# Risks associated with treated wastewater in greenhouse cooling system

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# ABSTRACT

The hot climate in the Gulf region is forcing many farmers to use controlled environment agriculture by using evaporative coolers inside greenhouses. These coolers are consuming around 60% of the water used for the greenhouse. Replacing groundwater with treated wastewater will have a good impact in saving freshwater for other different applications. The problem is that treated wastewater is rich of nutrients that can support algae growth, block the cooling pads, and reduce the efficiency of the cooling system. Moreover, it is unclear if the water can be a source of any airborne diseases that could affect human health and crop quality. Unfortunately, few or no data is available related to the applications of treated wastewater in greenhouse cooling system. Therefore, the aim of this study is to evaluate the possibility of using treated wastewater in greenhouse cooling system and asses any potential risk to the environment that could affect human health and crop quality and safety. The greenhouse cooling system was connected to tertiary treated wastewater and the system was left to run for two months. Algae growth was observed in cooling pads. Samples from cooling pads, air, and water were taken for microbial analysis. The same sampling was made from other greenhouse running with freshwater. Using treated wastewater in cooling system did not show any negative impacts in plant growth. However, rapid growth of algae in cooling pad of treated wastewater was noticed compared to the groundwater cooling system. This could be minimized by covering cooling pads with shade net, or adding some anti-algae growth (CaSO<sub>4</sub>) in cooling tanks, or using plastic cooling pads, or cleaning cooling pads from time to time. Moreover, more types of bacteria were found in treated wastewater cooling system but were not harmful for human and plants. Almost similar microbes were found in the air and water running in all greenhouses. Therefore, the study recommends the use of treated wastewater in the greenhouse cooling system with application of antifouling compounds or using plastic cooling pads that can be easily cleaned.

Keywords: Treated wastewater; Non-conventional water resources; Cooling pad; Algae growth; Gulf region

### 1. Introduction

Oman is categorized as an arid to semiarid country with a temperature exceeding 45°C in summer and an average annual rainfall of 100 mm. Therefore, Oman's climate is not highly suitable for open-field cultivation, especially in summer time that is almost 8 months of the year [1]. In addition, around 52% of the agricultural lands became unsuitable due to salt accumulation that came from saline irrigation as a result of seawater intrusion, which mainly happened in Batinah Region [2]. To overcome the problem of hot conditions, Controlled Environment Agriculture (CEA) was adopted, to improve the quality and quantity of crops through controlled environment in greenhouses [3]. Tabook and Al-Ismaili [4] reported that greenhouse production was 12 times more than open field grown crops in Oman. The number of greenhouses in Oman had increased from 782 in 2001 to 2491 greenhouses in 2008. The common cultivated crops in Oman greenhouses are cucumber (around 90%) and then tomatoes (about 5%–9%).

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Greenhouses in arid and semi-arid areas utilize auxiliary fans and evaporative coolers to cool down the greenhouse and reach optimum temperature for plant growth. The evaporative coolers are made of honey comb cardboard pads that should be wetted with any source of water. When the hot and dry air from outside the greenhouse passes through the coolers, the water absorbs some of the heat from the air and therefore it evaporates and consequently it cools down (the temperature decreases). Then, the air inside the greenhouse becomes humid and cool and the ambient air temperature inside the greenhouse decreases [5]. The problem is that too much water is used for the cooling purposes, in some cases, it can be double the amount of water used to irrigate the crops inside the greenhouse. A study was done in United Arab Emirates (UAE) to estimate the consumed water and energy in a tomato greenhouse. They found that the total amount of water consumed during one season was 837,662 L in the cooling pad and 615,052 L in irrigation. This means that 58% of water was used only for cooling the greenhouse and the rest was for crop needs [6]. Another study was done in Sultanate of Oman and reported that 2/3 or 67% of water used in greenhouse is used only for cooling the temperature of the greenhouse using cooling pad system [3]. Furthermore, a study was conducted to compare water and energy consumptions of three greenhouses located in different places in Oman and the amount of water consumed for cooling purposes during cucumber production season were 11.88, 15.48, 14.4 m<sup>3</sup> and for the same three greenhouses, irrigation waters were 9, 9, 20 m<sup>3</sup>, which means 57%, 63% and 42% of total water were used in cooling pad, respectively [4].

In Oman, "Haya Water" is a governmental company that is responsible for building, operating, and managing wastewater projects in most Governorates in Oman. Treated wastewater (TWW) is a by-product of sewage treatment process with total flow of 149,940 m<sup>3</sup>/d, treated effluent of 77,503 m<sup>3</sup>/d; where 52% of this treated wastewater is utilized [7]. It is a good source of good and clean water that can support agricultural lands with input of different nutrients. It helps in improving the fertility of different soils, increasing the yield of different crops and reduce usage of groundwater (GW) resources. Reuse of treated wastewater will result in the conservation of higher quality water that can be used for different purposes other than irrigation. However, the safe disposal of the treated wastewater is considered as a major concern for Haya Water and relevant authorities. Furthermore, reusing of treated wastewater produced from the wastewater treatment plants in sustainable, economic and environmental friendly ways are very important for a country like Oman. Therefore, there is a need to implement a comparative study on the potential reuse options for the treated wastewater produced by Haya Water.

Combining the need for large volumes of water in the greenhouses' cooling system with surplus of treated wastewater can be beneficial, taking into account the expected environmental and health impacts and how this treated wastewater will affect the human health and quality of crops grown inside the greenhouse. Therefore, the aim of this study was to evaluate the possibility of using treated wastewater in greenhouse cooling system and asses any potential risk to the environment that could affect human health and crop safety and quality.

## 2. Methodology

Two small greenhouses (3 m × 6 m) were constructed at Agricultural Experiment Station, College of Agricultural and Marine Sciences, Sultan Qaboos University (SQU), Oman (Fig. 1). One of them was connected with treated wastewater for irrigation and cooling systems. The other one was connected with groundwater, as a control, for comparison.

#### 2.1. Experimental design

The experiment was conducted in two greenhouses for a total area of  $18 \text{ m}^2$ , were used to grow three species of roses, Rosa (Omani flower, Syrian flower and Pakistani



Fig. 1. Greenhouses connected with treated wastewater and groundwater.

flower) and lettuce in soil. Four replicates from each type of the flowers and twelve lettuces were planted in each greenhouse. Each greenhouse was running completely either by GW or TWW. Plant growth parameters were monitored and measured. Moreover, samples were taken from the air and cooling system of both greenhouses.

### 2.2. Plant growth

Treated wastewater is rich of different elements. Therefore, as expected, production of flowers was higher for the three different flower types in the greenhouse irrigated with treated wastewater compared to groundwater. The average number of flowers produced by Omani flower, Pakistani flower and Syrian flower in GW were 2, 1 and 2, respectively, while the average number of flowers produced by Omani flower, Pakistani flower and Syrian flower in TWW were 5, 2 and 5, respectively (Fig. 2). The result indicated that there was no significant difference in number of flowers between GW and TWW (P-value = 0.0578) and between the flowers species in both treatments (P-value = 0.234) (Table 1). The same observation was noticed with lettuce growth. The average weight of lettuce in treatment 1 and 2 were 277 and 308.5 g, respectively (Fig. 3). Whereas, there was no significant difference in weight of lettuce at different treatments ( $P(T \le t)$  two-tail = 0.524) (Table 2).

Table 1

ANOVA test of number of flower produced by Omani flower, Pakistani flower and Syrian flower in GW and TWW

Source of variation	SS	df	MS	F	P-value	$F_{\rm crit}$
Sample	28.03	1	28.03	3.97	0.058	4.26
Columns	21.8	2	10.9	1.54	0.234	3.40
Interaction	6.87	2	3.43	0.49	0.621	3.40
Within	169.6	24	7.07			
Total	226.3	29				

Water quality is one of the most important parameters affecting plant growth. The aim of the study was to determine the impact of treated wastewater in greenhouses cooling system and irrigation of different plants. Plants growth was better in TWW than GW. According to Irénikatché Akponikpè et al. [8], treated wastewater is more useful for plants irrigation due to the presence of different nutrients especially  $NH_4^+$  and  $NO_3^-$ , which are considered as major ions needed for plant growth. According to Faizan et al. [9] and Urbano et al. [10] treated wastewater is more profitable for plants growth than groundwater.

Average flower yield of Omani roses, Pakistani roses and Syrian roses were higher in TWW (5, 2 and 5, respectively) compared to GW. However, in GW, the number of Omani roses, Pakistani roses and Syrian rose flowers were (2, 1 and 2, respectively). Similar result was found by Marinho et al. [11]. They found, the highest mean production of commercial rose stems was observed in rose that was irrigated by treated wastewater and the production increased by 31.8%. Whereas Nirit et al. [12], found no significant different in rosebushes productivity irrigated by treated wastewater and groundwater. According to Feigin et al. [13], treated wastewater contains organic and ammoniacal forms which contribute to the increase of flower yield. In addition, the average fresh weight of lettuce increased in TWW (308.5 g) due to the improvement in soil fertility.

### 2.3. Algae growth in greenhouse cooling system

Treated wastewater is rich with nutrients that could support algae growth and block the cooling system. Therefore, algae growth was studied and monitored in all greenhouses and it was observed that treated wastewater enhanced algae growth which could minimize the life-time of cooling pads (Fig. 4).

Algae samples were taken from different locations of both cooling pads. All samples were studied under microscope (Inverted microscope) (Fig. 5). Same type

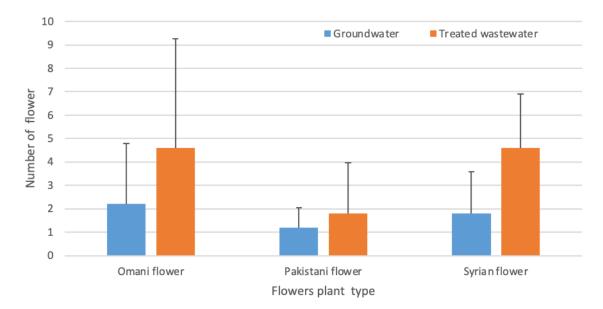


Fig. 2. Average number of flowers produced from each type of water (GW and TWW).

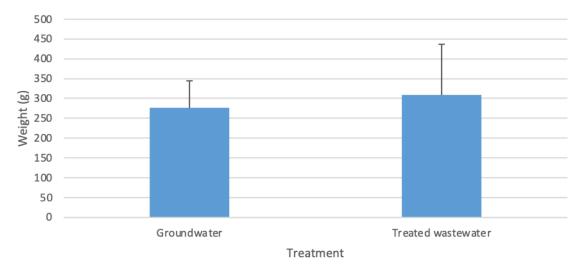


Fig. 3. Average weight of lettuce irrigated with GW and TWW.

Table 2 *t*-Test of lettuce weight in GW and TWW

	Groundwater greenhouse (GW)	Treated wastewater greenhouse (TWW)		
Mean	277	308.5		
Variance	5,101.11	18,111.39		
Observations	10	10		
Hypothesized				
mean difference	0			
df	14			
t-Stat	-0.653			
$P(T \le t)$ one-tail	0.262			
t-Critical one-tail	1.761			
$P(T \le t)$ two-tail	0.524			
t-Critical two-tail	2.14			

of algae (*Tetrastrum* sp.) was found in both greenhouses cooling systems (groundwater and treated wastewater). *Tetrastrum* sp. was reported as the major algae presented in both cooling systems. However, it had higher growth in treated wastewater cooling pads. According to Palmer et al. [14], *Tetrastrum* sp. is a type of algae and its presence is one of the most common issues of using treated wastewater, which is characterized as freshwater algae with four cell and flat surrounding a hole in the middle. Cells are shaped like triangle, ovoid or heart, and mostly found with one to four spines and usually with or without a phytoplankton or pyrenoid of lakes [14].

Algae growth can be minimized by different applications (options) such as:

# 2.3.1. Option 1: light effect

Algae from five random sampling points (around  $1.5 \text{ cm} \times 1 \text{ cm}$ ) of greenhouse cooling system were collected for both systems (treated wastewater and groundwater) and mixed with 10 mL of cooling system water. Distilled

water was used as a control. Light samples were incubated at 27°C and 12 h light while, dark samples were covered with aluminum foil. Every day during 4 d, total amount of Chlorophyll a was measured. Indicator of Chlorophyll a absorbance at wavelengths of 647 nm was measured using spectrophotometer.

The results indicate that Chlorophyll a of cooling pads in TWW and GW were different. However, Chlorophyll a was higher in TWW than GW in the presence and absence of light. Light increased algal growth due to enhancement of photosynthesis process (Fig. 6).

Based on this finding, all greenhouses were covered with green net so algae growth could be minimized as one step to reduce the sunlight and the biofouling problem (Fig. 7).

# 2.3.2. Option 2: addition of different chemicals ( $CuSO_{4'}$ ZnSO<sub>4'</sub> chitosan and clorox) to the cooling tanks as antifouling compounds for controlling algal growth

Algae from five random sampling points (around 1.5 cm × 1 cm) of greenhouse cooling system were collected for both systems (treated wastewater and groundwater) and mixed with 10 mL of cooling system water. Samples were centrifuged at 5,000 rpm for 5 min. 100  $\mu$ m of algae was mixed separately with four different chemicals at four different concentrations (copper sulfate and zinc sulfate 0.1 g/100 mL, 0.2 g/100 mL, 0.3 g/100 mL and 0.4 g/100 mL, chitosan 0.1 mg/100 mL, 0.2 mg/100 mL, 0.3 g/100 mL and 0.4 g/100 mL and 0.4 g/100 mL and clorox 100  $\mu$ L, 200  $\mu$ L, 300  $\mu$ L and 400  $\mu$ L). All samples were incubated in well plats at 27°C in light condition for 12 h (Fig. 8).

Lethal concentration LC50 (50% of the tested population) provides a data for the percentage of the organisms that were killed by different concentrations of each toxicant after specified lengths of exposure [15].

It was found that chitosan has more antifouling activity with less dose compared to other antifouling compounds (Figs. 9–10). According to Guo et al. [16], chitosan is a natural product from the shell of shellfish which is widely used as antifungal due to non-toxic properties.

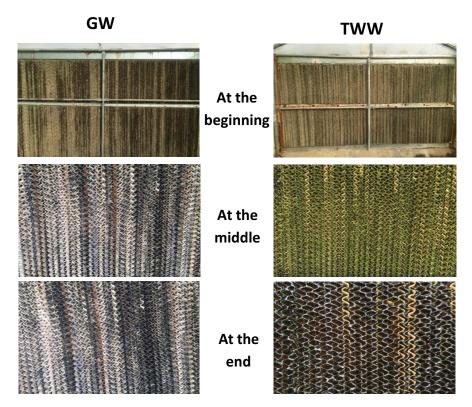


Fig. 4. Growth of algae in cooling system in both treatments during the experiment.

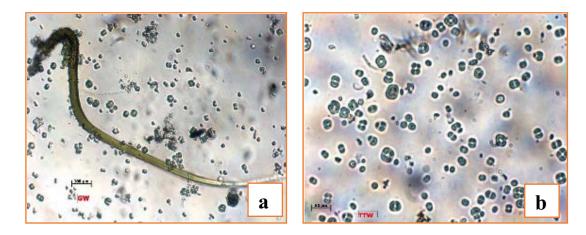
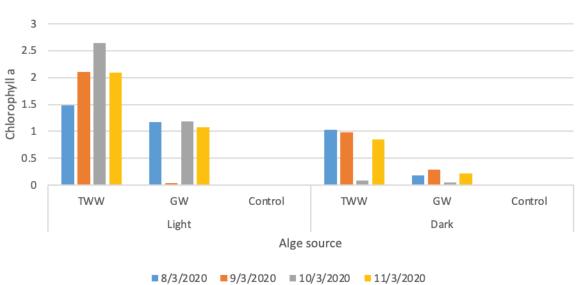


Fig. 5. Tetrastrum sp. algae in greenhouses cooling pad in GW and TWW: (a) groundwater algae and (b) treated wastewater algae.

Based on the lab findings, all four chemicals (copper sulfate, zinc sulfate, chitosan and clorox) were tested under field condition using greenhouse cooling system. However, chitosan is a natural product but it is costly and need some technical knowledge in application. Therefore, it will be difficult for the farmer to apply it. Clorox had a short term effect and later algae was growing faster than control treatments. Therefore, copper sulfate was the best option under field conditions. It was added in concentration of 0.31 mg/L in treated wastewater tank. Every week, algae from five sampling points (around 1.5 cm × 1 cm) from greenhouse cooling system were collected in all treatments (T1: cardboard cooling system running with treated wastewater, T2: cardboard cooling system running with groundwater and T3: plastic cooling system running with treated wastewater). For measuring the Chlorophyll a, samples were left in a filter paper of 0.74  $\mu$ m mesh size and then 90% acetone was adding to all samples and kept for overnight. After 24 h, the extractions were purified using centrifuge (5,000 rpm) for 5 min. Chlorophyll a absorbance at wavelengths of 750, 664, 647 and 630 nm was measured using spectrophotometer. Total amount of Chlorophyll a was calculated using trichromatic equation: Chlorophyll a = (11.85 × A664) – (1.54 × A647) – (0.08 × A630) (Fig. 11) [17]. Moreover, air temperature was measured weekly inside all three greenhouses.



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Fig. 6. Average Chlorophyll a of cooling pads in treated wastewater (TWW) and groundwater (GW) in light and dark conditions.



Fig. 7. Greenhouses covered with green shade.

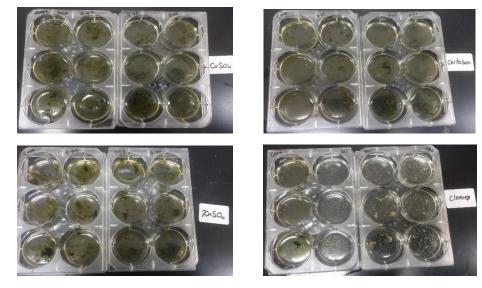


Fig. 8. Lab experiment for algae growth with different chemicals.

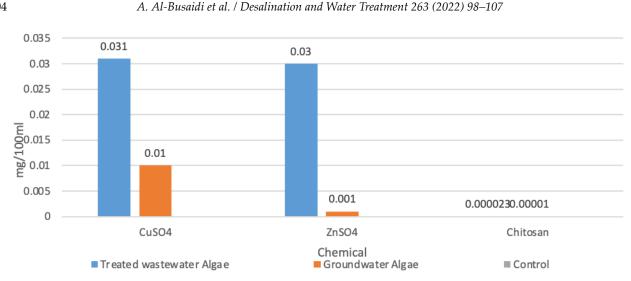


Fig. 9. Lethal concentration (LC50) of  $CuSO_4$ ,  $ZnSO_4$  and chitosan in algae growth of treated wastewater and groundwater cooling pads.

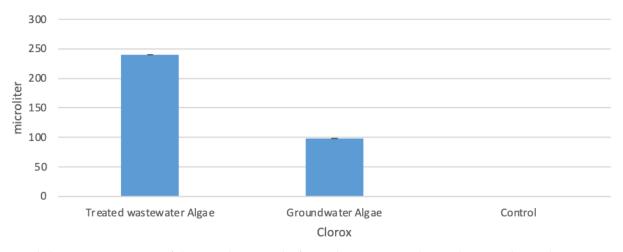


Fig. 10. Lethal concentration (LC50) of clorox in algae growth of treated wastewater and groundwater cooling pads.

It was found that algae growth decreased in cooling system that was running by treated wastewater with copper sulfate compared to the cooling system that was running by groundwater and plastic cooling system. Plastic cooling system had the highest values of Chlorophyll a which could be due to the shape of cooling pads (Fig. 12). According to Lauer [18], the optimal dose for killing *Tetrastrum* sp. algae is 0.02 mg/L of CuSO<sub>4</sub>. However, we found that 0.31 mg/L of CuSO<sub>4</sub> is the optimal concentration for killing 50% of *Tetrastrum* sp. and that is because the difference in the experiment condition and period.

### 2.3.3. Option 3: cleaning effect

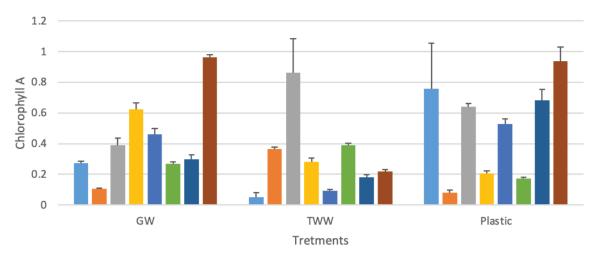
The easier method to overcome the algae growth was cleaning the cooling pads using brush and pressurized water. Cleaning process for the area of  $3 \text{ m} \times 2 \text{ m}$  in each system took around 10 min. Algae samples were taken before and after cleaning.

After cleaning, the growth of algae decreased by 90.93% in groundwater cooling system cardboard, 90.63% in

treated wastewater cooling system cardboard and 98.47% in plastic cooling system (Fig. 13).

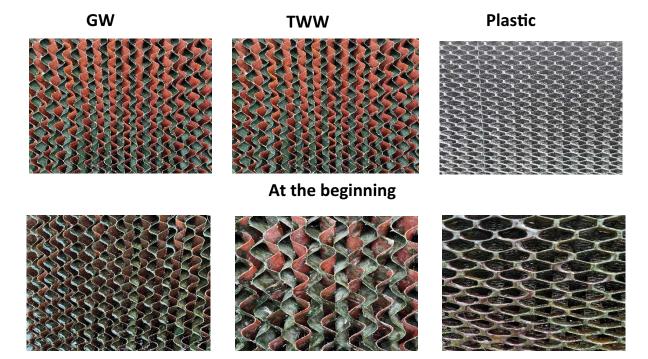
Treated wastewater is rich with nutrients that could support algae growth and cause biofouling [19]. Biofouling is undesirable deposition and growth of microorganisms on wetted surfaces and is considered an abiotic form of biofouling [20]. Biofouling blocks the cooling pads and affects the efficiency of the greenhouse cooling system and reduce the lifetime and efficiency of cardboard using in the cooling system. Moreover, it is unclear if the water could be a source of any diseases by spreading harmful microbes inside the greenhouse, affecting human health and contaminating crops. Although the wastewater treatment systems are designed to minimize the concentration of contaminants, including pathogens, many wastewater treatment plants release such effluents without chlorination [21]. One example is Escherichia coli which has been commonly used in aquatic environments as a faecal pollution predictor and most strains of it considered as a non-pathogenic while, some strains may be pathogenic [21]. As well, Pseudomonas mendocina, Enterobacter kobei and Pseudomonas alcaligenes

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Fig. 11. Chlorophyll a level in cooling system pads in GW cooling system, TWW cooling system and plastic cooling system (Experiment 2).



# At the end

Fig. 12. Differences in algae growth with time in each greenhouse cooling systems.

are soil and water bacteria and considered as pathogenic bacteria [22,23].

## 2.4. Micro-organisms in water, air and cooling system

*Tetrastrum* sp. is a type of algae and it is one of the most common issue related to treated wastewater which is characterized as freshwater algae with four cell and flat surrounding a hole in the middle. Cells are shaped like triangle, ovoid or heart, mostly found with one to four spines and with or without a phytoplankton or pyrenoid of lakes [14]. Algae growth increased in the second month due to the start of summer season and then decreased due to the addition of  $ZnSO_4$  to the cooling system tank of treated wastewater. According to Lauer [18], the optimal dose for killing *Tetrastrum* sp. algae is 0.02 mg/L of  $CuSO_4$ . However, we found that 0.31 mg/L of  $CuSO_4$  is the optimal concentration for killing 50% of *Tetrastrum* sp. In addition, algae growth

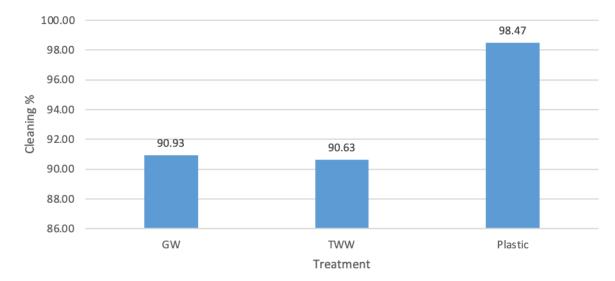


Fig. 13. Cleaning efficiency of the cooling systems in removing the algae.

was increased in the present of light compared to the dark conditions so more photosynthesis and higher growth.

Regarding microbial contaminants, treated wastewater from tap and cooling system had more bacterial growth. Pseudomonas mendocina, Stenotrophomonas maltophilia. Microbacterium liquefaciens, Pseudomonas stutzeri, Pseudomonas oleovorans and Stenotrophomonas nitritireducens are different bacteria found in treated wastewater. Whereas, Pseudomonas mendocina, Aeromonas hydrophila, Bacillus infantis, Aeromonas jandaei, Enterobacter asburiae, Enterobacter kobei, Enterobacter ludwigii and Pseudomonas alcaligenes were found in cooling system using treated wastewater. According to Al-Jassim et al. [24], treated wastewater include more pathogenic bacteria and species of bacteria were replaced by others due to changing bacteria environment. According to Wehr et al. [22] and Brooke [23], Stenotrophomonas maltophilia, Pseudomonas stutzeri, Pseudomonas oleovorans, Stenotrophomonas nitritiredu-Pseudomonas mendocina, Enterobacter kobei cens. and Pseudomonas alcaligenes are soil and water bacteria and are considered as a pathogenic bacterium. According to Al-Jassim et al. [24], microbial risk will decrease when plants are irrigated for 20 min per irrigation and four times per week. In addition, reducing storage time in the tank will reduce the growth of pathogenic microbes. For air contamination, bacteria growth was higher within a distance of 6 m from cooling pad, which means it was close to the cooling fans and may enter the greenhouse through the fans.

### 3. Conclusion

This study indicates that treated wastewater is more productive for agricultural production compared to groundwater. Omani rose, Pakistan roes, Syrian roses and lettuce grew better in treated wastewater due to the presence of some nutrients needed for plant growth. *Tetrastrum* sp. was the algae species found in cooling system of both greenhouses. Using treated wastewater in cooling system did not show any negative impacts in plant growth. However, rapid growth of algae in cooling pad of treated wastewater greenhouse was noticed compared to the groundwater cooling system. Moreover, more types of bacteria were found in treated wastewater cooling system but there transfer to human and plants were limited due to no direct contact between them. Therefore, the study recommends the use of treated wastewater in the cooling system with some application of antifouling compounds. Further studies are suggested to find out the impacts of antifouling compounds to the surrounding environments.

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