QATAR UNIVERSITY

COLLEGE OF ARTS AND SCIENCES

GRASS ESTABLISHMENT, WEED POPULATIONS AND SOIL MICROBIAL SUCCESSION IN TURF GRASS SYSTEM AS INFLUENCED BY PRODUCED WATER IRRIGATION.

BY

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ABSTRACT

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Title: Grass Establishment, Weed Populations and Soil Microbial Succession in Turf Grass System as Influenced by Produced Water.

Supervisor of Thesis: Dr. Mohammed Abu-Dieyeh.

Agricultural water use is high in a world marred with water scarcity, thus necessitating alternative water resources such as wastewaters. This study attempted to use produced water (PW) to irrigate turf grass - *Cynodon dactylon* and *Paspalum* sp. Assessment on established grasses, microbial succession, heavy metal accumulation and germination tests for weeds and turf grass seeds were conducted to evaluate the effects of PW irrigation. *C. dactylon* depicted lower tolerance while *Paspalum* sp. showed better tolerance capacity towards PW. *C. dactylon* grown from seeds under greenhouse conditions were not able to tolerate more than 30% concentration of PW. Microbial succession study presented that PW irrigation had caused changes in the fungal species present in PW irrigated soil. *Paspalum* sp. was found to accumulate higher concentrations of V and Pb in shoots and Cr, Ni and As in roots in comparison to tap water treated turf grass. Germination tests recommended irrigation with PW to be performed after establishment of turf grass. Tests also revealed that PW could encourage growth of weed - *Chloris virgata* while discourage growth of *Amaranthus viridis* and *Launaea mucronata*. This study suggests PW can be used as an alternative water resource but after further research.

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CHAPTER 1 - INTRODUCTION

The United Nations predicts that by 2050, the human population would expectedly rise above 9 billion. This increased population would add pressure on vital resources such as water. The world will face an increased demand for freshwater supplies for both agricultural and consumer use (Taft, 2015). Apart from increased population, heightened industrialization and pollution have also contributed to the formation of a situation of water scarcity in almost every continent. The World Health Organization, in 2015 reported that 748 million people globally still lack drinking water resources (Tong et al., 2017) suggesting the grave situation of water scarcity.

While the usage of available water is varied, it has been reported that 86% of the current water usage is devoted to irrigation for agriculture (Tsoutsos et al., 2013). This suggests that a hefty percentage of the limited freshwater resource available is used up for agricultural needs. In the U.S. for example, the USDA reports that agriculture is the major user for both surface water and groundwater, accounting for almost 80% of the country's consumptive water use (USDA, 2015). Around the Gulf too, water consumption for irrigation is high. This is attributed to the fact that most Gulf countries are arid lands with very low fertility hence requiring high amount of irrigation to sustain any form of agriculture. In 2015, it was reported that the state of Abu Dhabi in UAE used 56% of its water resources for agriculture. In addition, it was reported that ineffective irrigation practices such as over irrigation and use of improper irrigation systems lead to wastage and further add to over utilization of water resource for agriculture (The National, 2015). Most Gulf countries are facing similar situations. With further plans and strategies to become food secure, excessive pressure is being put at growing food locally. This requires intensive use of water and suggests that water use for agriculture in the Gulf is set to increase further.

Given such a scenario, water conservation is the answer to manage scarcity. Water re-use is an apt conservation strategy. Water reuse strategies are known to decrease fresh water system withdrawals. In addition if reuse occurs through wastewater reclamation it helps alleviate wastewater volume and the pollutant and nutrient loads that are associated with it (Garcia-Cuerva et al., 2016). On a global scale, the world is producing around 330 km^3 wastewater annually (Mateo-Sagasta et al., 2015). Researchers have been actively studying ways to channelize these wastewaters for re-use. Since wastewaters are reservoirs of nutrients and organic matter apart from water, they can be utilized for socio-economic and environmental activities which include: high water consuming process of irrigation. Given risks associated with growing food crops, non food crops such as turf grasses are ideal for wastewater treatment.

Turf grasses cover huge areas around the world. In U.S. lawns and turf grasses constitute 164,000 - 202, 000 km^2 of area. This area is three times higher than the area covered with corn (Sevostianova & Leinauer, 2014). Landscaped areas and turf grass systems provide varied environmental benefits including phytoremediation, erosion control and mitigation of heat island effects. They also provide safe, shady and cool places for athletic activities and exercise and provide area for outdoor gatherings (Sevostianova & Leinauer, 2014). Use of wastewater for turf grasses has been conceptualized in the last decades and also applied in various parts of the world. For example, in the state of Nevada in the U.S. more than 30 of the 53 golf courses are utilizing recycled water to irrigate greens, fairways and landscape plants (Wright et al., 2012).

Turf grass systems are recognized as systems well suited for irrigation with grey water, considering the range of salt concentrations that they can tolerate. Studies on turf grass irrigation with grey water are many given that grey water is known to have low pathogen levels and expectedly increase crop yields (Mohamed et al., 2014). Hence, turf grass systems provide a means to decrease use of freshwater while utilizing wastewater.

For the State of Qatar, the only source of natural freshwater resources are the ground water and precipitation. The 'Water Statistics in the State of Qatar 2013' reported that the theoretical volume of maximum exploitable groundwater is 47.5 million m^3 per year but current extractions stood at a staggering 250 million m^3 per year (MDPS, 2016). It was reported that this over extraction is lowering groundwater levels and also increasing salinity. Apart from ground water, desalination of seawater and re-use of treated sewage effluent are the other two sources of water in Qatar with each contributing 57% and 33% volume respectively as per data for the year 2012 (MDPS, 2016). Desalination of water is a costly affair requiring input of money and energy. Data provided in 'Water Statistics in the State of Qatar 2013' suggests that a significant portion of water sourced in Qatar is used in agriculture (Figure 1) i.e. a considerable amount of desalinated water is used for irrigation purposes.

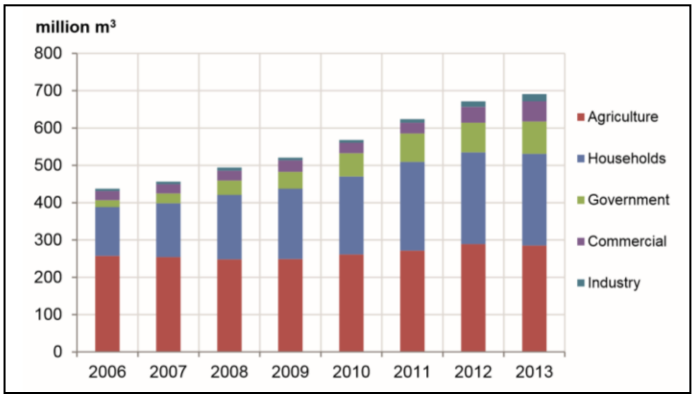


Figure 1. Water usage per sector in Qatar (MDPS, 2016)

Thus, for Qatar utilization of wastewater for irrigation of turf grasses is ideal. One type of wastewater produced in large volumes in Qatar is produced water. Qatar's major economic activity is the Oil and Gas industry, thus large volumes of produced water is produced every day. Produced water results when hydraulic fracturing is performed to extract oil and gas from deep shale formations. The process involves using large volumes of water to unblock the target formation (Li et al., 2016). The water hence generated after the process - a mix of formation water, treatment chemicals used while drilling, production, stimulation-process and oil-water separation process and with the water that is re-injected is together termed as produced water (Zheng et al., 2016). The higher the age of the reservoir, higher is the amount of water used. The typical oil to produced water ratio being 1:3 for most of the oil wells (Munirasu et al, 2016). Produced water is of concern to environmentalists and waste management institutions due to its components and large volumes. Current method of its disposal involves, injecting the water back into deep wells, which too is cost intensive. Therefore, using produced water for irrigation provides an alternative to its disposal apart from the recent alternative of attempting to re-use the produced water for new wells as 'fracturing fluids'.

Such a scenario suggests that identification of ways to utilize produced water to grow turf grasses in Qatar is required. However, such applications also have associated risks. Therefore, studying changes in microbial community, succession, diversity and abundance becomes vital to allow understanding of changes in produced water irrigated soil and associated micro biota. In addition, study on changes in weed community associated with turf grasses is also eminent. Although previous studies have evaluated and applied irrigation of turf grass with wastewater, a study testing produced water is novel and has been attempted to be assessed in this study.

CHAPTER 2 - LITERATURE SURVEY

2.1. Arid land and water security

Water security is currently a hot topic globally with discussions taking place at various conferences and forums. Steps are being recommended to ensure water availability and security in every nation. Many groups under UNESCO have dedicated their work to water security and more and more publications are being made to assess the water situation and look for solutions (Malekian et al., 2017). Global warming, pollution and population growth have threatened water resources and although 71% of the Earth's surface is covered with water, the world is seeking to save the only 0.3% that is available for human use (Dubreuil et al., 2013).

The Middle Eastern region has abundant water resources in the form of seawater but highly lacks freshwater source. The region is an arid area lacking major natural fresh water resources such as rivers and lakes. In most Middle Eastern countries, the only sources of fresh water being - ground water reserves and the infrequent precipitation. Hence, although being surrounded by seas and oceans Middle East is water insecure. Few countries (mainly in the Gulf) are dependent on the costly process of desalination to fulfill the water needs of their respective countries.

The Middle Eastern region is categorized under areas that are water deficit (Falkenmark et al., 2009). Figure 2 illustrates the expected freshwater status of countries around the world in 2050, with Middle Eastern countries including Qatar expected to have only 1-1000 m^3/capita/annum of freshwater resources (Falkenmark et al., 2009). Thus, the future of freshwater availability in the Middle East is grim.

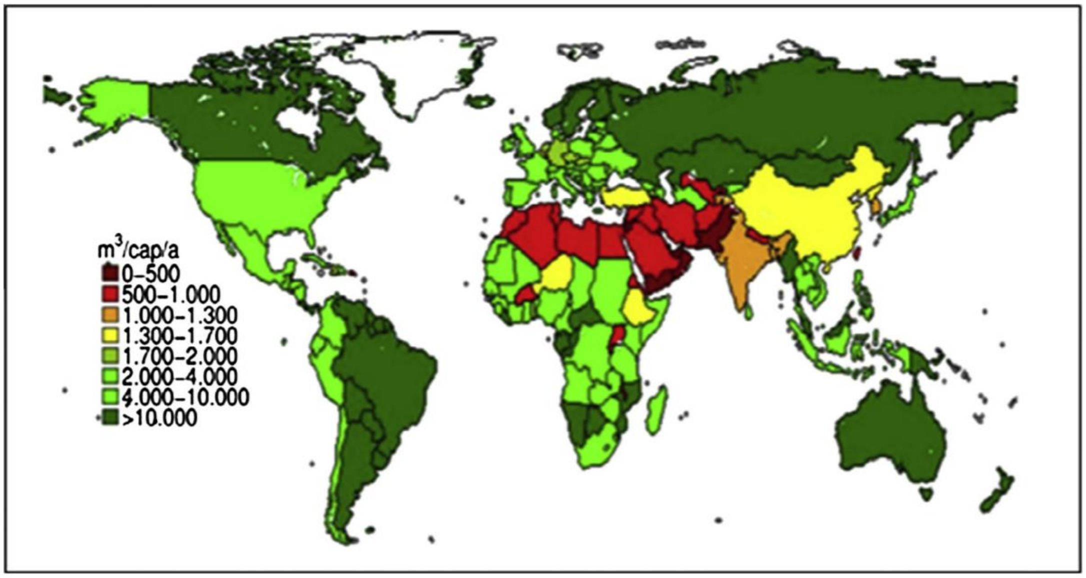


Figure 2. Expected water status of countries around the world in 2050 (Falkenmark et al., 2009)

Currently, of the low amount of water that is available in the Middle East, water usage for agriculture is particularly high given extreme climatic conditions and more importantly the lack of fertility in the region. The drought conditions and lack of essential macro and micronutrients limit productivity. Studies have indicated a lack of zinc (Zn) and iron (Fe), and an excess of boron (B) in the soils of certain countries in the region (Ryan et al., 2013). This means high input of both water and fertilizers is required to be able to produce agricultural products in the region.

This scenario applies to Qatar too. The data provided by the government shows high water input for the agricultural sector. The graph (Figure 3) shows the estimated amount of water used in agriculture in Qatar from 1990-2013. The water requirement has almost doubled from 1990 to 2013 reaching close to 300 million m^3 in the latter year. While the source has always primarily been groundwater, an insignificant portion now also comes from Treated Sewage Effluent (TSE). TSE has been incorporated as a source since 2004 to meet the growing demand for water. TSE produced in Qatar has multiplied more than 3.4 times in the last seven years

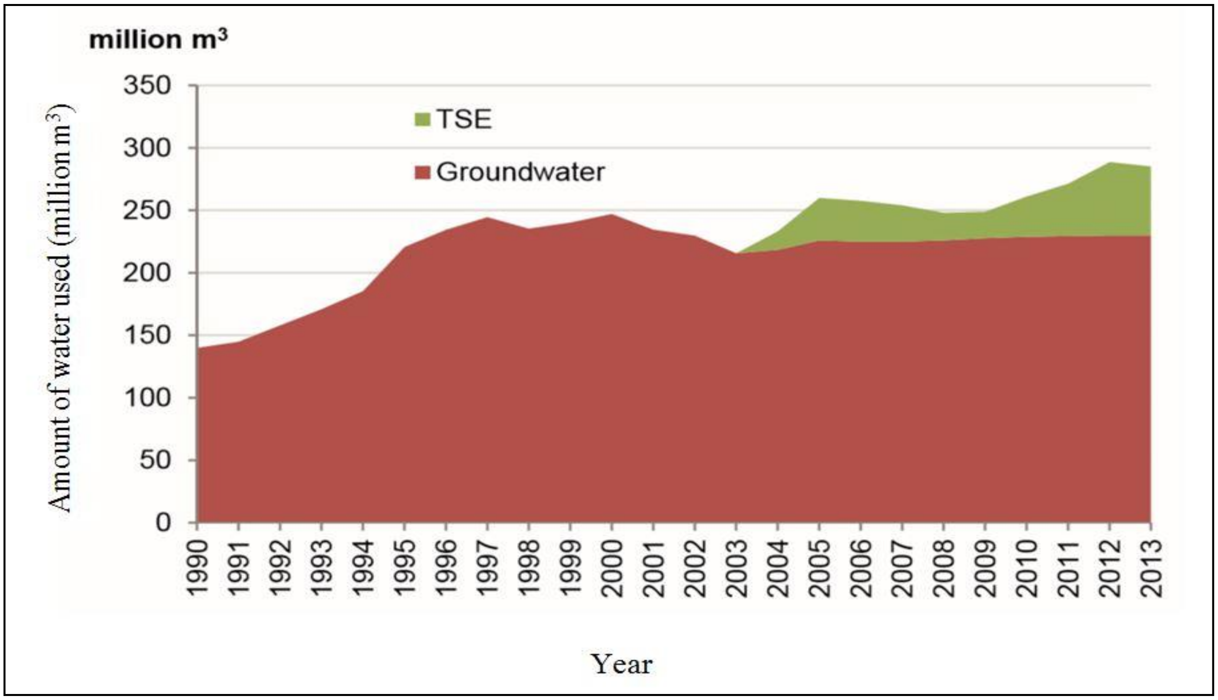


Figure 3. Qatar's water use in agriculture and its sources - 1990-2013 (MDPS, 2016)

escalating to 117 million m^3 in 2012, of which approximately 67% is used for landscaping and agricultural purposes today (Jasim et al., 2016).

Even with such high water input, the agricultural productivity in Qatar has not been significant. The water use efficiency is pretty low. As per reports to produce 1 Qatari Riyal of GDP (for agriculture), 559.1 liters of water input was required in 2013 (MDPS, 2016). This volume is staggering and suggests that high monetary and water resources have not been able to generate high productivity. Given such a scenario, it becomes imperative to look for alternate resources that lower freshwater use and also costs. The incorporation of TSE for irrigation has already been performed and is suggestive that further steps and strategies need to be formulated in this direction to achieve productivity aims without comprising freshwater use. Water re-use for irrigation is thus the need of the hour in Qatar.

2.2. Wastewater re-use in agriculture

Around the Middle East steps are being taken to re-use water for agriculture needs. Saudi Arabia has strategized to recycle and re-use more than 90% of its wastewater. The Environment Vision 2030 of Abu Dhabi also proclaims targets of recycling 100% of its wastewaters while Tunisia (in North Africa) has set goals to recharge its aquifers by irrigating more than 25,000 hectares of land with 30 million m^3 of treated wastewater (Jasim et al., 2016). Thus, while attempts are in swing to channelize water re-use, wastewater use for irrigation has been studied for long.

The most commonly utilized wastewater is the treated wastewater. It is known to contain nutrients that can enhance plant growth, decrease freshwater demand and reduce environmental impacts of wastewater disposal. In 2015, Biswas et al. (2015) reported that in under-developed countries such as Bangladesh, clean water is a highly valuable resource and use of wastewater for irrigation allows more water to be available to the public for domestic use. Therefore, they assessed the use of wastewater filtered through low-cost methods, for irrigation of Red Amaranth fields in Bangladesh. They concluded that the wastewater after filtration had the potential to be re-used for irrigation (Biswas et al., 2015).

Similarly, given the amount of wastewater being produced everyday various other attempts have also been made by researchers to investigate the use of wastewater as an irrigation source. In Nigeria where water is scarce, Abegunrin et al. (2016) found wastewater as a valuable resource that improved soil fertility and crop growth but it required pre-treatment and caution before use. Similar studies in Greece also found wastewater irrigation beneficial for growing olive trees (Petousi et al., 2015). Jeong et al. (2016) mentioned that in Korea, given the constant volume of wastewater available and the fertilizer components present in it, wastewater is being widely used in the country to irrigate paddy fields.

Khaya senegalensis, commonly known as African mahogany that gives excellent timber was attempted to be grown using wastewater sourced from sewage (Ali et al., 2013). The researchers tested both primary and secondary treated wastewater and evaluated the chemical composition and vegetative growth of the *K. senegalensis*. Assessment conducted upon 6, 12 and 18 months of treatments showed that primary wastewater had significantly improved parameters of plant growth such as fresh and dry weights of shoots/roots/leaves, leaf area, stem diameter and plant height. This had been caused due to improved soil properties and enhanced uptake of Pb, Cd, Na, K, P, N, Ni and Fe in the parts of the plants. Secondary wastewater's effects in terms of improvement were lower than that of primary but higher than tap water. The authors suggested that sewage wastewater could be used as an alternative source for growing *K. senegalensis*. In addition, given its high absorption capacity, it could be used as a phytoremediator in areas that have heavy metal polluted soils with double benefit of controlling land degradation and also improving biomass of wood.

Similar to this study, a previous research conducted in 2011 in Egypt, had also assessed effects of primary and secondary sewage wastewater on *Swietenia mahagoni*, another species of mahogany (Ali et al., 2011). Pot experiments treating mahogany seedlings and noting effects at 6, 12 and 18 month durations, depicted results that were highly similar to the study of Ali et. al (2013). Primary effluent had caused improvement in fresh and dry weights of shoots/roots/leaves, leaf area, stem diameter and plant height while also causing high total uptake of Fe, Ni, Pb, K, P, N and Cd. Here again secondary effluent proved to be more beneficial than tap water but lower in comparison to primary effluent. They concluded that growing mahogany trees with the sewage effluent was a safe and economically sound way to dispose of wastewaters while also improving timber production (Ali et al., 2011; 2013).

However, the topic of concern with using wastewater for irrigation is the ability of the plants to withstand and be able to grow using it. The quality of wastewater used and the concentration of its components significantly determines plant growth, they either encourage it or suppress it. The higher concentrations could either support and enhance growth or could prove to be detrimental. Also, different species of the plant are expected to grow differently. Amongst the same species too, seedlings of certain age may be able to withstand wastewater higher than other age seedlings. Hence, these scenarios require investigation.

Both positive and negative effects of wastewater use have been reported. Wastewater is known to commonly increase N, P, K, Mg and organic carbon (OC) thus being advantageous in certain aspects. In 2015, researchers in the African nation of Djibouti reported that the dry and arid climate of the country had caused farmers to use wastewater as a resource for irrigating crops (Abdoulkader et al., 2015). The researchers undertook a study to determine the effect the wastewater had on *Panicum maximum*, grasses commonly grown in the African continent. They found that irrigation with wastewater improved the stem height and aerial dry matter as compared to potable water. While such reports suggest the use of wastewater, others add negative connotations to wastewater mainly due to bioaccumulation issues (Abdoulkader et al., 2015).

Bioaccumulation of components sourced from the irrigation water is a common phenomenon. For instance, researchers depicted that lettuce plants irrigated with wastewater containing antibiotics, were taken up by the plant and this uptake was dependant on environmental factors such as soil (Zhang et al., 2016). With wastewater, the danger of bio-accumulation could be higher given that its chemical components include organic compounds, metals and heavy metals. These could include iron, barium, lead, manganese and zinc (Veil et al., 2004). While, accumulation could deter health of plants it could also serve as a risk to humans and animals.

Constraints in obtaining freshwater for irrigation has caused China to utilize wastewater to irrigate farmland in the last three decades. A case study was undertaken in the country to understand the long-term impact of wastewater irrigation on heavy metal concentrations in both plants and soil (Meng et al., 2016). The result suggested that the soil that was irrigated with the wastewater had high concentrations of Hg, As, Ni, Cr, Cu, Zn, Pb and Cd in comparison to soil irrigated with freshwater. Wheat plants to which the wastewater was applied had accumulated Pb, Cr, Cd and As in the roots suggesting high absorption capacity of the roots for these metals. The authors suggested that in order to ensure food safety the use of wastewater requires continuous monitoring and also control of pollution is important (Meng et al., 2016).

An Indian study evaluated accumulation of metal in tomatoes and soil that were irrigated with wastewater (Alghobar & Suresha, 2015). They reported that concentrations of SO_4 , Mg and Ca were higher than non-wastewater treated sites. The total nitrogen content in the soil irrigated with the wastewater and the treated wastewater was much higher than groundwater irrigated site. The tomato crops grown on wastewater irrigated sites had significantly higher concentrations of Zn, Na, K, total N and P, and Ca mg/kg in comparison to groundwater treated crops. They concluded that the quality of the wastewater was the determinant of the accumulation process.

In another study in Saudi Arabia, Balkhair and Ashraf (2016) also studied bioaccumulation in okra crops that were irrigated with wastewater during a year long period (2010-2011). The researchers reported that concentrations of Cr, Cd, Ni and Pb were above the allowable limit in the edible parts in 63%, 83%, 90% and 28% of the samples respectively. The authors stated that the bio-contamination was higher as compared to other studies conducted in Saudi Arabia and around the world and indicated that utilization of vegetables grown using that wastewater was a source of slow human poisoning by heavy metals.

Mahmoud and Ghoneim (2016) in Egypt, evaluated heavy metal contamination in El-mahla El-Kobra area that had discharges of untreated wastewater. They reported that shoots of rice and maize grown in soils that were irrigated with these discharged waters, bioaccumulation factors for Mn, Cu, Pb, Zn and Cd were more than 1.0. In addition, contamination levels in *Phragmites australis, Cynodon dactylon* and *Typha domingensis* were also reported to be high. Therefore, the authors suggested that these species could be used as hyper accumulators to decontaminate waters (Mahmoud & Ghoneim, 2016).

Spinach plants (*Spinacia oleracea* L.) through a greenhouse study were evaluated for their heavy metal accumulation when irrigated with sewage sludge (Eid et al., 2016). The spinach root and shoot accumulated all tested heavy metals except lead. The concentrations of the heavy metals were below phytotoxic levels except for iron and chromium. However, based on the translocation factor (TF), the authors stated that heavy metal transport to the edible parts was restricted and hence the sewage could be used as fertilizer to grow spinach in Saudi Arabia (Eid et al., 2016) .

A study conducted in Pakistan reported that poor economic conditions had forced farmers in the country to irrigate their fields using wastewater (Raja et al., 2015). The authors analyzed wastewater irrigated crops to determine heavy metal accumulation. Gourd, cauliflower, spinach, cabbage, mustard leaves, maize, berseem, sugarcane, wheat, lucerne, rice were reported to have Zn, Pb, Mn and Cr concentrations higher than recommended safe limits. Thus, the researchers concluded that by using wastewater for irrigation, farmers were not only risking their health but also the health of consumers by supplying heavy metal contaminated vegetables and crops (Raja et al., 2015).

Carrots, radish and lettuce irrigated with treated municipal wastewater in UAE, were found to have accumulated higher concentrations of Zn, Cu, Fe and Cr but concentrations were found to be lower than standards set by EU and WHO (Qureshi et al., 2016). The trend for bioaccumulation factors (BAF) of heavy metals followed the trend Fe > Zn > Cu > Cr which if compared to trends of estimated dietary intake (EDIs) are Fe > Zn > Cr > Cu. The low uptake of heavy metals by the vegetables suggests that there insignificant risks to human health from metal accumulation. Also, since accumulation of the heavy metals is dependent on soil properties, nutrient management and absorption capacity of the vegetables, human exposure and risk to contamination of heavy metals can be reduced significantly by choosing appropriate crops (Qureshi et al., 2016).

Hence, in order to properly utilize wastewater, a balance needs to be struck between the advantages and disadvantages. Based on the afore mentioned studies one can interpret that utilizing wastewater to grow non food crops such as turf grass, would limit it's usage in growing human consumables thereby diverting wastewater irrigation to safer options and reducing human exposure and risk. In addition, cultivation of forage crops too should be avoided as bio-accumulation may affect the livestock that feed on them.

2.3. Wastewater and soil microbial system

Wastewater, apart from bringing about changes in the soil structure may as well cause changes on the soil micro biota. Risks of soil pollution at the sites irrigated with the wastewater are also probable, thereby affecting soil quality.

Soil micro biota, establish themselves through complex networks and interactions between the biotic and abiotic factors. The exogenous micro biota that are introduced through the use of the wastewater can create a competition with endogenous micro biota. This competition can also lead to elimination of the endogenous species. The introduced water can additionally cause physio-chemical changes in soil. This too could cause a change in the microbial community in the region (Becerra-Castro et al., 2015). Bacteria and fungi are key players in maintenance of soil fertility and health. They form the foundation of the biotic community structure. Therefore, studies to understand the changes that the use of wastewater would bring to these microbial communities have been imperative.

Microbial succession in a system is common when inputs to the system are modified. When a system is begun to be provided with wastewater it adds newer input such as excess nutrients and heavy metals. These are bound to cause changes in the microbial community, wiping out certain communities while encouraging the growth of others. Microbial succession has been studied in various systems. Faryal et al. (2007) reported that use of textile wastewater altered soil chemistry and also caused changes in both bacterial communities and also in Vesicular Arbuscular Mycorrhizae (VAM).

Tian et al. (2014), studied microbial succession in reed rhizosphere soils that were irrigated with oil-polluted water. It was reported that after 5 months of irrigation, the oil contents of the water had caused decrease in the abundances of actinomycete, bacteria and fungi in the reed rhizosphere soils. However, given the contents of the irrigation water such as Poly Aromatic Hydrocarbons (PAH), the water promoted the growth and reproduction of micro-organisms capable of hydrocarbon degradation. They also reported that although the bacterial community structure had not been affected much but the dominant flora had been modified. Phyla such as *Cyanobacteria, Firmicutes and Actinobacteria*'s growth and reproduction were reduced by the oil pollution. In totality, they concluded that the pollution had caused the simplification of the functions present in the communities of the micro-organisms (Tian et al., 2014).

Guo et al. (2017) studied the effects that use of reclaimed water had on the chemical properties of soil. In addition, they also studied changes in microbial community of the irrigated soil. The experiment was performed through collection of soil samples from *Brassica campestris* l.sp. grown in greenhouse pot culture. They reported that use of reclaimed water caused the soil to have higher electrical conductivity (EC) and also caused higher water retention thereby increasing the soil water content. More importantly, they suggested that the type of irrigation water used could highly influence the structure of soil microbial community. Their findings showed that soil irrigated with reclaimed water led to abundance of Bacteroidetes, Gemmatimonadetes and Proteobacteria as compared to soil irrigated with clean water.

In another study, succession was assessed in an area in China where tidal wetlands had been converted to paddy field microbial community changes were studied. The authors reported that there were changes in bacterial community composition and soil physiochemical characteristics in an orderly manner. The succession of bacterial communities was linked with significant increase in relative abundance of *Firmicutes* and *Alphaproteobacteria* and significant decrease in *Planctomycetes* and *Gammaproteobacteria.* In addition, the long term rice cultivation caused enrichment of populations such as *Clostridiaceae* and *Rhodospirillaceae.* The authors rationalized that the change in cation exchange capacity and pH due to the paddy soil development caused the shift in bacterial community structure (Ding et al., 2016)

Truu et al. (2009) also reported similar changes of soil microbial parameters when secondary treated wastewater was used to irrigate short-rotation willow coppice. Two samplings over a period of 3 years showed that the irrigation regime had increased microbial community diversity and affected the biochemical and chemical properties of the irrigated soil. Succession has been commonly observed in bioremediation studies too. A site contaminated with weathered petroleum hydrocarbons was supplemented with nutrients. Aerobic heterotrophic bacterial counts, soil water content and total petroleum hydrocarbons were determined. The authors reported a sharp rise in plate counts during the beginning 3 weeks and a slowdown in the remaining 21 weeks. Based on their results they concluded that different phases of petroleum degradation were associated with specific phylotypes of bacteria (Kaplan & Kitts, 2004).

2.4. Produced water

The oil and gas industry has been booming in Qatar and has been the prime reason behind the high GDP growth. Petroleum production has increased to 3,163,000 barrels/day (US EIA, 2016). Techniques used to extract oil and gas are varied with unconventional techniques such as hydraulic fracturing being used these days. This technique requires millions of gallons of water that has been integrated with chemical additives to be injected in the oil well. The additives are mostly surfactants that help oil to release from the rock matrix while high pressure water that contains particulates and other additives is used to increase the porosity of rocks (Venkatesan &

Wankat, 2017). The water injected comes back through the well bore to the surface and is termed as flowback water. However, it gets mixed with the naturally present water of the reservoirs (known as formation water) before returning. This formed mixture is termed as 'produced water' (Torres et al., 2017).

Produced water is the largest stream of waste generated in the oil and gas industry and is regarded as the primary source of pollution in the industry. With each barrel of oil/gas, three barrels of produced water are generated (Fard et al., 2017). Hence as per this ratio, given that 3,163,000 barrels/day of petroleum is produced in Qatar (US EIA, 2016), one could estimate that 9,489,000 barrels/day of produced water should be produced in Qatar. Worldwide it has been reported that produced water production would reach to more than 15 billion gallons per day by 2017. Data suggests that generation of produced water in U.S alone is 1 trillion gallons per year (Camarillo et al., 2016).

Produced water is known to have unique characteristics due to its components. Its constituents also make it difficult to dispose it or recycle it. Commonly, produced water is known to contain - organics, dissolved salts and also naturally occurring radioactive materials (NORM). However, the composition of water may differ from well to well/place to place. The geological location, chemicals employed, operating conditions of the field, hydrocarbons produced and the lifetime of the field determine the water's physical and chemical properties (Sheikhyousefi et al., 2017). For example, cooler reservoirs are expected to have lower TDS as compared to hotter reservoirs (Stewart & Arnold, 2011).

2.4.1. Chemical composition of produced water

Produced water contains organic compounds, dissolved salt, suspended solid particles, emulsified oil and fracturing chemical compounds. The suspended solids include asphaltenes, formation solids, scale and corrosion products, bacteria and waxes (Li et al., 2016). They also include metals and heavy metals in addition to petroleum hydrocarbons such as benzene, toluene, ethyl benzene and xylene (BTEX) (Zheng et al., 2016; Zha et al., 2017). Other studies also report presence of dissolved gases, production chemicals and dissolved formation minerals (Chew et al., 2017). Multiple inorganic salts are also present such as $CaCl_2$, $MgCl_2$ and NaCl. The salinity resulting from these may be as low as few ppm to as high as 300g/L (Sheikhyousefi et al., 2017).

Reports suggest that produced water also contains high concentrations of barium (Ba). In addition, sodium (Na^+), lithium ions (Li^+), magnesium (Mg^{2+}), calcium ions (Ca^{2+}) and strontium ions (Sr^2+), silica (SiO_2), chloride (Cl^-) and sulphate (SO_4^{2-}) are also generally present in high concentrations (Fard et al., 2017; Venkatesan & Wankat, 2017). Boron too is suspected to be present in produced water as it is usually used as a cross linker. Its concentration can range from lower than 5 mg/L to as high as 400 mg/L (Floquet et al., 2016). The produced water obtained from an oil field in the State of Sergipe, Brazil was reported to contain 0.003-4540 mg/L of metals, 0.9-10.3 µg/L of PAHs and 96.7-1397 µg/L of BTEX (Dórea et al., 2007). The TDS in produced water can be as high as 400,000 mg/L while typically sea water has a TDS of about 35,000 mg/L (Li et al., 2016).

In Qatar, produced water samples were analyzed for different metals and other chemical constituents (Atia et al., unpublished data, 2017). The water analyzed was the same water used in this study. Varied concentrations of the produced water (diluted using tap water) sourced from TOTAL company's Halul Island station were analyzed. TDS values, pH, chemical constituents, metal concentrations were measured. A comparison of the values obtained for Qatari produced water with data provided by Collins (1979) and Fakhru'l-Razi (2009) is provided in Table 1, 2 and 3 (Atia et al., unpublished data, 2017).

Table 1

A Comparison of Chemical Constituents of Produced Water and Tap Water (Atia et al., unpublished data, 2017)

All parameters in mg/L if not mentioned beside it	Conc. of Qatari produced water							Collins, 1979	Fakhru'l-Razi et al, 2009	
	100% PW	50%PW	40%PW	30%PW	20% PW	10%PW	Tap water	seawater	Oil PW	Gas PW
EC (mS/cm)	240	175.0	116.6	85.1	63.5	33.6	0.1785			4200-180000
TDS	310,000	155,000	124000	93093	62000	31000		35000	1,200 – 10,000	2600 - 310000
TSS	6760	3380.0	2704.0	2030.0	1352.0	676.0			1.2 - 1000	14 - 800
pH	6.54	6.6	6.7	6.7	6.7	6.7	7.5		4.3 - 10	4.4 - 7
F	4	2.0	1.6	1.2	0.8	0.4	ND			
Cl	122,000	61000.0	48800.0	36636.6	24400.0	12200.0	5.1	19,353	80 – 200,000	1,400 - 190,000
Br	710	355.0	284.0	213.2	142.0	71.0	<0.1			150 - 1149
NO_3	500	250.0	200.0	150.2	100.0	50.0	ND			
PO_4	4	2.0	1.6	1.2	0.8	0.4	ND			
SO_4	50	25.0	20.0	15.0	10.0	5.0	1	2,712	2 - 1,600	<0.1 - 47
CO_3	134	67.0	53.6	40.2	26.8	13.4	ND	142	77 - 3,990	
Na	61,000	30500	24400	18318	12200	6100	3	10,700	132 – 97,000	520 - 120,000
K	1850	925.0	740.0	555.6	370.0	185.0	0.15	387	24 – 4,300	149 - 3870
Ca	10,700.00	5350.0	4280.0	3213.2	2140.0	1070.0	39.2	416	13- 25,800	9,400 - 51,000
Mg	2,200	1100.0	880.0	660.7	440.0	220.0	2.5	1,294	8 – 6,000	0.9 - 3,900
NH_4	126	63.0	50.4	37.8	25.2	12.6	ND		10 - 300	
SAR (meq/L)	139.94	69.97	55.98	42.03	27.99	13.99	0.13	58.01		
Ionic strength	3.79	1.91	1.50	1.14	0.76	0.31	0.00232	0.695		

Table 2

A Comparison of Metal Concentration in Produced Water and Tap Water (Atia et al., unpublished data, 2017)

Unit mg/L	Conc. of Qatari produced water							Fakhru'l-Razi et al., 2009	
	100% PW	50% PW	40% PW	30%PW	20%PW	10%PW	Tap Water	Oil filed PW	Gas PW
Li	4	2.0	1.6	1.2	0.8	0.4	0.011	3 - 50	18.6 - 235
B	38.6	19.3	15.4	11.6	7.7	3.9	ND	5 – 95	ND - 56
Ba	5.5	2.8	2.2	1.7	1.1	0.6	0.013	1.3 - 650	ND - 26
Bi	0.3390	0.1695	0.1356	0.1018	0.0678	0.0339	ND		
Al	0.1360	0.0680	0.0544	0.0408	0.0272	0.0136	ND	310 - 410	0.5 - 83
As	0.0137	0.0069	0.0055	0.0041	0.0027	0.0014	ND	<0.005 - 0.3	0.004 - 151
Ag	0.0240	0.0120	0.0096	0.0072	0.0048	0.0024	ND	<0.001 - 0.15	0.047 - 7
Cd	0.0007	0.0004	0.0003	0.0002	0.0001	0.0001	0.0002	<0.005 - 0.2	<0.02 - 1.21
Co	0.0009	0.0004	0.0004	0.0003	0.0002	0.0001	0.001	ND – 0.010	
Cr	0.0111	0.0056	0.0044	0.0033	0.0022	0.0011	0.001	<0.02 - 1.1	ND - 0.03
Cs	0.0240	0.0120	0.0096	0.0072	0.0048	0.0024	ND		
Cu	0.0182	0.0091	0.0073	0.0055	0.0036	0.0018	ND	<0.002 - 1.5	ND - 5
Fe	0.8414	0.4207	0.3366	0.2527	0.1683	0.0841	ND	<0.01 - 100	ND - 1100
Mn	0.2760	0.1380	0.1104	0.0829	0.0552	0.0276	0.0075	< 0.004 – 175	0.045 - 63
Pb	0.0525	0.0263	0.0210	0.0158	0.0105	0.0053	0.009	0.002 - 8.8	<0.2 - 10.2
Sr	750	375	300	225	150	75	0.021	0.02 - 1000	ND - 6,200
V	0.01	0.005	0.004	0.003	0.002	0.001	ND	ND – 0.290	
Zn	0.063	0.0315	0.0252	0.0189	0.0126	0.0063	0.619	0.01 - 35	0.02 - 5

Table 3

A Comparison of Hydrocarbon Constituents of Produced Water (Atia et al., unpublished data, 2017)

Unit mg/L	Conc. of Qatari produced water							Fakhru'l-Razi et al., 2009	
	100% PW	50% PW	40% PW	30%PW	20%PW	10%PW	Tap Water	Oil filed PW	Gas PW
Benzene	0.0395	0.0198	0.0158	0.0119	0.0079	0.0040	ND	0.39 - 35	0.01 - 10.3
Toluene	0.0720	0.0360	0.0288	0.0216	0.0144	0.0072	ND		0.01 - 18
Ethyl Benzene	0.0300	0.0150	0.0120	0.0090	0.0060	0.0030	ND		
Xylene	0.0150	0.0075	0.0060	0.0045	0.0030	0.0015	ND		
Total Diesel	0.1180	0.0590	0.0472	0.0354	0.0236	0.0118	ND	N/a	
Total PAHs	0.2925	0.1463	0.1170	0.0878	0.0585	0.0293	ND	0.04 to 3 (Jerry et al., 2011)	
TOC	2430	1215.0	972.0	729.7	486.0	243.0	n/a	0 - 1,500	67- 38,000
BOD	10	5.0	4.0	3.0	2.0	1.0	n/a		75 - 2,800
COD	8983	4491.5	3593.2	2697.6	1796.6	898.3	n/a	1,220	2,600 - 120,000
Phenols	165.5	82.8	66.2	49.7	33.1	16.6	n/a	0.009 - 23	
CN	0.50	0.25	0.20	0.15	0.10	0.05	n/a		

2.4.2. Treatments of produced water

Countries that are water stressed but endowed with oil and gas reserves are trying to find ways to protect their freshwater resources by cost effectively and efficiently treating produced water. Releasing produced water to the environment has been widely criticized due to the pollution that is caused in underground water, water ways and soil. Management of produced water is a complex task given it's afore mentioned components.

Physical, biological and chemical methods such as filtration, sand filtration, demulsifiers and electrochemical processes have been used. Sometimes treatments utilize a combination of methods. Traditionally management techniques involved- re-injection into the same formation well or another suitable well, release in the environment and treatment of the water followed by reuse by the industry itself. The re-injection is an expensive process costing about US $ 0.3-10 per barrel (Li et al., 2016). In recent years, produced water for beneficial purposes such as for irrigation and for consumption have also taken centre stage.

Biological treatments are considered highly effective. However, the salinity of the produced water often limits this type of treatment. Use of microbial fuel cells (MFC) that simultaneously clean produced water while also produce electricity is widely studied (Sheikhyousefi et al., 2017). Basic treatment steps involve use of gravity settling that allows removal of particulate matter, separation of gas and recovery of oil. Additionally, coagulation-flocculation or dissolved air/gas flotation are also used. To remove residual oil nut shell filters are utilized as coalescing media (Camarillo et al., 2016). Electrocoagulation and hydrocyclones are other commonly used techniques but are high in operation costs due to requirement of high voltages (Venkatesan & Wankat, 2017).

In the past decades common treatment methods for produced water have consisted membrane processes. These include forward osmosis (FO), nanofiltration (NF), ultrafiltration (UF) and microfiltration (MF) (Chew et al., 2017). Newer technologies are also emerging. Membrane distillation for instance is being tested to treat produced water. It is proclaimed to use lower-grade waste heat and produce high quality water (Chew et al., 2017). In this technique, water vapors pass from a higher temperature section to lower temperature section through a membrane that is porous. The vapors are driven through due to the difference in vapor pressure. Membrane distillation has varied advantages. It requires moderate operating costs, has high recovery, is expected to completely remove non-volatile components and requires low operating hydrostatic pressure. In addition, as mentioned above it uses lower-grade waste heat which are abundantly found in oil and gas industries. The membrane distillation has four varied configurations, however, direct contact membrane distillation (DCMD) is the one most studied one (Chew et al., 2017).

Hybrid electrobiochemical treatments to remove hydrocarbons from the produced water are also being tested these days as they simultaneously remove sulfate and the petroleum hydrocarbons (Mousa et al., 2016). Application of ceramic membranes is also being attempted and are being favored as the process is robust and has good oil and grease separation efficiency (Weschenfelder et al., 2015).

As mentioned previously, since barium is particularly high in produced water, treatment of produced water involves barium removal. This is because barium causes formation of brite scale formation during injection and affects the environment tremendously (fish and aquatic organisms absorb barium) if the water is released without treatment to the environment. Methods used to remove barium include - precipitation, adsorption, ion exchange and membrane filtration.

Recent studies are propagating use of nano carbon technology for effective barium adsorptions (Fard et al., 2017). Given high salinity, newer desalination techniques such as hydrate based desalination is also being suggested (Fakharian et al., 2017).

2.4.3. Use of produced water in agriculture

As mentioned the wastewater readily available in Qatar is the produced water. Produced water is normally injected back into the petroleum reserves. However, returning produced water back into the ground has been associated with inducing seismic activity and frequent earthquakes in Texas and Okhlahoma (Pica et al., 2017). Both these states have a significant number of injection wells. Therefore, stakeholders handling produced water are looking for ways to channelize this water for safer and good use.

However, usage of produced water for irrigation is a new concept and thus studies on the topic are recent and few. A new study in U.S. has attempted to utilize produced water for irrigation in similar fashion as this current study (Pica et al., 2017). Given high salinity and total organic content (TOC) levels of produced water the authors tested non-food biofuel crops - Switch grass (*Panicum virgatum* L.) and Rapeseed (*Brassica napus* L.). These two crops are known to be salt tolerant plants. In a greenhouse experiment, the crops were irrigated with four different TOC concentrations and three different total dissolved solids (TDS) concentrations.

The authors investigated seedling emergence, plant height, plant uptake, biomass yield and leaf electrolyte leakage. They reported that the highest concentrations tested (salinity and TOC), significantly lowered growth, health and physiological characteristics of both species. The organic content of the produced water negatively affected physiological parameters and biomass yield of both crops. The authors concluded that removal of TOC to below 5 mg/ml and organic matter removal to less than 50 mg/ml to be able to utilize the produced water to maintain a sustainable biomass production rate (Pica et al., 2017).

Burkhardt et al. (2015) studied effects of produced water sourced from the Powder River Basin in Wyoming and Montana in U.S. They studied soil characteristics, secondary metabolites and plant biomass. A 2-year field study was conducted to study the effects of the produced water on switchgrass and corn, and four biofuel species - lemongrass, Japanese corn mint, common wormwood and spearmint. The results obtained showed that essential oil content in spearmint and lemon grass were significantly affected by produced water. The oil levels in spearmint in two harvests were significantly different suggesting differences based on growth stage. They concluded that prolonged use of produced water could have long term deleterious effects on the soil and also the plants, except if the produced water was treated or diluted with clean water (Burkhardt et al., 2015).

Similar to produced water, researchers have also worked on coal bed methane water (CBMW) that is formed in a manner similar to that of produced water. Coal bed methane is filled with water and hence to reduce pressure and release methane, the water is required to be pumped out and this is known as CBMW. CBMW is known to have high concentrations of sodium ions, sulfur ions, magnesium ions, sulfate ions, calcium and bicarbonate ions. Zheljazkov et al. (2013) tested the effect of irrigation of CBMW on peppermint (*Mentha x piperita* L.) and spearmint (*Mentha spicata* L.) crops. 0% (tap water), 25%, 50%, 75% and 100% of CBMW were tested. The researchers reported that 50% CBMW conc. and below did not suppress fresh herbage yields while 75% and 100% conc. decreased fresh herbage yields of both species and also the oil yields for peppermint as compared to the control.

2.5. Turf grass systems

2.5.1. Importance of turf grass vegetation

Turf grasses add green value to our surrounding. They provide areas for leisure and sport activities and more importantly contribute to carbon sequestration. They are grown in sports arenas such as sport fields and golf courses. Homes, schools, parks and public areas are commonly laid with turf grass. In addition, they are also used for land reclamation activities in areas that have been contaminated or are industrial sites. They occupy spaces around highways, roadsides and airfields. They also are key in terms of aesthetics. They add aesthetic value to areas and attract people for activities such as camping, hiking and photography. In recent times, turf grasses are being experimented as a means of renewable energy production. A replacement of hydrocarbons is being researched through obtaining biomass from turf grasses (Kopecký & Studer, 2014). Some species of turf grass such as *Cynodon dactylon* are known to have additional properties such as wound healing properties. *Cynodon dactylon* is categorized as medicinal plant and is used to treat skin diseases, diarrhea, burning sensations, vomiting and fever in Ayurveda. It is known to contain compounds such as terepenoids, alkaloids, palmitic acid and vitamin C. In addition, it also contains volatile oils, phenolics, flavonoids such as orientin, vitexin, apigenin and carotenoids such as neoxanthin, beta-carotene (Biswas et al., 2017).

Turf grasses today, are an important vegetation. Their coverage area is on the rise with more and more landscaping and sport projects. Water requirement for turf grass hence would simultaneously increase. Given the water scenario around the world and especially in Middle East, it becomes imperative to find alternative water resources to irrigate turf grass. Alternative sources such as wastewater for growing turf grass hence seems an apt strategy. However, most wastewaters have increased concentrations of salts apart from heavy metal content. Wastewater such as the produced water are specifically known to have high salinity due to the mixing of the water with seawater. Although some species are known to be highly salt tolerant utilization of wastewater for turf grass irrigation is challenging.

2.5.2. Use of waste water in turf grass agriculture

In most arid climates, turf grasses can grow all year round thereby allowing high wastewater utilization. Additionally, turf grass's requirement of water is very high which allows higher utilization of wastewater effluent. The high root and shoot density allows increased removal of pollutant from the wastewater. More importantly, turf grasses allow use of wastewater to grow non food crops hence reducing public risk (Kenna, 1994). Hence, irrigation of turf grasses with wastewater has been performed worldwide in the last few decades. As a whole, wastewater reuse has increased globally. In California alone, in 2009, 894 million m^3 of reclaimed water was reused. In Beijing too, in 2010, 680 million m^3 of wastewater was reused and accounted for almost 20% of water supplied to the entire city (Chen et al., 2013). Aside from saving freshwater, use of reclaimed water provides soils with organic matter and nutrients. Reports from both Beijing and California have shown that reclaimed water use improved soil enzyme activities that involve soil microbial biomass carbon and nutrient cycling. Hence, use of reclaimed water for irrigation can promote sustainability of soil, particularly barren urban soils (Chen et al.,2013).

Wastewater is often referred to as fertile water as it supplies turf grass with low amount but constant dosage of nutrients. It has been reported that most irrigation strategies add 24.5 to 97.8 kg of nitrogen per hectare from the wastewater (King et al., 2000). Notably, turf grass systems are very effective in utilizing this nutrient source and hence reduce the need for fertilizer application. This reduced fertilizer application increases efficiency of nutrient uptake and more importantly decreases the environmental load of nutrients added to the soil (King et al., 2000). Treated wastewater use to irrigate turf is also on the rise in US. Sidhu et al. (2015), reported that treated wastewater contains Endocrine Disrupting Compounds (EDCs). Irrigation with treated wastewater hence may increase exposure of EDCs to humans. They studied exposure risk and reported that risks could be decreased if contacts with turf grasses that have been irrigated recently are avoided.

Studies pertaining to the irrigation regimes, their effect, assessment of turf grass growth and suitability have been carried out far and wide. The wastewaters used and studied include - reclaimed water, grey water and treated waste water effluents. Mohamed et al. (2014) studied the effects that grey water (sourced from laundry and bath tub) had on turf grass. They specifically wanted to assess nutrient leaching and hence treated turf grass with three different irrigation regimes - untreated bath tub water, untreated laundry water and 100% potable water. The species of grass studied by the researchers was *Cynodon dactylon* L. Using mass balance, the authors studied nine elements - Na, Cl, P, K, Mg, Ca, Zn, Al and B. Their findings showed that at the end of the study the soil had stored K, P, Na and Cl, while Al and Mg had significantly leached in the soil. Additionally, the turf grass had up taken B, Na and Cl which the authors concluded had affected its growth (Mohamed et al., 2014).

A study in Portugal, assessed the quality of turf grass as a response to varied irrigation regimes (Costa et al., 2011). They also studied Bermuda grass *(Cynodon dactylon)* since it is the major species of grass grown in most golf courses in southern Portugal. The irrigation regimes tested were potable water and two types of treated wastewater. Their results showed that turf grass quality was independent of salinity of water used for irrigation for electrical conductivity levels of below 2.1 dS m^{-1}. They concluded that treated final effluents could be considered as an alternative to potable water for irrigation of golf courses. Additionally, using the wastewater for irrigation provides a better environmental alternative to dumping of the water in seas, lakes and rivers (Costa et al., 2011). Singh et al. (2013) report that *Cynodon dactylon* has high ability to grow in high sodium containing - severe sodic soils. It hence can be adopted as an ecological tool for rehabilitation of lands that have been degraded.

Pilot scale studies have also been conducted to assess turf grass growth when irrigated with wastewater. A research conducted in Sicily, Italy studied the reuse of urban-treated wastewater for irrigation of Bermudagrass through a pilot-scale study that used horizontal subsurface flow system (Licata et al., 2015). The wastewater they obtained was from constructed wetlands. After irrigation, qualitative and biometric parameters of Bermuda grass, as well as physical-chemical properties of soil were studied. A comparison was done between freshwater irrigated turf grass and wastewater irrigated turf system. The authors concluded that although the wastewater treatment did not significantly increase soil pH, it did increase salinity and organic matter content thereby confirming that wastewater provided an additional source of water and fertilizer to area that lack freshwater supplies (Licata et al., 2015).

Turf grass systems have also been studied as a means to remove metal ions from wastewater. Dar et al. (2012) studied Ni(II) ion removal from wastewater using lawn grass. They aimed to study and report the adsorption effectiveness of lawn grass in Ni ions removal in order to consider it for wastewater purification. Through studying effects of temperature, contact time, metal concentration, adsorbent loading weight and pH they concluded that lawn grass is apt for adsorbing Ni ions from wastewater.

Turf grass irrigated with saline wastewater effluent can cause injury and stress due to water deficiency, ion balances as well as ion toxicity. Water stress that is induced due to salt is known as "osmotic" or 'physiological" drought. The drought causes limited water uptake that decreases photosynthesis, leaf size, rooting, carbohydrate storage and cell turgor. All of these effects could result in low performance in turf grass and lead to poor tolerance and low recovery from wear (Evanylo et al., 2010).

Some species of turf grass however, are known to be salt resistant. Turf grass species such as *Pennisetum clandestinum* Hochst, have been known to resist high salinity, thus making this grass ideal for growing using saline water and also for cultivation in salinized areas. The grass is known to have a well developed root system and high growth rate. Muscolo et al. (2013) evaluated the biochemical and physiological basis that provides the turf grass its salt resistivity. Parameters including root morphology, biochemistry, metabolism, growth, germination and nutritive properties were studied. Based on these the authors concluded that *Pennisetum clandestinum* Hochst. could germinate and grow well in salinized regions. Thus, concluding that the turf grass could be grown using recycled saline wastewater and additionally it could also be used to reclaim salt affected arid zones.

Yet, salinity tolerance of turf grass is complex. It often differs based on the stage of plant development. In addition, temperature and relative humidity both also influence plant response to salinity. It is known that plants become more sensitive to salinity when exposed to dry and hot conditions as compared to humid and cool conditions. This can be attributed to evapotranspiration demand that cause increased salt uptake. Salinity of soil is also known to differ with both time and depth. The soil salinity at the surface is known to have salinity similar to that of the irrigation water while salinity at the roots is several times higher (Marcum, 2006).

Determination of seed germination, plant survival, root weight/length, shoot weight/length, physical appearance have been commonly used to asses salinity tolerance in turf grasses (Marcum, 2006).

Water's total salinity comes from cations - sodium, magnesium, calcium and potassium and from anions chloride, carbonate/bicarbonate and sulfate (Marcum, 2006). Wastewater commonly contain high levels of the aforementioned ions thus giving it its high salinity. These ions upon accumulation are known to cause high soil alkalinity causing the pH to rise above 8.5. This could subsequently cause adverse changes on structural properties of soil that can lead to formation of compaction layer that has low infiltration capacity (Stenchly et al., 2017). In 2016, Shakir et al. (2016) reported that use of wastewater had caused soil to become highly saline hence affecting it's quality.

2.5.3. Weeds in turf grass system as influenced by wastewater irrigation

As with microbial community structure, plant structure too can be affected by the use of produced water. Weeds are a nuisance to fields of crops and also to areas where turf grass are grown. They are unwanted plants that compete with the cultivated crop for nutrients, water and space. Weeds are known to have rapid growth and maturation (Ferguson et al., 2016). More importantly, their pollination and reproduction processes happen with ease. Weeds are also adaptive, have resistance to climatic adversities with ability to resist decay of seeds. They can also remain dormant for extended period of time in the soil (Flamini, 2012). Weeds commonly associated with turf grass system include large crabgrass (*Digitaria sanguinalis* (L) Scop.), dandelion (*Taraxacum officiniale*) and ground ivy (*Glechoma hederaceae* L.) (Ferguson et al., 2016). *Malva neglecta*, knotweed, blue grass, quack grass and *Polygonum sp.* are some of the other commonly occurring turf grass weeds. Weeds require continuous treatment and management. Manual removal and use of herbicide add to management costs. A thorough understanding of the life cycle of weeds, their seed productivity and dispersal can aid in their management and minimize associated problems (Dille, 2017).

A change in the nutrient availability, soil structure and microbial community can induce a change in the weed community of the specific area. Since produced water contains organic matter and metals, the weed community structure is expected to change based on these parameters as they could lead to enhancement of weed growth.

The change in weed community would subsequently through succession, also bring about changes in species abundance in the irrigated area. The resource available may favor one species over the other with time. Galal and Shehata (2015) studied weeds associated with rice *(Oryza sativa* L.) that had been irrigated with varied water sources. Five farms were studied of which three were irrigated with wastewater and two receiving groundwater. They reported that while rice grew better with wastewater irrigation, biomass of weed species *Convolvulus arvensis* and *Echinochloa crus-galli* followed a reverse trend. Biomass of *Eclipta alba* in wastewater irrigated fields was lower as compared to *Cyperus deformis* in the same field. Water source had differentiated weed biomass (Galal & Shehata, 2015). Wafula et al. (2015) also reported similar results.

Hence, use of produced water may slowly influence the growth of certain plants over the other and bring about change in abundance of certain species as compared to the species abundance before the produced water was applied. In general, the community structure of weeds in the region is expected to be affected. Thus, studying effect on turf grass associated weeds is crucial to predict the changes brought about by the irrigation source.

Germination tests based on parameters that define the irrigation source could be an ideal way to understand changes that weeds associated with turf grass would have. If the irrigation source is produced water for instance, determination of effect of salinity, heavy metal concentration and hydrocarbons allows pre-understanding of weed dynamics. This helps to minimize issues that would come once the produced water irrigation is performed. It would allow precautions to be taken and allow better management techniques to be applied to reduce weed growth and their abundance.

2.6. Qatari turf grass systems and rationale of study

In Qatar too, turf grass systems are a common sight. Parks, sidewalks, road dividers, golf courses are laden with turf grasses. Statistical data has already proved the water insecurity of the country. Given that as an oil and gas based economy, we have an alternate source of water in hand that is not only abundant but is also readily available - produced water. Utilization of this resource for alternative purposes not only helps remove burden from fresh water resource but more importantly resolves issues of management of produced water. It additionally, helps prevent negative effects on the environment and reduces cost. Qatar's turf grass area accounts for 701,628 m^2 (Ministry of Municipality & Environment, Qatar, personal communication, 2017) which implicates the high water requirement for its maintenance.

Thus, re-using produced water as an irrigation source with specific attempt to irrigate turf grasses is an ideal task and hence, is the rationale behind this study with the objectives as listed in chapter 3.

CHAPTER 3 - OBJECTIVES OF THE STUDY

A. Assessing turf grass seed germination and turf grass species establishment that has been irrigated using produced water.

B. Investigating the effect of produced water on seed germination of weed species.

C. Evaluating effects of produced water use on soil micro biota's relative abundances and the microbial community succession upon produced water use.

CHAPTER 4 - METHODOLOGY:

4.1. Produced water samples

The produced water tested in this study was provided by TOTAL Qatar. The water was sourced from their station at the Halul Island and transferred in a big container ($1m^3$) to be kept stored outdoor, after consideration of all safety issues due to leaking (Figure 4).

In each sampling the salinity and pH was determined before experimental work. Prior to any assessment, the stock produced water samples were diluted according to the respective solution using tap water.

Figure 4. Produced water container.

4.2. Assessing the effect of different concentration of produced water on turf grass (*Cynodon dactylon)* establishment.

2 g of turf grass seeds (Cêsped gobi, *Cynodon dactylon,* Semillas fito, Spain) were sown in 30 pots (20 cm diameter) containing 60% sandy loam soil and 40% Peat moss soil. The pots were left for a period of 2 months to let grass get established prior to treatment (Figure 5). The experiment was one factor with a completely randomized design (CRD) and set up inside a greenhouse (24 ± 2 °C with 15 hr of light/day at photon flux density minimum of 350 ± 50 µmol m^{-2} s^{-1}). 6 replicates were designated for each treatment and the pots were irrigated once a week with 200 ml of the assigned treatment. Treatment volume was determined based on irrigation regimes during establishment period. The treatment levels were - 0% (tap water), 25% produced water, 50% produced water, 75% produced water and 100% produced water concentrations. The dilutions were prepared using tap water. Two weeks post treatment the irrigation was alternated

with tap water to de-stress the turf system and reduce the accumulative effects of heavy metals, salinity and other produced water components.

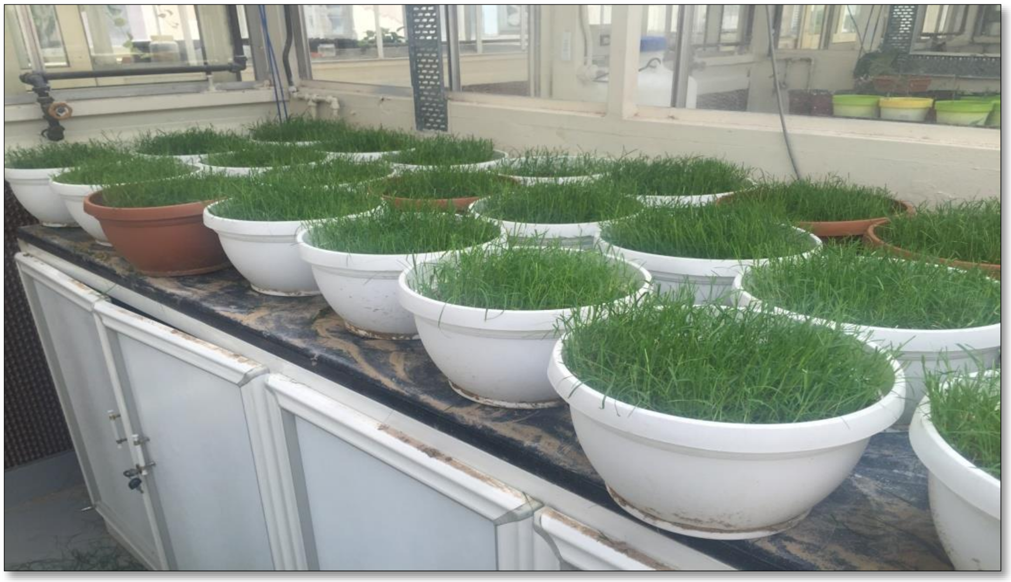


Figure 5. Established pots at pre-experimental stage.

The percentage of green biomass was visually estimated every week using a scale rank of 0-100%, 0% for no green grass in the pot, 100% for complete green and healthy grass in the whole pot and a % of green grass compared to control treatments for other results. Soil samples (~ 50 g) from a depth of 5 cm were collected for each treatment at week 3, 5 and week 7 to assess for soil micro biota. After a period of 14 weeks, the green biomass for each pot was collected and put into a labeled paper bag. The root system of each pot was carefully cleaned from soil, washed with tap water and then put in a separate paper bag. All paper bags were placed inside an oven for 3-4 days 80°C. The dry weight of the above ground biomass and the below ground root biomass were determined for each pot.

4.3. Assessing the effect of different produced water and saline water concentrations on turf grass (*Cynodon dactylon)* establishment.

Based on the results obtained for part experiment 4.2, lower concentrations of produced water were tested in order to reach the proper dilution of produced water needed to keep healthy grass. In addition, different concentrations of saline water treatments were also added in order to determine if the negative effect was due to the salinity of the produced water.

2 g of turf grass seeds (Cêsped gobi, *Cynodon dactylon,* Semillas fito, Spain) were sown in 44 pots (20 cm diameter) containing a mixture of 60% sandy loam soil and 40% Peat moss soil. The pots were left for a period of 2 months to let grass established prior to treatment (Figure 6). The experiment was one factor and 4 replications with a completely randomized design (CRD) and set up inside a greenhouse at a similar conditions like experiment 4.2. The treatment levels were: 0% (tap water), 10% produced water, 20% produced water, 30% produced water, 40% produced water and 50% produced water.

Figure 6. Established pots at pre-experimental stage.

The saline water concentrations were matched to the produced water concentrations. The calculations were made using 100% salinity as 150 g/L (average of total measured salinity of produced water). The saline water concentrations tested were - 15 g/L saline solution (designated as 1.5% S) , 30 g/L saline solution (designated as 3% S), 45 g/L saline solution (designated as 4.5% S), 60 g/L (designated as 6% S) and 75 g/L (designated as 7.5% S). The irrigation was alternated with tap water to de-stress the turf system and prevent accumulation of heavy metals and other produced water components.

The irrigation was accomplished using 200 ml per pot of the assigned treatment once a week. Two weeks post treatment, the irrigation was alternated with tap water to de-stress the turf system.

The percentage of green biomass was visually estimated) every week using a scale rank of 0-100%. 0% for no green grass in the pot, 100% for complete green and healthy grass in the whole pot and a % of green grass compared to control treatments for other results. Soil samples (~ 50 g) from a depth of 5 cm were collected for each treatment in week 3, 5 and 7 to assess for soil micro biota. After a period of 14 weeks, the green biomass for each pot was collected and put into a labeled paper bag. The root system of each pot was carefully cleaned from soil, washed with tap water and then put in a separate paper bag. All paper bags were placed inside an oven for 4 days at 80°C. The dry weight of the aboveground biomass and the belowground root biomass were determined for each pot.

4.4. Assessing the effect of different produced water concentrations and saline water concentrations on established turf grass *(Paspalum* sp.*)* on a larger scale and outdoor conditions.

Ready turf grass rolls of *Paspalum* sp. commonly used in Qatari turf grass systems were obtained upon request from the Ministry of Environment, Public parks department. 21 large pots of the size - 65 cm x 25 cm x 20 cm were procured. Although they have drainage systems the pots were designed in a way to prevent water leakage and thus had no outlets (Figure 7). 7 treatment levels were designated - 0% (tap water), 10% produced water, 20% produced water, 30% produced water, 15 g/l saline solution (designated as 1.5% S), 30 g/l saline solution (designated as 3% S) and 45 g/l saline solution (designated as 4.5% S). Three replications were assigned for each treatment. The experiment followed Completely Randomized Design(CRD) and was set up outdoors to simulate natural conditions.

The pots were irrigated twice weekly with 250 ml/pot of assigned treatment each time. The irrigation was alternated with tap water (in the third week) to de-stress the turf system. The percentage of green biomass was visually estimated biweekly using the afore mentioned method. Soil samples (~50 g) from a depth of 5 cm were collected from each pot to assess microbial abundance and diversity at biweekly intervals.

After a period of 10 weeks, the green biomass for each pot was collected and put into a labeled paper bag. The root system of each pot was carefully cleaned from soil, washed with tap water and then put in a separate paper bag. All paper bags were placed inside an oven for 4 days at 80°C. The dry weight of the aboveground biomass and the belowground root biomass were determined for each pot.

Figure 7. Pots laden with ready turf grass prior to treatment.

4.5. Assessment of microbial abundance and diversity

The collected soil samples from the above three experiments (4.2/4.3/4.4) were processed to determine changes in microbial community through time and among treatments. 1 gram of soil sample from a treatment was aseptically added to 9 ml of sterile distilled water and serial dilutions were performed to reach 10^{-3} and 10^{-4} dilutions. 1 ml of 10^{-4} dilution was plated using pour plate method on nutrient agar plates with 4 replications each. The plates were kept at 30°C for 48 h for to determine CFU of bacteria/ml.

To assess for soil fungi, 100 µl of 10^{-3} dilution was plated using Rose Bengal Agar (chloramphenicol incorporated) with 4 replications each. The plates were kept at 25°C for 5 days to determine CFU of fungi/ml. Number of colonies of each species were counted and then a colony of each species were purified in a separate plate to be used for identification purpose. All fungi were identified based on colony morphology and microscopic examination. These steps were repeated for each treatment. Species diversity and abundance calculations were performed for experiment 4.4. only due to high number of samples and time limitation.

4.6. Metal digestion and ICP Analysis

Shoot and root samples from treatments - 0%, 10% PW, 20% PW and 30% PW were collected at the end of experiment 4.4. The samples were dried at 100° C overnight following which they were manually ground to powder form. 0.25 g of sample was weighed and added to heat resistant tubes. To this 5 ml of conc. nitric acid was added. SRM 15151- Apple leaves was used as reference material. One sample (10% PW roots) was duplicated to ensure validation of measurements. A spike was prepared by taking 0.1 ml of standard 100 ppm (ICUS-2959) and diluted to 100 ppb. Two blanks were also prepared containing the acid only. All samples were capped and placed on a hot block set at 105 ° C. After 2 hours the sample tubes were uncapped jand the hot block temperature was increased to 130 ° C to allow evaporation. Following evaporation, the residue was mixed with 3 ml of conc. nitric acid and 1 ml of hydrogen peroxide and sample tubes were allowed to boil at 155° C in the hot block. The samples were then transferred to measuring flasks and their volume was completed to 50 ml using distilled water. The digested samples were then filtered twice using 0.25 µm filters to remove precipitates. The filtered samples were then analyzed for metals - vanadium, chromium, manganese, nickel, cobalt, zinc, arsenic, cadmium and lead through ICP Analysis.

4.7. Seed germination experiments

Results of produced water analysis showed that it contained - nickel – 3.2 ppb, zinc – 49.7 ppb, cobalt – 0.75 ppb, lead – 48.86 ppb (Fathy et al., unpublished data, 2017) in addition to a high salinity of 150 g/L. Therefore, germination tests on turf grass seeds (*Cynodon dactylon*) and weed species that are known to be associated with turf grass system were performed using concentrations of the above mentioned heavy metals in addition to produced water and saline water concentrations. Chloride salts of each of the metals was used to prepare the following concentrations nickel – 3.2 ppb, zinc – 49.7 ppb, cobalt – 0.75 ppb, lead – 48.86 ppb.

4.7.1. Germination test of turf grass (Cynodon dactylon) seeds

Three types of turf grass seeds were attempted to be tested - Cêsped sparring (Semillas fito, Spain), Cêsped costa (Semillas fito, Spain) and Cêsped gobi (*Cynodon dactylon,* Semillas fito, Spain). However, seeds of Cêsped sparring and Cêsped costa were found to be not viable and hence the experiment was continued with Cêsped gobi. Seeds of turf grass (Cêsped gobi, *Cynodon dactylon,* Semillas fito, Spain) were surface sterilized using 5% sodium hypochlorite solution for 2 minutes and were followed by washing with distilled water 3 times. 10 seeds of turf grass (*Cynodon dactylon*) were placed in a Petri dish layered with cheese cloth that was priory soaked with 3 ml of the assigned treatment solution and then sealed with parafilm (Figure 8). 4 replicates were prepared for each treatment. The treatments tested were - 0% (distilled water), 1% produced water, 5% produced water, 10% produced water, 20% produced water, 1.5 g/L saline water (designated as 0.15% S), 7.5 g/L saline water (designated as 0.75% S), 15g/l saline solution (designated as 1.5% S) and 30 g/l saline solution (designated as 3% S), nickel chloride – 3.2 ppb, zinc chloride – 49.7 ppb, cobalt chloride – 0.75 ppb and lead chloride – 48.86 ppb. The Petri dishes were placed in a growth chamber at 28°C for 14 days. Distilled water was added (~3 ml) upon requirement to prevent dry out. The plates were daily observed for seed germination. The appearance of the white radicle was used as an indicator of germination. The germinated seeds were counted on daily basis for 14 days. Accumulative of percent germination was documented for every treatment in all experiments.

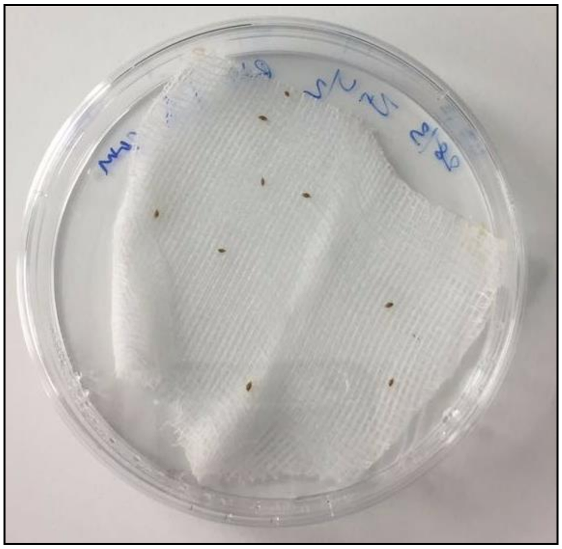


Figure 8. *Cynodon dactylon* seeds treated with Zinc chloride prior to incubation

4.7.2. Germination test of seeds of weed species associated with turf grass

Seeds of 6 common turf grass weeds were collected from turf grass fields inside Qatar University campus. The seeds were cleaned from husk and other residues and then kept in a labeled paper bags in a refrigerator. The tested species were: - *Amaranthus viridis, Malva neglecta, Launaea capitata, L. mucronata., Chloris virgata* and *Oligomeris subulata.* The seeds were soaked in 5% sodium hypochlorite solution for 2 minutes and were followed by washing with distilled water 3 times. 5-10 seeds (depending on the availability of seeds) were placed in a Petri dish layered with cheese cloth that was priory soaked with 3 ml of the assigned treatment solution and then sealed with parafilm. Four replicates were prepared for each treatment. The treatments tested were - 0% (distilled water), 1% produced water, 5% produced water, 10% produced water, 20% produced water, 1.5 g/L saline water (designated as 0.15% S), 7.5 g/L saline water (designated as 0.75% S), 15g/l saline solution (designated as 1.5% S) and 30 g/l saline solution (designated as 3% S), Nickel chloride – 3.2 ppb, Zinc chloride – 49.7 ppb, Cobalt chloride – 0.75 ppb and Lead chloride – 48.86 ppb. The Petri dishes were placed in growth chamber at 28°C for 14 days. Distilled water was added (~3 ml) upon requirement to prevent dry out. The plates were daily observed for seed germination. The appearance of the white radicle is used as an indicator of germination. The germinated seeds were counted on daily basis for 14 days. Accumulative of percent germination was documented for every treatment in all experiments. During the experiment seeds of *Malva neglecta, Launaea capitata* .and *Oligomeris subulata* were found to be not viable and the experiment was continued with *Amaranthus viridis*, *L. mucronata.* and *Chloris virgata.*

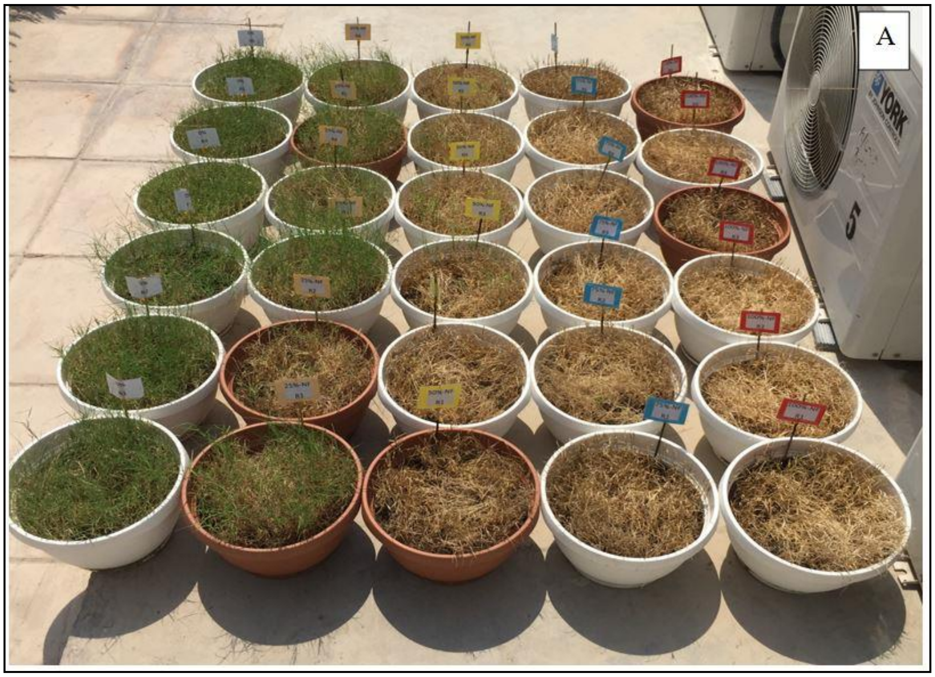
4.8. Data Analysis

SigmaStat 4.0 was used to perform data analysis. Tukey test was used for mean comparisons at $P \geq 0.05$.

CHAPTER 5 - RESULTS

5.1. Effect of produced water on grass (greenhouse) and salinity (greenhouse and outdoor experiment)

Cynodon dactylon grass were established from seeds and grown under greenhouse conditions. Starting from the second week post treatment of produced water irrigation, the grasses started to dry without future recovery (Figure 9). Only pots irrigated with 25% Produced water were tolerant and survived up to the end of experiment (14 weeks). Although the grass was able to survive at 25% PW irrigation, the green biomass (Figure 9 & 10) and the dry matter of both above and belowground were significantly reduced (Figure 11) compared to turf grass biomasses subjected to tap water irrigation.



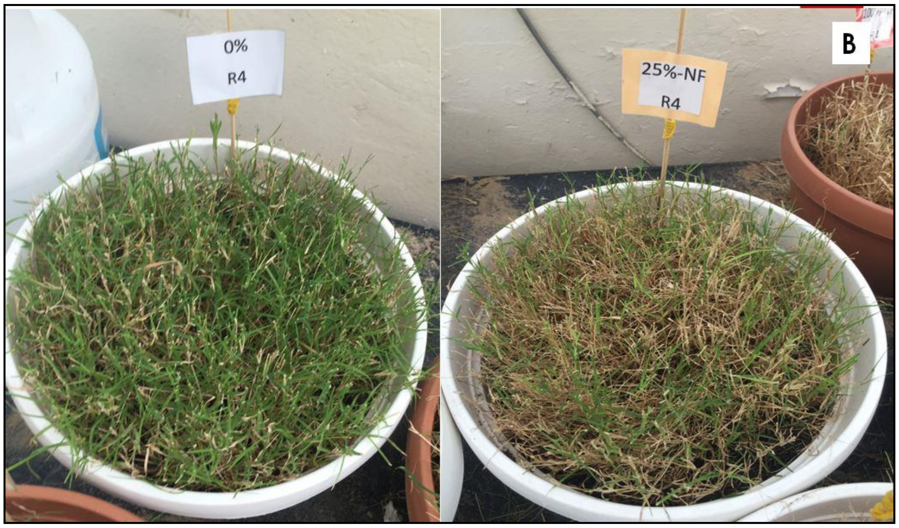


Figure 9. Photos for the effect of concentration of produced water (L-R, 0% (tap water) up to 100% produced water) on turf grass (*Cynodon dactylon*) coverage (%) after being subjected to 14 weeks of treatment [A]. A replicate each of 0% treatment and 25% PW after 14 weeks of treatment [B]. The grass was grown in 20 cm pots and placed under greenhouse conditions.

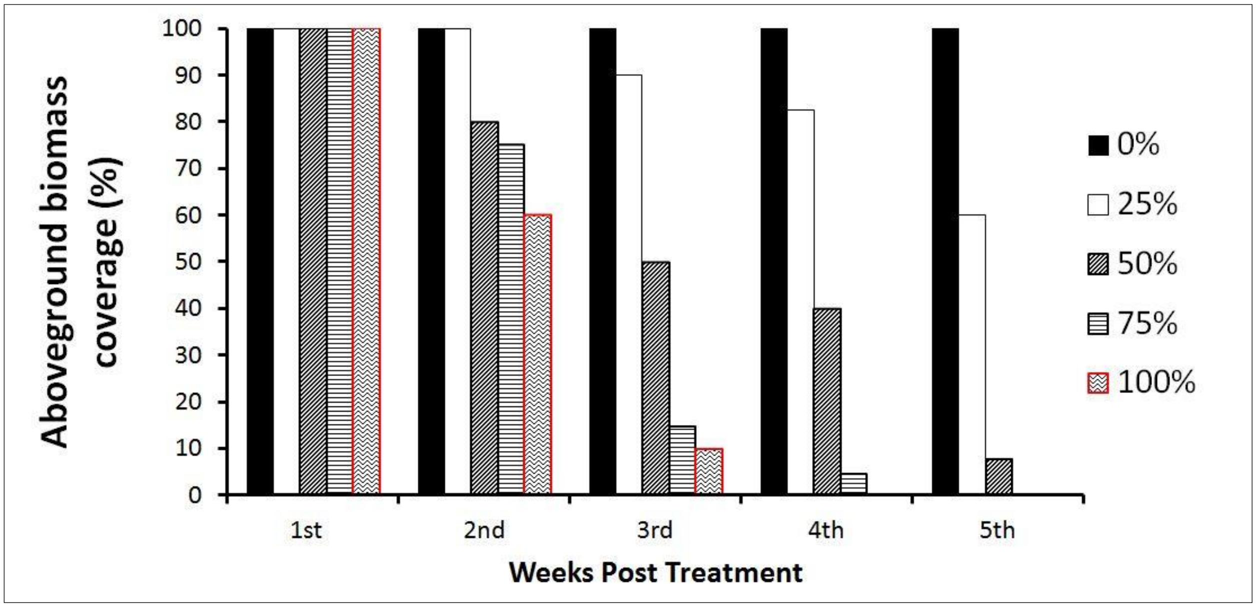


Figure 10. Effect of concentration of produced water (0% (tap water) up to 100% produced water) on turf grass (*Cynodon dactylon*) coverage (%) after being subjected to 5 weeks irrigation regimes. The grass was grown in 20 cm pots and placed under greenhouse conditions.

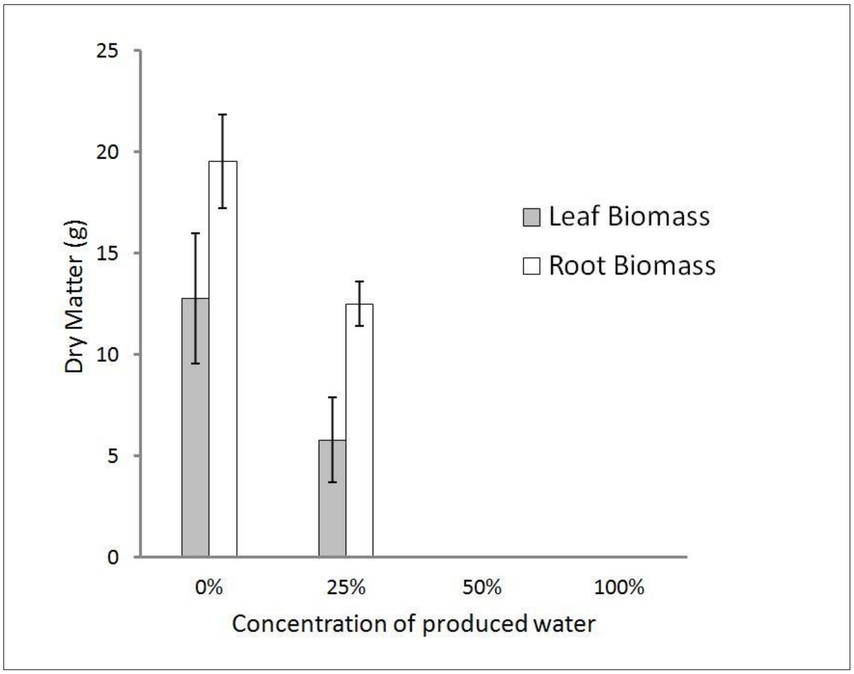


Figure 11. Effect of concentration of produced water (0% (tap water) up to 100% produced water) on biomass (dry matter) of above and belowground of turf grass (*Cynodon dactylon*) after being subjected to 14 weeks irrigation regimes. The grass was grown in 20 cm pots and placed under greenhouse conditions. Error bars represent the standard error of the means.

Based on the above results of experiment-1, one of the research questions was, is the decline in grass biomass due to salinity factor or other toxic substances available in the raw produced water. Therefore, the experiment was repeated under similar conditions however the treatments were different. Treatment levels included irrigation with 0% to 50% produced water in addition to salinity treatments of 0% up to 7.5%. The salinity treatment levels were prepared based on the concentration of salts in the 50% produced water. Results obtained were almost similar to results of the first experiment as above 30% PW irrigation killed the grass (Figure 12). Interestingly, salinity treatment of 4.5% (equivalence to salinity in 30% PW) showed similar grass biomass like the 30% PW treatment (Figure 13). Almost similar trends were obtained on coverage% (based on visual estimation) and again the salinity treatment effects are almost matching the effects obtained from produced water treatments (Figure 14).

After 14 weeks of irrigation, results from dry matter biomass, indicated a significant reduction in leaf dry weight starting from 20% PW and the matching 3% salinity treatments while the significant reduction in root biomass started from 30% PW or the matching equivalence of salinity treatment (4.5%) (Figure 15).

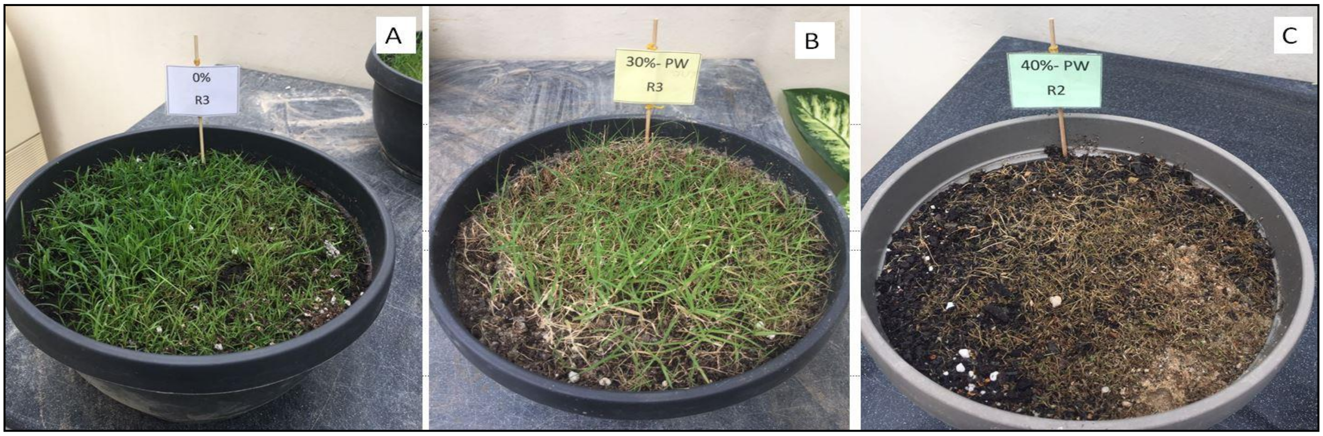


Figure 12. Photos for the effect of concentration of produced water 0% (tap water) [A], 30% PW[B] and 40% PW[C] on turf grass (*Cynodon dactylon*) coverage (%) after being subjected to 14 weeks of irrigation regimes. The grass was grown in 20 cm pots and placed under greenhouse conditions.

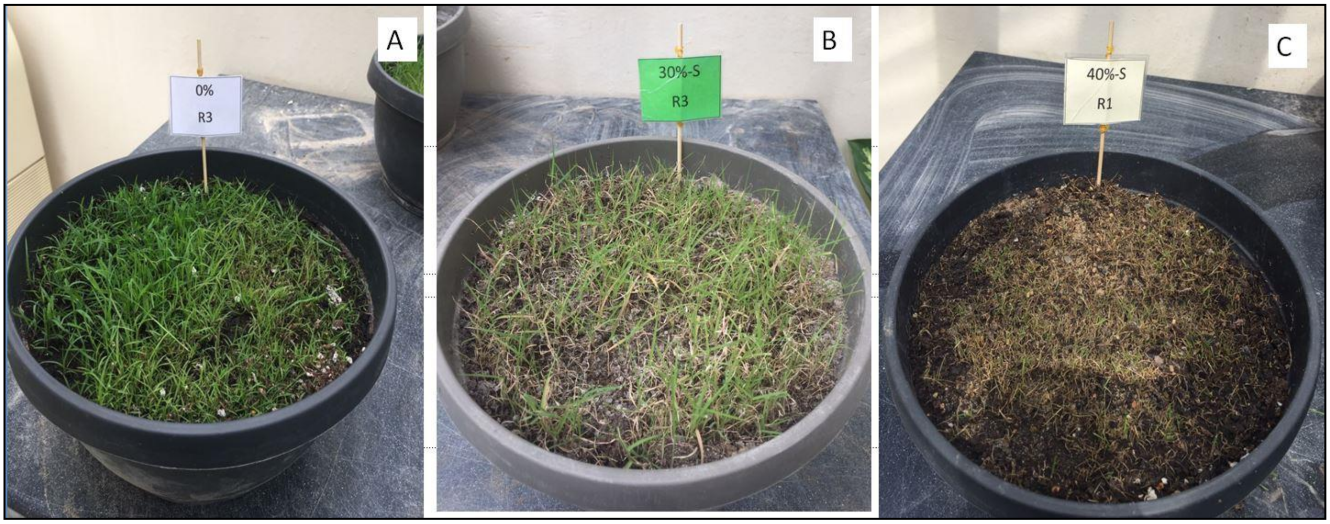


Figure 13. Photos for the effect of concentration of produced water 0% (tap water) [A], 4.5% S (shown as 30% S) [B] and 6% S (shown as 40% S)[C] on turf grass (*Cynodon dactylon*) coverage (%) after being subjected to 14 weeks of irrigation regimes. The grass was grown in 20 cm pots and placed under greenhouse conditions.

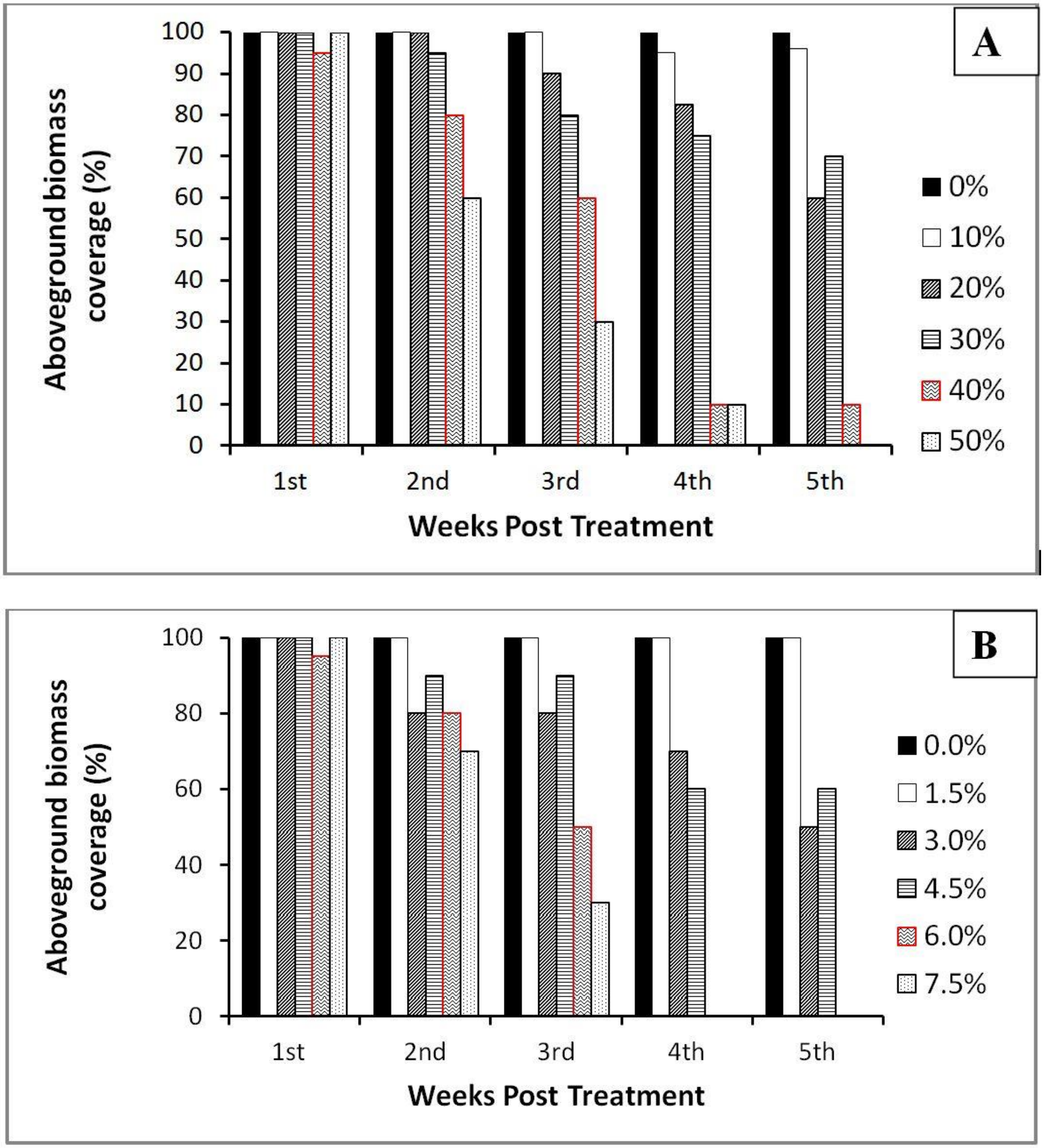


Figure 14. Effect of concentrations of produced water (0% (tap water) up to 50% produced water) [A] and saline water concentrations (0% (tap water) up to 7.5% NaCl solution) [B] on turf grass (*Cynodon dactylon*) coverage (%) after being subjected to 5-weeks irrigation regimes. The grass was grown in 20 cm pots and placed under greenhouse conditions.

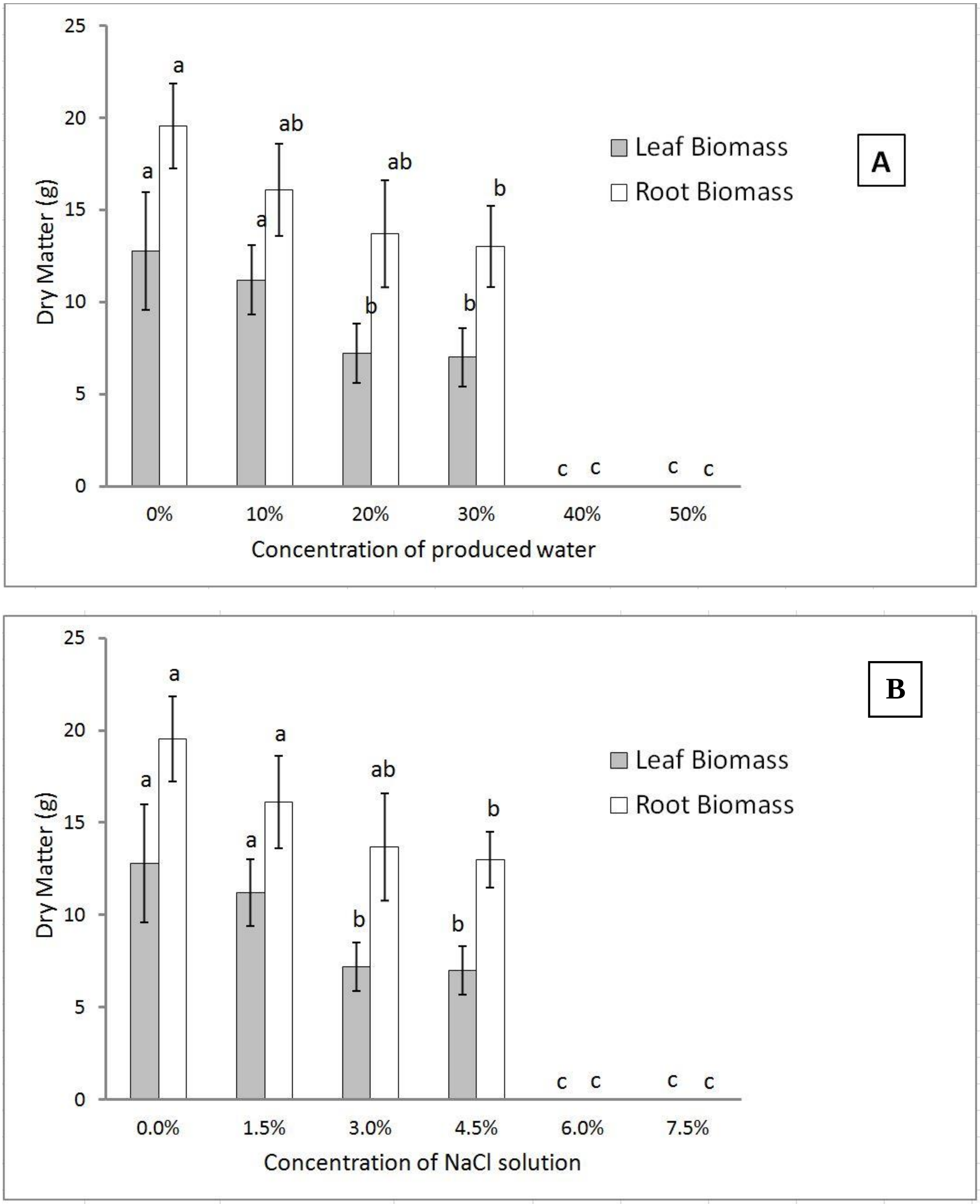


Figure 15. Effect of concentrations of produced water [A] and saline water concentrations [B] on turf grass (*Cynodon dactylon*) biomass (%) after being subjected to 14-weeks irrigation regimes. The grass was grown in 20 cm pots and placed under greenhouse conditions. Error bars refers to standard error of the means. Within leaf or root biomass, any common letter between treatments refers to no significance at $P \geq 0.05$ using Tukey's test.

Furthermore, a new experiment was established in outdoor conditions and using the turf grass species *Paspalum* sp. which is commonly used in most Qatari turf grass systems. The used grass was planted from rolls of seedlings and under outdoor conditions. In this experiment, we aimed to simulate the natural conditions of turf grass growth in Qatar.

The obtained results indicated a better tolerance of *Paspalum* sp. to produced water and salinity treatment. Figure 16 shows the appearance of turf grass under all treatments after 10 weeks of irrigation. It's clear that there are neither negative effects on the green biomass due to produced water nor due to saline irrigation (Figure 16 and 17). However, Figure 18 shows a significant reduction on dry weight of above and belowground parts under both treatments of produced water and salinity treatments.

Figure 16. *Paspalum* sp. after 10 weeks of treatment with (R-L) 0% (tap water), 10% PW, 20% PW, 30% PW, 1.5% S, 3% S and 4.5 % S grown in 65 cm x 25 cm x 20 cm pots and outdoor conditions.

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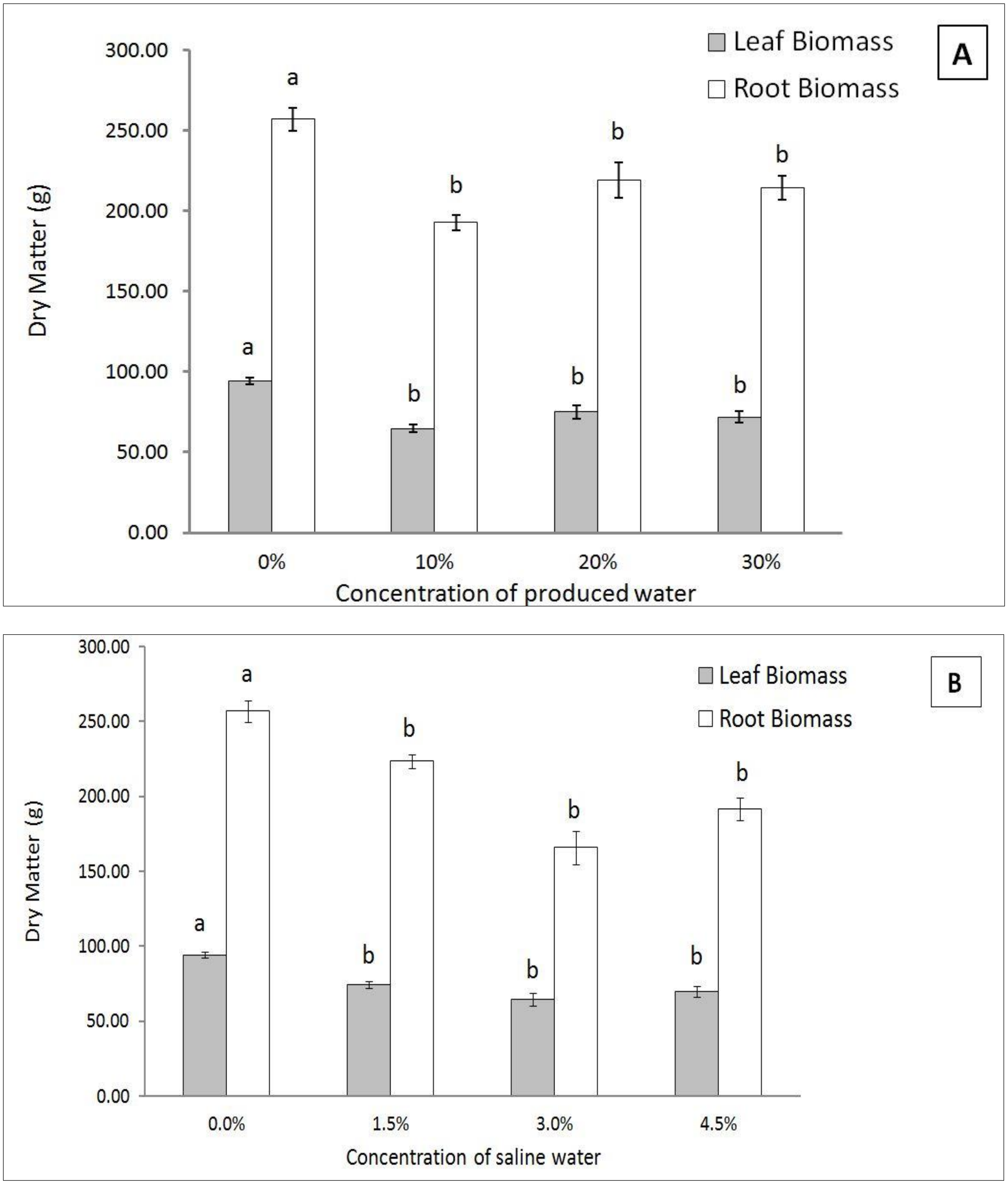

Figure 17. Effect of concentrations of produced water [A] and saline water concentrations [B] on turf grass (*Paspalum* sp.) biomass (%) after being subjected to 10 weeks irrigation regimes. The grass was grown in 65 cm x 25 cm x 20 cm under outdoor conditions. Error bars refers to standard error of the means. Within leaf or root biomass, any common letter between treatments refers to no significance at $P \geq 0.05$ using Tukey's test.

5.2. Effect on soil microbiota (greenhouse and outdoor experiment)

Results on soil microbiota from greenhouse experiment using *Cynodon dactylon* turf grass and after 14-weeks of produced water irrigation regimes are presented in Figures 18 & 19.

Bacterial colony forming units(CFU) were significantly reduced at 25% PW treatment. Fungal colony forming units significantly increased at 50% PW and (Figure 18 & 19). For the second trial interestingly, saline treatments (without produced water) showed same trends as compared to trends in produced water (Figure 20 & 21) for both bacterial and fungal growth. A decrease in both fungal and bacterial CFU was observed as higher concentration of salinity could have exerted less bacterial fungal colony counts (Figures 22 & 23).

The outdoor experiment of *Paspalum* sp turf grass was also studied for change in soil microbiota. Through time the densities of bacteria were increased in all treatments with time but were significantly lower than 0% treatment (Figure 24). Fungi results indicated different pattern of growth as CFU were decreased through time in each of the treatments and 20 to 30% PW had higher growth of fungi (Figure 25).

Salinity treatments had similar trends of growth for bacteria (Figure 26) with an anomaly in 3.0% S whose bacterial densities matched that of 0% treatment. Fungal CFU trend was similar to that of produced water treatment (Figure 27).

Investigation of succession of soil fungi in the outdoor experiment of *Paspalum* sp turf grass was accomplished in the current study. Table 4 shows the composition of fungal genera obtained under PW and tap water treatments. Results indicated different composition of fungal genera although 3-4 fungal species were common in all treatments. Under PW treatments a general trend of more fungal species were recorded compared to tap water treatment. Figures 28-

31 represent the succession of different genera of soil fungi under different treatments of produced water. Dynamics of fungi population seems to have different patterns of succession after the treatment with produced water. The most abundant genus was *Cladosporium* which showed a pattern of succession that was different between tap water and produced water. Richness of fungal genera was increased in all treatments through time up to the 6th week post treatment (Figure 32).

Total fungal colony counts increased in all treatments up to the 4th week and then started to decline with time (Figure 33).

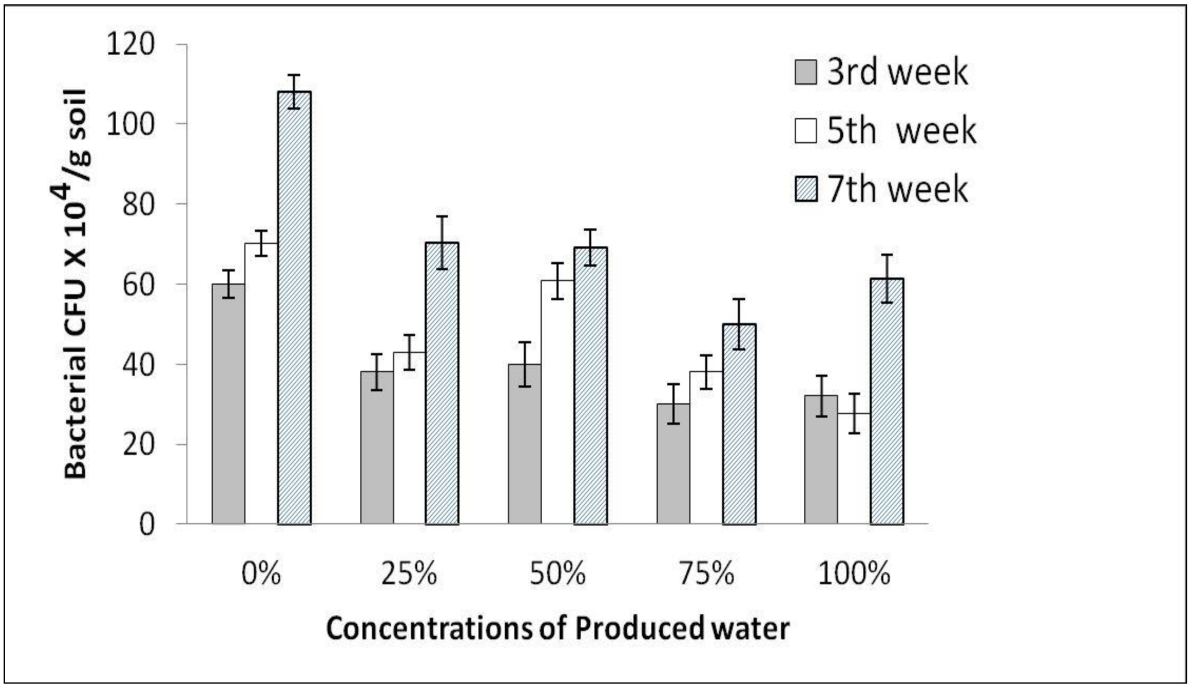

Figure 18. Effect of concentrations of produced water used to irrigate turf grass (*Cynodon dactylon*) on population densities of soil bacteria through time. Error bars represent the standard error of the means. The grass was grown in 20 cm pots and maintained under greenhouse conditions.

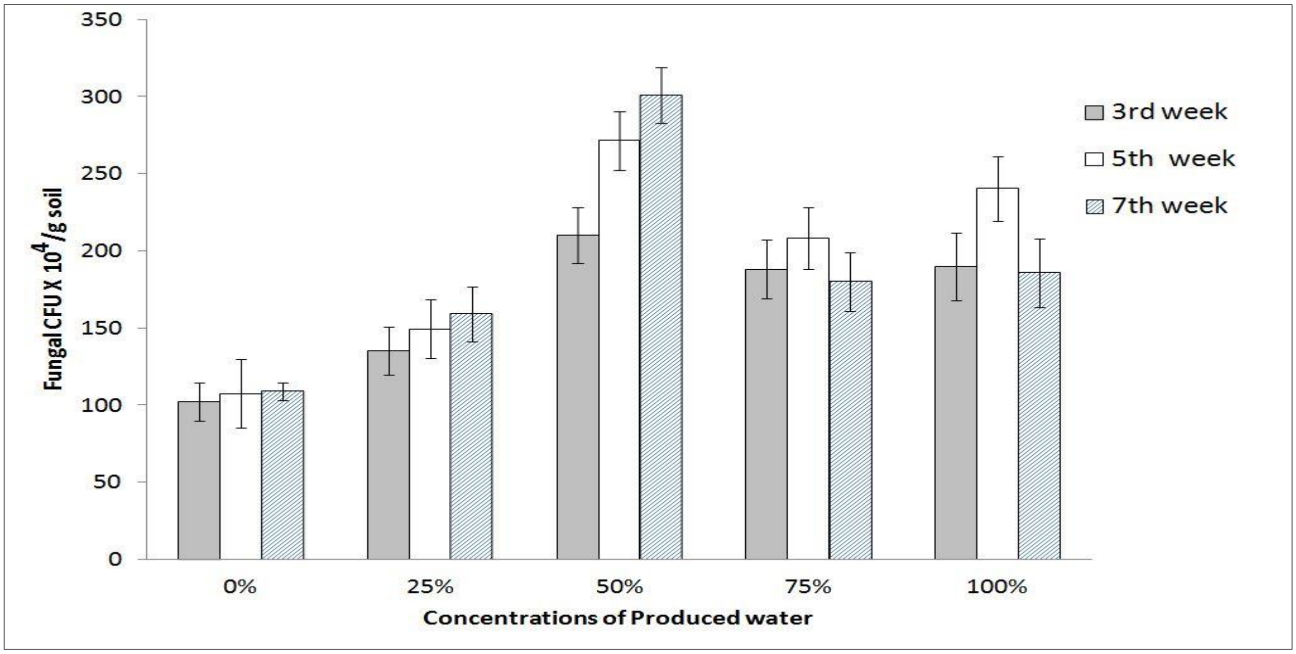

Figure 19. Effect of concentrations of produced water used to irrigate turf grass (*Cynodon dactylon*) on population densities of soil fungi through time. Error bars represent the standard error of the means. The grass was grown in 20 cm pots and maintained under greenhouse conditions.

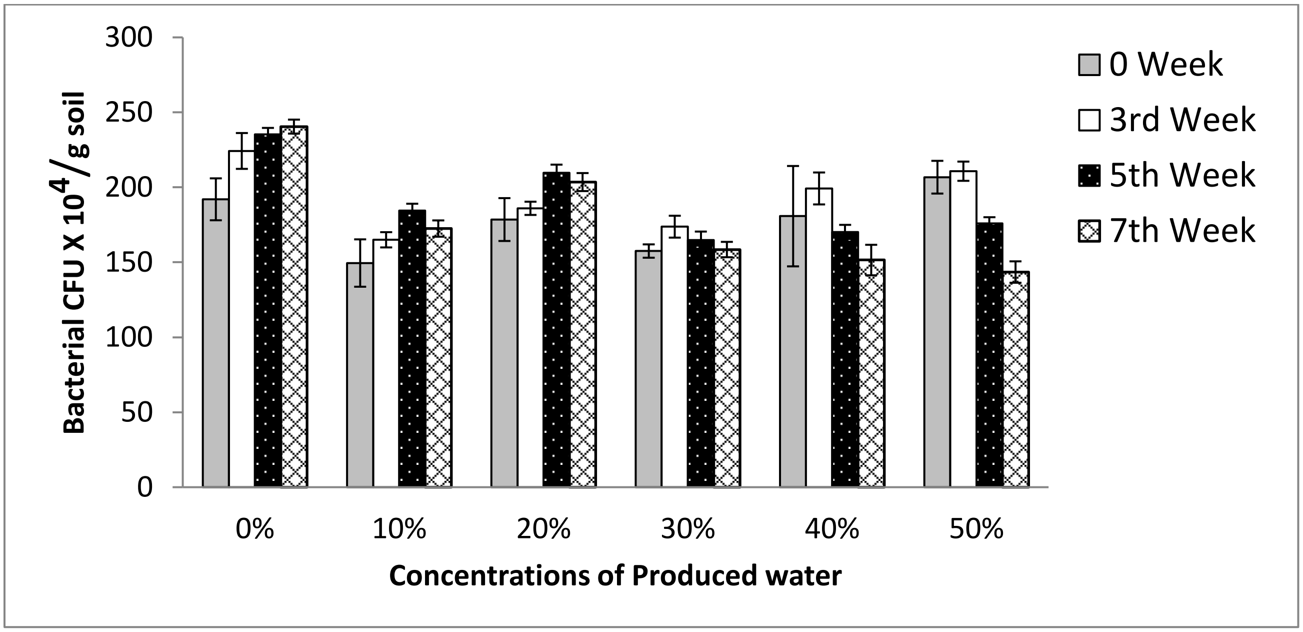

Figure 20. Effect of concentrations of produced water used to irrigate turf grass (*Cynodon dactylon*) on population densities of soil bacteria through time. Error bars represent the standard error of the means. The grass was grown in 20 cm pots and maintained under greenhouse conditions.

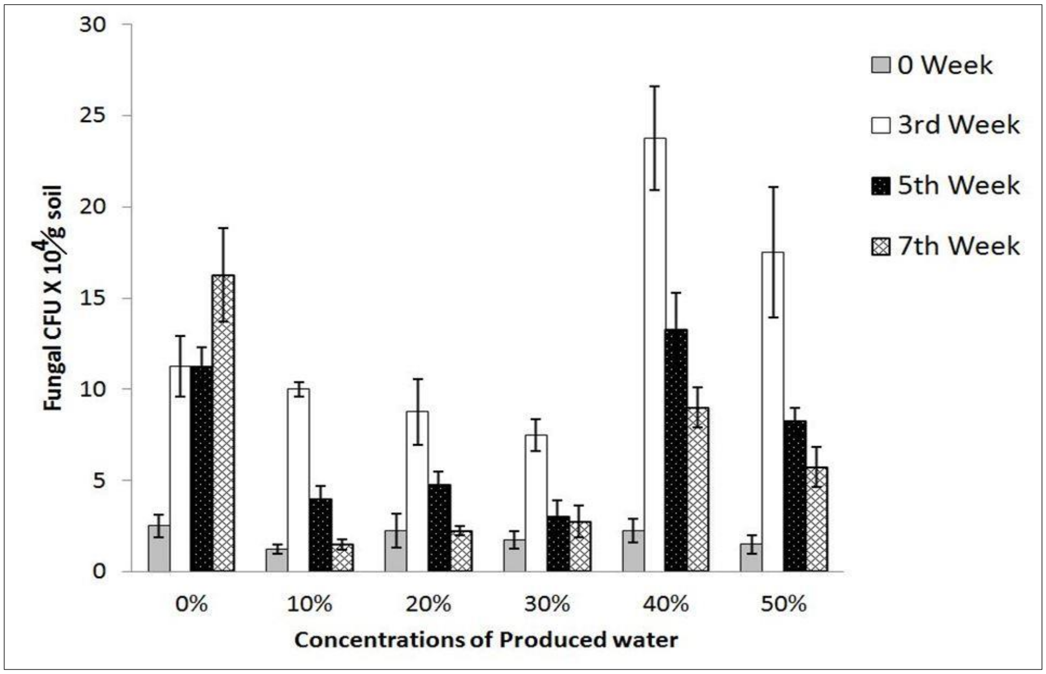

Figure 21. Effect of concentrations of produced water used to irrigate turf grass (*Cynodon dactylon*) on population densities of soil fungi through time. Error bars represent the standard error of the means. The grass was grown in 20 cm pots and maintained under greenhouse conditions.

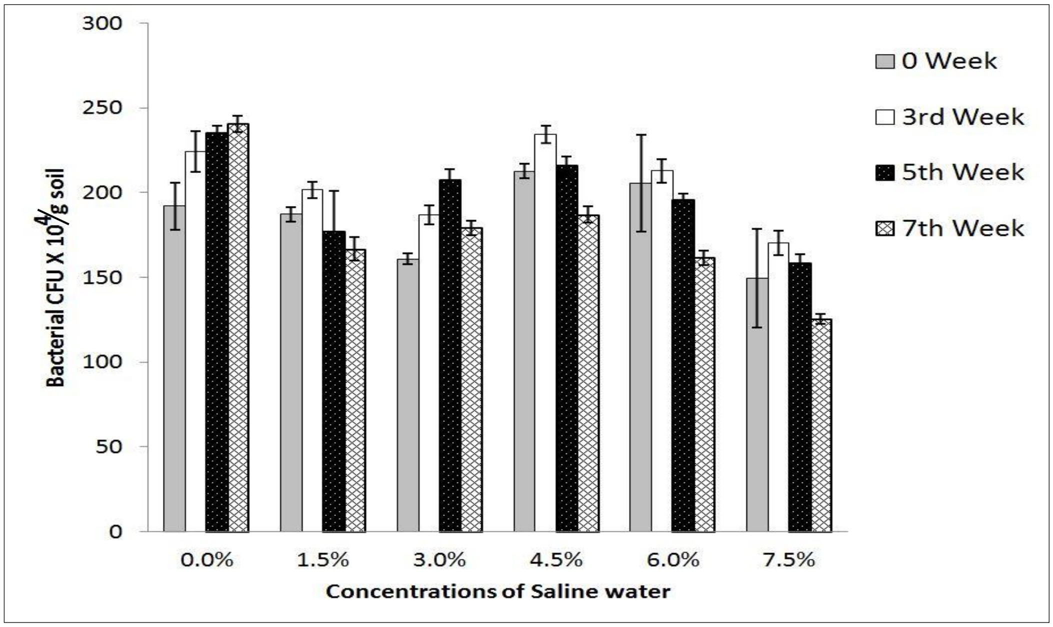

Figure 22. Effect of concentrations of saline water used to irrigate turf grass (*Cynodon dactylon*) on population densities of soil bacteria through time. Error bars represent the standard error of the means. The grass was grown in 20 cm pots and maintained under greenhouse conditions.

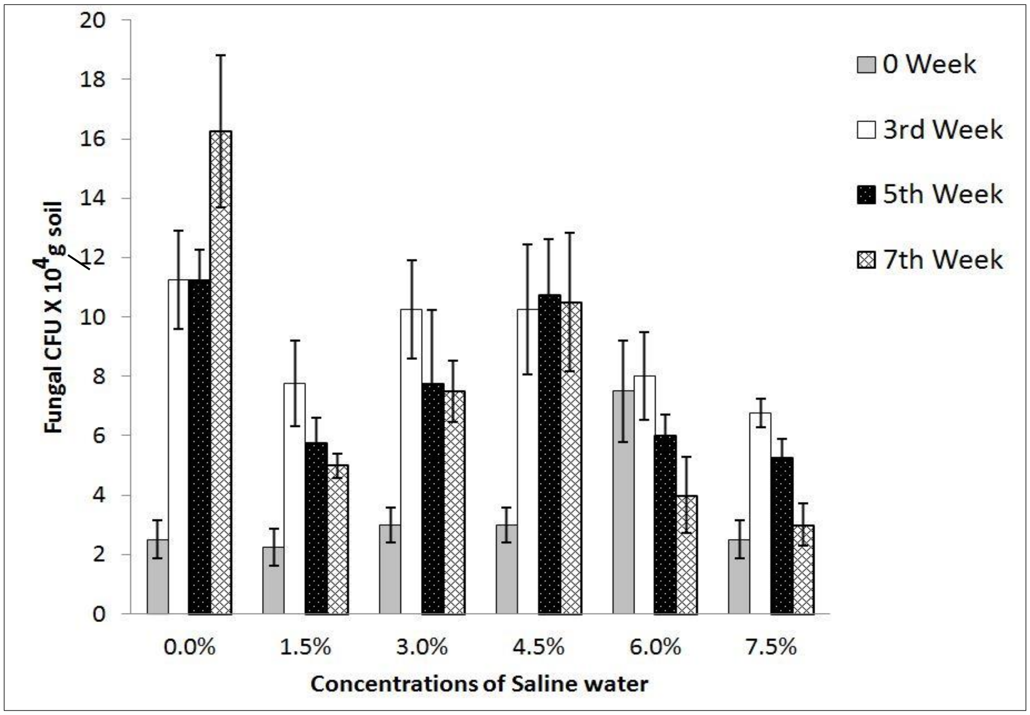

Figure 23. Effect of concentrations of saline water used to irrigate turf grass (*Cynodon dactylon*) on population densities of soil fungi through time. Error bars represent the standard error of the means. The grass was grown in 20 cm pots and maintained under greenhouse conditions.

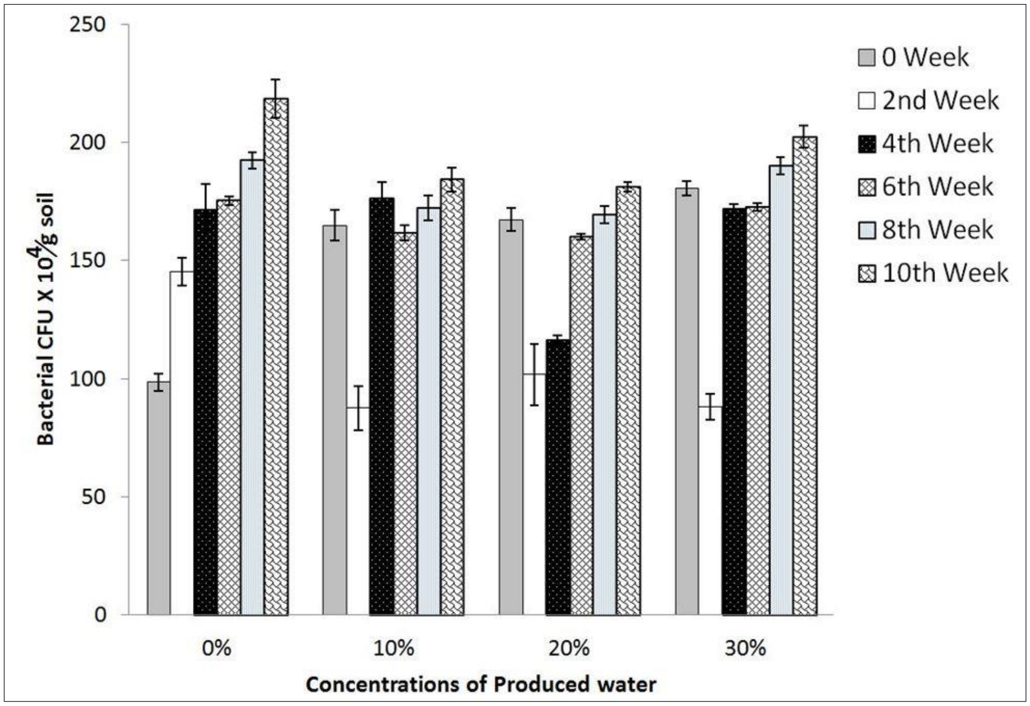

Figure 24. Effect of concentrations of produced water used to irrigate turf grass (*Paspalum* sp.) on population densities of soil bacteria through time. Error bars represent the standard error of the means. The grass was grown in 65 cm x 25 cm x 20 cm pots and placed outdoors.

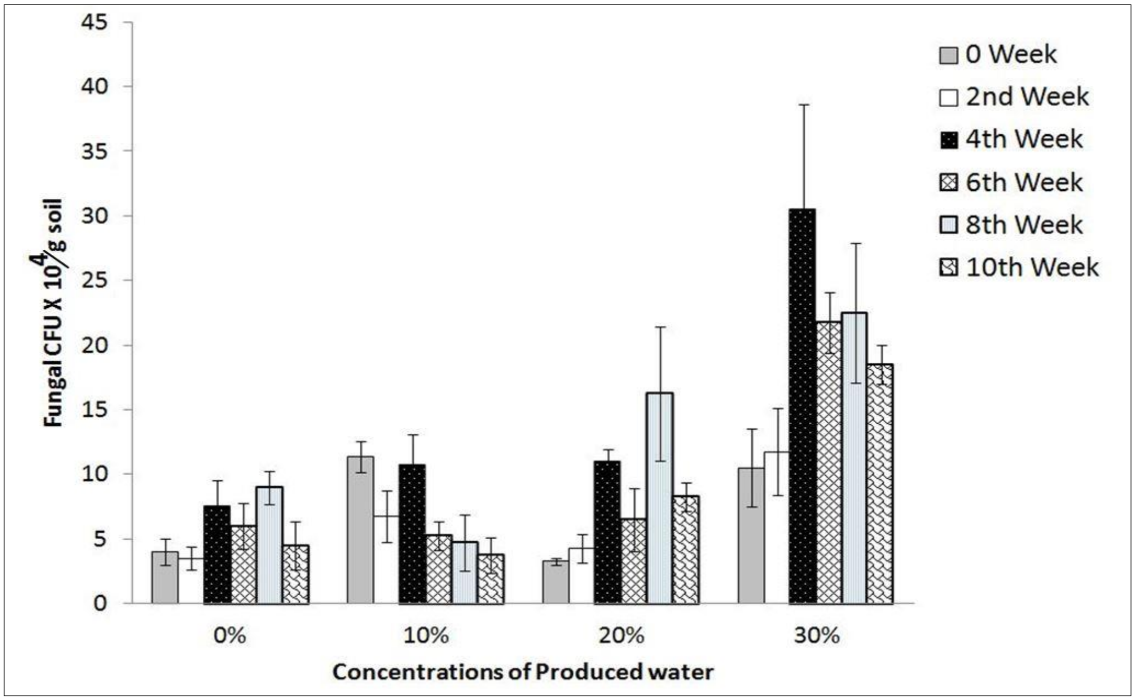

Figure 25. Effect of concentrations of produced water used to irrigate turf grass (*Paspalum* sp.) on population densities of soil fungi through time. Error bars represent the standard error of the means. The grass was grown in 65 cm x 25 cm x 20 cm pots and placed outdoors.

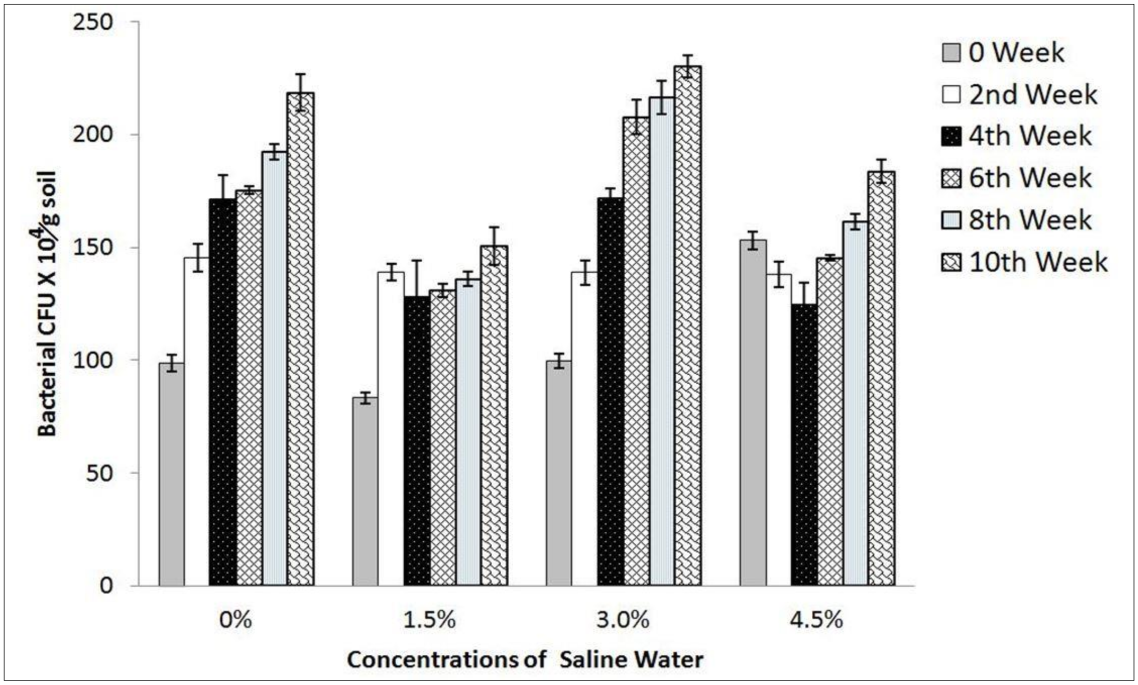

Figure 26. Effect of concentrations of saline water used to irrigate turf grass (*Paspalum* sp.) on population densities of soil bacteria through time. Error bars represent the standard error of the means. The grass was grown in 65 cm x 25 cm x 20 cm pots and placed outdoors.

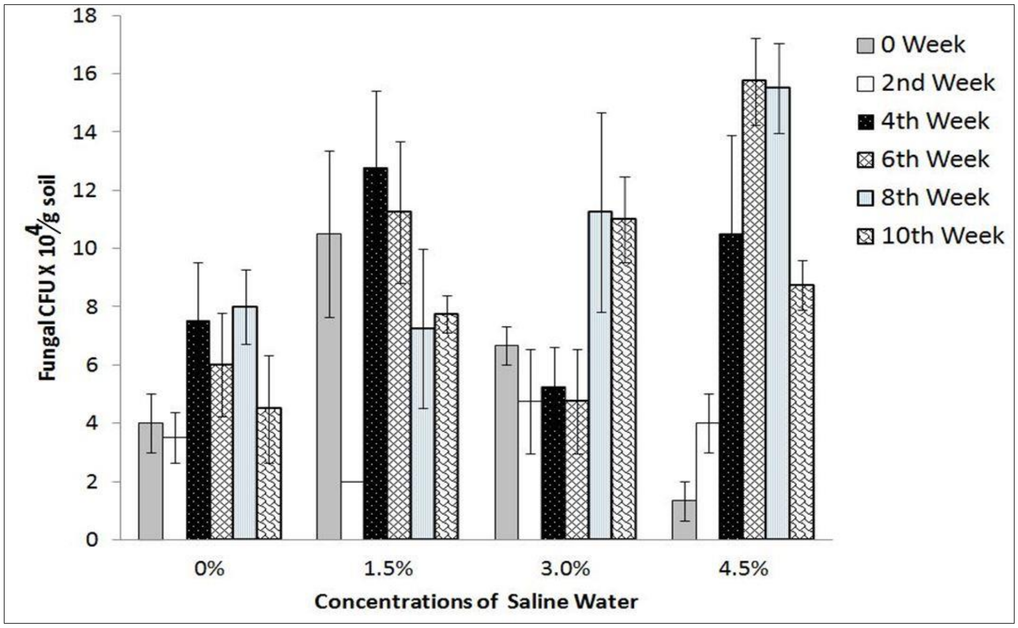

Figure 27. Effect of concentrations of produced water used to irrigate turf grass (*Paspalum* sp.) on population densities of soil fungi through time. Error bars represent the standard error of the means. The grass was grown in 65 cm x 25 cm x 20 cm pots and placed outdoors.

Table 4

List of all Fungal Species Reported in Soil Samples after being Irrigated with Different Concentrations of Produced Water (PW) versus Regular Water (0% PW)

0%	10% PW	20% PW	30% PW
Aspergillus flavus *Aspergillus terrus var. africans* *Aspergillus terrus var. terreus* *Cladosporium oxysporum* *Cladosporium tenuissimum* *Fusarium* sp. *Penicillium* sp.	*Aspergillus flavus* *Aspergillus terrus var. africans* *Cladosporium cladosporioides* *Cladosporium oxysporum* *Cladosporium tenuissimum* *Fusarium moniliforme* *Fusarium* sp. *Penicillium* sp. *Rhizopus* sp.	*Aspergillus* sp. *Aspergillus flavus* *Aspergillus terrus var. africans* *Aspergillus terrus var. terreus* *Chaetomium elatum* *Cochliobolus sativus* *Cladosporium oxysporum* *Cladosporium tenuissimum* *Fusarium moniliforme* *Fusarium* sp. *Penicillium* sp.	*Aspergillus* sp. *Aspergillus flavus* *Aspergillus terrus var. africans* *Aspergillus terrus var. terreus* *Cladosporium oxysporum* *Cladosporium tenuissimum* *Fusarium* sp. *Fusarium moniliforme* *Gibrella sp.* *Penicillium* sp. *Rhizopus* sp.

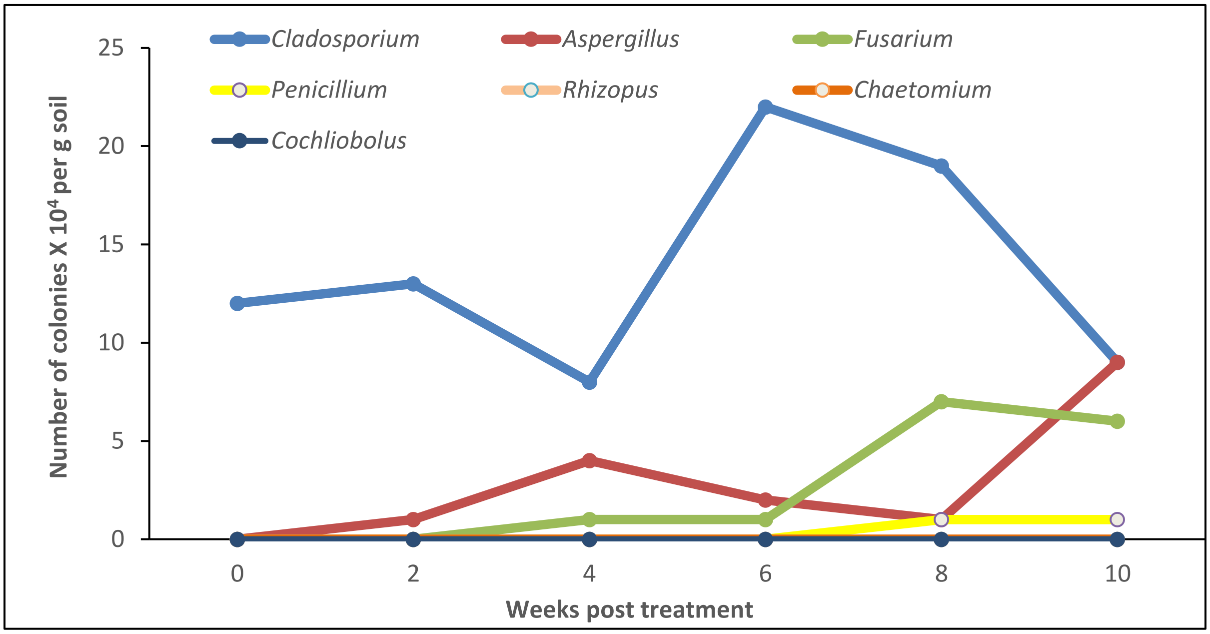

Figure 28. Succession of fungal genera in soil after irrigation with regular tap water.

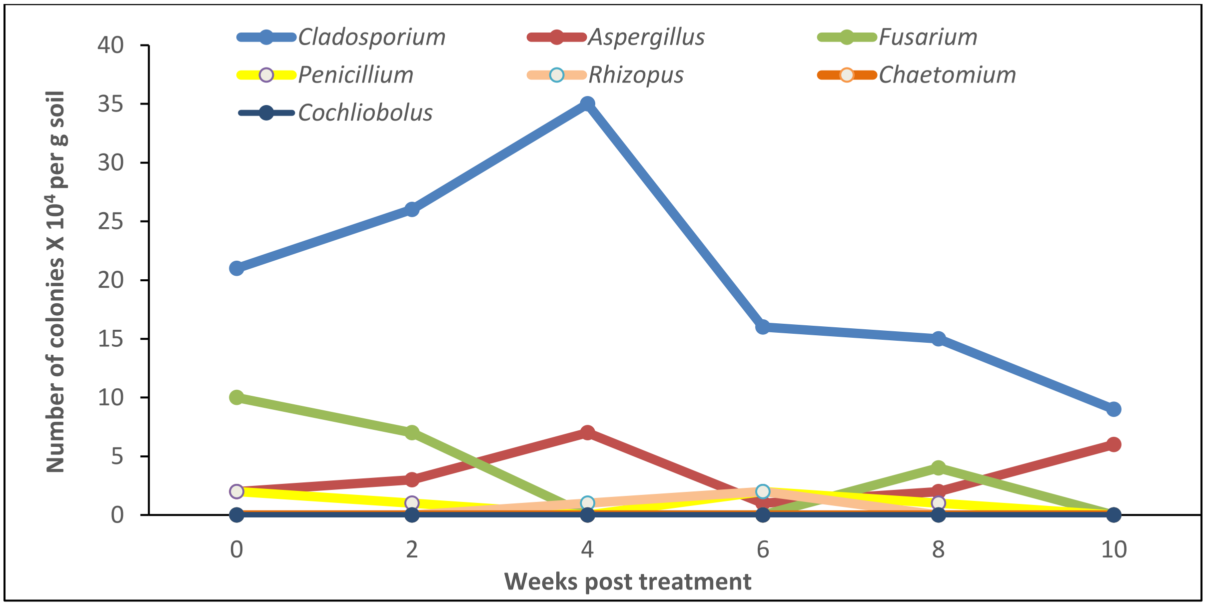

Figure 29. Succession of fungal genera in soil after irrigation with 10% produced water.

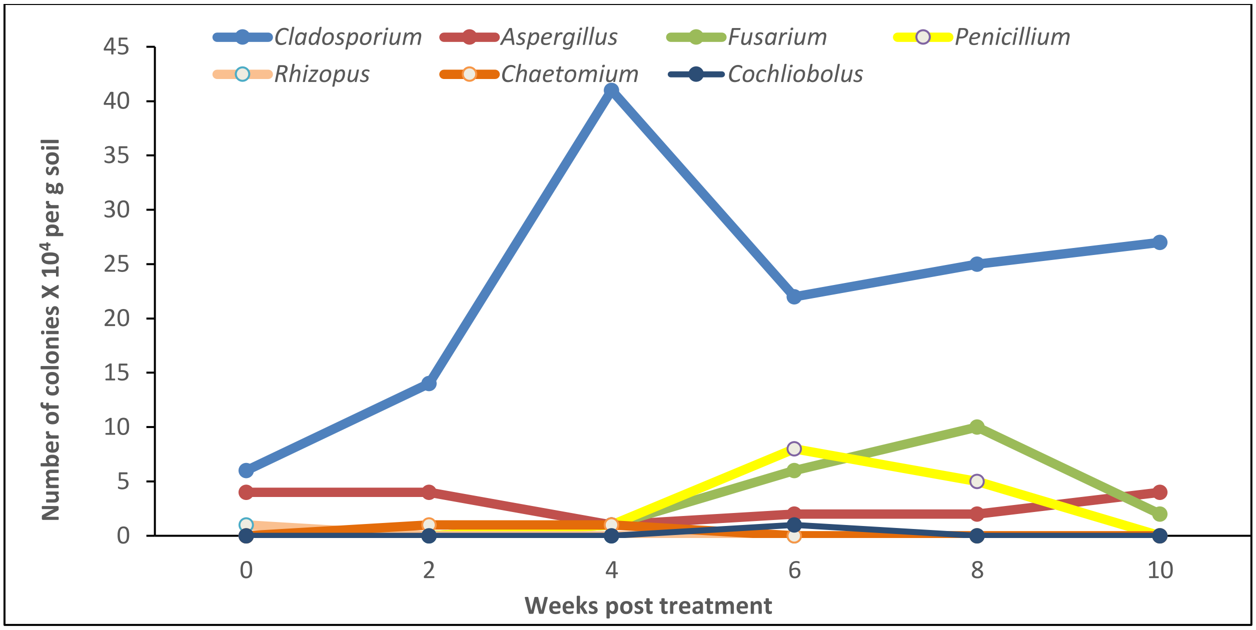

Figure 30. Succession of fungal genera in soil after irrigation with 20% produced water

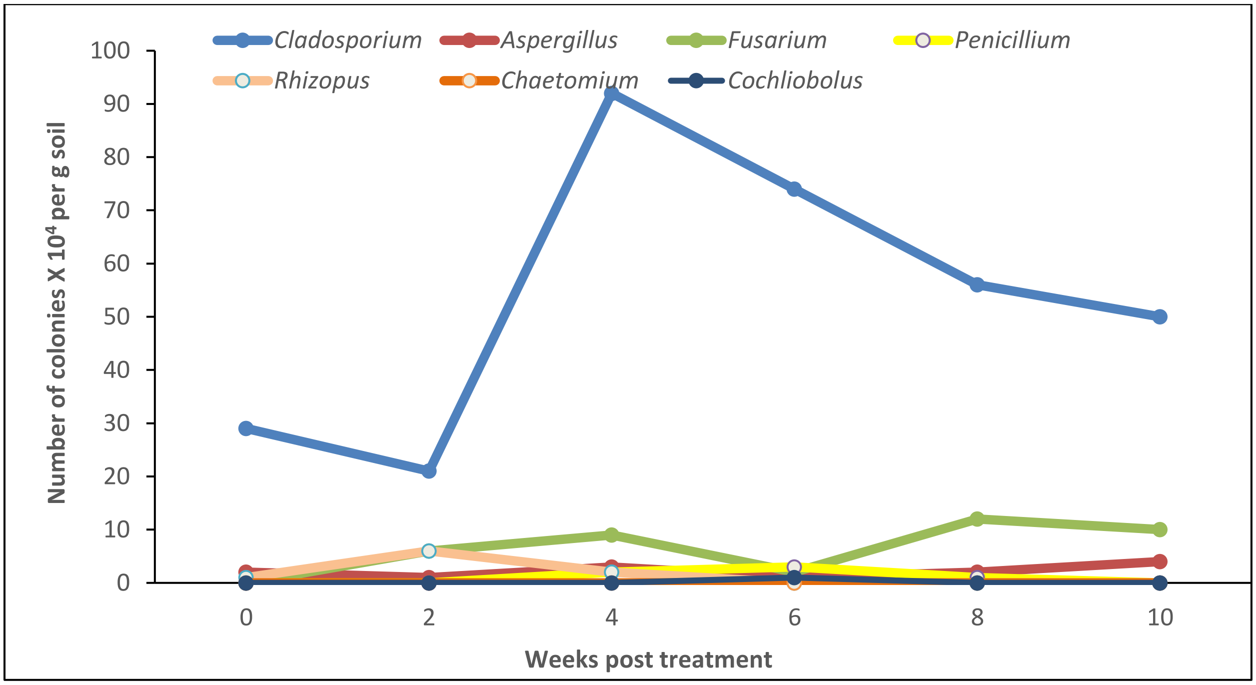

Figure 31. Succession of fungal genera in soil after irrigation with 30% produced water

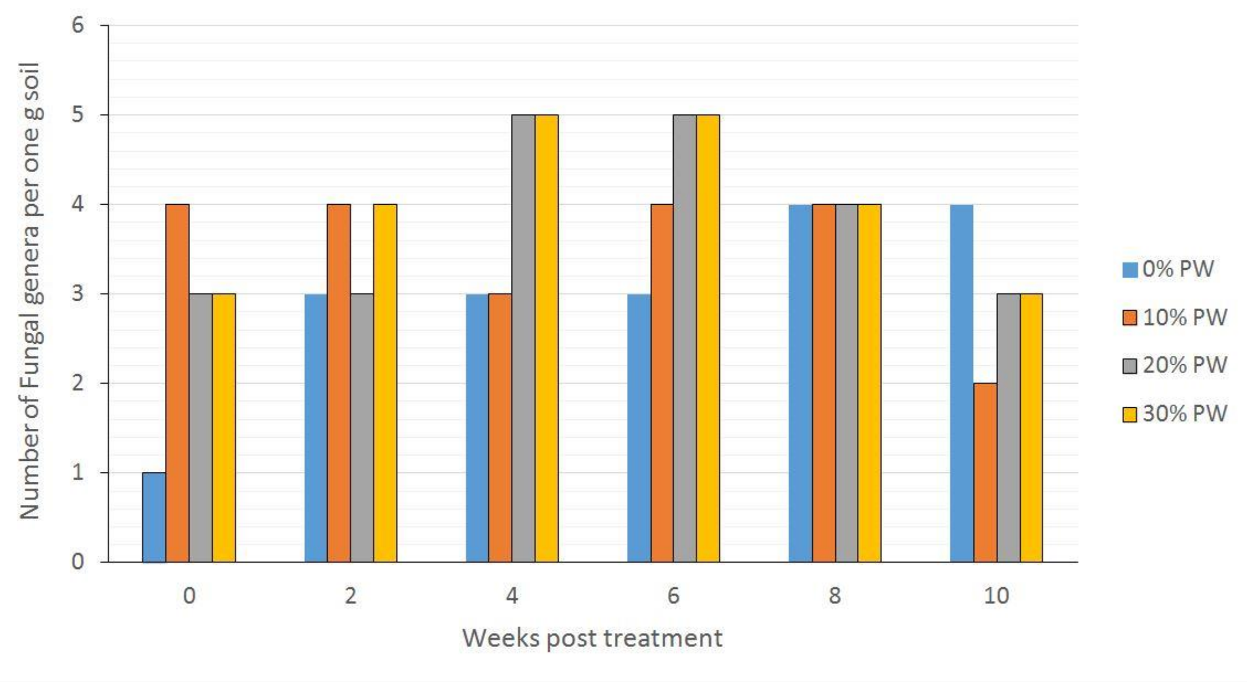

Figure 32. Succession of fungal genera in soil after being subjected to produced water treatments (PW)

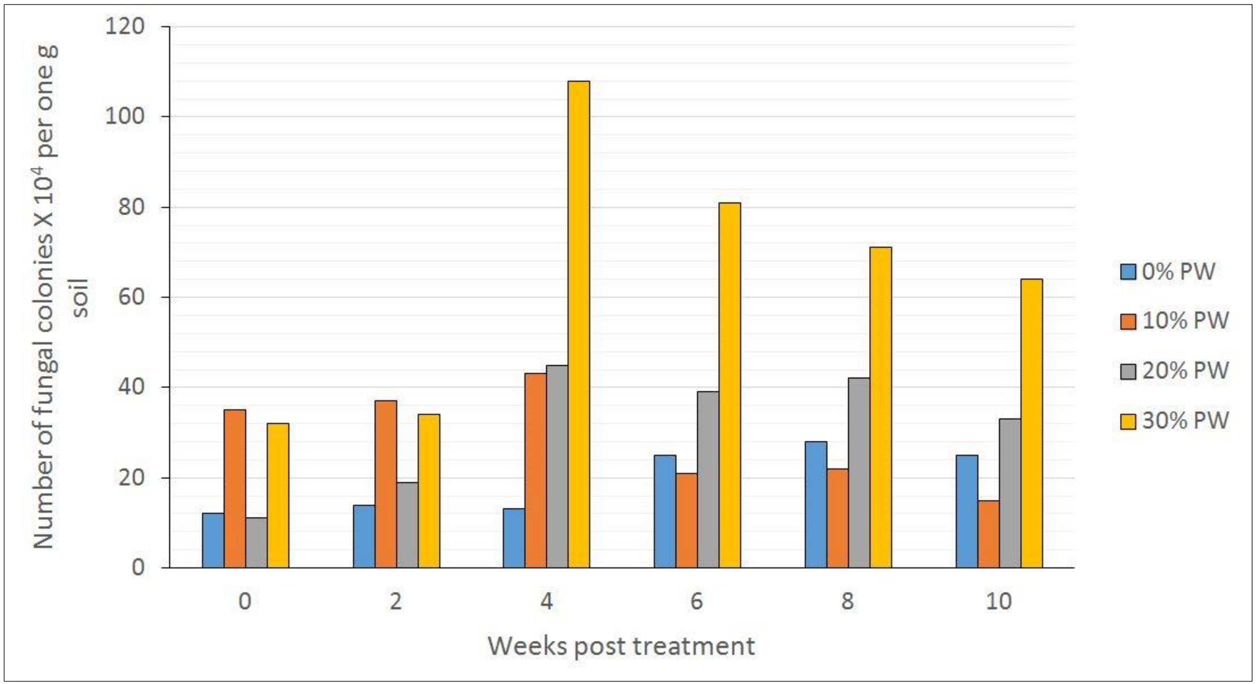

Figure 33. Succession of fungal densities in soil after being subjected to produced water treatments (PW).

5.3. Metal digestion and ICP Analysis

Preliminary investigation of the fate of heavy metals in the plant parts after being subjected to produced water irrigation was performed and results are shown in Table 5 for shoots and Table 6 for roots. According to the results of the ICP analysis, some elements were mainly accumulated in shoots i.e. V and Pb and others were more accumulated in the roots i.e. Cr, Ni, and As (Figure 34).

Table 5

ICP Analysis of Shoots of Paspalum sp. Treated with Produced Water conc.

Metal	blank 1	blank 2	Ref	0% Shoots	10% PW Shoots	20% PW Shoots	30 % PW Shoots
V	0.092	ND	ND	0.582	11.55	11.9	7.734
Cr	4.467	0.597	4.249	27.89	100.5	21.12	54.17
Mn	1.572	2.012	222	598.8	476.2	700.1	490.8
Ni	1.558	0.892	5.295	16.37	52.27	32.5	28.78
Co	ND	ND	ND	5.052	4.188	4.694	3.485
Zn	292.1	191.7	400.4	356.6	305.1	447.5	477.2
As	ND	ND	ND	3.1	3.958	3.498	ND
Cd	0.221	0.323	0.464	0.414	0.315	0.31	0.227
Pb	ND	ND	17.38	4.363	30.99	25.6	33.35

Table 6

ICP Analysis of Roots of Paspalum sp. Treated with Produced Water conc.

Metal	**blank 1**	**blank 2**	**Ref**	**0% Roots**	**10 % PW Roots**	**20% PW Roots**	**30 % PW Roots**
V	0.092	ND	ND	27.81	70.5	86.79	67.07
Cr	4.467	0.597	4.249	23.57	151.4	112.8	111.4
Mn	1.572	2.012	222	694.3	552.1	805.9	579.9
Ni	1.558	0.892	5.295	22.21	91.91	74.84	68.84
Co	ND	ND	ND	18.99	17.49	33	17.05
Zn	292.1	191.7	400.4	445.2	376.8	352.3	464.8
As	ND	ND	ND	7.459	18.81	22.34	9.911
Cd	0.221	0.323	0.464	0.677	0.807	1.128	0.657
Pb	ND	ND	17.38	12.55	43.63	66.84	54.47

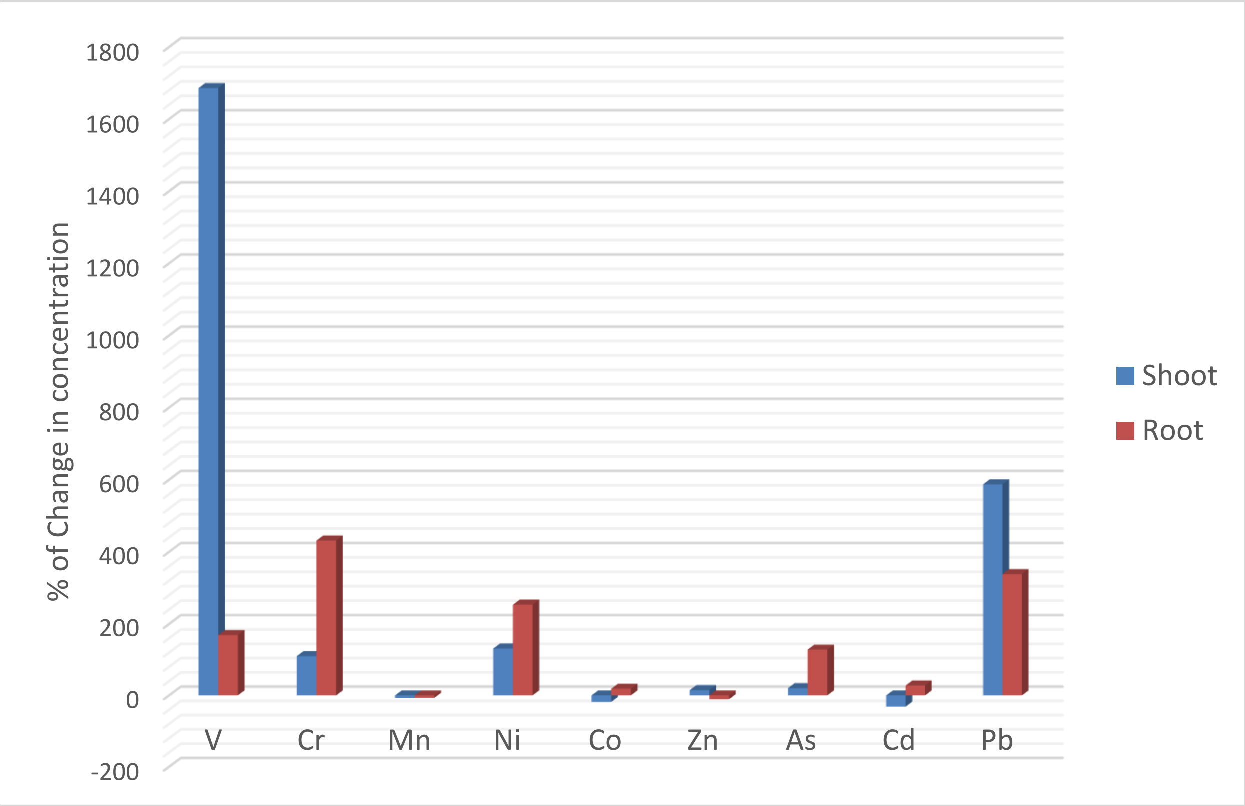

Figure 34. Change in concentration of heavy metals in the below (roots) and aboveground (shoots) parts of turf grass after 10 weeks of irrigation regime using produced water.

5.4. Effect on germination of turf grass seeds

Seed germination experiments were established to investigate the effect of heavy metals available in produced water on germination of turf grass seeds (Figure 35). All kinds of heavy metals decreased significantly the germination potential of the seeds of *Cynodon dactylon* turf grass compared to control (Figure 35).

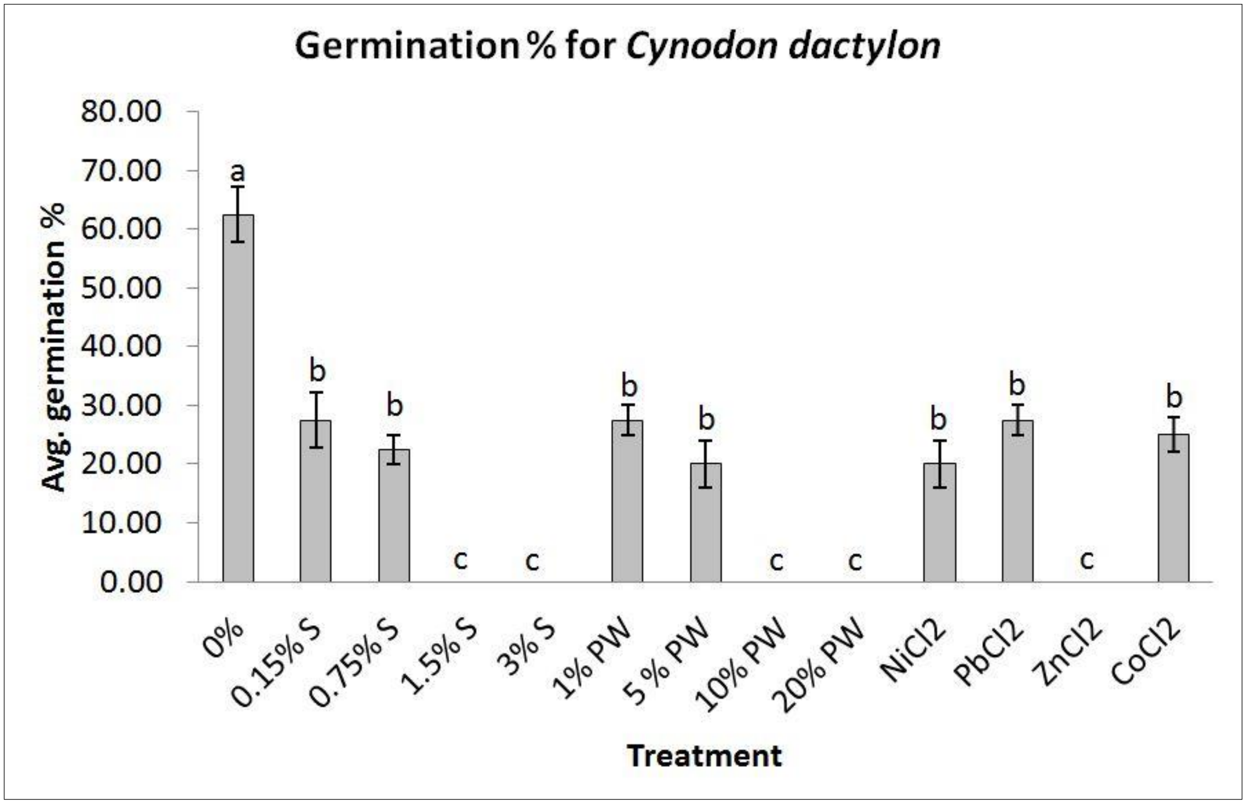

Figure 35. Average percentage of germination at 14 days for *Cynodon dactylon* subjected to varied treatments. Error bars refers to standard error of the means. Any common letter between treatments refers to no significance at $P \geq 0.05$ using Tukey's test.

5.5. Effect on germination of weed seeds

Seeds of weeds encountered in the turf grass were collected to be assessed for germination under different heavy metal treatments. The selected metals and their concentrations were chosen based on the chemical composition of the produced water. Different weeds showed different germination potentials after being treated with different heavy metals (Figures 36-38). For example, the most common weed species grown in turf grass system of Qatar is *Amaranthus viridis*. The germination of the species was significantly reduced under all treatments (PW, salinity and heavy metals).

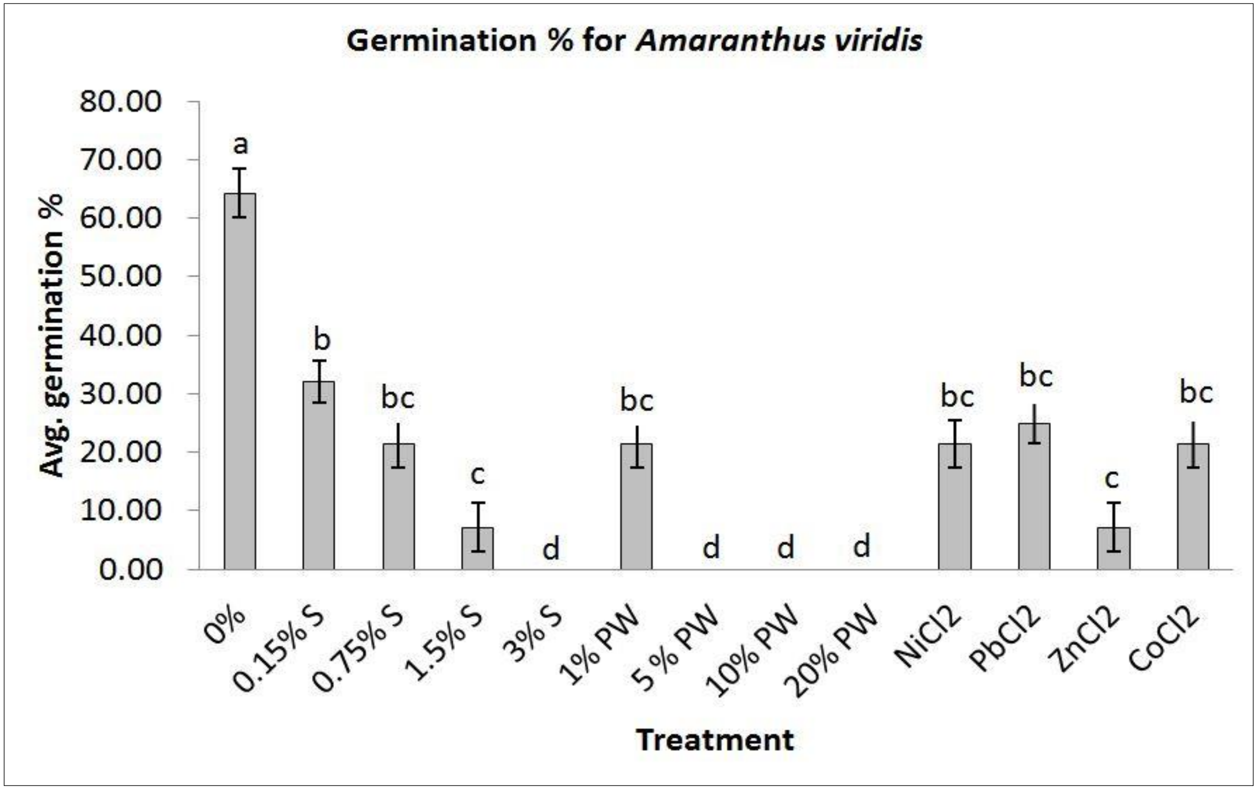

Figure 36. Average percentage of germination at 14 days for *Amaranthus viridis* subjected to varied treatments. Error bars refers to standard error of the means. Any common letter between treatments refers to no significance at $P \geq 0.05$ using Tukey's test.

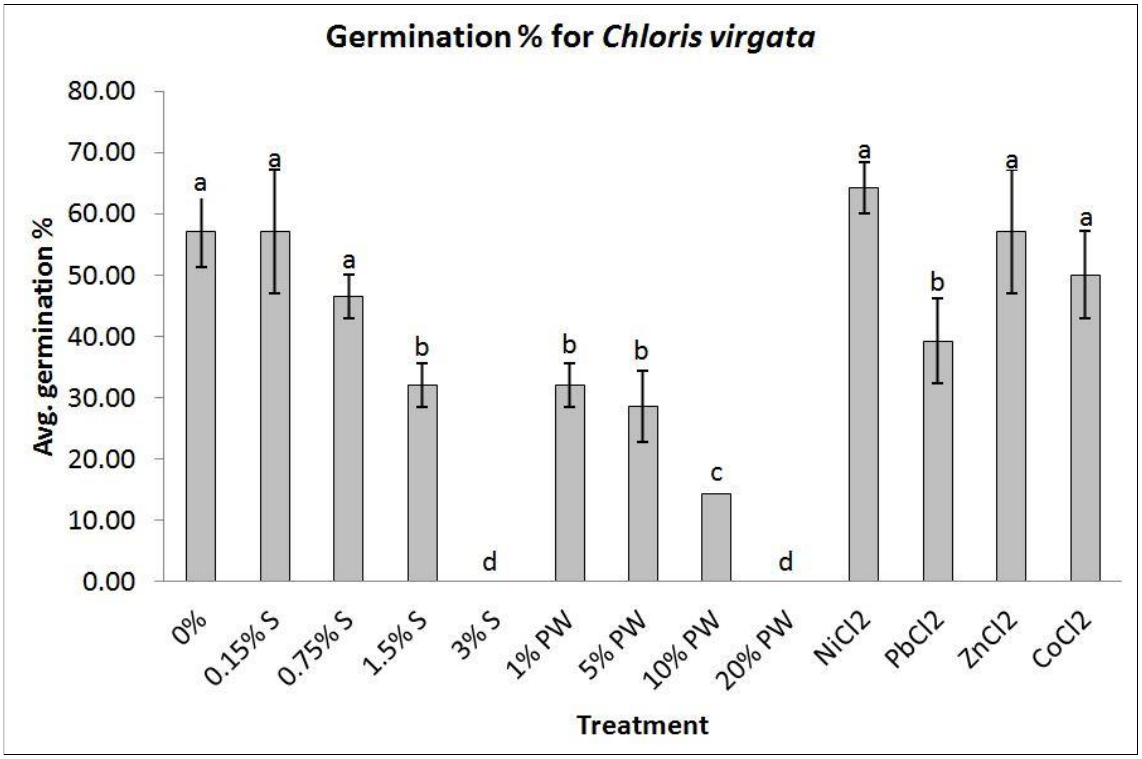

Figure 37. Average percentage of germination at 14 days for *Chloris virgata* subjected to varied treatments. Error bars refers to standard error of the means. Any common letter between treatments refers to no significance at *P* ≥ 0.05 using Tukey's test.

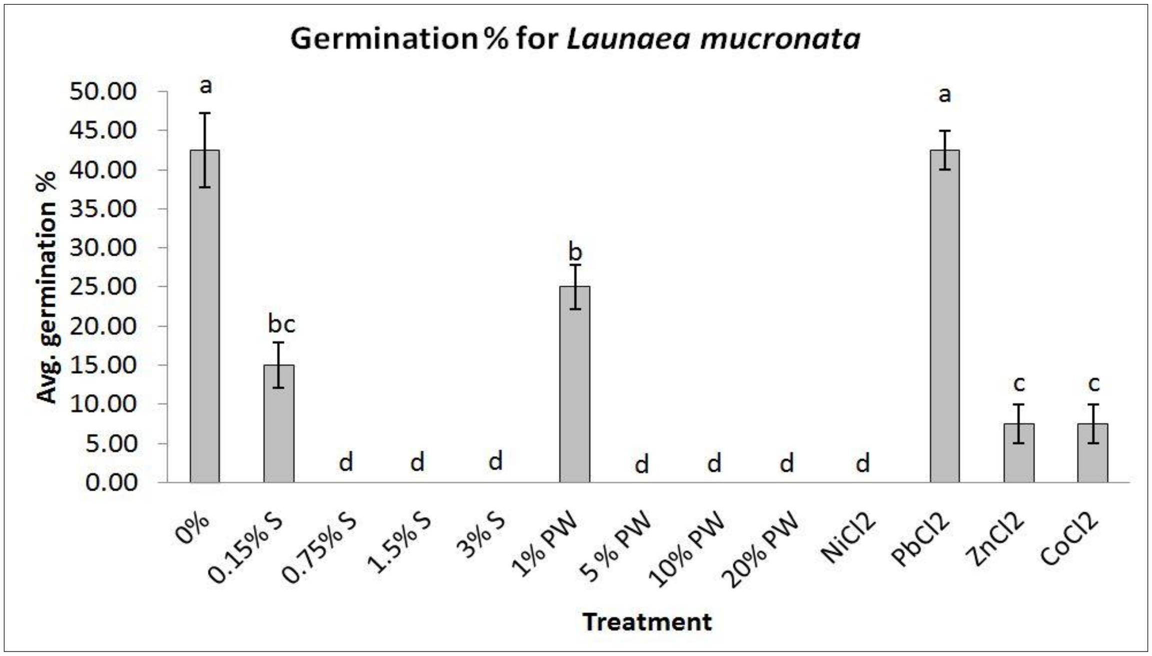

Figure 38. Average percentage of germination at 14 days for *Launaea mucronata* subjected to varied treatments. Error bars refers to standard error of the means. Any common letter between treatments refers to no significance at *P* ≥ 0.05 using Tukey's test.

CHAPTER 6 - DISCUSSION

6.1. Effect of produced water on grass (greenhouse) and salinity (greenhouse and outdoor experiment)

Experimentation on *C. dactylon* and the similarity in the results obtained in produced water treatments and salinity treatments was an indication that negative effects on the treated grass were primarily due to NaCl concentration of the raw produced water. Thus, to effectively utilize produce water removal of NaCl prior to irrigation can be recommended.

Based on obtained results, *C. dactylon* can tolerate up to 30% PW treatment but with loss in both aboveground biomass coverage% and biomass (leaf and root) while *Paspalum* sp. can at least tolerate 30% PW treatment with no loss in aboveground biomass coverage% but with reduction in biomass (leaf and root). A decrease in biomass of plants treated with produced water was an expected scenario. A change in irrigation regime is bound to bring about changes in biomass of treated plants. Interestingly, similar biomass reduction results have been reported by Burkhardt et al. (2015), and Pica et al. (2017) who also tested utilization of produced water for irrigation of varied plants. The findings of this study are consistent with their results.

King et al. (2000) reported that in general turf grass species are efficient in utilizing nutrients from sources like waste water. The differences in growth patterns observed in *C. dactylon* and *Paspalum* sp. study suggests that *Paspalum* sp. may have higher efficiency in utilizing the nutrient available in produced water as compared to *C. dactylon.* Different species have different tolerance capacities and have different growth requirements. The experimental conditions might also play a role in growth abilities of the turf grasses. Since, *C. dactylon* experiment was an indoor/greenhouse experiment and *Paspalum* sp. an outdoor experiment simulating natural condition, difference in their response to produced water is likely. In addition, *Paspalum* sp. may have an inherent capacity of higher tolerance. It has been reported that *Paspalum* sp. such as *Paspalum vaginatum* have high salinity tolerance (Pompeiano et al., 2016) and can be an explanation for its ability to better withstand both produced water and saline water concentrations in comparison to *C. dactylon.* These results give us an indication that for application of produced water for irrigation in Qatar, *Paspalum* sp. could be a better choice. This is advantageous as *Paspalum* sp. is the most commonly used turf grass system in Qatar.

6.2. Effect on soil microbiota (greenhouse and outdoor experiment)

Bacterial CFU of *C. dactylon* treated with 0% -100% PW followed a trend of increase with passage of time. Significant increase in CFU was observed in week 7 as compared to week 3 and 5 for all treatments. It can be hypothesized that while presence of good biomass would have led to increase in CFU for 0% treatment, the increase in CFU in 25% PW-100% PW treatments can be attributed to presence of PW's components such as metals (mentioned in Table 1-3) that could have been favorable and enhanced the growth of bacteria (Figure 18). The high density of fungal colonies in 50% PW treatment can be attributed also to presence of materials in produced water that encouraged fungal growth but the higher concentration of the materials in 75% PW and 100% PW discouraged their growth (Figure 19).

Meanwhile, bacterial CFU of *C. dactylon* treated with 10% -50% PW and 1.5%S -7.5% S followed a trend of initial increase followed by a decrease in CFU with passage of time. The decrease was observed to be sharp for 40% PW and 50% PW. In comparison, bacterial CFU of 0% treatment showed an increasing trend (Figure 20). Kaplan and Kitts (2004) reported a similar sharp increase in plate counts of bacteria in the initial 3 weeks followed by a decrease in waste water irrigated soil. Changes in fungal CFU followed a similar trend where CFU in 10%-50%

PW and 1.5%S -7.5% S treatments first increased significantly (week 3) and then fell sharply (week 5 & 7) (Figure 21). Also, increase in fungal CFU of 40% PW and 50% PW treatments increased very sharply in week 3 as compared to other treatments. This can be explained due to presence of metals and organics that could have enhanced the growth of fungi initially as with the previous experiments. However, presence of other factors, that could act as slow toxins to fungi such as heavy metals, may have led to decrease in fungal CFU after week 3.

Bacterial CFU of *Paspalum* sp. treated with 10% PW-30% PW followed an opposite trend to that of *C. dactylon*. Bacterial CFU first decreased sharply (week 2) and then continued to increase until week 10 (Figure 24). The differences can be attributed to the type of association the bacteria have with *C. dactylon* and *Paspalum* sp. of the species. As for saline water treatments bacterial CFU followed an increasing trend for all treatments (except for 4.5% S) treatment where bacterial CFU first decreased (until 4 week) and then increased (Figure 26). Fungal CFU *Paspalum* sp. treated with 10% PW-30% PW and 1.5% S- 4.5% S showed no apparent trend as compared to that of *C. dactylon*. Significant increase in fungal CFU was however observed in week 4 for both produced water and saline water treatments followed by a decrease. Bacterial and fungal responses to irrigation are expected to vary. It has been reported that bacteria and fungi both respond different to metal toxicity (Rajapaksha et al., 2004) and hence their behavior in presence of produced water is expected to differ as well. In addition, the behavioral trend of both fungi and bacteria in each of the experiment are bound to differ in some respect as it is highly dependent on the soil used, time and type of irrigation, species already existing in the soil prior to treatment, environmental conditions and most importantly bacterial and fungal associations with the plant.

A change in species of fungi present in soil of *Paspalum* sp. treated with 10% PW- 30% PW was witnessed. In comparison to soil from 0% treatment, a higher number of fungal species were noted in soil from 10% PW- 30% PW (Table 4). For example, analysis of soil from 30% PW pots before treatment depicted only 2 species. After treatment 9 more species *Aspergillus flavus, Aspergillus terrus var. africans, Aspergillus terrus var. terreus, Cladosporium tenuissimum, Fusarium* sp., *Fusarium moniliforme, Gibrella* sp., *Penicillium* sp. *and Rhizopus* sp. were found. Certain species' abundance differed when compared to 0% and PW treatments. Fungal densities decreased with time for 10% PW treatments while increased with time for 20% PW. Fungal density for 30% PW treatment initially increased sharply and then followed a descending pattern with time. These results are in contrast to Tian et al. (2014) who reported fungal abundance had decreased in oil-polluted soil but just like this study, the author also observed microbial succession and change in dominant flora. Guo et al. (2017) also reported a change in abundance of certain microbial species in soil irrigated with reclaimed water. Truu et al.(2009) also reported increased diversity of microbial community in soil irrigated with waste water.

Since, produced water contains varied types of organics, inorganics, metals it was expected that number and species of fungi could either be enhanced or lessened. PW irrigation can also encourage growth of species that may be harmful. Fungal succession studies are important to understand the modifications and are a requirement when plans to using alternative water resources such as produced water are charted. They give an indication of the kind of fungal species that were encouraged or discouraged by the irrigation source and the associated risks and issues they could cause. Similarly, bacterial succession study is also recommended in further researches. Succession studies can help minimize risks especially, if irrigation of produced water is performed in areas that are open to public and particularly to children (areas such as parks). The risk of exposure to components of the produced water (such as heavy metals) and also disease causing bacteria/fungi could threaten human health and hence require in-depth studies.

6.3. Metal digestion and ICP Analysis

Metal digestion and ICP Analysis of shoots and roots of *Paspalum* sp. treated with 10% PW - 30% PW depicted accumulation of certain metals in shoots while others accumulated in roots (Figure 35). Vanadium (V) and lead (Pb) were found to accumulate in the shoots of 10% PW - 30% PW in higher concentration as compared to 0% treatment. In contrast, chromium (Cr), nickel (Ni) and arsenic (As) accumulated in the roots in higher quantity as compared to 0% treatment. Interestingly, the 10% PW - 30% PW treated grass also lost certain metals making their concentration (shoots/shoots) lower than concentration observed in 0% treatment. Their shoots had lowered concentration of manganese (Mn) cobalt (Co), and cadmium (Cd) while their roots had lowered concentration of manganese (Mn) and zinc (Zn). Our results are similar to that of Dar et al. (2012) who studied Ni accumulation in *C. dactylon* and to that of Mohamed et al. (2014) who also studied element accumulation in *C. dactylon* that was irrigated with produced water. Ali et al. (2013) also reported similar accumulation of metals including Pb, Cd and Ni in parts of plants that were irrigated with wastewater.

6.4. Effect on germination of turf grass seeds

Germination capacity of *C. dactylon* was significantly lowered by all (Figure 35) it can be concluded that the seeds were affected by both, the presence of NaCl and the presence of tested metals in the produced water. Nickel is known to be toxic to plants in even low quantity (Nabais et. al., 2011). Lead is known to be phytotoxin (Amari et al., 2017). Cadmium too is reported to be phytotoxic while zinc has been reported to inhibit germination in certain species

(Ghodake et al., 2011) and thus could have inhibited germination capacity of *C. dactylon*. This data suggests that while already established *C. dactylon* may tolerate up to 30 % PW concentrations, but germinating *C. dactylon* using produced water is not recommended. Irrigation of *C. dactylon* with produced water should be performed after they are well established. However, removal of salinity and metals through produced water treatments may enhance germination capacity of *C. dactylon* seeds.

6.5. Effect on germination of weed seeds

Weeds are capable of disrupting vital ecosystem processes and out compete native species (Pickering et al., 2016). Having a successful germination is extremely crucial in the life cycle of seeds (Zhang et al., 2012). In modified environmental conditions, seeds that can modify their germination behavior are highly likely to survive and establish themselves (Zhang et al., 2012). Thus, germination capacity of weed seeds gives an indication of its survival rate when subjected to produced water irrigation.

Weed species *Amaranthus viridis* was discovered to be tolerant of salinity between 0.15% S-1.5% S but with lowered germination capacity (Figure 36). However, the seeds could not germinate in produced water concentrations higher than 1% PW suggesting factors other than salinity affecting its germination. Metal treatments also reduced germination capacity. All treatments significantly differed from 0% treatment (distilled water). Based on these results it can be assumed that fields that are irrigated with produced water would discourage the growth of *Amaranthus viridis* thus decreasing competition between turf grass and the weed species. Also, it would lower costs for its removal and management.

On the contrary, weed *Chloris virgata* was observed to germinate with no significant differences between 0% treatment, 0.15% S, 0.75% S, $NiCl_2$, $ZnCl_2$ and $CoCl_2$ treatments. 10% PW lowered germination percentage as compared to all treatments (Figure 37). No germination was observed in 3% S and 20% PW treatments. It can be suggested that *Chloris virgata* was affected primarily by salinity higher than 1.5% S since germination of seeds was noted in metal treatments. It can be concluded that weed *Chloris virgata* can germinate and grow in fields irrigated with produced water if the concentration used is below 20% PW. Concentration of produced water higher than 20% PW may discourage their growth but it would also deter growth of the turf grass. *Chloris virgata* is recognized as a halophyte species commonly growing in saline areas and degenerated grasslands. It is known to have high seed production (Zhang et al., 2012). It follows a C4 photosynthetic pathway giving it ability to grow in desert conditions and be drought resistant (Bhatt et al., 2016). Thus is its ability to germinate in both produced water and saline water. Hence, produced water concentration used for irrigation of turf grass needs to be chosen in a manner to maximize turf grass growth and minimize growth of *Chloris virgata.*

Seeds of *Launaea mucronata* could not germinate in 0.75% S - 3% S, 5% PW - 20% PW and $NiCl_2$ treatments (Figure 38). Interestingly, seeds germinated well in $PbCl_2$ solution with no significant differences as compared to 0% treatment while 0.15% S, 1% PW, and $ZnCl_2$ and $CoCl_2$ treatments significantly decreased germination in comparison to 0% treatment. Drawing conclusions, it can be said that fields that have been irrigated with produced water would have reduced growth of *Launaea mucronata* and hence decreasing competition between turf grass and the weed species. It would also lower costs for its removal and management.

This research is a preliminary study to assess utilization of produced water for irrigation purposes as no such study has been undertaken before in the Gulf region. Use of produced water for irrigation is a new concept and hence only few studies have undertaken assessment of produced water usage but irrigation of turf grasses with produced water has not been studied before. This study shows that utilization of produced water for irrigation is possible. Since, it has been understood that salinity is the key factor determining the effect the produced water has on irrigated grass, its removal can allow higher concentrations of produced water to be used. Treatment of produced water using treatments listed in 2.4.2 can further enhance produced water usage by removing components that deter plant growth. Rigorous studies of metal accumulation and microbial succession are key to understand dynamics of interaction between the plant, soil and irrigation source. A longer study is suggested to completely understand this objective. Turf grass associated weeds also need further investigation in order to allow insight of weed growth and management. More species of weeds need to be analyzed for their response to produced water. This will allow development of proper weed management strategies and management of cost. This study is a stepping stone in this particular research area, paving way for more in-depth analysis and research to ensure utilization of produced water with minimal risk to environment and human health.

CHAPTER 7 - CONCLUSION

The two tested turf grass species depicted varied degree of tolerance and growth ability. *C. dactylon* was reported to be able to withstand up to 30% PW concentration maximum. The incorporation of salinity factor in the experiment gave insight into understanding that NaCl concentration was primary cause of observed effects on *C. dactylon* turf grass. The experiments conducted on *Paspalum* sp. suggested that it has a much higher capacity to tolerate salinity and also produced water as a whole. It can withstand at least withstand 30% PW/4.5% salinity. It can be recommended that *Paspalum* sp. be used in areas in Qatar that are planned to be irrigated with produced water. Since *Paspalum* sp. is the type of turf grass that is used by the Ministry of Environment around Qatar's parks, golf course and road sides, utilization of produced water for their growth is a possibility. However, as mentioned the grass requires to be well established prior to treatment to maximize growth. In addition, a study conducted over a period of two seasons would further allow understanding of the ability of the studied turf grass species to withstand produced water treatments.

Microbial analysis conducted showed different trends of increase and decrease in bacterial and fungal CFUs. Fungal succession study showed presence of certain species in 10%PW-30%PW treated soil that were absent in soil treated with tap water, suggesting need for extensive research in this area to prevent risks to human health. Metal accumulation in *Paspalum* sp. treated with 10% PW- 30% PW varied, with increased accumulation of certain metals and lowered accumulation of others. A complete study evaluating accumulation of metals in *Paspalum s*p. could indicate its ability to be used as a means of metal removal or be utilized in bioremediation projects.

Use of produced water is expected to discourage growth of *Amaranthus viridis* and *Launaea mucronata* but may have no effect on growth and abundance of *Chloris virgata*. The concentration of produced water chosen for irrigation hence is the key determinant of the effect it would have on the growth of both turf grass and also weeds and their abundance. In conclusion, produced water is a viable alternative irrigation source that can be utilized for irrigating turf grass if suitable turf species are chosen, area requiring irrigation is well studied, appropriate concentration of produced water is used and public risk assessment is performed.

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