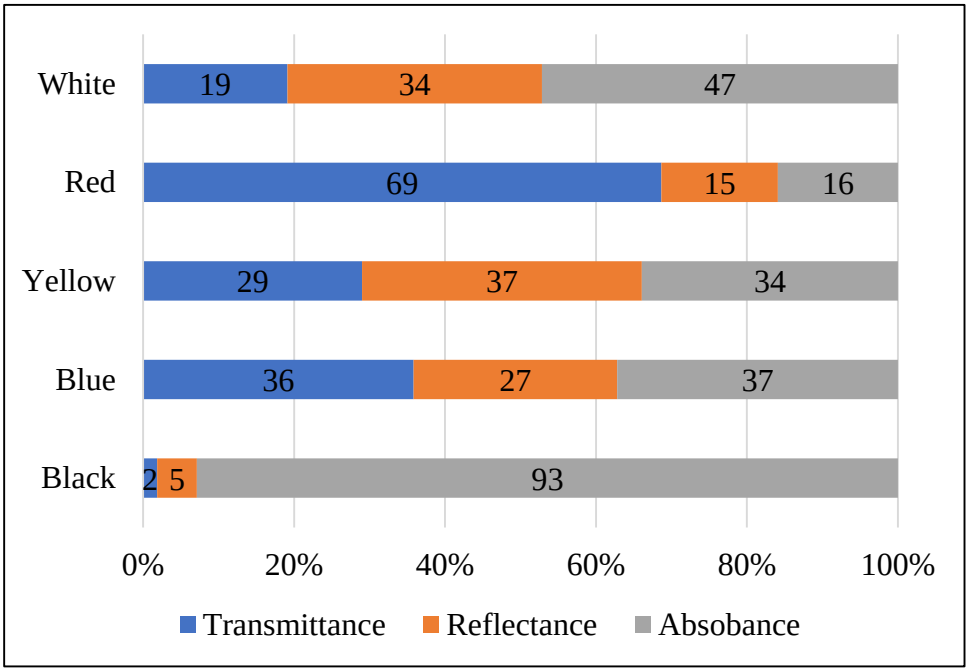

Figure 15. Optical properties (transmittance, reflectance, and absorbance) of the different colored storages

4.1.1.Measure of temperature

The temperature of inside the acrylic box was kept at 20°C. The daily variation of water temperature (with standard error ±0.8 °C) was recorded with a UA11-K Thermocouple K type (Radionode, Gyeonggi-do, Korea).

4.1.2.Bacterial enumeration

Water samples (1 mL) were obtained in a weekly manner by using a sterile pipette that was deep until the level of water. The bacterial colony forming unit (CFU) was determined by a 3M petrifilm Aqua Heterotrophic Count (AQHC) method. The water samples were serially diluted to get 15–

200 colonies on the plate. Using a sterile pipette, 1 mL of the diluted sample was dispensed on the AQHC, evenly spread with the plate spreader provided by the manufacturer, and incubated at 37°C for 48 hours. The bacterial colonies observed in the petrifilm after the inoculation were counted. The measurement was triplicated and the average number of bacterial CFU were expressed in CFU/mL.

The SAB concentration was also examined every week throughout the period. The Kobayashi et al. method was used to detach the bacteria from the surface. Briefly, the conical tube filled with 20 mL phosphate saline buffer (PSB) with the coupon was vortexed (SI-0246A Vortex-Genie-2, Scientific Industries Inc., USA) for three minutes. After that, it was immersed in an ultrasonic cleaner (DAIHAN, WUC-D3H, Korea) with a frequency of 40 Hz. The duration of sonication was determined during a preliminary study with different time intervals (5, 10, and 15 min). The water temperature began to rise after 15 minutes of sonication. Consequently, the water inside the sonicator was changed every 15 minutes. The detachment of bacteria was complete after two series of sonication for 15 minutes.

After the detachment of bacteria, the same method that was employed for SB, was also used here. The CFU value was multiplied by the volume of PSB and divided all with the surface of the coupon. The bacterial counts were expressed in CFU/cm^2.

4.1.3.Quantification of the photosynthesis pigment in rainwater and the extracellular polymeric substances

The rainwater stored for six weeks or the remaining PSB with cell and EPS was vacuum filtered through a cellulose acetate membrane filter (ADVANTEC 0.22 μm and 47 mm diameter, Millipore, Japan). The membrane filter was placed inside a 10 mL conical tube filled with dimethyl sulfoxide (DMSO, Daejung chemicals, Korea) and vortexed for two minutes.

After resuspension, the DMSO (Villa *et al.*, 2012) was heated to 65°C for one hour (Bell and Sommerfeld, 1987). The sample was transferred into a two mL sterile tube and centrifuged with micro centrifuge (Purispin 17R- Cryste, Gyeonggi-do, Korea) for 10 min at 7000 g and 20 °C. The supernatant was analyzed in a spectrophotometer (HS-3300, Humas, Daejeon, Korea). The chlorophyll-a (µg/g of biomass^{-1}) and total carotenoid (µg/g of biomass^{-1}) were estimated by the equation suggested by Wellburn (Wellburn, 1994).

4.1.4.Extraction and characterization of the extracellular polymeric substances

The method described by Villa et al. (2012) was used to extract the extracellular polymeric substances. The coupon removed from the storage tank was placed in a 50 mL conical tube that was prefilled with 20 mL 2% ethylenediaminetetraacetic acid (EDTA, Deajun Chemicals, Korea). The tube was sonicated for two series of 15 minutes to detach the EPS. After that, it was shaken at 300 rpm for 3 hours and centrifuged at 8000 g for 20 min at 4°C. The supernatant was filtered through a 0.2 µm polyethersulfone membrane syringe filter (SP25P020S HYUNDAI MICRO, Gyeonggi-do, Korea). The protein content was quantified by the Bradford method with Pro-Measure™ solution (Intron Biotechnology, Gyeonggi-do, Korea). Carbohydrates were determined using the phenol-sulfuric acid assay (Masuko *et al.*, 2005) with glucose as a standard. To normalize the value obtained, they were divided by the biomass and expressed in µg of proteins per g of biomass and µg of polysaccharides per g of biomass.

4.1.5.Bacterial community analysis

A total of six samples of three rainwater samples and three biofilm samples, each from black, blue, or red storage tank, were sequenced for the V3 and V4 region of the 16rRNA gene. The samples were taken at the end of the experiment (6th week) because the effect of the color would be greater with a longer exposure time (Angarano *et al.*, 2020). These colored storage

tanks (red and blue) were chosen because the effect of visible light was detected in them and the black storage was used as a control. Each water sample (10 mL of PSB and EPS for biofilm and 900 mL of rainwater for the suspended bacteria) was filtered using 0.45 µm filter paper (Whatman filter, Sigma-Aldrich, USA). A sterile vacuum filtration unit was used to filter the water. The filter paper was stored at -20°C for further analyses.

Half of each filter paper was used for DNA extraction. DNA extraction was performed using PowerMax Soil DNA Isolation Kit (Mobio Laboratory, Carlsbad, CA, USA) with the modification of adding 300 mg 0.1 mm diameter and 100 mg 0.5 mm dimeter glass beads to the microcentrifuge tube (Yamamoto et al., 2012). The samples were first homogenized by a bead beater (BioSpec. Inc. Bartlesville, OK, USA) for 4 min and then proceeded to the DNA extraction following the protocol. The samples were then eluted with 50 µl 10 µM Tris buffer and kept at -80 °C until sending to sequencing. All the DNA samples were sequenced at Macrogen, Inc. in Korea using an Illumina Miseq platform (Illumina, Inc., San Diego, USA) targeting the V3-V4 regions of the bacterial 16S rRNA gene with a pair of primers 314F (5'-CCTACGGGNGGCWGCAG-3') and 805R (5'-GACTACHVGGGTATCTAATCC-3') reported elsewhere (Albertsen *et al.*, 2015).

The obtained sequences were processed using QIIME2 version 2019.10 (Caporaso *et al.*, 2010). DADA2 (Callahan *et al.*, 2016) was used for denoising and quality control. Naïve Bayesian classifier (Wang *et al.*, 2007) was used to assign taxonomy with Greengenes database (http://greengenes.lbl.gov) (DeSantis *et al.*, 2006). For diversity analyses, libraries were rarefied sequence reads using "MicrobiomeAnalyst" package (Dhariwal *et al.*, 2017).

4.1.6.The statistical analysis

All statistical analyses were carried out with SPSS v 25.0 for windows (IBM, USA). A logarithmic data transformation was performed when it was not normally distributed. Data were subjected to repeated measures ANOVA and a Duncan post-hoc test with a *p*-value at a 95% confidence interval. The time, with six levels (1 level per week) and surface color, with five levels (white, red, yellow, blue, and black) were the fixed factors. In the case of correlation between time and color of storage tank, a pairwise comparison was performed.

4.2. Result

4.2.1.The water temperature stored rainwater in the different colored storages

The color of storage is known to affect the temperature of the water inside them. A higher temperature was observed in the storage tank painted in black (Figure 16), which is similar to the finding of previous researchers (Wilkerson *et al.*, 2011). The light absorbed by the black storage tank (Figure 4) was transformed to heat, and increased the water temperature (Evison and Sunna, 2001). Compared to the black storage tank, the differences in temperatures with the red storage tank ($\Delta T = 0 – 4$ °C) and blue storage tank ($\Delta T = -0.8 – 2.5$°C) was not significant, while in comparison with white storage tank ($\Delta T = 0 – 5.6$ °C) and yellow storage tank ($\Delta T = -0.7 – 4.3$ °C), there was a significant difference ($p<0.05$). Therefore, the color of storage induced a significant variation of water temperature between a storage tank transmitting a large spectrum white (400 nm – 800 nm) and yellow (526 nm - 799 nm) color and storage tank not transmitting light (black).

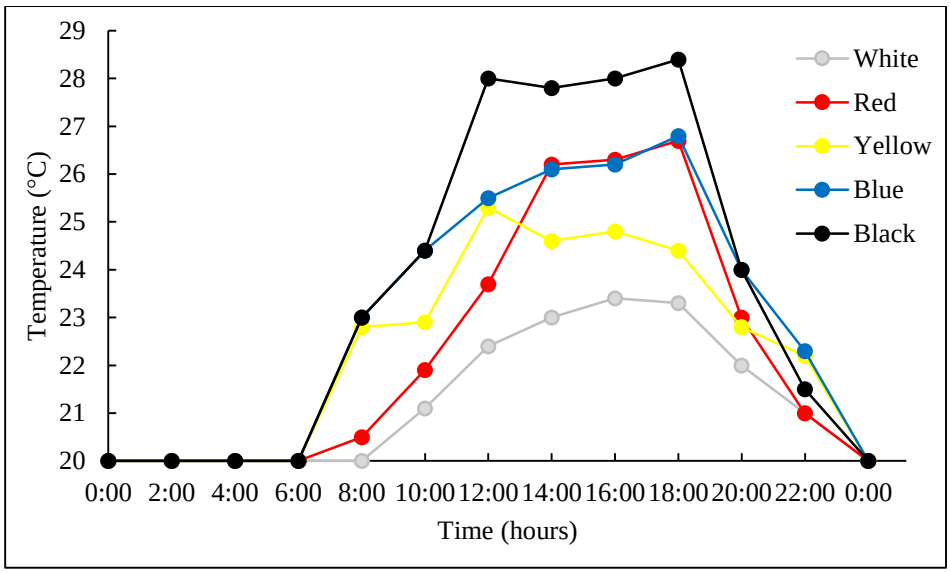

Figure 16. The change of temperature in one day at each colored storage

4.2.2.Bacterial counts of suspended bacteria in the different painted storages

The bacterial concentration was investigated by a culture-based method and shown in Figure 17. The bacterial count was 174 CFU/mL at the beginning of the experiment and at the end of experiment, it was 398 CFU/mL in white, 154 CFU/mL in red, 150 CFU/mL in yellow, 610 CFU/mL in blue, and 400 CFU/mL in black storage tank. The storage tank exposed to the 588 nm - 800 nm wavelength (red storage) had a slow bacterial growth. The statistical analysis showed that effect of the color of storage tank on overall bacterial count was significant (Greenhouse Geisser $F(4,10) = 9.16$, $p < 0.05$). The post-hoc test indicated that the heterotrophic bacteria concentration in the red storage tank was significantly low compared to the white, yellow, blue, and black storage tanks.

Furthermore, the variation of the number of planktonic bacteria over time was divided into two phases: bacterial growth, up to 1 to 3 weeks, depending on the wavelength irradiating the bacteria, and exponential decay of the bacteria. Statistical analysis revealed that the effect of the time on the

bacterial concentration was significant (Greenhouse Geisser F (3.30) = 37.53, $p<0.001$), with the highest number of bacteria at the 3rd week in white, blue and black storage tank. Besides, a pairwise comparison test showed that the difference of the average bacterial counts over time, irrespective of the color of storage tank, was significant after the 3rd week.

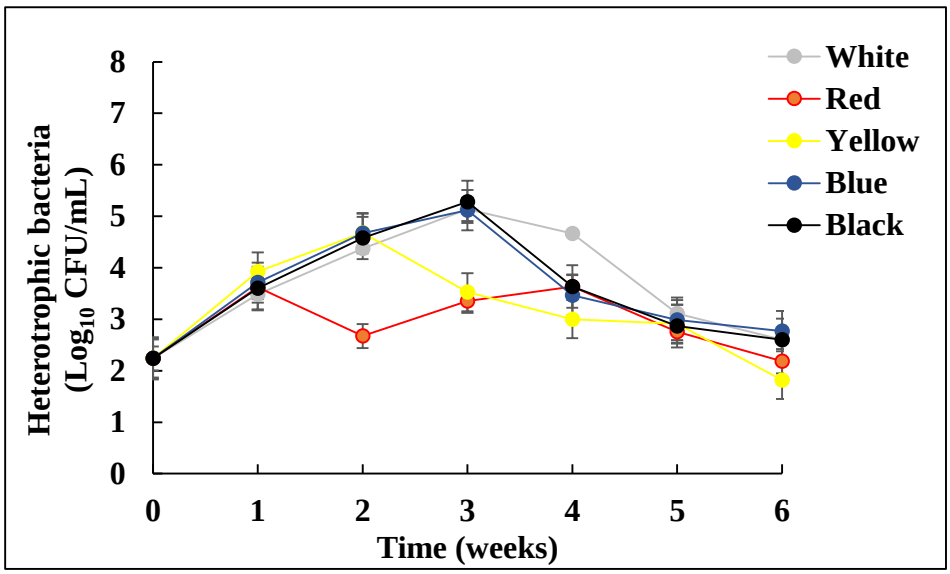

Figure 17. The change of bacterial concentration of suspended bacteria in each colored storage

4.2.3.Bacterial counts of surface-attached bacteria in the different colored storages

The number of bacteria attached to the surface of the coupon had two phases: the attachment phase and the lag phase (up to 2 – 3 weeks) giving the color of the storage tank, followed by a stationary or death phase (Figure 7). The effect of time on the colonization and development of SAB was significant (Greenhouse Geisser F (2.22) = 33.44, $p<0.001$), with the highest bacteria in the second week. Furthermore, there was a significant interaction between the time and color effect on the SAB counts (Greenhouse Geisser F (8.22) = 4.642, $p<0.001$). A follow-up of ANOVA analysis showed the

difference between bacterial counts in white and blue storage tank was significant in the second week ($p<0.01$). During the fourth, the SAB counts in red storage tank were significantly different compared to the bacteria in white, yellow, blue, and black storage tanks. Besides, in the 5th week, there was a significant difference in the bacterial counts in blue and red.

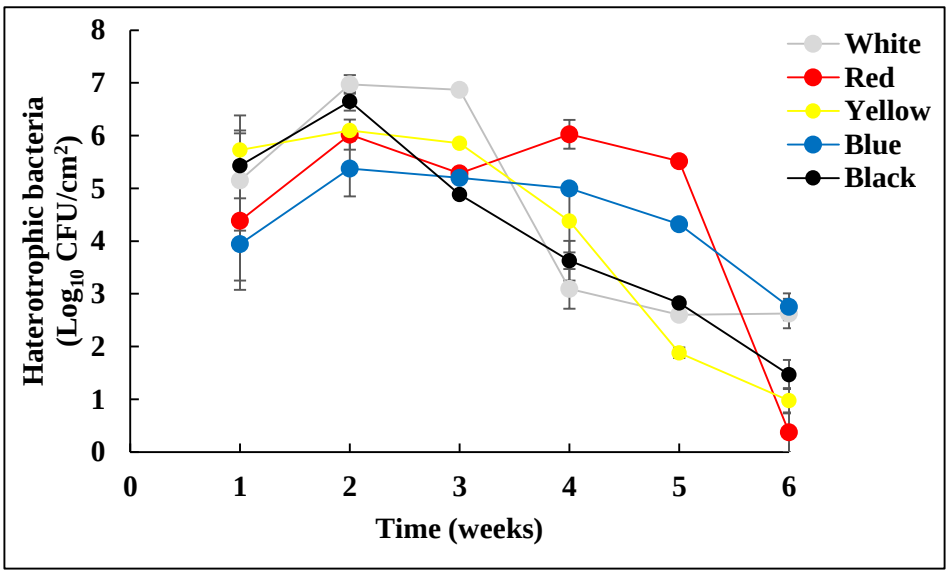

Figure 18. The change of bacterial concentration of surface-attached bacteria in each colored storage

4.2.4.Quantification of photosynthetic pigment in stored rainwater and the extracellular polymeric substance

The quantification of phototrophic biomass is usually assessed by the biomarkers chlorophyll-a (Gambino *et al.*, 2019). In our study, the chlorophyll-a content in the stored water, at the end of the experiment, was very low in the three storage tanks (white, red, and blue). Moreover, in yellow and black storage tank, it was not detected (Figure 19).

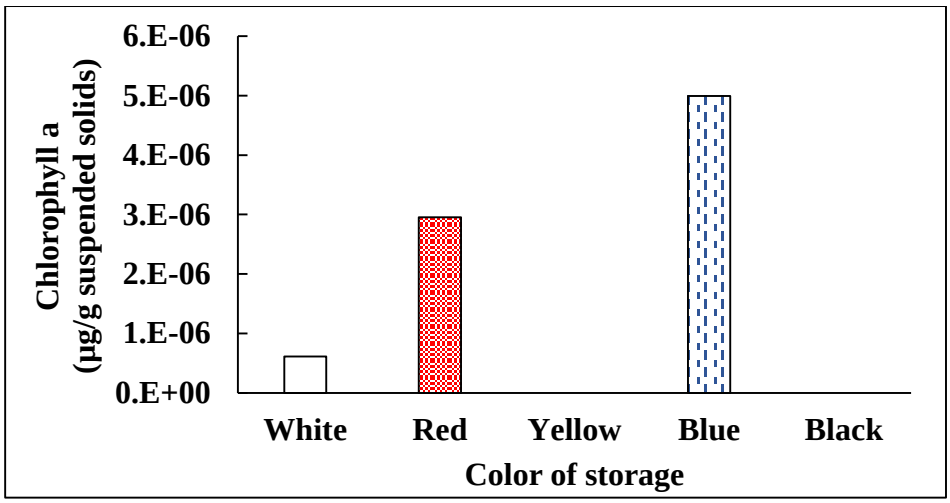

Figure 19. . Chlorophyll-a content in water inside each colored storage at 6th weeks

On the other hand, the chlorophyll-a content in the biofilm matrix was detected from the 4th week onwards and increased throughout the experimental period. The chlorophyll-a content in the white storage tank was the highest, followed by the chlorophyll-a in yellow and red storage tank to a lesser extent. The effect of color on chlorophyll-a was significant (Greenhouse Geisser F (4.5) = 703.21, $p<0.01$). The pairwise comparison showed a significant difference in content in white, yellow, and red compared to the chlorophyll-a in blue and black storage tank. The effect of time on chlorophyll-a content was also statistically significant (F (1.5) = 86.53, $p<0.01$), with the highest content at the end of the experimental period. Except for the black storage tank, there was a significant difference in the mean of chlorophyll-a over time.

The production of carotenoid is a way for phototrophic bacteria to protect themselves against ROS and is usually assessed along with the chlorophyll-a. (Gambino *et al.*, 2019). The carotenoids were increased over time (Figure 20). There is a significant difference in carotenoids content in white and other storage tanks (Greenhouse Geisser F (1.5) = 28.34, $p<0.01$).

However, the difference of chlorophyll-a and the carotenoids according to the color of storage tank were stable. Thus, the response of the bacteria to stressful condition did not involve the secretion of carotenoids.

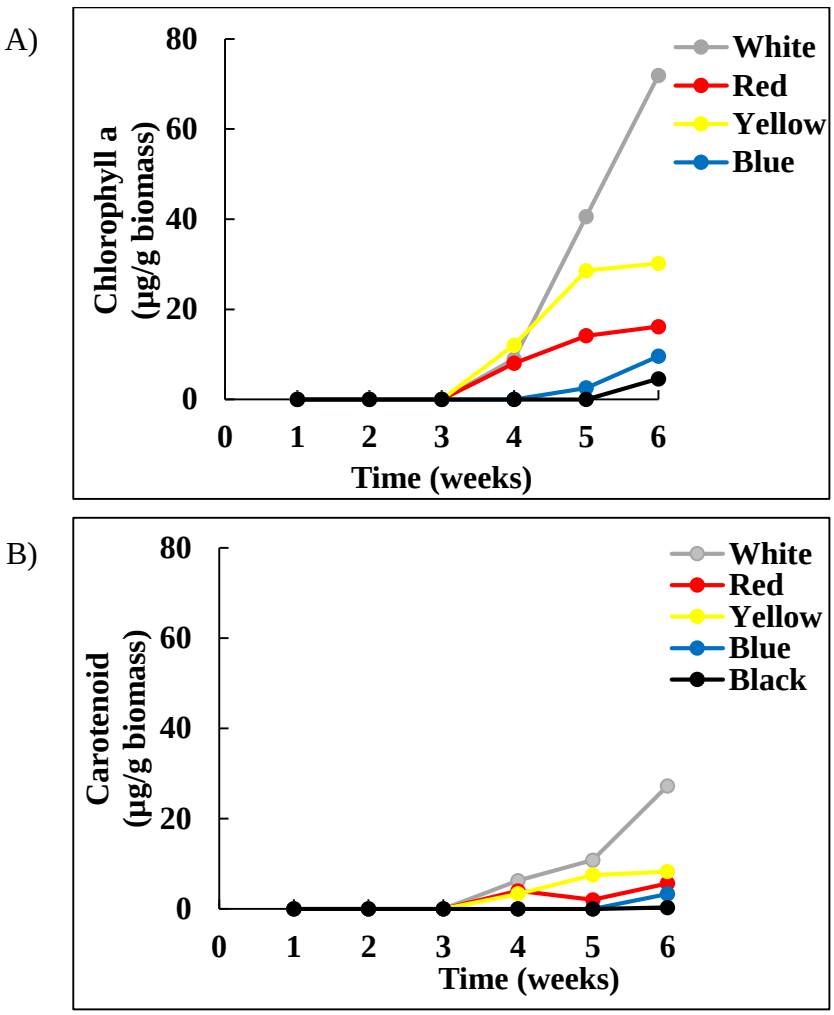

Figure 20. The change of chlorophyll-a (A) and carotenoid content (B) of biofilm matrix at each colored storage for 6 weeks

4.2.5.Matrix characterization of biofilm

The EPS plays a major role infeed and protecting the SAB from external environmental stress (Limoli, Jones and Wozniak, 2015a) and it has been

demonstrated that the composition of the matrix can be determined by the color of the light available to the cell (Gambino *et al.*, 2019; El Najjar *et al.*, 2020). The biofilm matrix was extracted then the main components were characterized: proteins and polysaccharides (Figure 21).

The EPS secreted by the SAB was rich in proteins in storage tank colored in red (from 45 to 1053 µg/ g biomass) and blue (from 170 to 795 µg/ g biomass). The content of protein in EPS, produced inside these two storage tanks, was significantly higher than in other storage tanks (white, yellow, and back).

The content of polysaccharides ranged from 28.58 to 1182.79 µg/ g biomass; it decreased over time. The difference in the polysaccharide content was not significant.

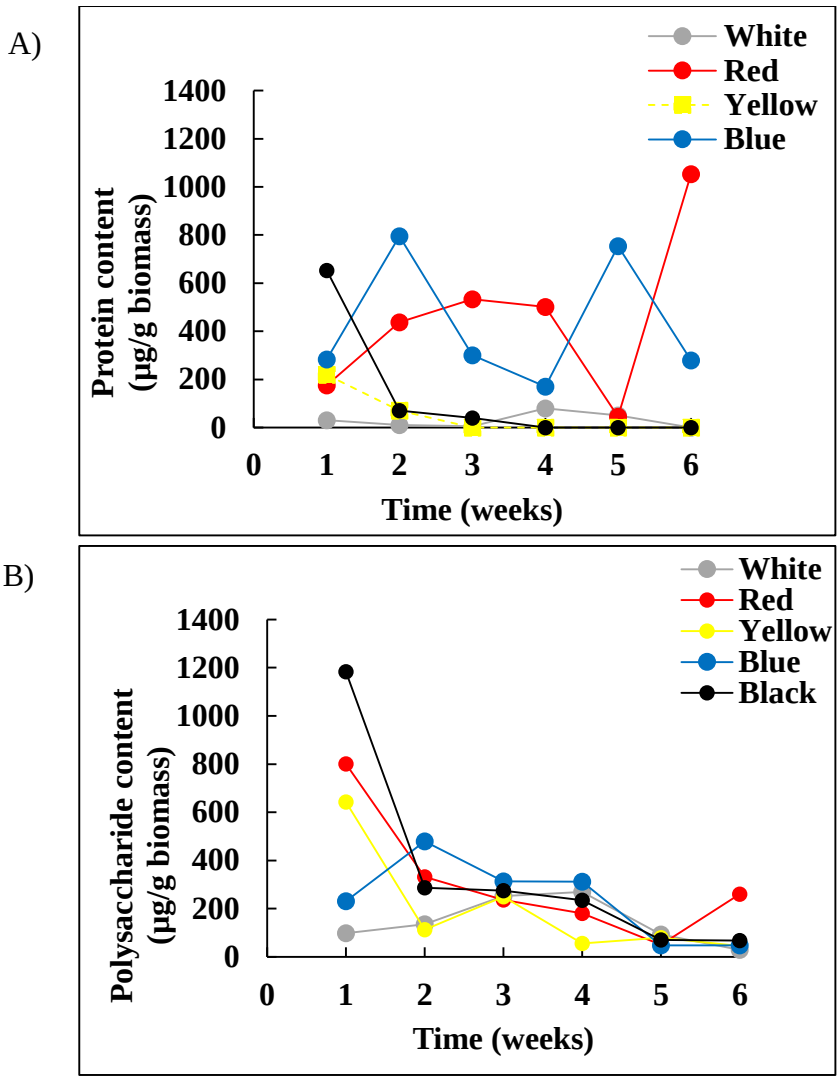

Figure 21. The change of proteins (A) and polysaccharide (B) of biofilm matrix at each storage for 6 weeks

4.2.6.Bacterial community analysis

4.2.6.1. Bacterial community structures

The 16S rRNA gene sequencing resulted in 89 927 sequence reads, where the number of sequences ranged from 8 182 to 19 279 reads per library. Taxonomic analyses were performed through MicrobiomeAnalyst (Dhariwal *et al.*, 2017), where 12 phyla, 23 class, 39 order, and 56 family were identified. At the phylum level, the bacterial community was markedly dominated by

Proteobacteria (86.8 ± 17.2%) and *Bacteroidetes* (6.5 ± 3.3%). *Actinobacteria* (3.7± 4%) and *Planctomycetes* (2.3± 4.1%), were only present in the planktonic bacterial community (Figure 22). The most abundant phylum in all the samples was *Proteobacteria*, of which β-*Proteobacteria* (69.1± 8.0%) was the most abundant, followed by α-*Proteobacteria* (8.1± 8.0%), and γ-*Proteobacteria* (6.0± 2.9%) (Figure 22)

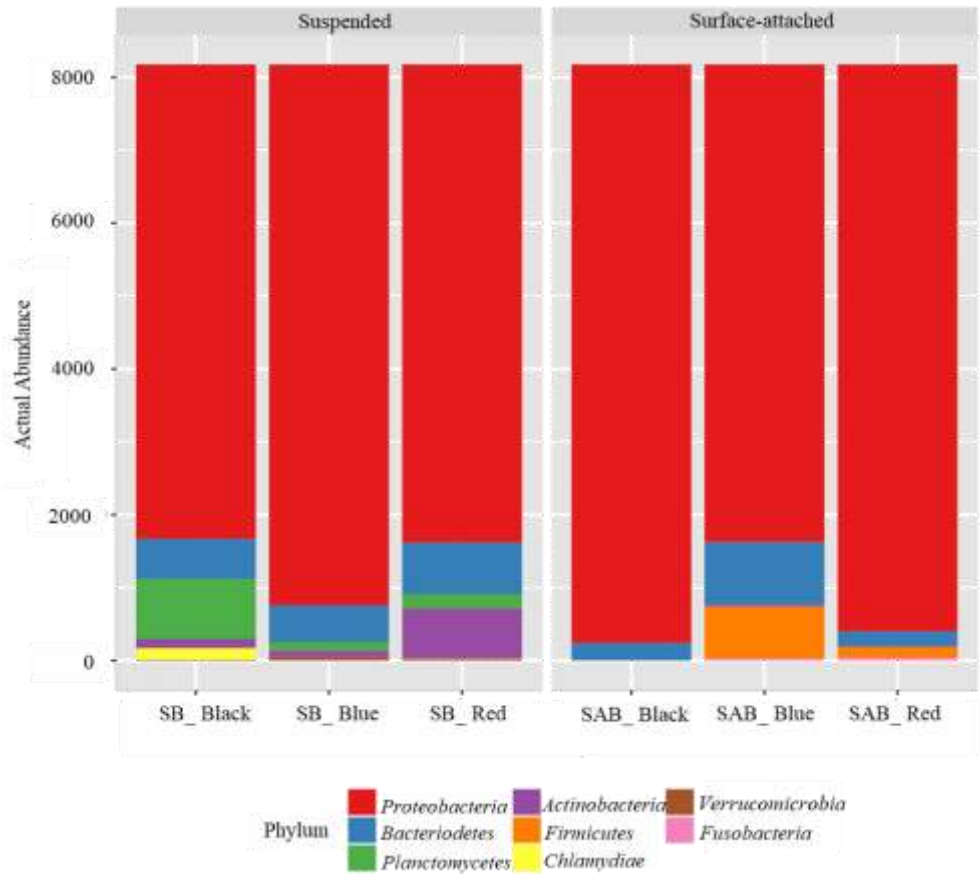

Figure 22. Bacterial community compositions at the phylum level

4.2.6.2. Bacterial diversity

Alpha diversity measured by either Shannon index or abundance-based coverage estimate (ACE) did not differ significantly ($p>0.05$) by sample (Table 5). That means the SB and SAB share a common bacterial community.

Table 5. Mean and standard deviation of Shannon and ACE of richness in samples collected

Sample	Shannon	ACE
Suspended bacteria	1.31 ± 0.32	11 ± 2
Surface-attached bacteria	1.13 ± 0.54	10 ± 3
P-value	0.30	0.54

The abundance of bacteria at the genus level is shown in figure 23. The type of SB such as Chelatococcus, Filimonas, and Reyranella in black storage tank had a high relative abundance. When the SB received light, some bacteria became more abundant, *Methylotenera* in blue storage tank and *Nocardioides*, *Planktophilia,* and *Trachelomonas* in red storage tank. The abundance of surface-attached bacteria was higher in blue and red storage tanks. The majority of the genera belong to β-*Proteobacteria* and *δ*-*Proteobacteria* in the black storage tank.

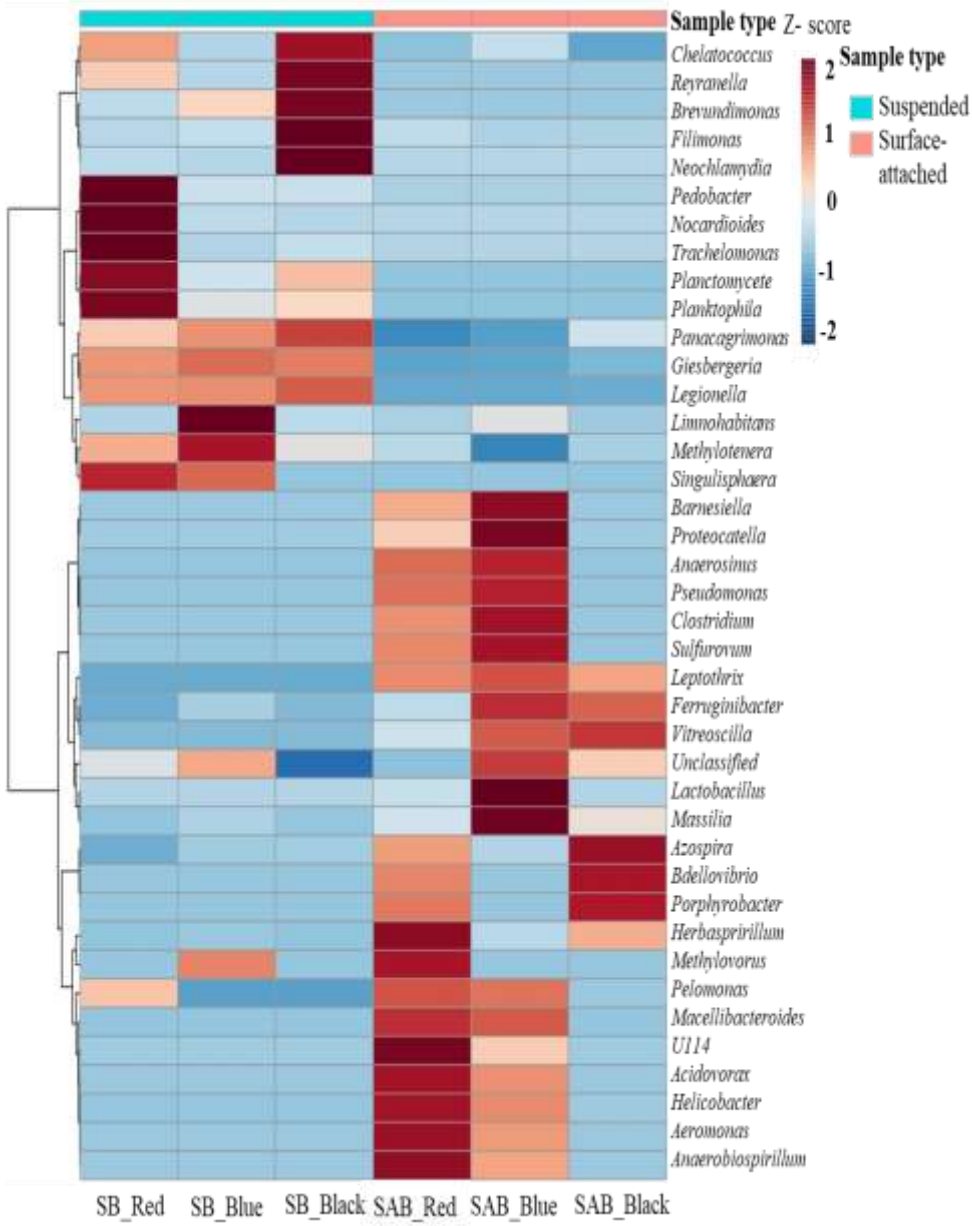

Figure 23. Composition and relative abundance of the bacterial at genus level (z-score displaced)

4.2.6.3. Detection of the potential pathogenic genera and species

The Biological Agents Code of Practice in 2020 was used to identify potentially pathogenic bacteria at a genus level (*2020 Biological Agents Code of Practice*, 2020). Two genera of potentially pathogenic bacteria

Helicobacter spp. and *Legionella spp.,* were detected at a low abundance. Among the genera *Legionella* was found in SB black (5.52%), blue (4.63%), and red (4.46%); whereas *Helicobacter spp.* was detected in SAB sampled from blue (0.28%) and red (0.44%) storage tank. The Clostridium thermosuccinogenes and Clostriduim cocleatum was classified as apathogenic bacteria (*Classification of Organisms: Pathogenicity classification of bacteria*, 2017).

4.3. Discussion

Storing harvesting rainwater is an alternative method to confront water scarcity (OECD, 2009; Yannopoulos, Giannopoulou and Kaiafa-Saropoulou, 2019). Recently, makeshift rainwater storage has become popular among people in developing countries (UN-Habitat, 2016b). From both public health and water supply points of view, investigating the effect of the color of storage on the microbiological water quality is of outstanding importance. However, the wavelengths of transmitted light impact were underestimated.

The influence of the color of storage on microbiological water quality has been investigated for groundwater and tap water and revealed that the response varies. For example, researchers observed a better reduction of bacterial counts of groundwater in transparent storage (Eniola *et al.*, 2007), while Ogbozige et al., found out that black plastic storage is the best color to maintain the good microbial quality of groundwater and tap water (Ogbozige, Ibrahim and Adie, 2018). Moreover, the color of the storage also has an impact on the colonization and development of SAB on its inner surface (Gambino *et al.*, 2019). The formation of biofilm on the surface has been known to influence the microbiological water quality of stored water (Coombes, 2006; Kim and Han, 2016). This broad range of responses to the color of storage highlights the need to assess this response.

The bacterial count was increased in accordance with the rise of temperature for the first three weeks. The result presented is conformed to the previous studies, reporting that a rise in water temperature induced by light absorption was responsible for microbial regrowth during storage period (Evison and Sunna, 2001). However, after three weeks, the bacterial concentration was diminished. This decline in the bacterial counts was probably due to the sedimentation and depletion of nutrients leading to the natural death of the bacteria (Eniola *et al.*, 2007; Amin *et al.*, 2013; Ogbozige, Ibrahim and Adie, 2018). The bactericidal effect of the light irradiation could influence the reduction of bacterial concentration (Ruiz-González *et al.*, 2013). It highlights the improvement of the microbiological water quality with the storage period (Spinks AT., 2007).

The bacterial count under the red light (588 nm – 800 nm) was low throughout the experimental period. This outcome is probably caused by the exposure of the 630 nm wavelength inducing sub-lethal damage of cells through photodynamic action (Nussbaum, Lilge and Mazzulli, 2002). Exposure to a red light produced ROS leading to the death of cells (Kamel, Saeed and Hassan, 2016; El Najjar *et al.*, 2020). In addition, the high transmittance, more than 60 % (Figure 15), of the red storage tank might have enhanced the bactericidal effect. The high light intensity formed more ROS and induced higher bactericidal activities (Kim *et al.*, 2013).

The growth of bacteria subjected to blue light (438 nm – 534 nm) is in discordance with the findings of Wang et al. 2017; they found that the bacterial concentration under blue light (400 nm – 470 nm) exposure decreased (Wang *et al.*, 2017). Our result can be explained by the low transmittance of the blue lid (Figure 15). Blue light has a better effective bactericidal activity at 400 nm and 425 nm (Halstead et al., 2016; El Najjar et al., 2020). However, only the 470 nm was transmitted by the blue storage tank

in our study (Figure 14). The high increase of bacterial count in white and yellow storage tank is conformed to the previous study, which indicated that "lighter color" has a less phototoxic effect and improves the growth rate (Kamel, Saeed and Hassan, 2016).

Regarding the bacterial community, the *Proteobacteria* and *Bacteroidetes* phylum had a high relative abundance in all the storage (Figure 11). The outcome of this study coincides with the previous researcher's findings (Evans et al., 2009; Kim and Han, 2015). They reported that stored rainwater has mostly *Proteobacteria*, *Bacteroidetes*, and *Firmicutes* (Spinks AT., 2007), which are part of the human gut microbiota; therefore, they are not harmful to humans (Rinninella *et al.*, 2019). A research conducted in South Africa on the bacteria community at a family level identified high relative abundances of *Comamonadaceae*, *Plantomycetaecae*, *Oxalobacteraceae*, and *Sphingomonadaceae* (Chidamba and Korsten, 2015). Another Australian study reported that *Comamonadaceae* and *Planctomycetaceae* were abundant in the rainwater sample from the tank (Ahmed et al., 2017). These outcomes are different from our result detecting high relative abundance of *Comamonadaceae* and *Oxalobacteraceae*. Dissimilarities in taxonomic composition might be the difference in sequencing technique and the geographical variation in rainwater associated with the climatic impact.

The result of the bacteria community of the SB in red corroborated the bactericidal effect of the 630 nm observed in the number bacteria fluctuation The heat map of the bacteria at the class level showed that the abundance of *Chlamydia*, *Planctomycetia*, *α-Proteobacteria*, γ-*Proteobacteria* irradiated by 630 nm wavelength (red storage tank) was reduced compared to the bacteria not receiving any wavelength (black storage tank). This result is similar to the findings of Yang et al., 2019, who reported that irradiation by

red light (630 nm) was harmful to *Planctomycetia* (Yang *et al.*, 2019). On the other hand, some types of bacteria identified in red storage tank had a low abundance in black storage tank. This bacterial proliferation is in accordance with previous researcher's findings. Red light (630 nm) stimulated the growth of photo heterotrophs such as *Flavobacteria, Actinobacteria,* and *Sphingobacteriia* (Hameed, W.-A. Lai, *et al.*, 2020).

The variation of the number of SAB in the different storages corresponds to the difference of wavelengths received by bacteria through the storage lid. The incorporated bacteria count in the red and blue storage were slightly constant, while those in black, white, and yellow storage decreased after the second and third weeks. The unaltered biofilm cell density in the red and blue storage contrasted with previous research (Aragão et al., 2019; Angarano et al., 2020). This disparity is probably due to the high matrix protein in red and blue storage. Protein in EPS participates in the structure, protection, and stability of biofilm (Fong and Yildiz, 2015a; Chi et al., 2019). Moreover, researchers reported that bacteria secreted a stress-induced protein under blue light and a high dose of red light, which is a physiological defense mechanism (El Najjar et al., 2020). In the 6th week, the number of attached cells decreased, and the protein content in the EPS increased. This augmentation of protein content is presumably due to the disruption of the dead cell and liberation of the protein cytoplasmic, inner, and outer membrane protein. Previous researches reported that cell lysis was involved in the increase of the protein content in EPS (Fong and Yildiz, 2015b).

The higher secretion of protein was associated with a low content of exopolysaccharide on the biofilm. Our finding is consistent with the previous research relating that more protein secretion inhibited the production of polysaccharides (Limoli, Jones and Wozniak, 2015b). Furthermore, the low content of exopolysaccharide in the EPS for white, yellow, black storage tank was probably due to the small number of incorporated cells. The EPS is

biosynthesized by the SAB, a small number of SAB will result in the low amount of EPS (Costa, Raaijmakers and Kuramae, 2018).

The bacterial reduction in white (400 nm – 800 nm) and yellow (584 nm) is in contrast with the finding of Angarano et al., they did not observe any impact of yellow (584 nm) color on the biofilm cell density (Angarano *et al.*, 2020). Our results are probably due to colonization of the phototrophic bacteria. The more mature the biofilm becomes; the higher number of phototrophic bacteria it contains. (Roeselers, Van Loosdrecht and Muyzer, 2007; Zancarini *et al.*, 2017). This argument is confirmed by the increase of chlorophyll-a in the matrix (Figure 9). The red far (700 nm- 750 nm) passing through the white, red, and yellow covers (Figure 3) triggered the photoreceptor of phototrophic bacteria to produce Chlorophyll-a (Gambino *et al.*, 2019).

There was no significant difference between the SB and SAB bacterial communities (Table 5), suggesting that they have a common source. The results of this present study are consistent with Spinks et al.,2007, which reported that the planktonic bacteria got attached to the surface of the rainwater tank storage and formed a biofilm (Spinks AT., 2007). Moreover, *Legionella spp*, was observed only in the biofilm bacterial community. It was removed from the water body and get attached to the surface. Consequently, biofilm improves the microbiological water quality of rainwater during the storage period; our findings are consistent with the previous research (Spinks AT., 2007; Kim and Han, 2015). Therefore, cleaning the tank is not needed, even counterproductive. The color of storage influences the abundance of bacteria attached to the surface. There is more diversity in blue and red storage compared to the bacteria in black storage. It is probably due to the protective layer of EPS rich in protein (Chi *et al.*, 2019).

Based on our results, the color of storage tank affects the microbial water quality and the aesthetical aspect of water. Consequently, the impact of each band of wavelengths should be categorized to propose the color of the plastic container preserving the water quality during storage period. The HPC is generally used to assess the microbial water quality of the drinking water, where HPC< 500 CFU/mL is the recommended limit (Pavlov et al., 2004). Moreover, the bacterial community analysis revealed the absence of pathogens in SB and SAB in red. The suitable color of plastic to store rainwater is red, then black, blue, yellow, and the least is white color for our source of light.

4.4. Limitation of study and further study

The influence of the storage color on the microbiological water quality of stored rainwater was assessed by using a fluorescent lamp as a light source and painted storage. Thus, these results may underestimate the sunlight exposure effect, which has different spectral composition and intensity distribution. Moreover, the storage capacity was small, so the physiochemical parameters except temperature could not be investigated.

Therefore, future work is required using color plastic storage, with the size used in the field, exposed to direct sunlight. Other parameters influencing the light properties and the microbiological water quality can be further investigation.

Chapter 5. Conclusion

The study experimentally investigated the effect of visible light on microbiological and physicochemical quality of harvested rainwater. The experiment was divided into two parts. First, two tanks, one exposed to sun and another not exposed to sun were used to assess the bacterial growth of SB and SAB. The bacterial count of SB decreased faster at TNES than TES case, resulting in a better microbial quality at TNES. The number of SAB decreased over time, when it was not exposed to visible light, while it remained stable when exposed to visible light. There was no big difference between TES and TNES in other physicochemical parameters except that the total organic carbon and concentration of dissolved oxygen were higher in TES than in TNES. Overall, the water quality can be maintained well without the effect of visible light.

Second, the effect of the color of storage on the microbiological water quality and biofilm formation on sorted rainwater. Results showed that the wavelengths passing through the cover influence the bacterial concentration of SB and SAB. There was bacterial growth in the first phase. However, when irradiation of 639 nm wavelength (red) the bacterial growth was small and bacterial concentration was low throughout the experiment. In the first phase, the color of storage did not affect the surface colonization by SAB. However, as the biofilm mature, a variation was observed among the SAB and matrix formation. The number of SABs decreased in all storages but earlier in black, yellow, and white storage. The EPS had a high protein content in red (630 nm) and blue (470 nm) storage. Furthermore, the chlorophyll-a concentration varied with the highest in a matrix formed in presence of red far band (700-750 nm) inside the white storage (400 nm – 800 nm); the chlorophyll-a content was also considerable in yellow (526 nm – 800 nm) and red storage

(588 nm- 800 nm). Red storage was the best storage to preserve the good microbial quality and aesthetic of water for the light used in this study.

From this research, it is possible to suggest a practical implication in the design and operation of typical household rainwater tanks to maintain a good water quality. The storage should be installed to avoid penetration of visible light by putting it under a shade or cover the opening. In case a shade area is not available the color of rainwater plastic storage should be carefully selected. Disinfection in the tank should be avoided to maintain the microbial balance and self-purification in the rainwater tank.

Some limitations of this research are identified, and further research is suggested to find easy ways to maintain good water quality in household rainwater harvesting tanks in developing countries and developed countries as well.

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국문초록

가시광선이 플라스틱 빗물저장탱크 내부의 미생물 수질 및 바이오필름 형성에 미치는 영향

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불충분한 상수도 시스템과 인구 증가로 인하여 물부족이 발생하는데, 빗물 수집이 잠재적 해결책으로 각광을 받고 있다. 하지만 저장시설의 비용이 비싸기 때문에 개발도상국에서는 임시방편으로 간단한 빗물 수집 시스템을 사용한다. 각 가정에서는 불투명하고 두꺼운 빗물저장조 대신에 재활용된 반투명의 작은 플라스틱 재질을 사용한다. 플라스틱 저장조는 태양빛을 받으면 가시광선을 반사, 전달 및 흡수한다. 플라스틱 저장조의 이러한 특성은 저장된 물에서의 세균의 생장과 미생물에 영향을 미칠 수 있다.

검은색 플라스틱 저장조는 가시광선을 흡수하여 열로 변환되어 수온을 높이며, 결국 부유성 세균(Suspended Bacteria) 증식을 촉진한다. 얇은 플라스틱 저장조는 색상에 따라 가시광선 파장의 영역을 투과시킨다. 적색의 저장조는 630nm 파장을 전송할 수 있으며, 이 파장은 강한 살균효과를 가진다. 파란색 저장조는 reactive oxygen species (ROS)를 생성시켜 세포에 해를 주어 부유성 세균의 증식을 억제하거나 사멸시킨다. 반면 흰색의 저장조는 가시광선 전체 파장의 영역을 투과시킨다.

본 연구에서는 일반적인 가정용 플라스틱 탱크에서 가시광선이 미생물학적 수질에 미치는 영향을 연구하고, 저장조의 색갈에 따른 미생물학적 수질과 미생물막의 형성에 미치는 영향을 연구하였다.

첫째, 작은 아크릴 저장조를 이용하여 가시광선의 유무에 따른 부유성세균과 부착성 세균의 성장에 미치는 영향을 알아보기 위하여 빗물을 넣고 3개월간 실험을 하였다. 하나는 태양에 노출시키고 (TES), 또 하나는 태양에 노출시키지 않았다 (TNES). 부유성 세균의 숫자는 노출되었을 경우보다노출되지 않았을 때 더빨리 감소하였다. 이것은 햇빛에 노출시키지 않았을 때 미생물학적으로 수질이 더 좋아지는 것을 나타낸다. 부착성 미생물은 가시광선에 노출되었을 때 안정화 되었지만, 어두운 조건에서는 감소하였다.

둘째, 저장조의 색갈이 미생물학적 수질과 바이오 필름 형성에 미치는 영향을 평가하기 위해 다섯 개의 1 리터짜리 원통형의 저장조에 흰색, 빨간색, 노란색, 파란색, 검은색으로 색칠을 하였다. 각 저장고에 빗물을 채워 6 주 동안 파란색 소형 형광등 밑에 설치하였다. 부유 세균과 부착 세균의 농도는 HPC (Heterotrophic Plate Count) 방법으로 측정하였으며 표면에 부착한 세균 구조의 총 바이오매스 양은 크리스탈 바이올렛 분석으로 측정했다.

빨간색 빛은 세균 성장을 억제하는 것으로 나타났다. 하지만 흰색, 노란색, 파란색, 그리고 검은색 빛은 부유성 세균의 성장률을 자극했다. 3주 후에 세균이 감소했고, 실험 종료 시점에서 빨간 저장고에서 세균 수가 가장 적었다. 게다가, 투과된 빛의 스펙트럼은 또한 표면에 바이오 필름의 개발에 영향을 미쳤다. 빨간색과 파란색 빛은 세포 외 기질에서 (extracellular polymeric substances) 있는 스트레스 인한 단백질을 유발했다. 단백질이 풍부한 EPS는 보호 계층이므로 표면에 부착한 세균의 수는 표면에 더 오래 남았다. 흰색과 노란색의 저장고에서는 EPS에 통합된 종속영양 생물은 감소했지만, 광합성 생물은 증가하였다. 검정 저장고에서는 바이오 필름의

형성이 낮았다. 결론적으로 플라스틱 저장고의 색의 우선순위는 빨강, 검정, 파랑, 노랑, 흰색 순이며 흰색이 가장 나쁘다.

본 연구에서 알아낸 내용은 플라스틱 빗물 저장조안에서 가시광선의 존재 여부는 미생물학적인 수질과 생물막의 형성에 영향을 미친다는 것이다. 또한 저장조의 색상은 저장된 빗물의 질을 유지하는 데 커다란 영향을 주기 때문에 색상 선택에 있어서 신중하게 고려되어야 할 것이다.

Keywords: 가시광선, 저장조의 색, 부유성 세균, 바이오필, 빗물저장조, extracellular polymeric substance, 세균의 다양성

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1 Corinthians 15:10

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