QATAR UNIVERSITY

COLLEGE OF ARTS AND SCIENCES

THE EFFECT OF TREATED WASTE WATER (TWW) ON THE RHIZOSPHERE MICROBIOMES AND THEIR IMPACT ON ARUGULA

BY

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ABSTRACT

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Title: The Effect of Treated Waste Water (TWW) on the The Rhizosphere Microbiomes and their Impact on Arugula

Supervisor of Thesis: Dr. Ipek Goktepe.

Qatar needs to achieve water and food security in an arid climate and maintain its fossil-fuel energy exporting status. Treated wastewater (TWW) can be an alternative source for vegetable crops irrigation in Qatar; however, no studies have been undertaken in Qatar to study the effect of local TWW on vegetable irrigation and soil microbiome.

Arugula (*Eruca sativa*) was chosen for this study due to its economic importance and profitability, fast maturation, and growing popularity as a salad green. A pinch of Arugula seeds were sprinkled uniformly in pots containing different soil mixtures (natural soil, peatmoss, sterilized peatmoss, and mixed soils), placed on counters inside of the Qatar University greenhouse at 25°C, with 10 hrs daylight, 14 hrs dark for 41 days. The plant samples were watered with either tap water (FW) or TWW throughout the study period. At days 0, 21, and 41, soil samples were analyzed for cations, anions, trace metals, total N, C, H, and S as well as for microbial growth.

Arugula seeds failed to germinate in natural soil and mixed soils due to high salinity in natural soil, making it unsuitable for agricultural purposes. The Microfauna in both natural soil and peatmoss exhibited changes in structure and abundance after irrigation with either FW or TWW. Changes in structure and abundance were due to time of sampling, soil type, competition and interaction between the different microbiota

naturally present in the soil. Results also showed a significant positive correlation in soil between aerobic mesophilic bacteria and plant growth (p=0.0255).

The growth of *Pseudomonas* spp, the *Streptococcus* spp., and nitrogen-fixing bacteria was significantly ($p \leq 0.05$) different in soil samples treated with TWW compared to FW. Total coliform counts were not significantly impacted by soil type, water regimen, or time (p=0.0845).

Further research should be undertaken to isolate and identify different species cultured and look into their activity to discern whether the TWW application improves the soil health by increasing the beneficial strains or increases the pathogenic ones. Microbial soil activity is another field that could also be investigated and its changes according to the irrigation method as another indicator of soil health.

ACKNOWLEDGMENTS

I would like to extend my heartfelt gratitude to my mother, family, and friends who helped me push through and get this far. A special thanks to Dr. Ipek Goktepe whose support and belief in me have been vital. I would also like to thank the advisory committee members, Dr. Nabil Zouari and Dr. Talaat Youssef for their valuable feedback and assistance. Special thanks go to Mr. Ahmad Arafat for his help in the greenhouse, Ms. Hajer Al-Naimi and her team at the Environmental Science Center and Mr. Fathy Atia and Mr. Ahmed Abdelsalam Easa at the Central Laboratory Unit for their assistance in the elemental analyses. Dr. Ahmed Moustafa's help in connecting me with the farms in Qatar and getting a better understanding of the farming situation in The State was crucial to the success of this project.

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CHAPTER 1: INTRODUCTION

Qatar's Arid Environment

Qatar is a peninsula in the Arabian Gulf and a member of the Gulf Cooperation Council (GCC) (The Cooperation Council for the Arab States of the Gulf-Secretariat General; 2012). Qatar is a desert country with less than 2% arable land (CIA World Fact Book, 2016) and is low in renewable natural resources. With an average total rainfall of 79 millimeters (Qatar Meteorological Department, 2016) as seen in Figure 1, it can even be considered a "hyper-arid zone" as defined by the FAO (1989) with rainfall below 100 millimeters.

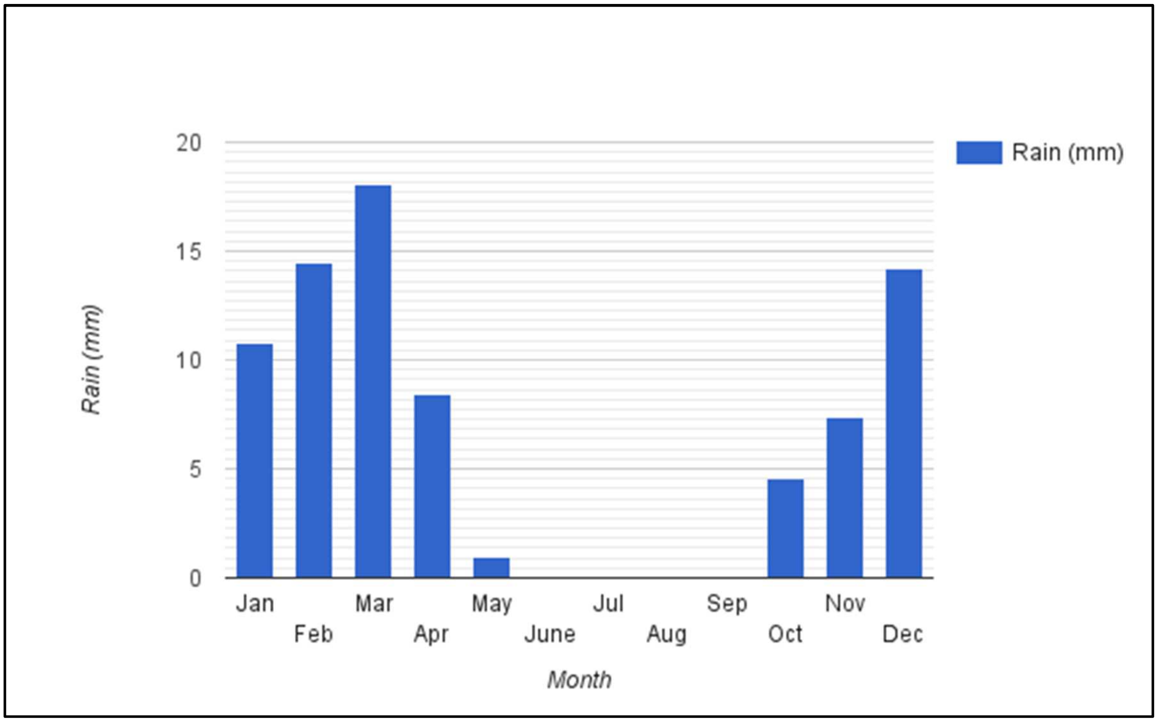

Figure 1: Average rainfall in Qatar (Qatar Meteorological Department, 2016)

The arid environment has a significant effect on the quality of the soil in the country making it dry, and with Qatar's dry sandy soil, there is a paucity of organic matter and microorganisms within the substrate (Shomar *et al.*, 2013). Out of Qatar's total land area, 1,161,000 Ha, only 1% of that land has been cultivated (Ministry of Development Planning and Statistics, 2014).

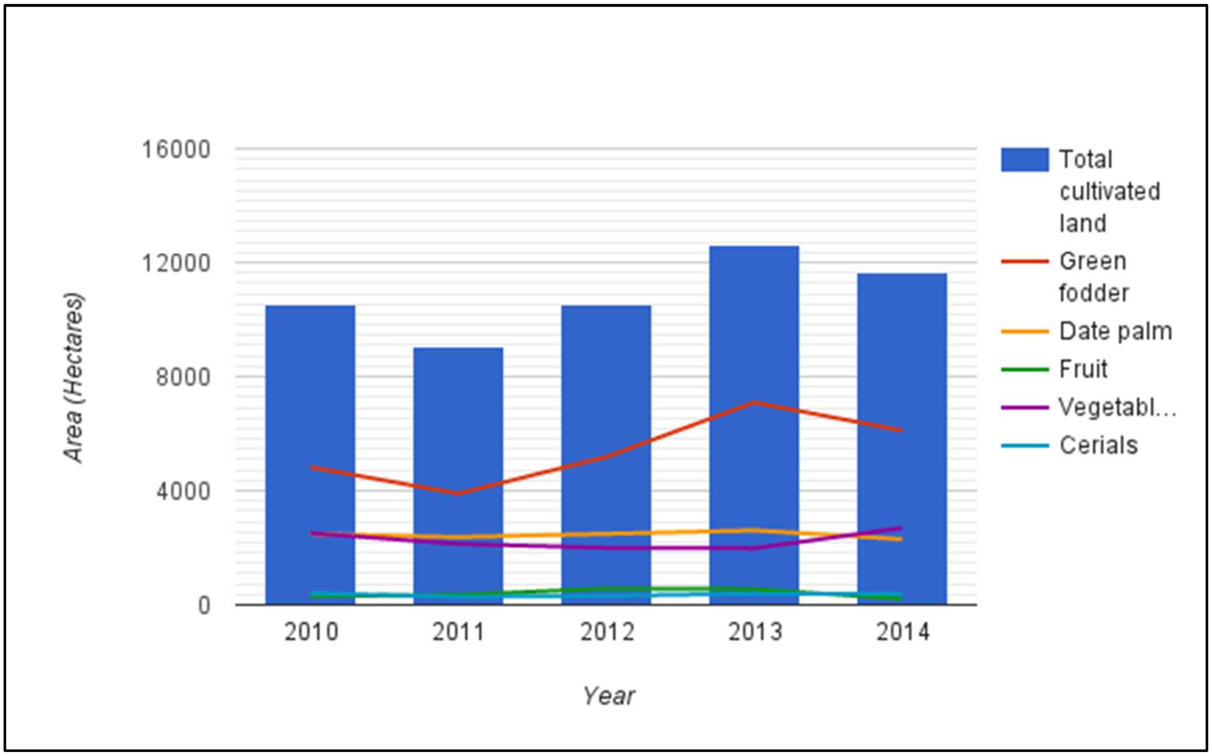

Figure 2: Total cultivated land in Qatar by area and general crop types cultivated (Ministry of Development Planning and Statistics, 2014)

As can be seen from Figure 2, there is a dropping trend in the areas of land cultivated, with most cultivation crops showing a similar trend except for vegetables which are showing a rising trend as perhaps more farms are switching towards vegetable cultivation. The trend of rising vegetable cultivation could be explained by calculating the value of crops cultivated per hectare of land. The value of vegetables was significantly higher and brought more returns per hectare than other cultivated crops, even when the value dropped in 2014, it was still much higher than all the other cultivated crops (Figure 3).

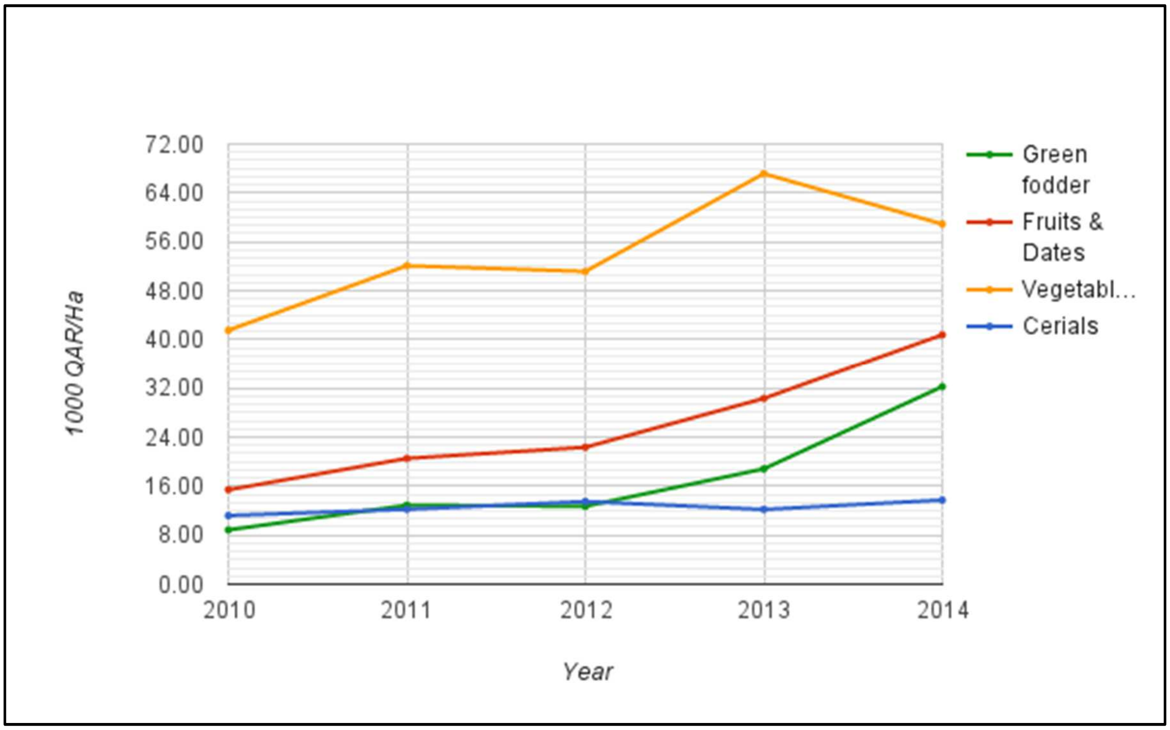

Figure 3: Value of produced crops in Qatar by 1000 QAR/Hectare (Ministry of Development Planning and Statistics, 2014)

Water-Food-Energy Nexus in Qatar

In recent years, the country witnessed a rapid economic and social development after the discovery of oil and natural gas, the third largest proven reserves in the world (CIA World Fact Book; 2016). The rapid growth has resulted in an unprecedented increase in population, especially through migration where Qatar ranked the first in the world for migrants coming in the country at a rate of 18.2 migrants per 1000 population (CIA World Fact Book; 2016).

The increasing net migration rate and rapid expansion has also put strains on the resources of the country, from water availability and use (Table 1), to food for the growing population and Energy production to support growing urbanization and local industry, while providing enough fossil energy (oil and gas) for export which is one of the main economic commodities in Qatar (Figure 4).

Table 1: Available renewable water with population increase in Qatar (FAO, 2016)

Year	Total renewable water resources per capita (m3/inhab/yr)	Total population (1000 inhab)
1972	449.6	129
1977	320.4	181
1982	210.9	275
1987	138.1	420
1992	118.9	488
1997	109.6	529
2002	92.95	624
2007	49.24	1178
2011	31.02	1870
2012	27.78	2016
2014	25.06	2235

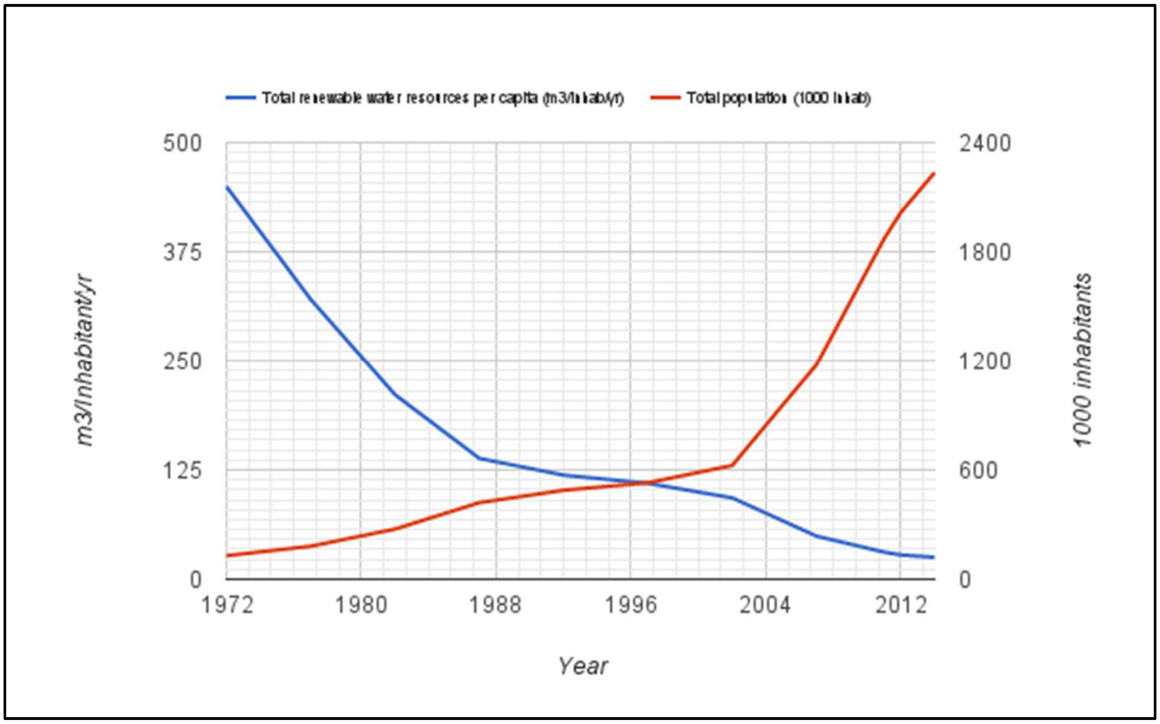

Figure 4: Total renewable water resources per capita and population increase with time (FAO, 2016a)

It is noteworthy note that Qatar cannot sustain its growing population with its scarce natural renewable water resources, ground water replenished from the annual rains and inflow from Saudi (FAO, 2016; Ministry of Development Planning and Statistics, 2014), alternate sources of water is required to sustain the growing population.

One emerging view is the, food, water and energy nexus. This means that energy is required to harness and distribute water and; therefore, produce food (Siddiqi and Anadon, 2011), and that water is often used and consumed to develop and deliver energy (Electricity, Figure 5) for consumption (Rio Carrillo and Frei, 2009). This suggests that there will be a need to assess and prioritize trade-offs between energy security, water security, food security, and the need for an infrastructure capable of making these decisions (Scott *et al.*, 2011).

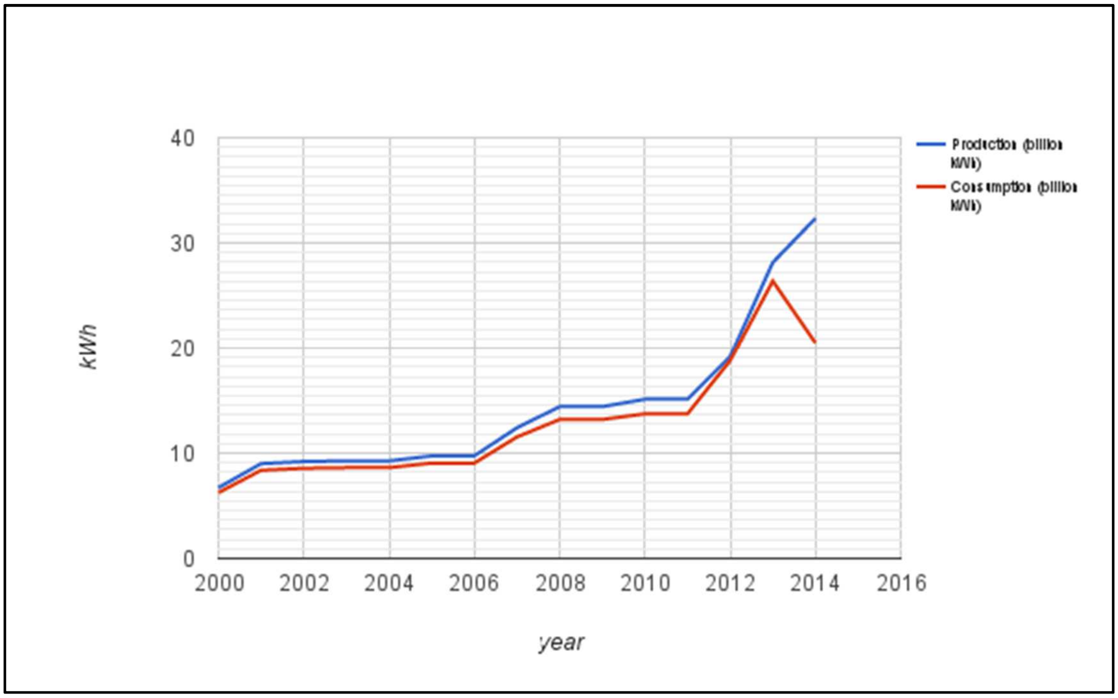

Figure 5: Electricity production and consumption (billion kWh) per year in Qatar (IndexMundi, 2016)

Looking at the oil production and consumption in Figure 6, it is clear that the oil production increased greatly as the demand locally and internationally for fossil energy increased.

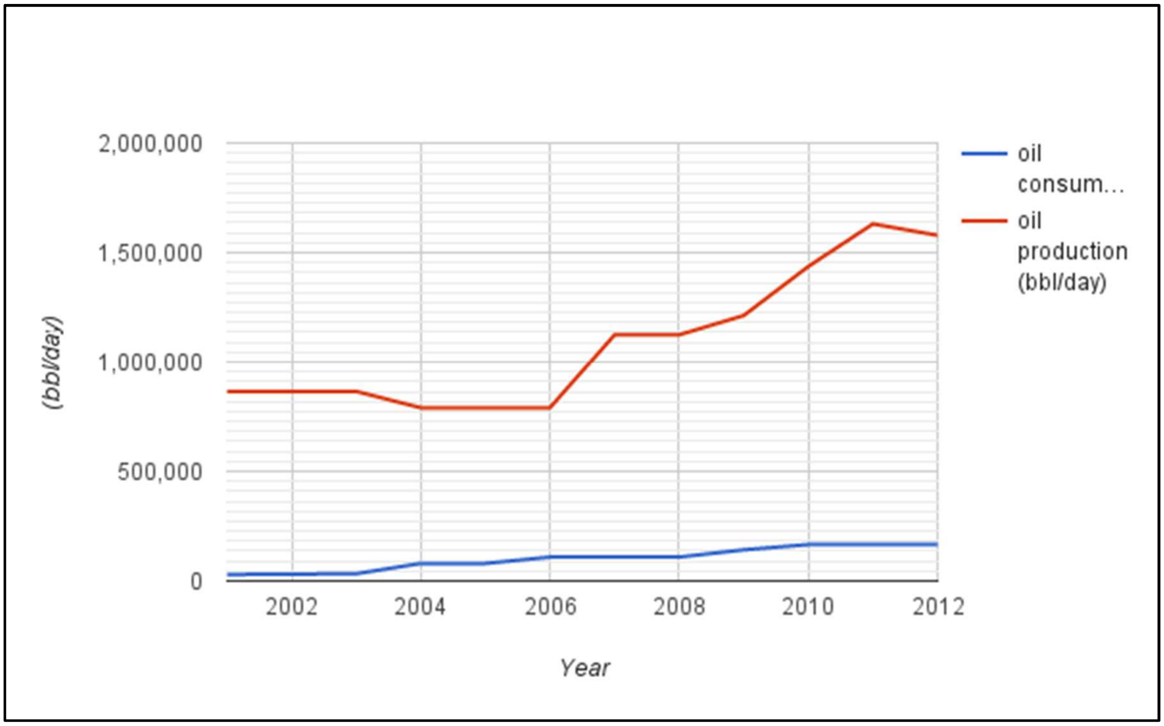

Figure 6: Oil consumption and production with time in barrels per day (IndexMundi, 2016)

Another emerging paradigm is the food and water nexus, and the concept of "virtual water" (Allan, 2002), which is the water used to produce food crops. Virtual water can be used as an indirect method towards water security, where a country with limited water resources can "save" their water and import virtual water as food which would cost much more if it had to be produced locally (Allan, 2002; Oki and Kanai, 2004). Qatar imports most of its food (Qatar Statistical Authority, 2011) as can be seen in Table 2 and Figure 7, indicating the transboundary movement of virtual water from exporting countries and highlights the importance of water for food security and the harnessing the water resources in order to secure food sustainability.

Table 2: Value of food produced in Qatar (Ministry of Development Planning and Statistics, 2014)

Value (1000 QAR)	2010	2011	2012	2013	2014
Green fodder	42701	50201	65855	133434	196899
Fruits & Dates	42701	55701	68059	95428	101724
Vegetables	103722	110730	101689	132485	157926
Cereals	4594	3431	4234	4815	5202
Meat	220812	206275	238077	285744	353898
Dairy	91065	106322	116383	167235	265750
Eggs	27079	30567	28348	30342	30156
Fish	158244	166336	165713	178874	241574

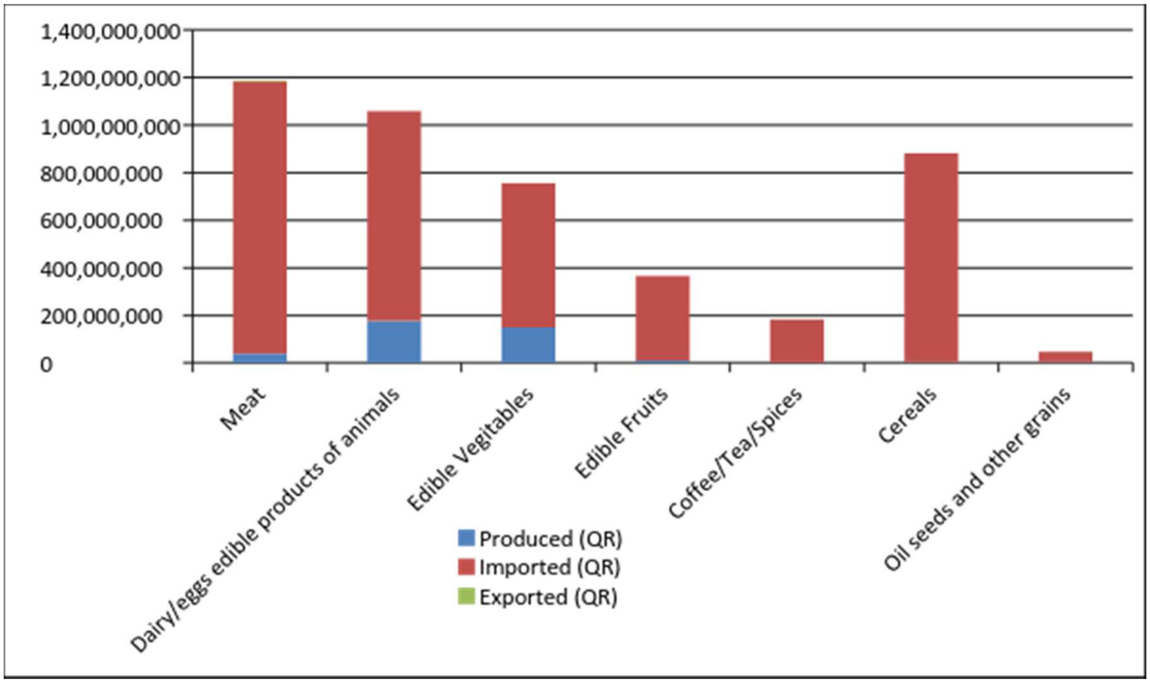

Figure 7: Value of traded foods in Qatar for 2011 (Qatar Statistical Authority; 2011)

Non-Conventional Water Sources

This brings us forward to the phenomena of "non-conventional" water resources (Qadir *et al.*, 2007) as tools towards water security for arid countries like Qatar. Virtual water has been discussed earlier, other examples of non-conventional resources include: Desalination: the process of converting seawater or highly brackish groundwater into good quality freshwater. There are several different desalination methods including distillation and reverse osmosis. Both are energy intensive with distillation methods being more energy intensive but yielding higher quality water (Qadir *et al.*, 2007). Qatar currently relies heavily on desalination to provide for its freshwater needs as the process has been optimized with electricity production and the availability of cheap non-renewable energy to fuel the production.

Desalination has its environmental implications, where it is a heavy carbon emitter as well as the effluent it generates which comprises of highly saline brine and chemicals used for the pre-treatment of the feed water. It also relies heavily on the use of non-renewable fuel, and this raises the issue of sustainability of this method once resources are depleted (Qadir *et al.*, 2007).

Wastewater recycling: Due to population increase and greater standards of living with increased demand for good-quality water from both public and industrial sectors, there has been an increase in wastewater production (Qadir *et al*., 2007). The use of treated wastewater (TWW) decreases the demand for freshwater in agriculture with the added benefit of less need for fertilizer.

However, some challenges include the social acceptance of the use of wastewater for food crop production by both the public and the policy makers (Shomar and Dare, 2015). Another challenge would be the training and setting up the framework for safe practices in agriculture with treated wastewater.

Figure 8 shows the total amount of water produced from desalination, with TWW being available since 2003 and growing in volume with time (TWW labeled TSE in the figure, as raw data could not be obtained, the figure was copied directly from the document).

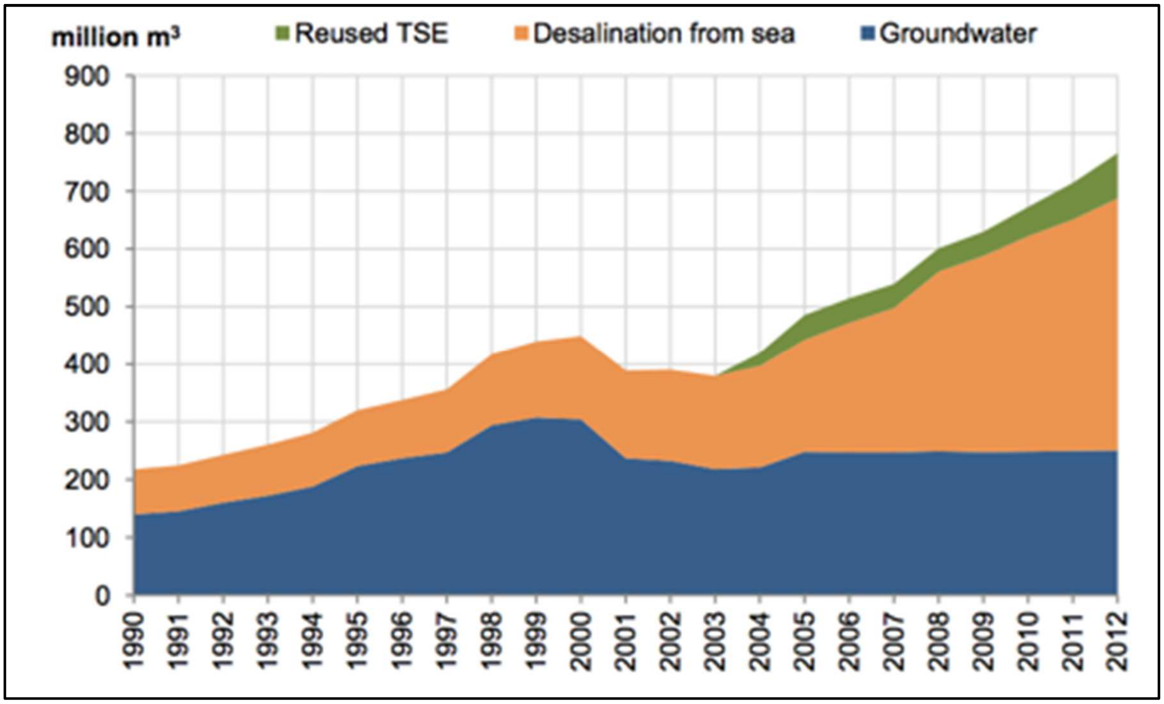

Figure 8: Total water production from 1990 to 2012 (Ministry of Development Planning and Statistics, 2013)

Figure 9 summarizes the current uses for TWW in Qatar. The majority is used for agricultural purposes, but due to the current ban on using TWW for commercial food production, the TWW used in agriculture is mainly for the production of animal fodder.

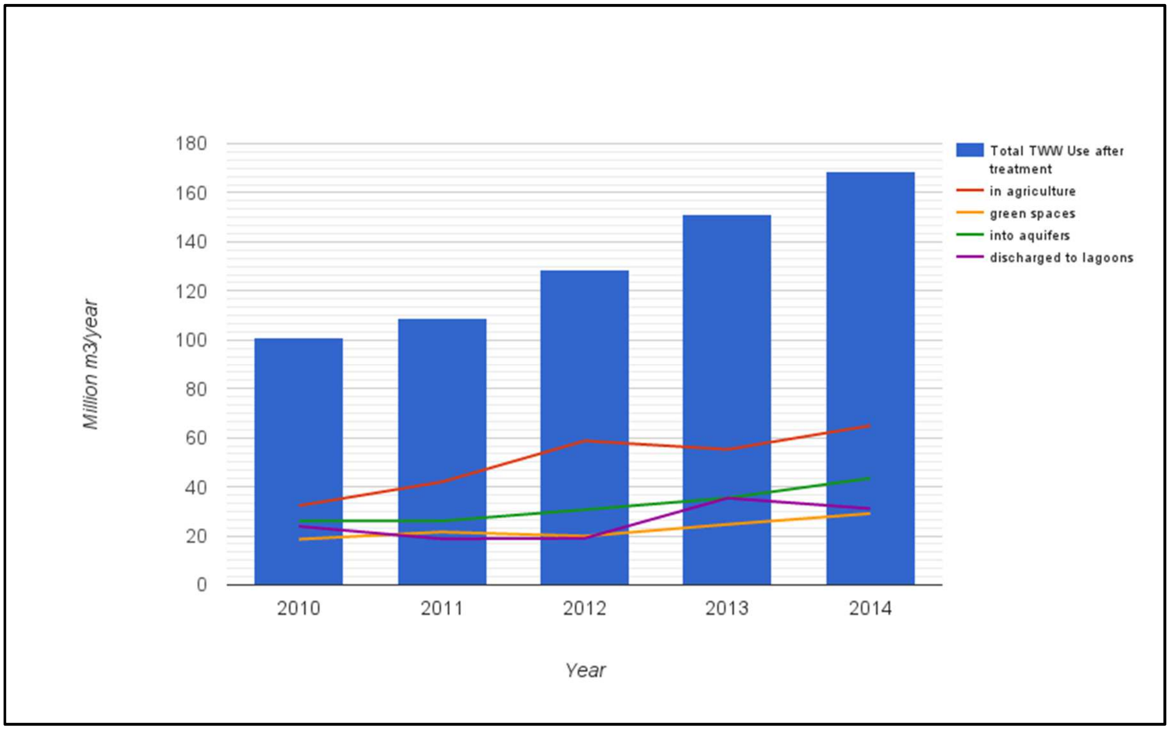

Figure 9: Current uses of TWW in Qatar (Ministry of Development Planning and Statistics, 2014)

Water Security in Qatar

Qatar is a country which is highly vulnerable to issues of food security and food price volatility. To address this problem, the responsible Qatari agencies have issued a "Master Plan" to help reduce Qatar's vulnerability (QNFSP, 2013). One of the strategies mentioned in the plan was to improve Qatar's international trade and investment in food; however, this has caused a political backlash from other international communities (QNFSP, 2013). An example is the leasing out of Kenyan land for Qatari farms has been described as "Neo-Colonialism" and that local farmers will get limited or no benefit since many fertile lands are owned by political families in Kenya (Daily Nation, 2009 Quote: Jacques Diouf, Head of FAO). The master plan also addressed the state of food production in Qatar, noting that it was below regional benchmarks by more than half the productivity (QNFSP, 2013). The plan also emphasized the need for the country to adopt "*high tech sustainable agricultural practices* and modernization of transport, market, *water* and energy infrastructure" in order to transform local agricultural production (QNFSP, 2013).

Farmers in Qatar are the heaviest users of water (FAO, 2016) and have a very strong interest in the issue of water security and water availability since their activities are heavily dependent on water. The farmers are constricted by the availability of less than 2% arable land (CIA: World Factbook; 2016), and the depletion of groundwater. They are also affected by the regulations from the government against the use of TWW for food production. Currently, 37% TWW is used for irrigation of public parks, 27% is used for forage production, 25% is injected in aquifers, and 11% is released into the lagoons in Qatar (El Emadi K and El Emadi H, 2014; Ministry of Development Planning and Statistics, 2014).

In a country where water shortages are at a critical level for agricultural production, the trend is to use TWW as alternative to address water scarcity issue. Although the volumes of TWW produced are small, the cost and environmental consequences of aquifer depletion for agriculture, like salt water intrusion, is high, and thus any move to reduce aquifer depletion would be highly recommended. However with advancement in agricultural practices and the slow move towards advanced farming techniques such as in Al-Sulaiteen Agricultural & Industrial Complex, Research & Development (SAIC) farms (SAIC, 2010), the demand for water for agriculture may decrease while productivity may increase.

Figure 10 summarizes the different factors affecting the agricultural sector from a water security perspective. It is evident that many different factors influence agriculture, including public's perspective about locally grown products and the water used to grow them. Although imported food does not face stringent constraints on the use of TWW, local agriculture faces many difficulties on the use of TWW for crop production in Qatar.

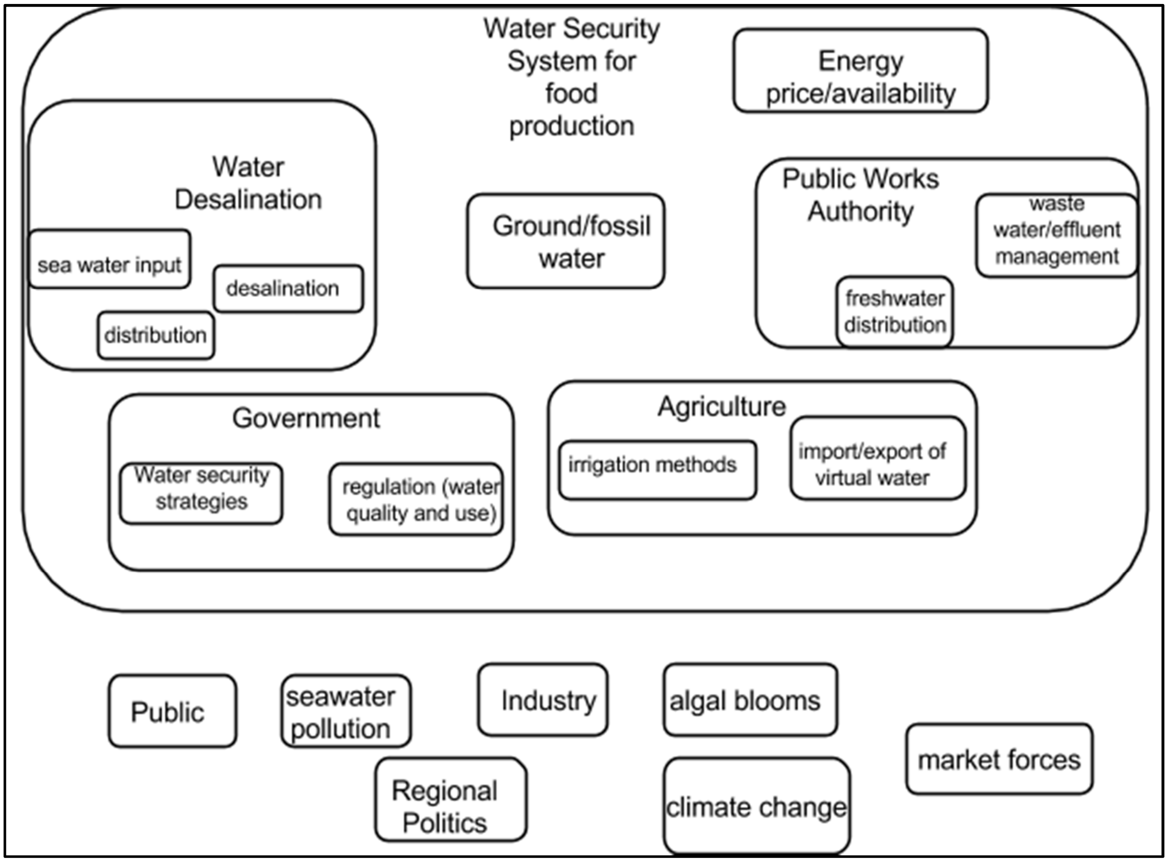

Figure 10: Water Security System Map for Food Production in Qatar

Soil in Qatar

As mentioned previously, Qatar's soils are generally poor in organic content and the majority is not arable (Shomar *et al.*, 2013; Shomar, 2015), this is due to the frequent dust storms (Shomar and Dare, 2015), low precipitation and high evaporation with the high temperatures and the high levels of urbanization and industrialization in the country (Shomar, 2013; Shomar and Dare, 2015). Shomar (2015) found that soil chemistry in Qatar varied due to anthropogenic factors, like the elevated levels of lead, molybdenum, vanadium, zinc and sulfur is possibly due to the oil and gas industrial activities depositing and/or leaching them into the soil. Another anthropogenic factor to the soil is the addition of foreign soils to increase the arability of some locations (Shomar, 2015) which alters the chemistry of the soil in the areas they were added in. High salinity of the soil was also noticed and attributed to seawater intrusion into the groundwater, which then through capillary action rise to the surface soil, increasing its salinity and/or due to aerosol deposition from the sea (Shomar, 2015).

An investigation into the biological soil crust of Qatar was also undertaken by Richer *et al*. (2012) to benchmark the microfauna in Qatar in the face of rapid urbanization and soil disturbances. Biological soil crusts are assemblages of bacteria, microfungi, and lichen and are important in desert soils for maintaining the soil structure against wind erosion and facilitating the establishment and development of vascular plants in the soil by assisting in nitrogen and carbon fixation (Powell *et al*. 2015). It was found that there was great variability in the surface coverage of biological soil crusts in Qatar ranging from 0 to 87% depending on soil type and topography with cyanobacteria being the main microorganisms found (Richer *et al*., 2012). Richer *et al*. (2012) also noticed that agricultural practices in sampled areas affected the composition and coverage of the microbia in the soil crust increasing their abundance and variety.

Problem Definition

Qatar is faced with the interconnected issues of achieving water and food security while trying to maintain its status as a fossil fuel energy exporting country which is the main economic resource of the country. TWW can be an alternative source for irrigation of vegetable crops in Qatar, however no studies have been undertaken or published yet in the country for use of the local TWW in vegetable irrigation.

Although some studies have looked into the possibility of uptake and accumulation of toxic metals and chemicals from TWW into plants, few studies have looked into the effect of TWW on rhizosphere and bulk soil microbiomes, as it was observed that agricultural practices affected biological soil crust assemblages, which play a large part in plant health, and none that looked into this issue in Qatar for the growth of vegetable crops (e.g. Arugula/Rocca) to address food security.

As it was highlighted in the "Master Plan" the need to use alternative sources of water with added benefits of nutrient recovery, TWW might be a beneficial alternative for agriculture in improving soil fertility and condition. Therefore, utilization of TWW in agriculture needs to be studied to investigate its effect on the soil and plant quality.

Arugula (*Eruca sativa*) was chosen for this study due to the economic importance and profitability of vegetable crops/hectare, its fast seeding to maturation phase which allows for its study in the short time allocated for this research, its growing popularity as a salad green. As a quickly perishable vegetable, it is important to have local sources to reduce shipment costs of imported arugula. Arugula is also tolerant to drought conditions yet also sensitive to any water pollutants that can affect its growth, thus making it easy to observe any negative effects of Qatari TWW use.

Objectives

This study was designed to:

1) Evaluate the effect of TWW and different soil on the growth of *E. sativa*,

2) Determine if the use of TWW significantly changes the composition of rhizosphere and bulk soil microbiomes, and

3) Assess the reliability of TWW for agricultural use in terms of addressing food security in Qatar.

CHAPTER 2: LITTERATURE REVIEW

Agriculture in Arid Lands

Farmers in arid countries face many challenges in crops that can withstand the harsh environments, lack of water availability, and low quality of soil. They also have a range of mitigation tools that can alleviate some of the problems they face. Figure 11 is a summary of these issues and challenges and possible mitigation solutions.

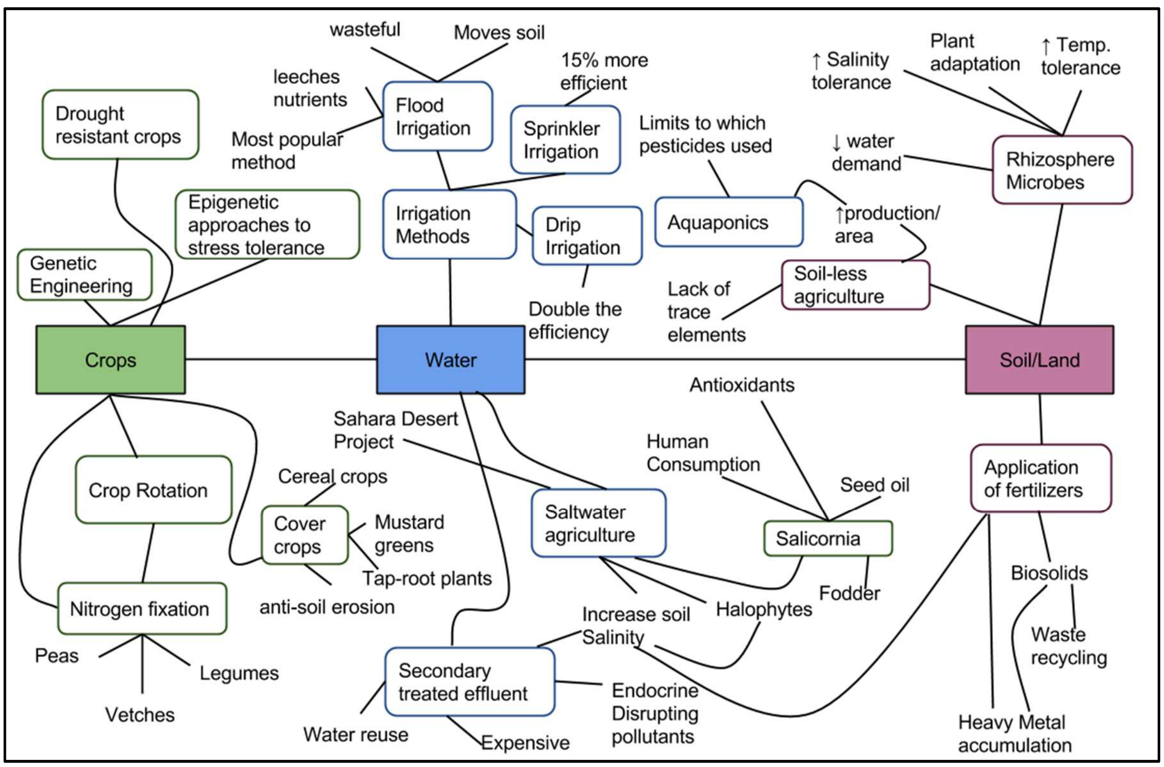

Figure 11: Agriculture in arid lands: challenges and some solutions (KAUST, 2016; Hunter et al., 2014).

Figure 12 highlights the interrelated and complex factors that need to be factored in when food and water security issues are addressed. One prime example is the increase in number of farms which would satisfy the increase in food security, but conversely reduce the amount of water available for agriculture. Similar forces include desalination for agriculture, where it would increase the availability of water; however, it would decrease the affordability of food.

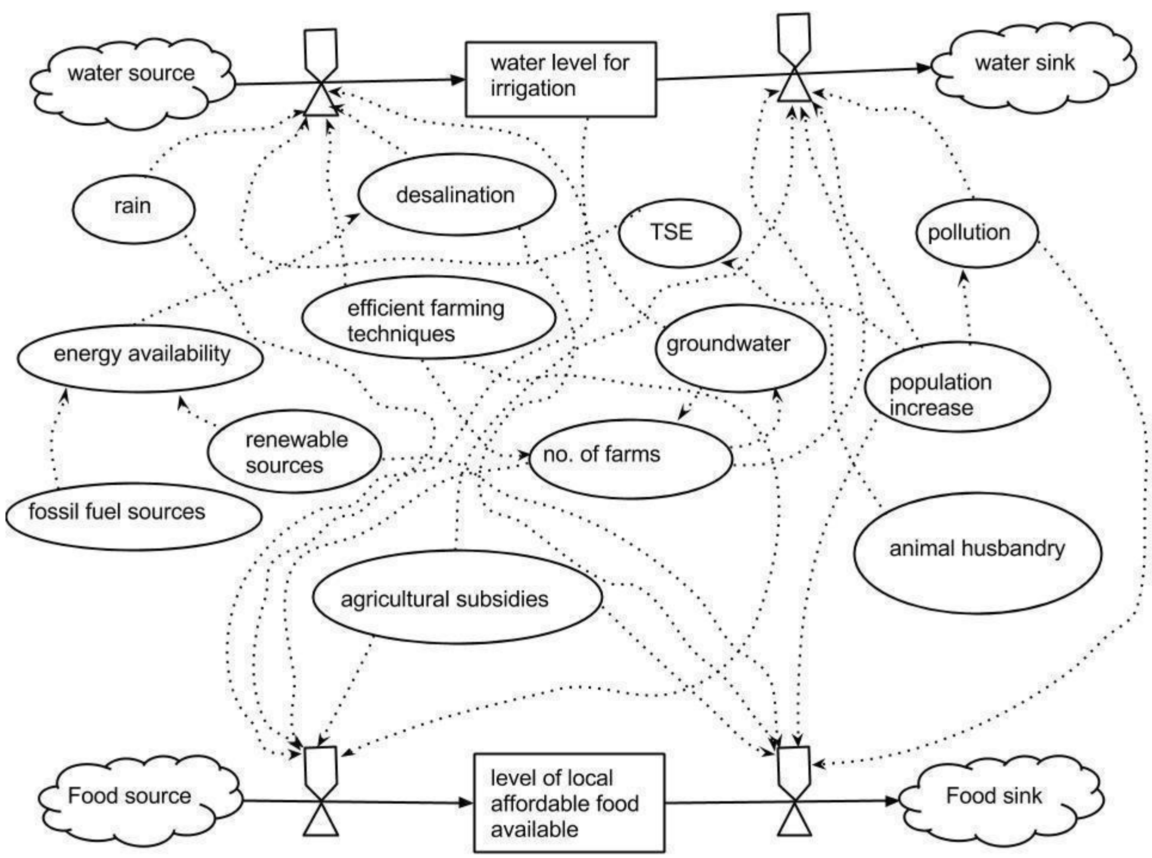

Figure 12: Systems dynamic diagram of Qatar's food-water nexus

The Use of TWW in Agriculture

Many countries are currently using TWW for direct irrigation of crops as well as landscape and forestry. Table 3 and Figures 13 & 14 summarize the list of countries using TWW for irrigation (perhaps not food crops specifically for some) with Asia having the greatest ratio of TWW irrigation, and Saudi Arabia and China being the most users for irrigation. It is also interesting to note that many countries that are using TWW for irrigation are countries that tend to have water shortages, e.g. Cyprus, Greece, and Italy of the European nations.

Table 3: Direct use of TWW for irrigation by State (FAO, 2016)

Direct use of treated municipal wastewater for irrigation purposes	Latest value(s)	$10^6 m^3/yr$	
Algeria	2012	10	**Africa Total: 416 ($10^6 m^3/y$)**
Djibouti	2000	0.1	
Egypt	2011	290	
Libya	2008	40	
Morocco	2008	2	
Namibia	1997	1.1	
South Africa	2009	6	
Tunisia	2009	67	
Brazil	2008	8	**Americas Total: 991 ($10^6 m^3/y$)**
Chile	2008	138	
Mexico	2010	401	
Peru	2011	114	
United States of America	2004	330	
Bahrain	2008	9	**Asia Total: 2471 ($10^6 m^3/y$)**
China	2008	480	
Iran (Islamic Republic of)	2010	328	
Iraq	2012	5	
Israel	2004	279	
Japan	2009	11.6	
Jordan	2010	103	
Kuwait	2012	109	
Lebanon	2011	4	
Oman	2004	24	
Qatar	2012	78	
Saudi Arabia	2010	535	
Syrian Arab Republic	2009	365	
United Arab Emirates	2012	140	
Cyprus	2010	11	**Europe Total: 167 ($10^6 m^3/y$)**
Greece	2010	69	
Italy	2006	87	
Australia	2013	280	**Oceania Total: 280 ($10^6 m^3/y$)**
Total:		**4324.8**	

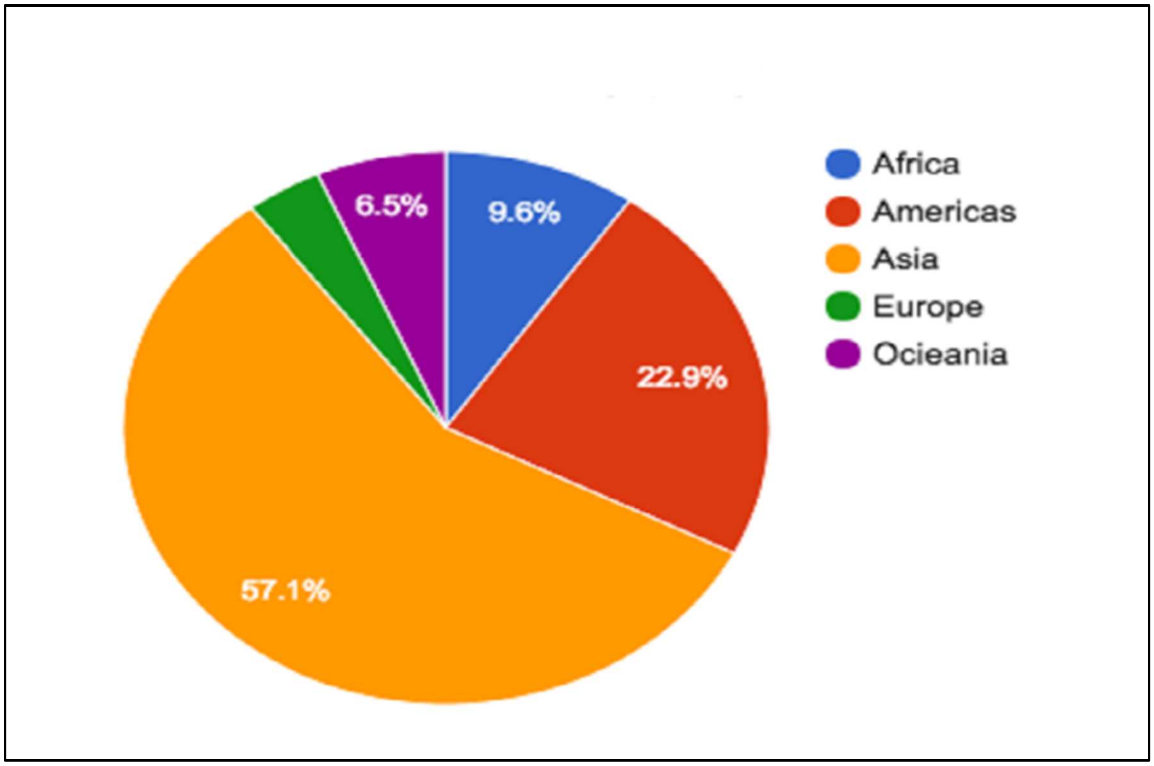

Figure 13: Ratio of TWW use by region, where Asia is leading in the amount of TWW use (FAO, 2016)

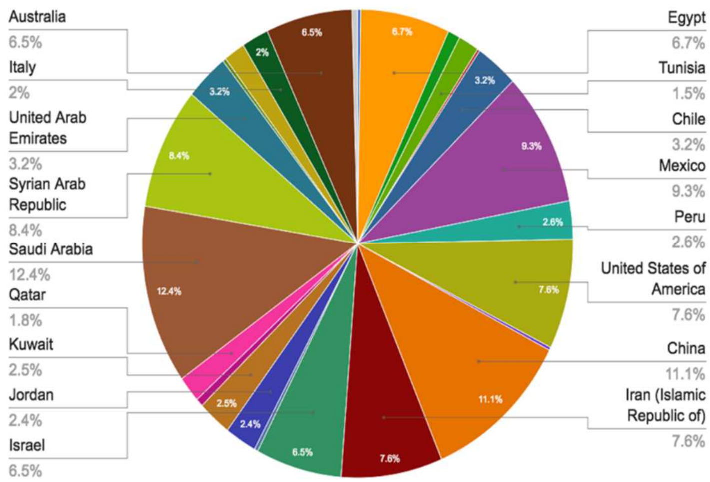

Figure 14: Ratio of TWW use by country, Saudi Arabia and China are the most users of TWW (FAO, 2016)

Asgharipour *et al.* (2012) noted that irrigation with sewage, especially 50% diluted sewage provided a stimulatory effect on the vegetative growth of millet grown in the Zabol region of Iran and that both macro and micronutrient content of the millet increased with the diluted sewage irrigation. It has also been found that sewage irrigation can increase the concentrations of nitrogen and phosphorus and other nutrient in soil (Thapliyal *et al.*, 2013, and Hua *et al.*, 2016) which aids in plant growth. Hua *et al.* (2016) demonstrated that using diluted sewage (30-60% dilution with freshwater) enhanced kale crop yields as well as increased the plant's total nitrogen content.

Regarding the use of TWW for irrigation, a three year study was conducted by Lonigro *et al.* (2016) on the possibility of contamination of food crops with fecal coliforms, *Escherichia coli, Salmonella,* protozoa *Giardia and Cryptosporidium*. It was found that despite the heavy load of bacteria in the TWW that is beyond the legal limit in Italy, *Salmonella, Giardia* and *Cryptosporidium parvarium* were not determined in the TWW. While, crops and soil were free from fecal pollution that may be harmful to consumers at the time of harvest.

As widely used as TWW may be, it has its risks, containing microbial contaminants, toxic heavy metals, high loads of nitrates and phosphates, etc. It might also contain residues of pharmaceutical and personal care products (PPCPs), and sometimes pathogens (Golovco *et al.*2014; Karnjanapiboonwong *et al.*, 2011; Prosser & Sibley, 2015). Shomar et al. (2013) noted that Qatar uses a small percentage of its TWW and have emphasized the importance of studies on the long-term impact of TWW on soil quality (Kamizoulis *et al.*, 2010), as part of Qatar's integrated water resources management system (Shomar *et al.*, 2013). Qatar's current standards for TWW are summarized in Table 4. Ashghal (the local authority in charge of TWW) also has expressed interest in improving their future standards, reducing total nitrogen and total dissolved solids by more than half, in order to widen the uses for TWW beyond landscape irrigation to other industrial processes like construction projects, concrete, and district cooling facilities (Ashghal-PWA, 2014).

Table 4: Qatar's TWW standards for reuse (Ashghal-PWA, 2014)

Standard effluent criteria	Current standard	Future standard
Suspended solids	5 mg/l	5 mg/l
BOD	5 mg/l	5 mg O_2/l
COD	50 mg/l	50 mg O_2/l
pH	6-9	6-9
Ammonia	1 mg/l	1 mg/l
Phosphate	1/2 mg/l	2 mg/l
Total nitrogen	10 mg/l	5 mg/l
Dissolved oxygen	2 mg/l (min)	2 mg O_2/l (min)
Chlorine (free residual)	0.5 – 1.0 mg/l	0.5 – 1.0 mg/l
Turbidity	2 NTU	2 NTU
Total dissolved solids	2000 mg/l	500 mg/l
Most probable number of fecal coliform/100 ml	0	0
Intestinal nematodes (no. of eggs/1 liter)	< 1.0	0
Enteric viruses (no. of plaque forming unit (PFU) /40 liters)	< 1.0	< 1.0
Gardia (no. of cysts /40 liters)	< 1.0	< 1.0

Chemical and Physical Effects of TWW irrigation on Plants and Soil

Several studies have been carried out to determine the chemical and physical effects of TWW irrigation on plants and soil. One study on the use of TWW compared with potable water and salt water for sorghum and sunflower plants in Qatar was carried out by Ahmed and Al-Hajri (2009). The authors concluded that some heavy metals were present in the TWW, however, they were all within the acceptable range, and that after irrigating the soil with TWW treatments, there was a significant difference in the amount of Cr, Mn and Zn. Additionally, there was selective absorption of Zn from the soil by Sorghum, reducing the amount available in the soil as compared to sunflower which was more selective for Cr. It is important to highlight that none of the heavy metals tested in this study reached toxic levels within the plant for human and animal consumption.

In a similar study in the Eastern Province of Saudi Arabia, Al-Omron *et al.* (2012) compared the long-term effect of TWW irrigation on date palm cropped soil versus irrigation with groundwater wells for 13 years. Their findings were that soil was being affected by saltwater intrusion in groundwater, where salinity of the soil samples were higher on average than samples irrigated with TWW. Thus, TWW may be an alternative irrigation method to highly saline/brackish groundwater. Another benefit from TWW irrigation is that they found an increase in soil organic matter when compared with groundwater. The added benefits of increased organic content in soil is the enhancement of plant growth, as well as increasing the soil's capability to retain water and be more stable (Bot & Benites, 2005).However, Al-Omron *et al.* (2012) and Ahmed and Al-Hajri (2009) found that heavy metals in the soils irrigated with TWW were higher on average than soils irrigated with groundwater, Zn (130%), Pb (55%), Fe (82%), Ni (84%), Mn (30%), Cu(40%), Cr (75%), Co (78%) and As (67%), yet all still within the acceptable range as well as a slight drop in pH when irrigated with TWW. In a different study carried out by Adrover *et al.* (2012), it was found that there were no negative effects on the soil when the land in Mallorca, Spain was irrigated with TWW for more than 20 years. Furthermore, they also determined that the soil exhibited better soil water-soluble organic carbon, soil microbial biomass, and soil microbial activity when compared to soils not irrigated with TWW. However, it must be noted that the TWW irrigated soil was mainly used for growing alfalfa, which is a plant that may have intrinsically improved soil conditions, while non-TWW irrigated soils were sampled from different sites with different cropping systems.

A study in Jordan (Rusan *et al.*, 2007) investigated the soil quality and forage plant samples in sites that have been irrigated with TWW for varying time scales, 2 years, 5 years, and 10 years. The authors indicated that as the wastewater irrigation period increased, soil salinity, organic content, and plant nutrients increased in the soil. Interestingly, they have also found that plant biomass increased when compared to control after being irrigated with TWW. However, it is important to emphasize that there was a marked decrease in biomass in lands that have been irrigated for longer period (10 years), yet the biomass was still higher than the control. They also noted that the levels of Cu, as well as Zn, Fe, Mn in plant samples increased after irrigating with wastewater initially, however, the levels decreased after longer periods of irrigation with TWW as these nutrients got leached away from the root zone of the plant. There is plenty of literature available to prove the uptake and accumulation of toxic metals like lead and arsenic TWW into plants, however, very few studies have looked into the effect of TWW and heavy metals on rhizosphere microbiomes which play a large part in plant health.

Other than heavy metal contamination, due to our modern lifestyles, using PPCPs as well as the range of new pharmaceutical products distributed to patients and from hospitals, many of these compounds find their way to the sewage systems. A study in Japan has looked into the degradation of pharmaceutical products in the sewage system before and after biological sewage treatment, then followed by ozonation as a second step of treatment (Azuma *et al*., 2016). It was revealed that many pharmaceutical products that have been detected in the sewage treatment facility influent were near similar levels of concentration after treatment, with relatively few being removed by conventional treatment (Azuma *et al.*, 2016). However, by adding an ozonation process after treatment managed to remove the majority of these recalcitrant products. It was also found that anti-cancer, psychotropic, and anti-pruritic drugs were still present after ozonation of treated sewage effluent prompting Azuma *et al.* (2016) to suggest even further treatment to remove these persistent drugs using methods like membrane and/or electrochemical treatments. Xu *et al.* (2014) also found that secondary treatment of wastewater failed to remove 12 of the 33 Endocrine Disrupting Chemicals (EDCs), with 4-nonylphenol (NP), a degradation product of many surfactants, and bisphenol A (BPA), an additive in plastics and polymers, being the highest concentrations in their Hong-Kong study. 4-nonylphenol has been found to reduce the growth rate of testes in some fish (Lech *et al.*, 1996) and BPA is known to lower the sperm count in mice, delay egg hatching, and suppression of growth in some juvenile fish (Aluru *et al.*, 2010). Xu *et al.* (2014) also demonstrated that out of the sewage treatment plants, the ones using activated sludge had better EDC removal rates than plants using biological filters. They also concluded that NP and BPA in TWW drains into the marine environment and have prompted gene transcription responses related to endocrine disruption pathways in the Japanese killifish in lab experiments. While most research has been looking into PPCPs uptake by plants (Prosser & Sibley, 2015) which indicated that indeed there was some uptake but below the limits, relatively little research has looked into the effects of PPCP contaminated wastewater on the microbial communities in the plant rhizosphere. In a study conducted by Yang *et al.*, (2009), it was found that wheat rhizosphere microbial assemblages changed due to exposure to oxytetracycline, a broad spectrum antibiotic. Yang *et al.* (2014) were concerned about the horizontal transfer and spread of antibiotic resistant genes originating from hospitals and domestic wastewaters to sewage treatment plants that receive them and subsequent spread of these genes through TWW to the overall environment. They have identified 271 subtypes of antibiotic resistant genes originating from 18 types in the sewage influent. However, they indicated that the secondary treatment process was 99.8% efficient at removing these genes from the water. Once again, activated sludge demonstrated to be the most efficient when compared to anaerobic digestion sludge (Yang *et al.*, 2014). However, the antibiotic resistance gene removal efficiency was drastically lower in the actual sludge than in the water (Yang *et al.*, 2014), this raises concern over the passing of these genes to pathogenic bacteria especially if sludge was used to condition agricultural soils.

Rhizosphere Microbiome Ecology

The Rhizosphere is the narrow zone surrounding each of the plant's individual root (Figure 15) and is one of the most complex ecosystems to be studied recently (Mendes *et al*., 2013,). Just as the microbial flora in the human gut affects human health and well-being, it has been identified that plant root systems and their surrounding soil also have a similar relationship with their microbial flora. Recent studies have started looking more closely into this relationship. It has been identified that many different microorganisms can have a profound impact on the plants, like affecting seed germination, plant growth, and development as well as disease prevention capabilities (Mendes *et al*., 2013).

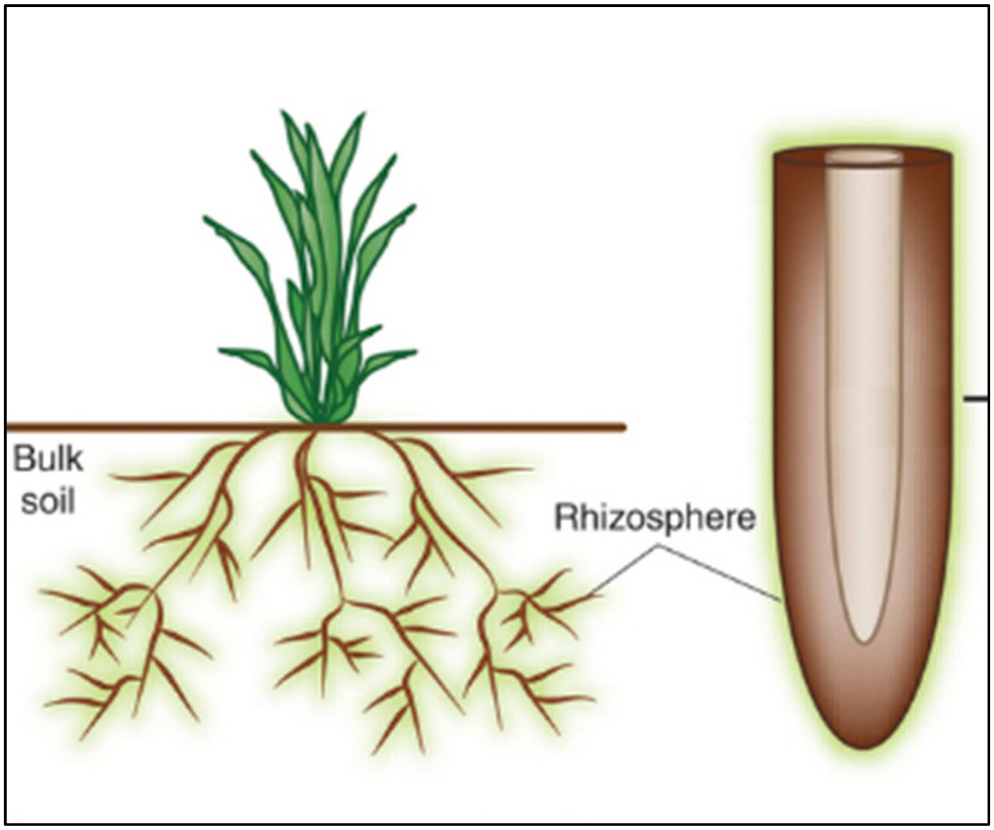

Figure 15: The Rhizosphere zone (Hirsch and Mauchline, 2012)

Maurhofer *et al.* (1992) have categorized the types of microorganisms into three main groups, the good, where the microorganisms help in immune responses against diseases, enhanced tolerance to abiotic stressors, aids in nutrient acquisition etc. which are generally seen as the bulk of the rhizosphere microorganisms. The second group is the bad microorganisms that can cause diseases to the plant, reducing plant productivity and vigor or indeed kill the plant. The final group is the pathogenic microorganisms that contaminate the plant and can be transferred to humans during their consumption, causing deleterious effects and illness. A good example to this group is shiga toxin poisoning from *E.coli* contamination occurred as a consequence of contaminated cucumbers which caused a subsequent economic disaster for the Spanish farming industry where other countries refused to buy their products after they have been blamed (Govan, 2011). More than 852 people got hemolytic uremic syndrome (HUS) due to the shiga toxin and it resulted in 32 deaths associated with the HUS (CDC, 2011). Another recent example of death due to contaminated cucumbers from Mexico city resulted in 888 people being infected with *Salmonella poona*, and resulted in 6 deaths (CDC, 2016) in the US in 2015.

The fear of opportunistic pathogens in vegetables that can harm humans is on the rise and resulted in many opinions in the scientific community. Berg *et al*. (2014) highlighted the importance of microbial biodiversity in the agricultural soil to mitigate these dangers, as many of the incidents were due to microbial imbalances. The changing practices in farming are attributed as one of the causes for these microbial imbalances (Berg *et al*., 2014). Therefore, it would be of interest to see the impact of the use of TWW in Qatar on the growth of possible opportunistic pathogens in the agricultural sector due to the changes to the soil microbiomes that the application of TWW may induce. Also, it is important to understand that these rhizosphere biome communities are not static, they are fluid and changing according to the different biotic and abiotic factors that influence them, from the plant's chemistry and exudates, to the type of water used for irrigation. Kayikcioglu (2012) has noted that rhizospheric microbial activity decreased when irrigated with TWW, compared to irrigation with freshwater, sometimes decrease was by 50%, all within one crop season of irrigation. This was attributed to the presence of heavy metals that were in the TWW which had an inhibitory effect on these organisms (Kayikcioglu, 2012). In contrast, Alguacil *et al.* (2012) found an increase in microbial biomass in the soil as well as microbial activity. It is inferred that microbial biomass and activity increase in C, N and P balancing microorganisms, is due to stimulation of nutrient rich wastewaters (Becerra-Castro *et al.*, 2015). Although some increase in microbial biomass and organic content of soil is desirable in water retention capacity of soil, too much of it has negative effects on soil properties like pore clogging (Becerra-Castro et al., 2015; Bot & Benites, 2005; Magesan *et al.,* 1999). Some studies focused on the application of different antibiotics and PPCPs, usually found in wastewaters, on soil. The authors concluded that these compounds affected microbial communities in the rhizosphere by disrupting their structures (Ding and He, 2010; Müller *et al.,* 2002) and their activities (Ding and He, 2010; Liu *et al.,* 2012). A study in Mexico investigated the direct effects of untreated sewage on soil microbiomes, compared with rainfed soils. It was demonstrated that wastewater irrigation increased the abundance of potentially pathogenic *Gammaproteobacteria* (*Stenotrophomonas* strains) as well as *Pseudomonas*, *Stenotrophomonas*, and *Acinetobacter* species, whereas rainfed soils were mainly abundant with *Bacillus* (Broszat *et al.,* 2014). It was also found that six different antibiotic resistance genes in soil bacteria exist, sulfamethoxazole resistance and oxacillin resistance being the most abundant in wastewater irrigated soils (Broszat *et al.*, 2014).

Arugula (*Eruca sativa*)

Arugula, *E. sativa*, is an angiosperm from the family of Brassicaceae, a group of plants commonly known as mustard greens due to the strong pungent aroma and strong flavor (Morales & Janik, 2002). *E. sativa* has been consumed as a food and medicine in the Mediterranean region since antiquity. It continues to be a popular plant, and is gaining more popularity as a gourmet salad around the world with many publications on growing methodologies in different climates, especially in Americas (Morales & Janik, 2002; Schuler *et al.,* 2004).

E. sativa is a fast growing herb and an important food source which is cultivated both at the industrial level and at home gardens. The edible portion of this vegetable is the leaf, which can be harvested after 20 days of growth and sequentially harvested after re-growth (Marsoni *et al*., 2014). The Brassicaceae family including *E. sativa* has been shown to contain thioglucosides which are readily converted to active isothiocyanates during digestion (Latte *et al*., 2011). Fuentes *et al.* (2014) have noted *E. sativa*'s ability as an antiplatelet and antithrombotic agent, to prevent cardiovascular diseases. Gründemann *et al*. (2015) have also demonstrated the medicinal properties of *E. sativa*. as a preventative as well as a treatment from cancer (Clarke *et al.*, 2011). The chemical composition of *E. sativa* is provided in Table 5.

Table 5: The Chemical Composition and Nutritional Value of Arugula/100g (USDA, SR-28, 2016)

Nutrients	Amount
Total Omega-3 fatty acids	170 mg
Total Omega-6 fatty acids	130 mg
Vitamin A	2373 IU
Vitamin C	15 mg
Vitamin K	109 µg
Folate	97 µg
Choline	15.3 mg
Calcium	169 mg
Magnesium	1.5 mg
Phosphorus	47 mg
Potassium	52 mg
Sodium	27 mg

Some studies have also shown that *E. sativa* extracts can be used as a non-toxic pesticide, especially against root-knot nematodes and a marked decrease in root-knot infections in tomato plants grown in soil conditioned with fresh arugula has been observed (Aissani *et al*., 2015). Thus, the plant would be an important food security commodity and highly desirable for its disease prevention properties when eaten as well as the plant's derivatives as pest control for growing other food products.

Studies on *E. sativa* irrigated with wastewater have been carried out in Adiyaman, Turkey to see the effects of irrigation with wastewater. Gezer (2013) concluded that irrigation with wastewater caused physiological changes to the plant as well as showing a high transfer factor for cadmium and other heavy metals, with preference in uptake in the following order: Pb>Cd>Cu>Cr>Ni. Kamran *et al.* (2015) have used these properties along with inoculation of *E. sativa* roots with *Pseudomonas putida* in order to enhance

Cd uptake and reduce phytotoxicity from the heavy metal; thus, using it as a means of phytoremediation of contaminated soil.

A study in Alexandria, Egypt (Khalil *et al*., 2015) has also exhibited significant amounts of shiga-toxin producing *E. coli* contaminating arugula and cilantro irrigated with TWW. Marsoni *et al.* (2014) has also shown that there is a possibility for arugula to accumulate some pharmaceutical and personal care products (PPCP) residues in its tissue when exposed to PPCP levels equivalent to irrigation river levels, however, the accumulated levels were low enough not to cause risk to humans after consumption.

Research Justification

From the review of the different scientific literature above, there are some conflicting findings regarding the effects of TWW irrigation on food crops where it enhanced growth in some and damaged growth in others. TWW irrigation also improved soil condition in some factors like increasing nutrients while also seen as a risk for increasing heavy metals and PPCP loads in the soil for uptake by the growing of food crops. These conflicting results may be due to differences in location of research where the soil quality is different, as well as the sources and qualities of TWW where the economic activities of the country affects the wastewater e.g. industrial vs. rural countries and the resultant wastewater generated. Therefore, there is a need to take a closer look at the TWW generated in Qatar and its effect on soil and plant growth, with arugula being the main crop of interest for this study. This study was specifically designed to observe if arugula is indeed negatively affected as has been observed in previous studies. Results from this study can help elucidate if further studies will be needed for the viability of TWW use for direct irrigation of food crops.

CHAPTER 3: METHODOLOGY

Soil preparation

Natural soil was collected from Qatar University campus biosphere reserve (coordinates: latitude at 25° 22’ 15.8869“ N, longitude at 51° 29’ 42.9598” E) where no previous agricultural or public space greening has occurred and soil has not been treated previously. Natural soil, non-agricultural, locally sourced, and without any artificial modifications or additions, was used to determine its health as well as to see if the addition of potting soil affects the microbial communities. The surface soil up to 10 cm depth was collected and kept in sterile ziplock bags.

Figure 16: Natural soil collection site

Two 20 liter commercial peat mosspeat moss “Plantafor SAB potting soil” was purchased from a local market and used for mixing with the natural soil. SAB potting soil is a German potting soil company where its potting soil is mainly composed of decomposed peat mosspeat moss mixtures.

Table 6: Potting soil nutrient composition as per “Plantafor SAB potting soil” packaging information

Organic Matter	85-95% dry matter
Nitrogen	100-400 mg/l
Phosphate P_2O_5	70-300 mg/l
Potassium K_2O	100-400 mg/l

Part of the peat moss was sterilized using an autoclave at 190°C for 20 minutes in order to remove microbiota, which might be present in the fertilizer, and to investigate if there are local microbiota in the peat moss that would change the microflora in the soil mix. Natural soil and unsterilized peat moss were mixed at a ratio of 4 kg to 4 kg in a sterilized bag and shaken until thoroughly mixed. Natural soil was also mixed at a 1:1 ratio by weight with the sterilized peat moss in a sterile ziplock bag and mixed thoroughly.

Arugula Planting

On 15th September 2015 and September 22, 2015, ten inch diameter plastic pots were set out and lined with Whatman® filter paper (12.5 cm diameter; pore size of 8 microns) to prevent soil escaping from the drainage holes. About 600 g of the prepared soils and soil mixtures as described in Table 7 were added to each pot making a total of 40 pots.

Table 7: Soil type and Soil mixture composition

Number of pots	Natural Soil (NS) g/pot	Sterilized Peat moss (SP) g/pot	Peat moss (PM) g/pot	Soil Type/Mixture total
10	0	600	0	SP 600 g/pot
10	600	0	0	NS 600 g/pot
10	300	300	0	NS+SP 600 g/pot
10	300	0	300	NS+PM 600 g/pot

A pinch of Arugula seeds purchased from a local farmer market supplier were sprinkled uniformly in each pot and placed on counters inside of the Qatar University greenhouse in order to be kept at constant temperature (25°C) and natural light conditions (November to December 2015; 10 hours daylight, 14 hours dark)

The pots were separated according to watering regime. Five pots for each soil type, sterilized peat moss (SP), natural soil (NS), natural soil mixed with sterilized peat moss (NS+SP), natural soil mixed with peat moss (NS+PM), were watered three times a week with potable tap water and five pots for each soil type were treated with TWW three times a week.

Freshwater (FW) was obtained from the greenhouse tap, while the TWW was obtained from the main-gate of Qatar University male campus where a municipal TWW distribution pipe is available.

Soil Sample Collection

Three pots out of the five from each treatment were randomly selected for soil and Arugula sampling. Soil samples (around 5 g) were collected from the pots near the root zone at three intervals:

- Day zero before watering the soil
- Day 21 middle of the vegetative growth of Arugula
- Day 41 when Arugula is usually harvested just before bolting

All soil samples were stored in sterile ziplock bags and kept at 4ºC until further analyses.

Figure 17: Greenhouse and pot arrangement

Figure 18: Arugula at day 21

Plant Sample Collection

The Arugula seeds only germinated in the sterilized peat moss; therefore, three Arugula plant samples were collected at days 21 and all the plants at day 41 from each of the selected pots to be weighed. The whole aboveground plants were cut, rinsed with deionized water, pat dried with tissue paper and the biomass weight was recorded. The length and width of the leaves of each sample were also taken to measure the leaf area and investigate if there were any changes in morphology of the plant due to the different water treatments.

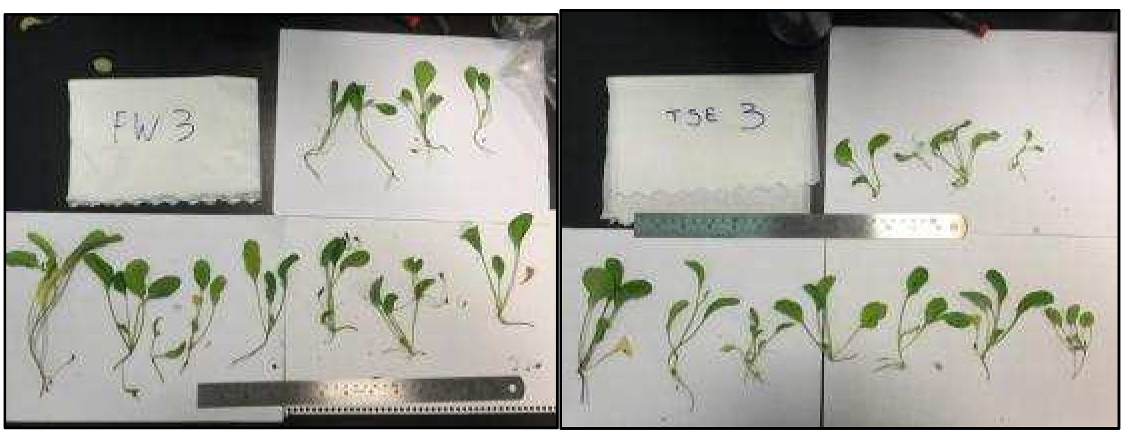

Figure 19: Harvested Arugula for weighing on day 41

Water Sample Collection

Water samples were also collected from the FW tap and TWW pipe in sterile 50 ml tubes for microbiological and chemical analyses.

Microbiological Analyses

Microbial Quality of Soil Samples:

Three types of media were used for the microbiological analyses: Plate Count Agar (PCA) for the aerobic mesophilic bacterial counts, Potato Dextrose Agar (PDA) for fungal colony counts, and MacConkey agar (MCA) for total coliforms and fecal coliforms counts. All media were prepared according to the Manufacturer's instructions, autoclaved at 121°C for 15 min, and 15 ml of warm (42 °C) agar was poured into sterile Petri dishes inside of a biosafety cabinet with a gas flame to prevent any contamination. Petri dishes with media were allowed to dry before being sealed in sterile plastic bags and stored at 4°C until further use. Brain Heart infusion Broth (BHB) was used as an enrichment broth for serial dilution of soil samples before culturing. One (1) g of soil sample was weighed in a sterile 15 ml tube, mixed with 9 ml of BHB, and vortexed to obtain homogenized sample. For determination of select microorganism, 0.1 ml of each decimal dilution was added to appropriate agar plates, then the PCA, PDA, and MCA plates were incubated at 25°C and 35°C, respectively, for 48 hr. A second batch of MCA plates was also incubated at 45°C for 48 hr for fecal coliforms determination. The bacterial colonies were counted and recorded as colony forming unit per gram (CFU/g).

<u>*Pseudomonas, Streptococcus,* and Nitrogen-fixing microorganisms' Counts in Sterilized Peat moss:</u>

After harvesting the Arugula at day 41, the rhizosphere soil was also further investigated for other microbiota using different media, including King's Medium B Base (KMB) to look at the differences in *Pseudomonas* spp. counts in the soil between freshwater irrigation and TWW irrigation, Starch Casein Agar (SCA) to test for *Streptococcus* spp. and Jensen Media (JM) for nitrogen fixing microorganisms. The plates were inoculated with 0.1 ml of the fourth serial dilution in replicates and incubated at 35°C for 48 hr before the colonies on plates were counted.

<u>Microbial Quality of Water Samples:</u>

For the water samples, 0.1 ml of each water sample was directly spread onto the appropriate agar plates. Then the PCA, PDA, and MCA plates were incubated at 25°C and 35°C, respectively, for 48 hr. A second batch of MCA plates was also incubated at 45°C for 48 hr for fecal coliforms analysis. The bacterial colonies were counted and recorded as colony forming unit (CFU/g).

Chemical Analyses:

<u>*Arugula Samples:*</u>

Harvested Arugula samples were processed to determine the concentrations of trace metals, namely Arsenic (As), Cadmium (Cd), Cobalt (Co), Chromium (Cr), Copper

(Cu), Iron (Fe), Manganese (Mn), Molybdenum (Mo), Nickel (Ni), Lead (Pb), and Zinc (Zn). The samples were digested with the addition of 9 ml of 70% Nitric Acid (HNO3) and placed into the hot-block at 95°C for 30 min inside a fumigation hood. Afterwards, 3 ml of 40% Hydrofluoric Acid (HF) were added to each sample which were kept on the hot-block for another 30 minutes. After 30 min, the hot-block temperature was raised from 95°C to 135°C for 1 hr before removing the tube covers to allow for acid evaporation. Once the acid evaporated to a gummy substance, 3 ml of nitric acid (70%) were added along with distilled water. The samples were transferred to volumetric flasks and more distilled water was added till the 100 ml mark. However, due to shut down of the Central lab and the malfunction of the Perkin-Elmer Optima 5300 DV Inductively Coupled Plasma Optical Emission Spectrometer at the environmental studies center of QU, it was not possible to process the samples during the MSc program's allocated time.

Soil Samples:

Soil samples were placed in aluminum trays and dried at 50°C in a gravity oven for 48 hrs. Afterwards, each sample was ground using a pestle and mortar until it was a fine powder. One (1) g of ground soil was weighed in a 15 ml tube and mixed with 10 ml of deionized water. The samples were shaken at 120 rounds per minute (RPM) on a rotary shaker for 4 hr at 22°C. The samples were then allowed to settle for at least 2 hrs after which they were filtered using Whatman #1 filter papers, and clear solutions were collected for anions, cations, pH, and electrical conductivity (EC) analyses.

pH and EC analysis:

Water samples were directly analyzed without prior processing using a “WTW Multi 350i” multi parameter electrode probes to measure pH and electrical conductivity(Reference method for pH, ASTM E70-07). The “WTW Multi 350i” probe was also used to measure the pH and electrical conductivity of soil samples.

Analysis of Total Ions:

The concentrations of cations (Na, K, Ca, Mg, Mn and St) and anions (F, Cl, Br, NO_3, PO_4 and SO_4) in the soil solutions and the water samples were measured using an Ion Chromatography (IC, Metrohm 850, Herisau, Switzerland) equipped with a conductivity detector. The columns used for detecting anions was “Metrosep A Supp 5 - 250/4.0” which was made of positively charged poly vinyl with quaternary ammonium groups, and one for detecting cations “Metrosep C 4 - 150/4.0” made of negatively charged silica gel with carboxylic groups. The eluent used for the detections of anions was 3.2 mM Na_2CO_3 and 1 mM $NaHCO_3$ at a flow rate of 0.700 ml/min at 22.4°C under 12 MPa pressure, and a run time of 30 min for each sample (Reference method USEPA 300.0) A known standard solution (CRM IC-MAN-02-1 multi-component anion mix 2, AccuStandard) was used to calibrate the machine before each use. Whereas 2.5 mM nitric acid was selected as eluent for the determination of cations in soil samples at a flow rate of 0.700 mlL/min at 22.4°C under 8 MPa pressure and a run time of 30 min for each sample (Reference method ASTM D6919.9) with the use of CRM Multi-component Cation mix 1 (VWR Chemicals) as calibration mix. The results were normalized to a standard curve by comparing them with a blank sample.

Total Nitrogen (N), Carbon (C), Hydrogen (H), and Sulfur (S) Analyses:

The total N, C, H and S analysis was only carried out on the dried ground soil samples. The soil samples were weighed and wrapped in tin foil capsules and then inserted into the "Thermo Scientific™ FLASH 2000 CHNS/O Analyzer." For every 10 samples, a Cystine standard (Certificate No. 218806/07/01/2015) was added to check for any shifts in the analyzer.

The standard method of taking the samples from room temperature to 400°C then 900°C, for flash combustion and the elemental gasses produced from the combustion were determined. Combustion results of the FLASH 2000 CHNS/O Analyzer were analyzed by the Eager Xperience Software to calibrate the sample results according to the cysteine standards. The results of N, C and H were given in percentages; no Sulfur was detected in the samples.

Trace Metal Analysis:

For quality control, known concentrations of trace metals [Arsenic (As), Cadmium (Cd), Cobalt (Co), Chromium (Cr), Copper (Cu), Iron (Fe), Manganese (Mn), Molybdenum (Mo), Nickel (Ni), Lead (Pb) and Zinc (Zn)] was mixed with 0.25 g of ground soil sample. The samples were acid digested using the same method as explained earlier. The concentrations of heavy metals were analyzed using an Inductively Coupled Plasma Optical Emission Spectrometer (ICP-OES, Perkin-Elmer Optima 5300 DV, Waltham, Massachusetts, USA) using ESC-METH- IC001-Rev04 method. The trace metals were calibrated according to the following wavelengths: As: 188.979 nm, Cd: 228.802 nm, Co: 230.786 nm, Cr: 283.563 nm, Cu: 327.393nm, Fe: 238.204 nm, Mn: 259.372 nm, Mo: 203.845 nm, Ni: 232.003 nm, Pb: 220.353 nm and Zn: 206.200 nm.

Statistical Analysis

The data were analyzed using STATA statistical analysis tools. A three factor analysis of Variance (ANOVA) was carried out to test the significance and interactions between the following factors: Soil type, water treatment, and time on microbial counts. A Pearson Correlation test was applied to investigate the interactions between the concentrations of cations and anions in the soil, as well as to determine if there is an interactions between the different types of microorganisms and cations and anions.

Since no growth was observed in pots containing natural soils, a two sample t-test was carried out to test for a significant difference between the mean weight and leaf size of Arugula irrigated with tap water and/or TWW in peat moss at a significance level of $p \leq 0.05$.

CHAPTER 4: RESULTS

Chemical Analysis of Water Samples

Table 8 displays the results of chemical analyses of water samples. Although the pH of TWW and fresh water (FW) is relatively similar, the TWW has a much greater electrical conductivity, mainly due to higher levels of K and Ca cations, and F, NO_3 and SO_4 anions. These ions are present due to the incomplete treatment of the water, and thus some remnants of solutes from sewage, and detergents etc. are still present in the water.

Table 8: The pH and Electrical Conductivity of Water Samples

	pH	**EC µS/Cm**	**Na ppm**	**K ppm**	**Ca ppm**	**Mg ppm**	**St ppm**	**F ppm**	**Cl ppm**	**Br ppm**	**NO_3 ppm**	**PO_4 ppm**	**SO_4 ppm**
FW	7.2	190	5.7	1.6	ND	16.9	ND	0.03	ND	0.05	0.21	0.16	2.7
TWW	7.5	1230	5.1	15.5	104.7	15.2	1.6	0.13	ND	0.08	19.05	0.26	262.0

It was noticed that FW collected from the greenhouse contained higher levels of trace/heavy metals than the secondary treated TWW (Table 9). This may be due to the leaching of these elements from the piping material. One concern over TWW is the detection of As which is a known carcinogen. There is no local standard for As levels in the TWW; however, the EPA standards for As levels in drinking water to be 0.01mg/L. The detected level of 0.55mg/L is above the recommended EPA standard for drinking water, as well as above the threshold level for other domestic uses like bathing and washing which is 0.5 mg/L (EPA, 2016).

Table 9: The concentrations of trace metals in water samples (mg/L)

	As	Cd	Co	Cr	Cu	Fe	Mn	Mo	Ni	Pb	Zn
FW	0	0	0	0	114.71	3.27	14.485	0	0	1.405	46.83
TWW	0.55	0	0	0	0	0	0.22	0	0	0	0

Microbial Analyses of Water Samples

The results indicated that there may be some microbial contamination of the water obtained from FW and the TWW pipe (Figure 20). However, the contamination level was reduced when the water was allowed to settle in a jug before irrigation. The microbial load of the TWW was higher, perhaps some of the extra nutrients in the TWW like K, Ca, F, NO_3 and SO_4 allow for bacteria to maintain their numbers even after settling in the watering jug. The difference between Aerobic Mesophilic Bacterial counts of FW and TWW is highly significant ($P \leq 0.00002$), indicating the secondary treatment is not sufficient to reduce microbial load in TWW samples.

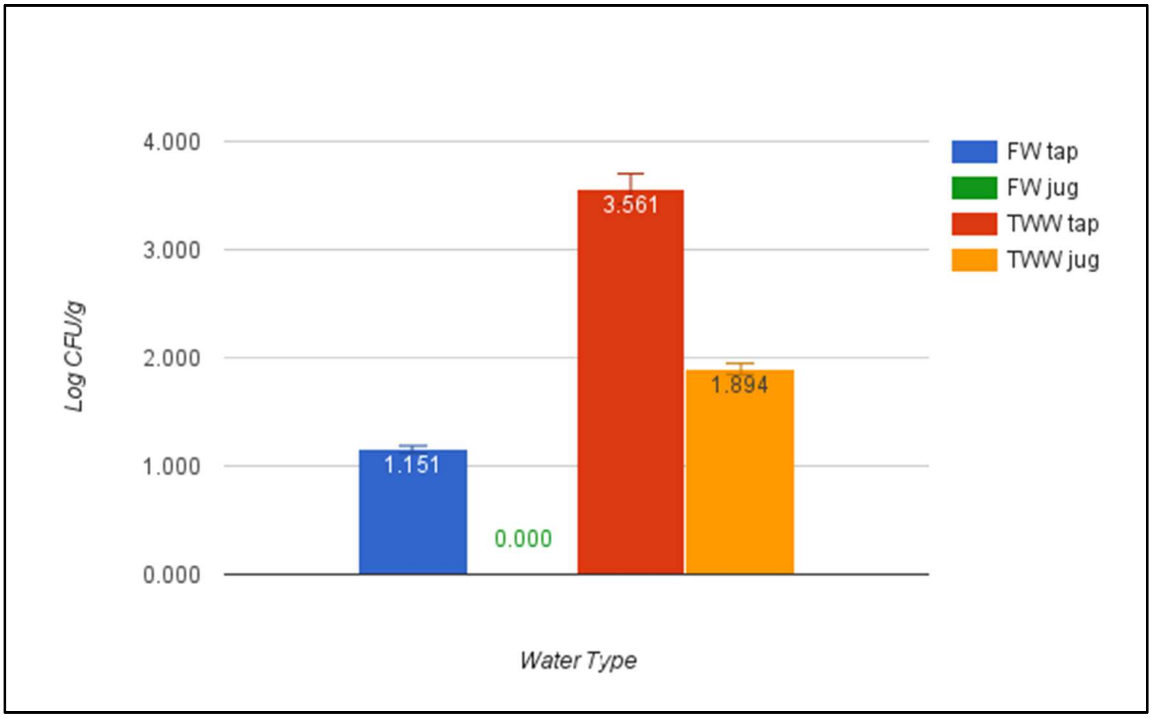

Figure 20: The Aerobic Mesophilic Bacterial Counts in Water Samples

It is also shown that both water samples were contaminated by fungi, however, there was no significant difference (*P*≥0.05) on the fungal colony counts in FW and TWW samples after the water samples were allowed to settle in the jugs (Figure 21).

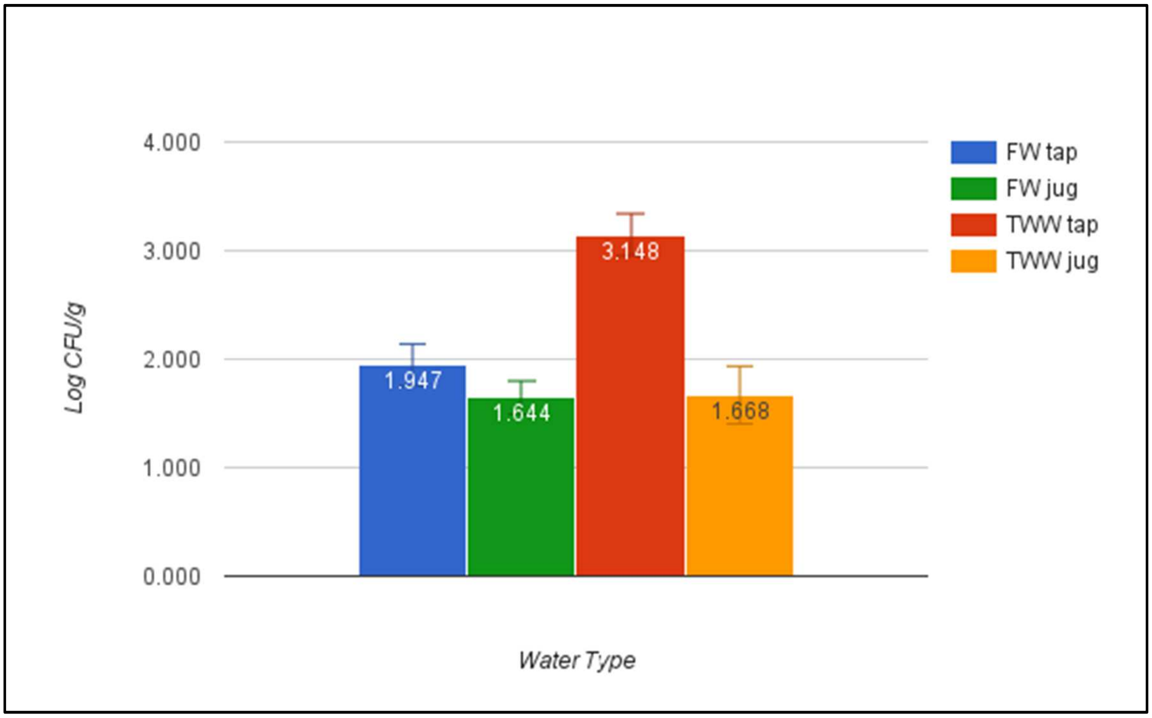

Figure 21: The total Fungal Counts in Water Samples

Figure 22 and 23 show that tap water is clear of coliforms, although fecal coliforms were detected in the water samples obtained from the TWW source. These colonies disappear once the water was allowed to settle in the jug before use. This may be due to the location of the TWW tap where it was placed in the ground near manure fertilized soil and plants that could cross contaminate the TWW pipe. The higher microbial counts in TWW have been proven to be significant ($P \leq 0.05$) for all different types of microorganisms.

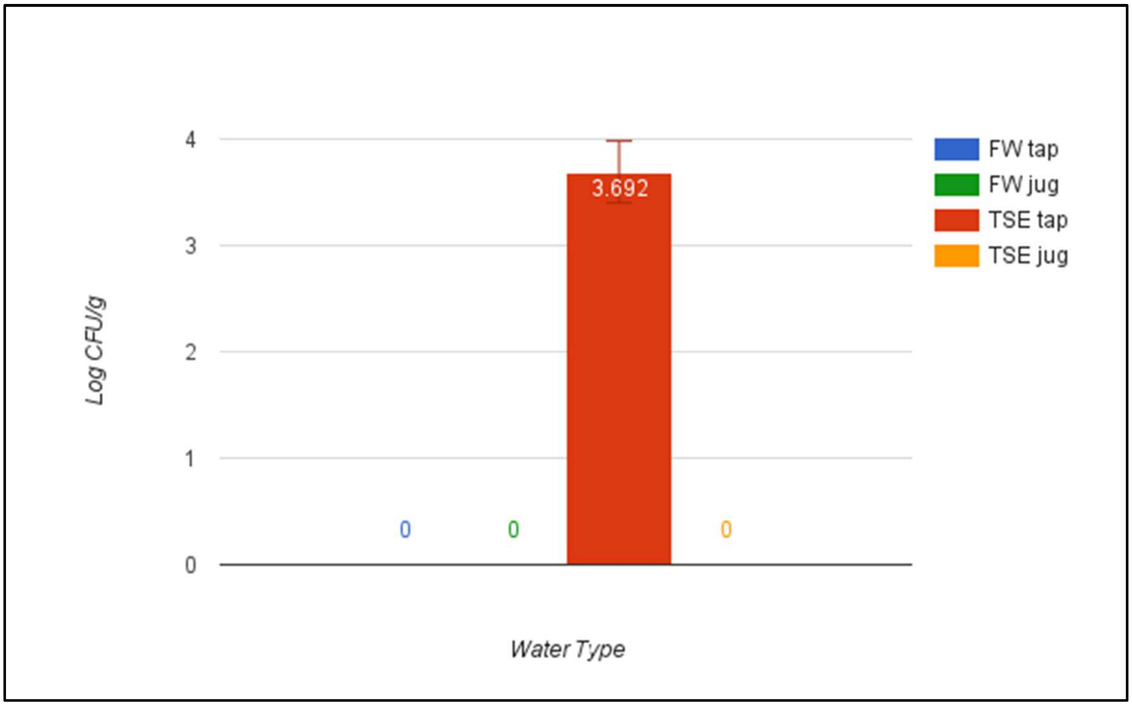

Figure 22: The Total Coliform Counts in Water Samples

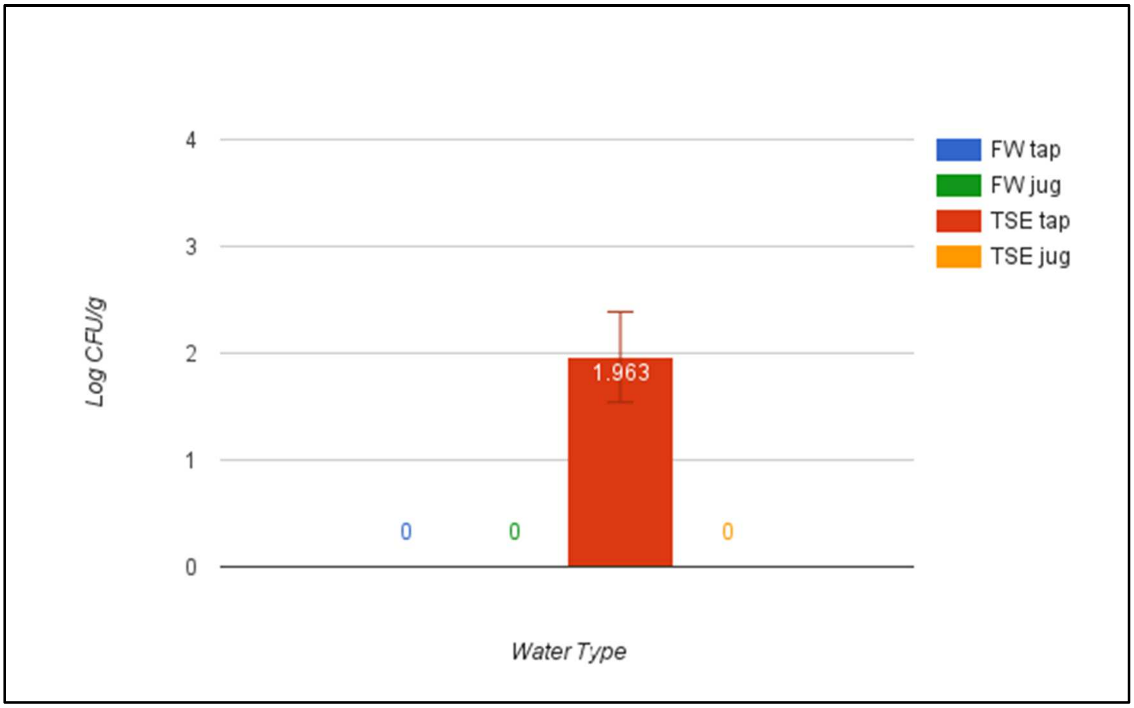

Figure 23: The total Fecal Coliform Counts in Water Samples

Chemical Analyses of Soil Samples

It can be seen that the pH of soil does not change with time nor by water treatment, and is mainly around pH 8 (Figure 24) which is a slightly alkaline pH and comparable to values cited in the literature.

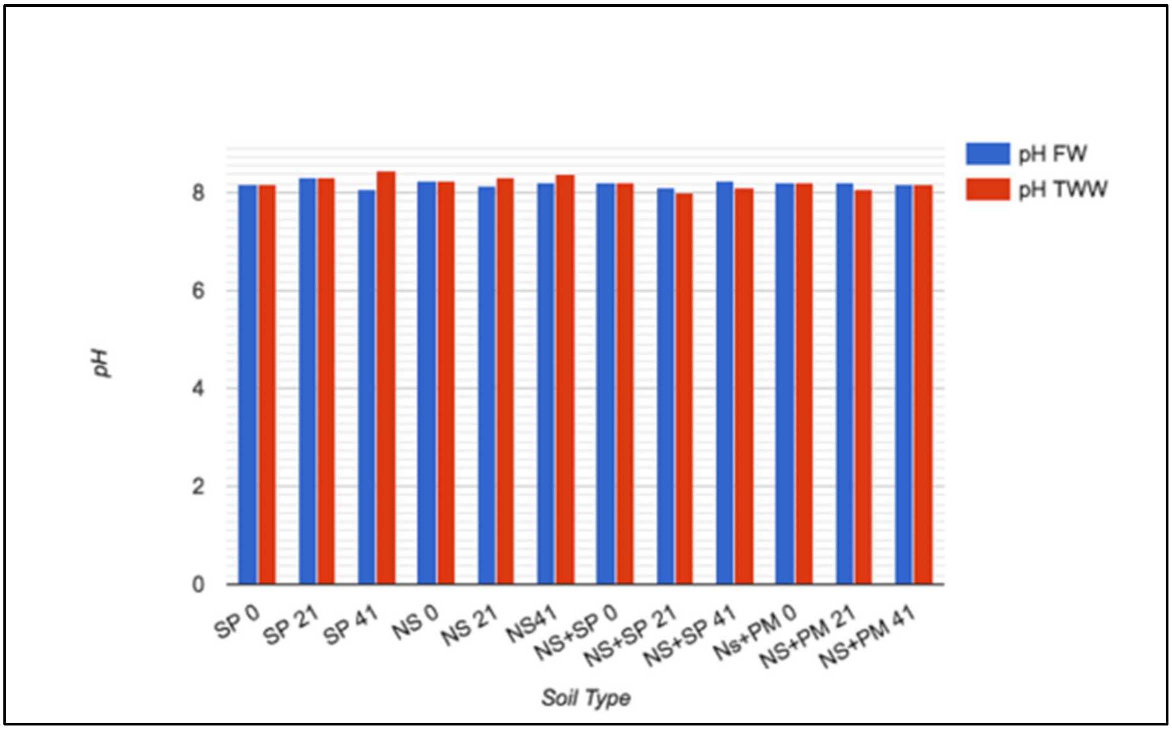

Figure 24: The pH values of soil types treated with FW or TWW during the study period

The electrical conductivity of different soils was significantly different ($P \leq 0.05$) with peat moss alone having the lowest conductivity due to the lower solutes level compared to the others (Figure 25). While, natural soil exhibited the highest conductivity due to the high levels of anions and cations naturally present in this soil type.

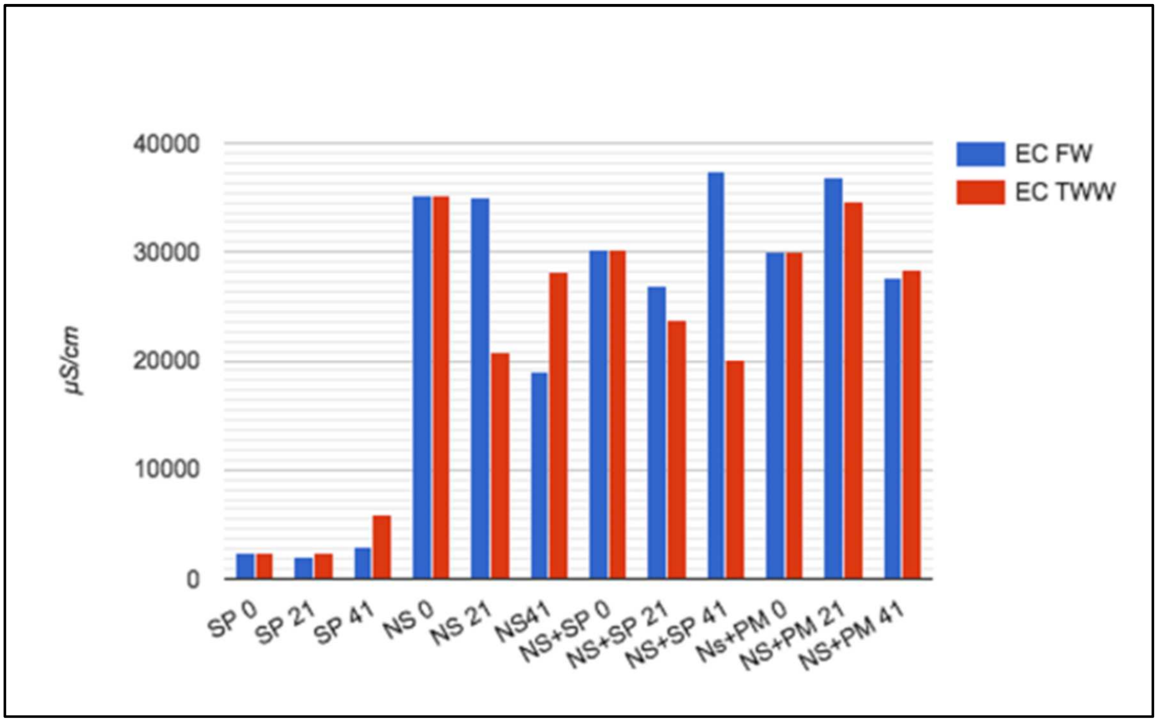

Figure 25: The Electrical Conductivity (EC) levels of soil types treated with FW or TWW during the study period

The levels of cations and anions in the soil after irrigation with different water type fluctuated throughout the study period (Table 10 and 11). These results are in alignment with the results of cations and anions in FW and TWW, where Ca cation was undetectable in FW but it was detectable at a rate of 104 mg/kg in TWW. Similarly, the concentration of SO_4 anion was 2.7 mg/kg in FW but 261.9 mg/kg in TWW. This eventually changed the soil's conductivity and elemental composition. When compared to the natural soil and mixed soil, the sterilized peat moss had significantly less solutes with Mg and K being the most dominant in the samples (Figure 27). Natural soil and its mixtures proved to be very high in solutes, with Na being the most prominent cation, indicating that the soil is highly saline, and its corresponding anion Cl also dominating the anions followed by SO_4(Figure 26). The levels of anions in the sterilized peat moss were so low that they were negligible compared to that of the natural soil.

Table 10: Cation and anion concentrations in soil samples irrigated with FW

FW	SP 0	SP 21	SP 41	NS 0	NS 21	NS 41	NS+SP 0	NS+SP 21	NS+SP 41	NS+PM 0	NS+PM 21	NS+PM 41
Na (mg/kg)	3.33 ±2.6	0.83 ±0.17	1.08 ±0.36	780.62 ±195.72	1289.92 ±579.76	511.56 ±203.16	578.46 ±62.16	403.83 ±111.6	459.17 ±172.96	544.9 ±34.92	679.51 ±213.02	663.13 ±42.71
K (mg/kg)	7.98 ±4.44	10.05 ±0.16	9.97 ±2.05	34.46 ±6.62	67.34 ±37.85	27.79 ±10.52	39.22 ±2.54	28.69 ±9.22	32.13 ±10.97	36.65 ±2.12	43.25 ±9.82	42.71 ±5.7
Ca (mg/kg)	0	0	0	154.34 ±31.59	214.68 ±76.11	118.21 ±37.74	154.67 ±9.6	83.01 ±64.94	125.82 ±43.13	141.97 ±7.14	176.99 ±52.14	158.52 ±29.99
Mg (mg/kg)	6.68 ±2.8	4.92 ±2.14	7.36 ±1.25	39.24 ±3.57	52.71 ±14.85	28.93 ±8.64	41.96 ±3.68	27.88 ±8.26	33.63 ±13.16	39.81 ±2.35	44.54 ±10.13	44.31 ±3.43
Mn (mg/kg)	0	0	0	0	50.85 ±58.72	0	0.01 ±0.02	0	0	0	0	0
St (mg/kg)	0.02 ±0.04	0	0.06 ±0.04	1.52 ±0.25	1.83 ±0.2	1.27 ±0.44	0.63 ±0.05	0.44 ±0.13	0.51 ±0.16	0.64 ±0.04	0.69 ±0.09	0.66 ±0.09
F (mg/kg)	0	0	0	0	0	0	0	0	0	0	0	0
Cl (mg/kg)	0	0	0	1297.95 ±345	2139.2 ±946	909.96 ±343	1037.1 ±94	736.43 ±208	821.79 ±346	938.65 ±109	1248.77 ±440	1177.67 ±135
Br (mg/kg)	0	0	0.02 ±0.03	1.82 ±0.86	3.42 ±1.78	1.03 ±0.55	1.42 ±0.32	1.04 ±0.37	1.11 ±0.75	1.17 ±0.63	1.84 ±0.94	1.27 ±0.72
NO_3 (mg/kg)	0.41 ±0.57	0.03 ±0.04	0.14 ±0.12	11.37 ±9.8	10.85 ±1.49	5.5 ±3.8	0.58 ±0.22	2.23 ±1.63	0.74 ±0.9	0.79 ±0.48	2.72 ±3.35	4.6 ±6.72
PO_4 (mg/kg)	2.15 ±2.83	2.74 ±0.38	4.18 ±2.04	0	0	0.02 ±0.04	0.83 ±0.52	0.26 ±0.15	0.35 ±0.30	0.46 ±0.28	0.38 ±0.13	0.31 ±0.23
SO_4 (mg/kg)	10.64 ±7.21	5.34 ±2.77	13.4 ±2.94	338.1 ±69.78	498.22 ±195	0.02 ±0.04	299.52 ±17.22	197.29 ±67.22	248.15 ±80.4	263.01 ±24.76	288.88 ±30.42	260.66 ±35.69

Table 11: Cation and anion concentrations in soil samples irrigated with TWW

TWW	SP 0	SP 21	SP 41	NS 0	NS 21	NS 41	NS+SP 0	NS+SP 21	NS+SP 41	NS+PM 0	NS+PM 21	NS+PM 41
Na (mg/kg)	3.33 ±2.61	1.43 ±0.6	6.89 ±13.13	780.62 ±195	654.81 ±90.93	311.44 ±224	578.46 ±62.15	518.48 ±193	431.04 ±132	544.9 ±34.92	680.99 ±33.25	527.69 ±83.46
K (mg/kg)	7.98 ±4.44	9.06 ±3.57	10.25 ±3.8	34.46 ±6.62	30.09 ±5.5	15.68 ±10.3	39.22 ±2.54	35.54 ±12.78	28.15 ±8.7	36.65 ±2.12	39.81 ±19.59	36.77 ±5.51
Ca (mg/kg)	0	0	0	154.34 ±31.59	136.85 ±11.09	135.05 ±18.7	154.67 ±9.6	74.53 ±86.05	121.13 ±26.35	141.97 ±7.14	157.01 ±78.06	132.56 ±24.4
Mg (mg/kg)	6.68 ±2.8	6.5 ±2.61	7.16 ±0.68	39.24 ±3.57	38.12 ±4.35	31.92 ±11.86	41.96 ±3.68	15.7 ±21.3	32.16 ±9.75	39.81 ±2.35	42.09 ±20.62	39.12 ±9.04
Mn (mg/kg)	0	0	0	0	0	0	0.01 ±0.02	0	0	0	0	0
St (mg/kg)	0.02 ±0.04	0.05 ±0.04	0.06 ±0.01	1.52 ±0.25	1.51 ±0.12	1.05 ±0.41	0.63 ±0.05	0.54 ±0.19	0.48 ±0.11	0.64 ±0.04	0.72 ±0.05	0.62 ±0.14
F (mg/kg)	0	0	0	0	0	0	0	0	0	0	0	0
Cl (mg/kg)	0	0	18.93 ±25.92	1297.95 ±345	1022.11 ±153	476.11 ±445	1037.1 ±94.02	908.65 ±331	780.76 ±250	938.65 ±109	1254.1 ±87.07	948.21 ±173
Br (mg/kg)	0	0	0.01 ±0.01	1.82 ±0.86	1.04 ±0.68	0.68 ±0.73	1.42 ±0.32	0.77 ±0.18	1.03 ±0.5	1.17 ±0.63	1.64 ±0.78	1.27 ± 0.51
NO_3 (mg/kg)	0.41 ±0.57	0	0.05 ±0.04	11.37 ±9.8	5.85 ±4.45	5.18 ±5.22	0.58 ±0.22	0.51 ±0.25	2.53 ±3.71	0.79 ±0.48	0.52 ±0.33	1.29 ±1.56
PO_4 (mg/kg)	2.15 ±2.83	3.19 ±2.51	1.27 ±1.83	0	0	0	0.83 ±0.52	0.15 ±0.02	0.23 ±0.16	0.46 ±0.28	0.56 ±0.36	0.21 ±0.08
SO_4 (mg/kg)	10.64 ±7.21	12.91 ±9.56	10.15 ±4.5	338.1 ±69.78	306.18 ±21.45	214.04 ±107	299.52 ±17.22	228.03 ±65.51	220.66 ±64.67	263.01 ±24.76	321.28 ±37.95	259.93 ±55.07

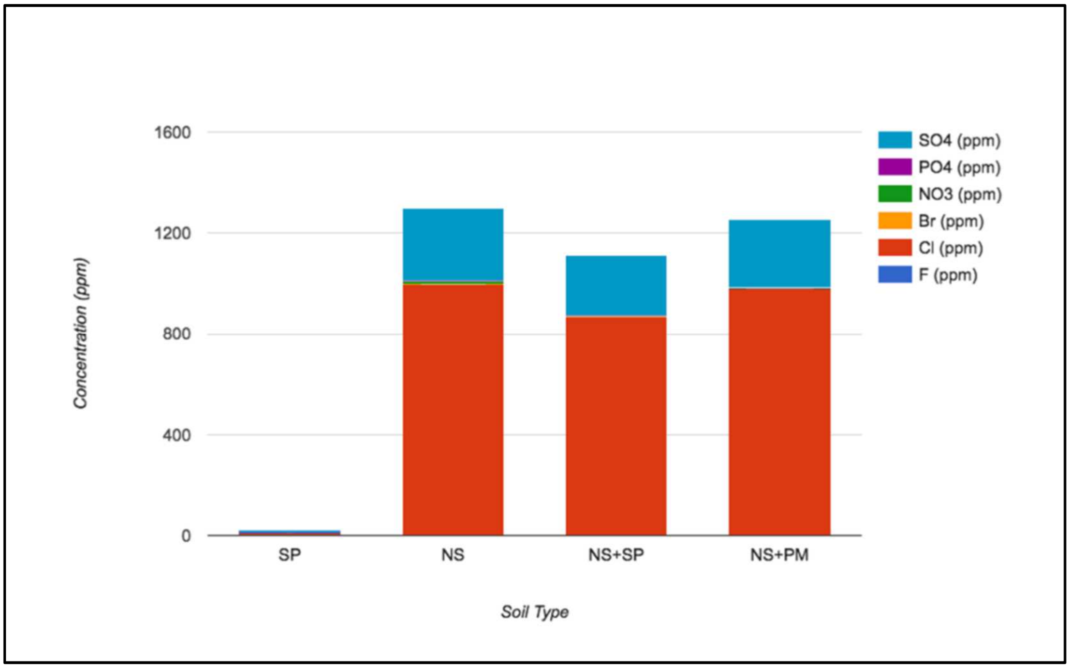

Figure 26: Concentration of anions by soil type

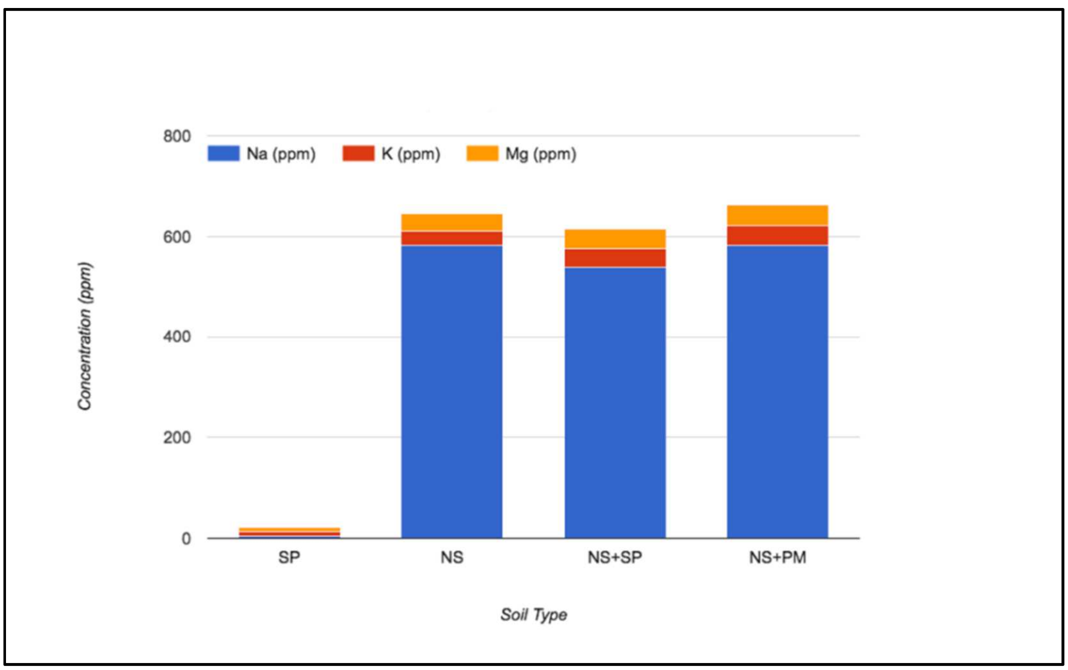

Figure 27: Concentration of cations by soil type

Although the levels of trace metals in different soil types fluctuated throughout the experiment duration, there was no link identified relating them to irrigation water type or duration of irrigation (Tables 12 and 13). It was clearly observed that both peat moss and natural soil types contained high levels of Fe. The mixed soils even exhibited higher levels of Fe due to the additive effect of Fe from the mixing of the natural soil and the peat moss.

Table 12: The concentrations of Trace Metals (mg/kg) in Soil Samples Irrigated with FW

	As	Cd	Co	Cr	Cu	Fe	Mn	Mo	Ni	Pb	Zn
SP41	0.58	0.08	0.30	1.95	20.52	1352	40.69	7.85	1.84	4.98	12.14
NS0	0.03	3.19	32.60	4.49	5995.7	155	1.50	17.29	0.57	10.37	0.00
NS41	2.16	0.01	2.93	36.22	5.22	5969	142.37	1.36	17.98	1.34	38.15
NS+SP0	1.73	0.01	1.91	21.40	8.10	4190	95.59	3.57	11.73	1.71	35.89
NS+SP41	1.97	0.02	1.91	20.64	11.28	4037	96.19	3.53	12.10	4.83	36.94
NS+PM21	1.89	0.01	2.37	26.53	8.51	4635	105.77	3.25	14.27	2.55	21.87
NS+PM41	2.05	0.05	2.09	20.54	8.36	4295	102.16	3.12	12.82	2.39	70.48

Table 13: The concentrations of Trace Metals (mg/kg) in Soil Samples Irrigated with TWW

	As	Cd	Co	Cr	Cu	Fe	Mn	Mo	Ni	Pb	Zn
SP21	1.00	0.10	0.44	1.85	22.74	1324	42.63	7.34	2.52	6.38	19.54
SP41	0.65	0.07	0.31	1.86	18.04	1349	42.27	7.95	1.86	5.14	12.37
NS0	0.03	3.19	32.65	4.49	5995.7	155	1.50	17.29	0.57	10.37	0.00
NS41	1.94	0.01	2.66	32.42	5.68	5471	133.13	1.36	16.63	1.13	13.32
NS+SP0	1.74	0.01	1.91	21.41	8.11	4190	95.59	3.57	11.74	1.72	35.90
NS+SP41	1.51	0.07	1.91	21.41	7.75	4024	96.21	2.77	12.31	1.85	20.63
NS+PM21	1.32	0.02	2.12	29.54	18.65	4674	100.14	3.73	14.27	4.21	31.10
NS+PM41	1.50	0.06	2.07	22.59	8.86	4233	105.85	3.47	13.08	2.86	20.62

Although the percentages of N, C, and H in different soil types changed throughout the experiment duration, there was no clear correlation ($P \geq 0.05$) to draw conclusion that irrigation water type has any effect on these components (Tables 14 and 15). It was clearly observed that natural soil was the poorest of all in terms of the concentrations of N, C, and Hpeat moss. S was not detected in any of the soil samples tested in this study.

Table 14: The Total Concentrations of N, C, H (%) in different soil irrigated with FW over time

FW	%N	%C	%H
SP 0	1.50	42.64	5.83
SP 21	2.15	46.14	5.01
SP 41	1.41	43.50	5.76
NS 0	0.00	4.83	0.73
NS 21	0.00	4.91	0.72
NS 41	0.00	4.55	0.65
NS+SP 0	0.38	19.00	2.42
NS+SP 21	0.36	17.44	2.33
NS+SP 41	0.40	19.49	2.55
NS+PM 0	0.30	17.34	2.46
NS+PM 21	0.33	19.15	2.79
NS+PM 41	0.43	24.55	3.44

Table 15: The Total Concentrations of N, C, H (%) in different soil irrigated with TWW over time

TWW	%N	%C	%H
SP 0	1.50	42.64	5.83
SP 21	1.36	42.47	5.63
SP 41	1.64	42.90	5.83
NS 0	0.00	4.83	0.73
NS 21	0.00	5.11	0.58
NS 41	0.03	5.03	0.71
NS+SP 0	0.38	19.00	2.42
NS+SP 21	0.39	18.31	2.54
NS+SP 41	0.66	23.25	3.83
NS+PM 0	0.30	17.34	2.46
NS+PM 21	0.33	19.15	2.79
NS+PM 41	0.38	23.24	3.29

Microbiological Analysis of Soil Samples:

It is evident that the aerobic mesophilic bacterial counts are much lower in the natural unmodified soil than in the peat moss or the mixtures (Figure 28). This may be due to Qatar's harsh conditions of extreme heat and aridity as well as the high salinity in the soil that may inhibit the growth of non-salt and heat tolerant organism.

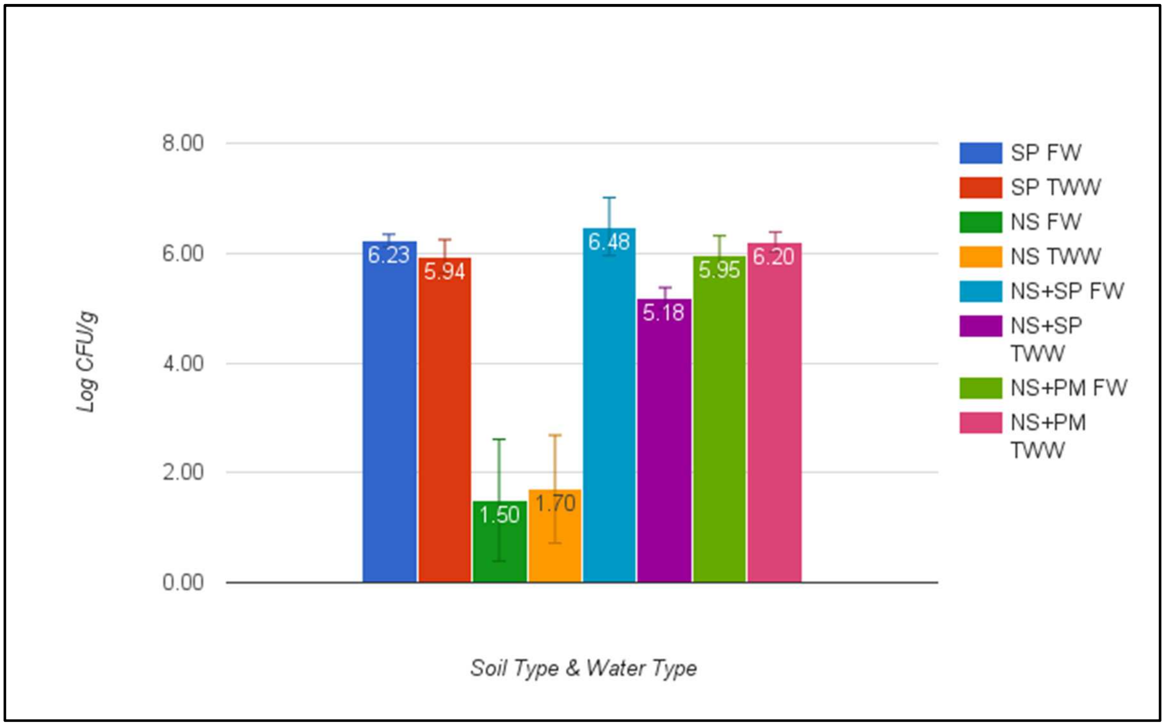

Figure 28: The average aerobic mesophilic bacterial counts (Log CFU/g) in different soil types treated with FW or TWW

The statistical analysis revealed that there is a strong interaction between all three factors (soil type, time, and water irrigation) on the aerobic mesophilic bacterial counts ($p<<0.05$). The natural soil exhibited the least fungal counts compared to the other soil types and mixtures (Figure 29). As observed in total aerobic bacterial counts, a strong three-way interaction was detected when samples were statistically analyzed for their fungal growth. This might be due to the presence of various microorganisms and the competitive exclusion among them. Another explanation could be that the natural conditions were not favoring the growth of fungi and yeasts.

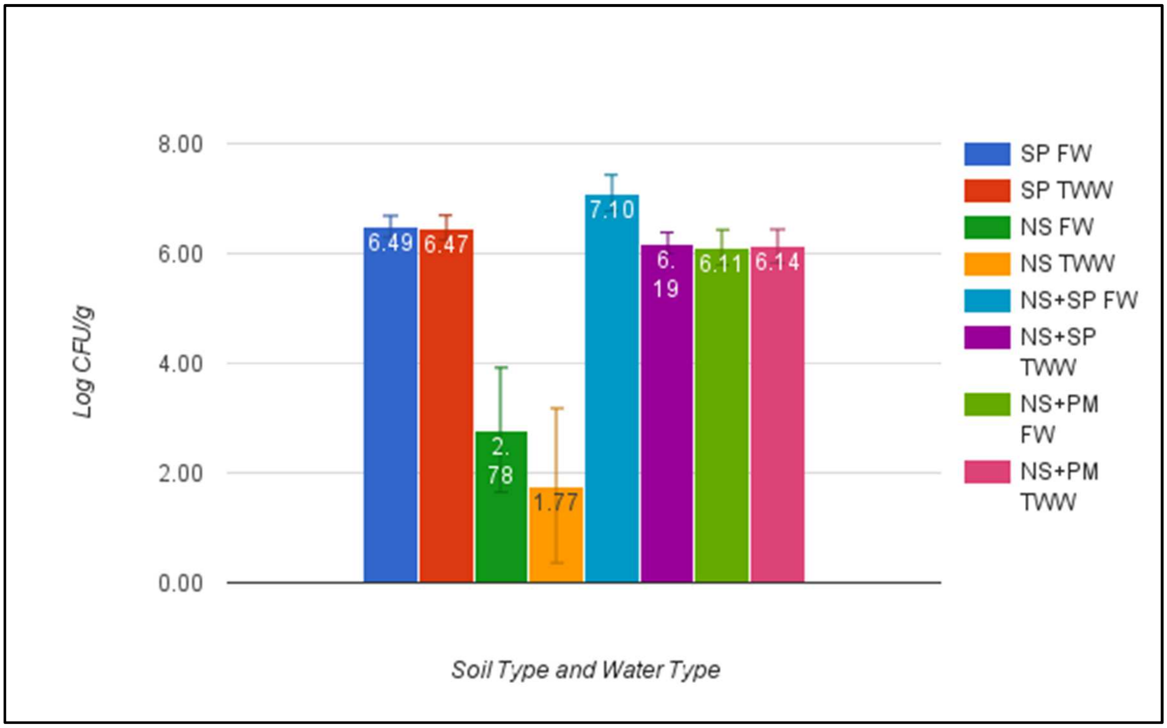

Figure 29: The average fungal counts (Log CFU/g) in different soil types treated with FW or TWW

Unlike the aerobic mesophilic bacteria and the yeasts and fungi counts; which exhibited significant interactions between soil type, water, and time on their counts; the total coliform counts were not significantly impacted by the type of soil, water, or time (p>0.05, p=0.0845), indicating that these bacteria can tolerate harsh conditions (Figure 30).

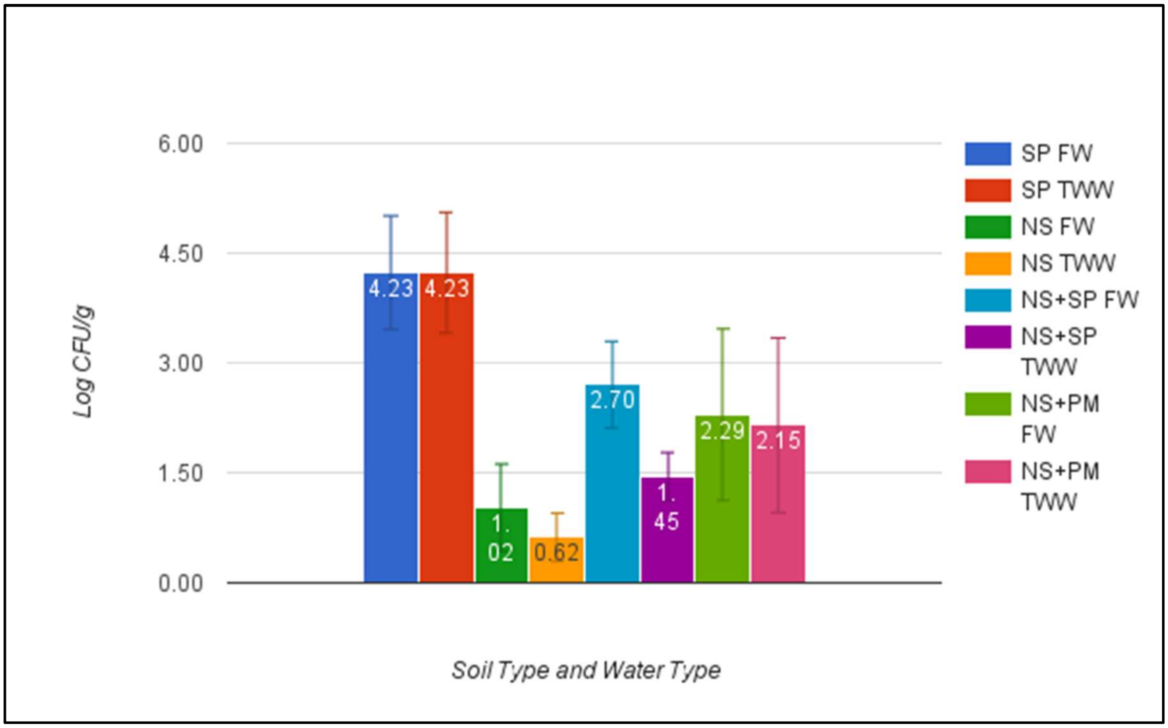

Figure 30: The average total coliform counts (Log CFU/g) in different soil types treated with FW or TWW

There was a significant interaction between the soil type and time on the total fecal coliforms counts (*P*=0.0001). The use of TWW surprisingly did not significantly affect the fecal coliform counts, which was one of the concerns of municipal TWW contaminating agricultural food products and soil. As can be seen in Figure 31, the sterilized unmixed peat moss had the heaviest load of fecal coliforms while natural soil had the least. This can be explained by the fact that the presence of salts in the natural soil mixed with the peat moss inhibited the growth of fecal coliforms.

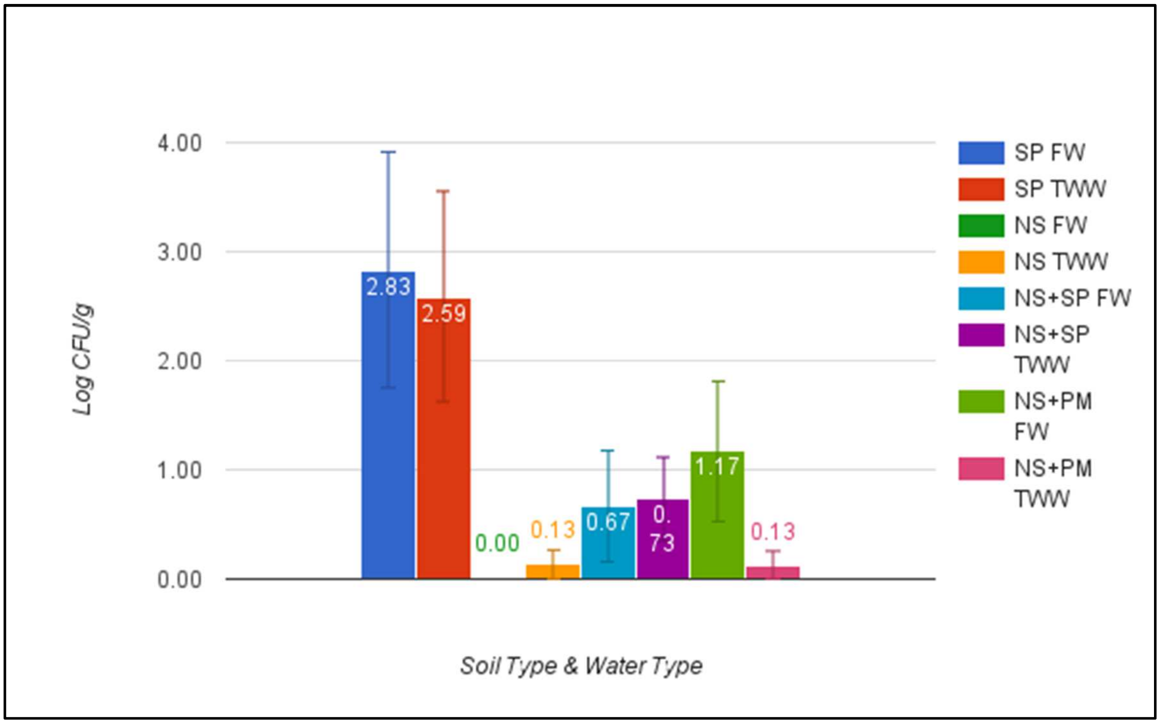

Figure 31: The average fecal coliform counts (Log CFU/g) in different soil types treated with FW or TWW

Overall, the Pearson correlation test indicated that there is a very strong positive correlation in the soil between aerobic mesophilic bacteria and the yeasts and fungi, r=0.9239, $p \ll 0.05$ ($p = 0.0000$). All of these factors eventually impacted the plant growth during the study period, indicating that the presence of aerobic bacteria is a positive factor to boost the plant growth.

Unlike the previous bacterial species studied, Rhizosphere cultures in soil showed very significant differences between treatment with freshwater and TWW. Figure 32 to 34 summarize the results of *Pseudomonas* spp, *Streptococcus* spp. and nitrogen fixing species. Overall, the soil samples treated with TWW exhibited significantly higher *Pseudomonas* spp, *Streptococcus* spp. and nitrogen fixing species counts compared to those of treated with freshwater, P = 0.001795, P = 0.000003, and P = 0.001204, respectively.

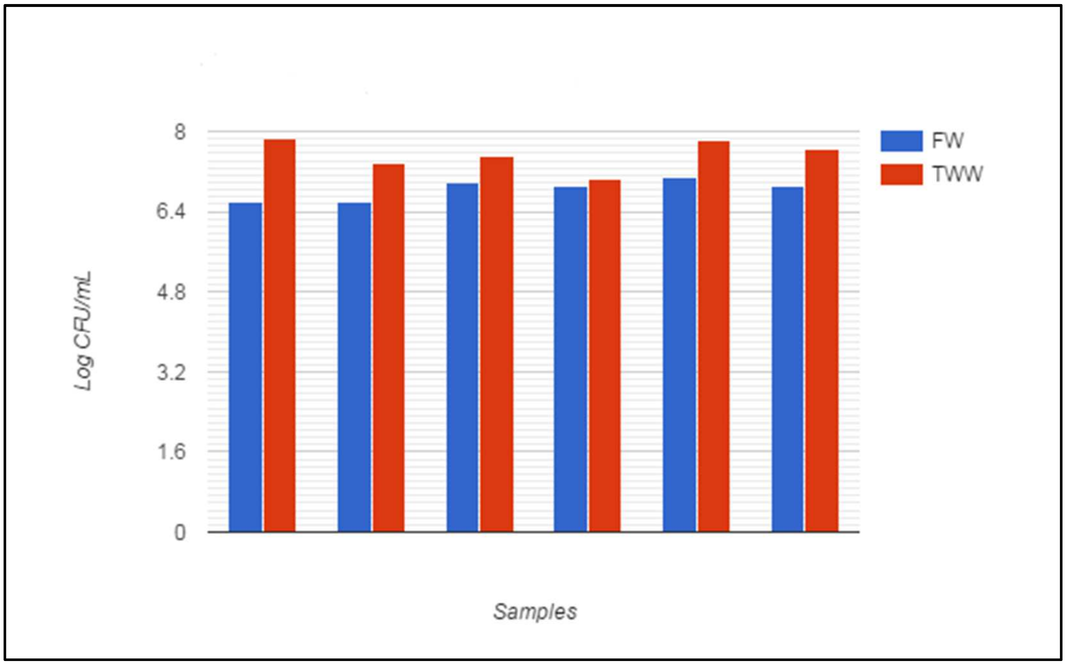

Figure 32: The total Pseudomonas spp. counts in soil samples treated with FW or TWW

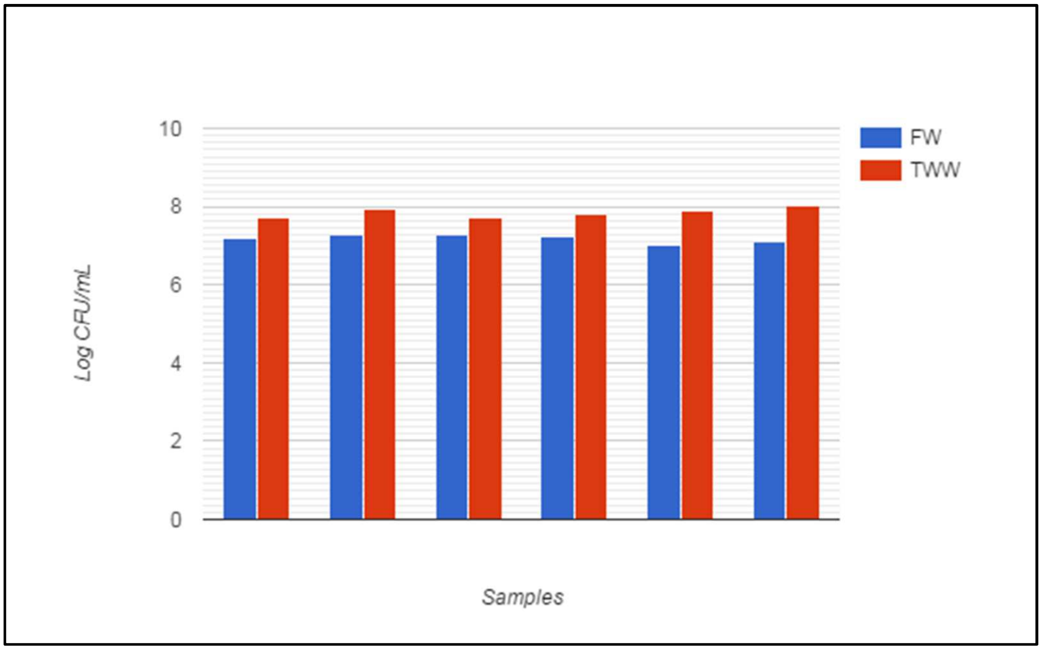

Figure 33: The total *Streptococcus* spp. counts in soil samples treated with FW or TWW

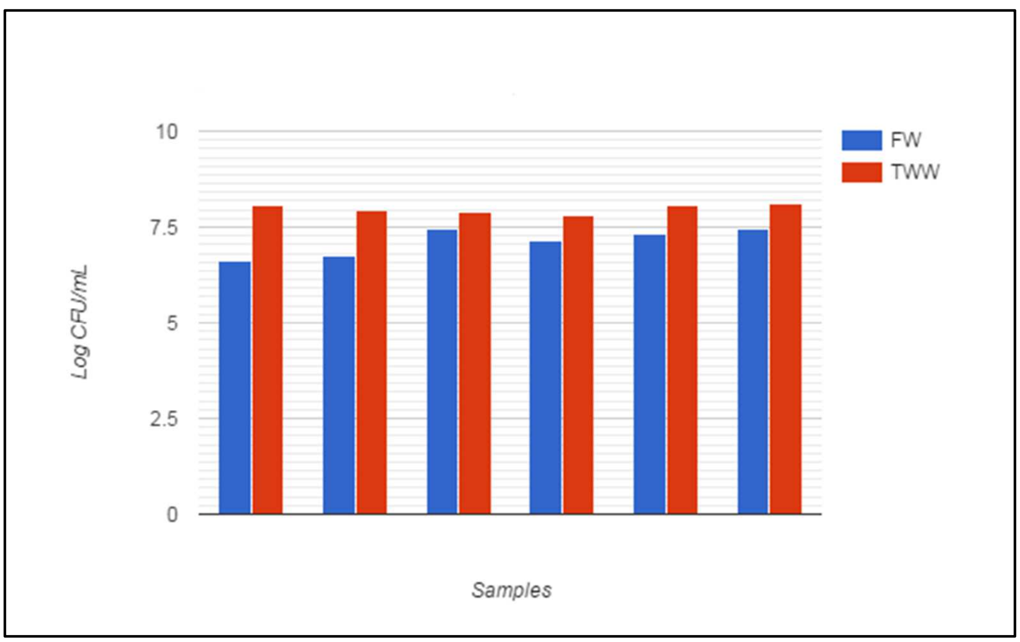

Figure 34: The total Nitrogen fixing bacterial counts in soil samples treated with FW or TWW

Plant growth

The leaf area measurements for the harvested Arugula showed no significant difference in size due to the water treatment as can be seen in Table 16.

Table 16: The Average leaf area (cm2) of Arugula at day 41

	N leaves	Leaf area (cm^2)
FW	60	5.45 ±3.89
TWW	61	4.64 ±1.86

However, when the weight of the plants was taken into consideration, a significant difference in plant weight was observed factoring the effect of water treatment and time. Time has a very significant impact on arugula's weight ($p<<0.05$), since at day 21 it is still in the process of growing and maturing and did not reach its full growth yet. Arugula irrigated with TWW during the growing phase weighed heavier than the Arugula irrigated with FW while at the mature stage (Table 17). . In conclusion, there is a significant effect ($p=0.0096$) of TWW on the growth of Arugula plant.

Table 17: The Average Arugula plant weight (g) with time

	N plants	Mean (g)
FW day 21	6	0.12 ±0.07
FW day 41	6	0.39 ±0.08
TWW day 21	6	0.05 ±0.28
TWW day 41	6	0.29 ±0.11

CHAPTER 5: DISCUSSION

The chemical analysis of the water shows that chemical composition of both FW and TWW are within the local standard limits for use in irrigating plants except for the elevated As found in the TWW. The microbial analysis of water indicated that TWW had slightly higher microbial load compared to that of FW, even before the start of the irrigation regimen. This corroborates with the previous studies by Lonigro *et al*. (2016) that indeed the water used for irrigation might be loaded with bacteria, but once in the soil, the interactions of the already present bacteria in the soil might be higher which eventually alters the soil chemistry. However, once the water has been used for irrigation the differences in microbial loads in the soil have shown to be influenced by other factors. The factors that influence the microbial growth include the type of soil the microorganisms inhabit which is proving to be the most important factor, the interaction between competing microorganisms, and time of sampling.

The chemical analysis of the soil indicates that salinity of the soil is an important factor to take into account when farming is considered in the country. The plants growing in the zone where the soil collection took place in this study have adapted to the soil's high salinity. There needs to be a careful planning about finding the best location, especially if soil-based agriculture is intended since some areas will be too saline impacting both the growth of plants as well as reducing the count of microorganisms in the soil microbiome. Peat moss is rich in all elements needed for the plant growth; therefore, it has been found as a suitable substrate to grow Arugula. This suggests that future farmers are better suited to opt for soil-less agriculture where plants can be supplemented with a little peat moss for initial growth then switch to hydroponics to compensate for the lack of arable land. High concentrations of salt in the natural soil make converting the non-arable soil to agricultural land very difficult even if other soils were added to it to increase its organic content. This study also proved the fact that natural soil conditioned with TWW did not support the seed germination nor plant growth, indicating that soil quality is an extremely important factor to be considered.

The total aerobic mesophilic bacterial counts reduced in general with the TWW irrigation. These results are in agreement with the findings of Kayikcioglu (2012) that a reduction of rhizosphere microbial activity was observed after TWW irrigation. The preliminary rhizosphere data on *Pseudomonas*, *Streptococcus* and nitrogen fixing bacterial counts provide a contradictory conclusion based on the data obtained from the experiments on the Aerobic Mesophilic bacteria and coliforms. The application of TWW in soil significantly increased such microbial growth in the soil. These findings were supported by the findings of Alguacil *et al.* (2012) that increased microbial biomass enhanced microbial activity in soil.

Pseudomonas is an aerobic mesophilic bacterium (Widmer *et al.* 1998) that is very common in soil, contributing towards the health of the soil from pathogens, although a few species can be pathogenic to plants and humans (Peix *et al*., 2009). Studies also show that *Pseudomonas* can withstand unfavorable conditions like presence of antibiotics, heavy metals, and PCPPs (Peix *et al*., 2009).In this study, the growth of *Pseudomonas* was enhanced after the treatment with TWW, indicating a competitive exclusion of other microorganisms. Further studies on the nature and origin of

Pseudomonas spp. is required in order to identify if the increase is from the pathogenic strains or the beneficial strains that can improve the soil immunity against pathogens.

Streptococci spp. are another common bacterial species found in soil, but it also can be used as an indicator of soil pollution especially when Fecal *Streptococcus* is being selectively looked for (Van Donsel *et al*., 1967 and Hagedorn *et al*., 1999). The results show that there is a significant increase in the number of *Streptococcus* spp. colonies in the soil samples irrigated with TWWpeat moss. This could be due to the presence of initial contaminants in TWW which was transported to the soil, as well as the extra nutrients that are available for the bacteria to utilize the growth of *Streptococcus* spp.

The significant increase in nitrogen fixing bacteria may be due to the higher content of nitrates in TWW which can be regarded as a positive outcome for soil since more nitrogen would become available for such bacteria. Eventually this positive impact will be translated into enhanced plant growth affecting the agricultural yield. These findings are supported by similar studies that there was a positive correlation between rhizosphere bacteria and Arugula growth (Mendes *et al*., 2013; Rashida *et al.*, 2016).

Arugula was shown to be sensitive to the Qatari TWW especially during the initial growth phase of its maturation, where it significantly weighed less than the corresponding plants irrigated with FW. This could be attributed to the higher levels of As present in the TWW or due to the lack of Cu, Fe, and Mn, minerals important for plant growth that are present in the FW but noticeably absent in TWW. However, these conclusions were not proven right after the completion of the growth phase and into the maturation phase as there was no significant difference between Arugula's weights. This could suggest that TWW may not be suitable for *E. sativa* at the initial seeding and germination phase. However, farmers can use a mixed system during the initial growing stages and then switching to 100% TWW at a later stage in order to conserve their freshwater and supplement the plant growth since *E. sativa* does not exhibit sensitivity to TWW in the late growth stage.

CHAPTER 6: CONCLUSIONS AND RECOMMENDATIONS

From the water treatment perspective, further research on the TWW quality should be undertaken with a look into a wider spectrum of heavy and trace metals as well as xenobiotics that are currently not tested for in governmental standards like PCPP. Specific considerations should be considered for the unique Qatari environment where lifestyle of the population and the economic activity of the country affects the quality of the wastewater produced.

Qatari natural soil in non-arable land was too saline and the negative effects of the salinity could not be mitigated by mixing with peat moss. Farmers may have to look into more intensive soilless farming techniques like hydroponics in order to improve food security. Further studies on soilless agriculture using TWW for irrigation are warranted to determine the effect of TWW on the growth of hydroponic plants and safety of plants irrigated with TWW for human consumption. The natural soil had also a poor microbiome, where it consistently showed the lowest counts for all the microbial cultures proving the degraded quality of the local soil for farming.

The rhizosphere microbiome is a complex ecosystem where many biological and chemical interactions taking place. For culture dependent soil microbiological research, there needs to be an expansion of the work to wider crops other than Arugula, including legumes and other leafy greens and vegetables. Further research should also be undertaken to isolate and identify the different species cultured and look into their activity to discern whether the TWW application improves the soil health by increasing the beneficial strains or increases the pathogenic ones. Microbial soil activity is another field that could also be investigated and its changes according to the irrigation method as another indicator of soil health. The growth of Arugula was affected by the microbia in the soil, specifically the aerobic mesophilic bacteria which enhanced its growth. Arugula was also found to be sensitive to TWW during its growth phase and it is not recommended to use TWW for irrigation of this food crop at the initial stage, however at the maturation stage TWW can be used in order to conserve freshwater.

REFERENCES

- Adrover, M., Farrús E., Moyà G., Vadell J., (2012), *Chemical properties and biological activity in soils of Mallorca following twenty years of treated wastewater irrigation*, Journal of Environmental Management, 95:S188-S192.
- Ahmed, T. A., Al-Hajri, H. H, (2009), *Effects of Treated Municipal Wastewater and Sea Water Irrigation on Soil and Plant Characteristics*, International Journal of Environmental Research, 3(4):503-510.
- Aissani, N., Urgeghe P. P., Oplos C., Saba M.,Tocco G., Petretto G. L., Eloh K., Menkissoglu-Spiroudi U., Ntalli N., Pierluigi Caboni P., (2015), *Nematicidal Activity of the Volatilome of Eruca sativa on Meloidogyne incognita*, Journal of Agricultural and Food Chemistry. 63: 6120−6125.
- Alguacil, M.M., Torrecillas, E., Torres, P., García-Orenes, F., Roldán, A., (2012), *Long-term effects of irrigation with wastewater on soil AM fungi diversity and microbial activities: the implications for agro-ecosystem resilience*. PLoS One, 7: 47680
- Allan, J.A., (2002), *The Middle East Water Question: Hydropolitics and the Global Economy*, L.B. Tauris Publishers, London., pp197
- Al-Omron A.M., El-Maghraby S.E., Nadeem M.E.A. , El-Eter A.M. ,Al-Mohani H., (2012), *Long term effect of irrigation with the treated sewage effluent on some soil properties of Al-Hassa Governorate*, Saudi Arabia, Journal of the Saudi Society of Agricultural Sciences, 11:15–18.

- Aluru, N., Leatherland, J.F., Vijayan, M.M., (2010), *Bisphenol A in oocytes leads to growth suppression and altered stress performance in juvenile rainbow trout*. PLoS One, 5(5): 10741.
- Asgharipour M. R., Azizmoghaddam H. R., (2012), *Effects of raw and diluted municipal sewage effluent with micronutrient foliar sprays on the growth and nutrient concentration of foxtail millet in southeast Iran*, Saudi Journal of Biological Sciences, 19: 441–449.
- Ashghal-PWA, (2014), *Public Works Authority Apply Sustainable Developments Concept with Sewerage Works, Qatar*, presentation, available at: http://wstagcc.org/en/wp-content/uploads/sites/2/2014/05/S6_L7_public-works-authority_Elemadi.pdf, last accessed 13 Dec 2016.
- Azuma T., Arima N., Tsukada A., Hirami S., Matsuoka R., Moriwake R., Ishiuchi H., Inoyama T., Teranishi Y., Yamaoka M., Mino Y., Hayashi T., Fujita Y., Masada M., (2016), *Detection of pharmaceuticals and phytochemicals together with their metabolites in hospital effluents in Japan, and their contribution to sewage treatment plant influents*, Science of the Total Environment, 548–549:189–197.
- Basem Shomar, Mohamed Amr, Khalid Al-Saad, Yasir Mohieldeen, (2013), *Natural and depleted uranium in the topsoil of Qatar: Is it something to worry about?*, Applied Geochemistry, 37:203–211.
- Becerra-Castro C., Lopes A.R., Vaz-Moreira I., Silva E.F. Manaia C.M., Nunes O.C, (2015), *Wastewater reuse in irrigation: A microbiological perspective on*

implications in soil fertility and human and environmental health, Environment International, 75: 117–135.

- Berg G., Erlacher A., Smalla K. and Krause R., (2014), *Vegetable microbiomes: is there a connection among opportunistic infections, human health and our 'gut feeling'?*, Microbial Biotechnology published by John Wiley & Sons Ltd and Society for Applied Microbiology, Microbial Biotechnology, 7: 487–495.
- Bot A., Benites J., (2005), *The importance of soil organic matter Key to drought-resistant soil and sustained food and production*, Food and Agriculture Organization of The United Nations Soil Bulletin,80, 2005, Rome, ISBN 92-5-105366-9.
- Bot A., Benites J., (2005), *The importance of soil organic matter Key to drought-resistant soil and sustained food and production*, Food and Agriculture Organization of The United Nations Soil Bulletin,80, 2005, Rome, ISBN 92-5-105366-9.
- Broszat M., Nacke H., Blasi R., Siebe C., Huebner J., Daniel R., Grohmann E., (2014), *Wastewater Irrigation Increases the Abundance of Potentially Harmful Gammaproteobacteria in Soils in Mezquital Valley*, Mexico, Applied and Environmental Microbiology, 80(17): 5282–5291.
- CDC, Center for Disease Control, (2011), *Outbreak of Shiga toxin-producing E. coli O104 (STEC O104:H4) Infections Associated with Travel to Germany (FINAL UPDATE)*, Posted July 8, 2011, available at:

http://www.cdc.gov/ecoli/2011/travel-germany-7-8-11.html, last accessed 5 Mar 2016.

- CDC, Center for Disease Control, (2016), *Multistate Outbreak of Salmonella Poona Infections Linked to Imported Cucumbers*, Posted January 26, 2016, available at: http://www.cdc.gov/salmonella/poona-09-15/index.html, last accessed 5 Mar 2016.
- CIA: World Factbook, 2016, *Qatar*, CIA: World Factbook website available at: https://www.cia.gov/library/publications/the-world-factbook/geos/qa.html , Last accessed 22 Nov 2016.
- Clarke N.A, Donsel D.J., Geldreich E.E., (1967), *Seasonal Variations in Survival of Indicator Bacteria in Soil and Their Contribution to Storm-water Pollution*, Applied Environmental Microbiology, 15:61362-1370.
- Daily Nation, *Kenya defends plans to parcel out 40,000 hectares to Qatar*, published 1 November 2009, available at: http://www.nation.co.ke/News/-/1056/513528/-/view/printVersion/-/3wecp8z/-/index.html;www.arabianbusiness.com/543415?tmpl=print&page=J, last accessed 12 Dec 2016.
- Ding, C., He, J., (2010), *Effect of antibiotics in the environment on microbial populations*, Applied Microbiological Biotechnology, 87: 925–941.
- FAO, (1989), *Arid zone forestry: A guide for field technicians*, ISBN 92-5-102809-5 available at: http://www.fao.org/docrep/t0122e/t0122e03.htm, last accessed 10 Dec 2016.

- FAO, (2016), AQUASTAT, website, Food and Agriculture Organization of the United Nations (FAO) available at: http://www.fao.org/nr/water/aquastat/data/query/results.html last accessed 10 Dec 2016.
- FAO[2], (2016), AQUASTAT, website, *Direct use of municipal wastewater for irrigation*, available at: http://www.fao.org/nr/water/aquastat/data/query/results.html?regionQuery=true&yearGrouping=SURVEY&showCodes=false&yearRange.fromYear=1958&yearRange.toYear=2017&varGrpIds=4265%2C4490%2C4512%2C4533%2C4534&cntIds=&regIds=9805%2C9806%2C9807%2C9808%2C9809&edit=0&save=0&query_type=WasteWpage&lowBandwidth=1&newestOnly=true&_newestOnly=on&showValueYears=true&_showValueYears=on&categoryIds=-1&_categoryIds=1&XAxis=VARIABLE&showSymbols=true&_showSymbols=on&hideEmptyRowsColoumns=true&_hideEmptyRowsColoumns=on&lang=en, last accessed 12 Dec 2016.
- Fuentes E., Alarcón M., Fuentes M., Carrasco G., Palomo I., (2014), *A Novel Role of Eruca sativa Mill. (Rocket) Extract: Antiplatelet (NF-κB Inhibition) and Antithrombotic Activities*, Nutrients, 6:5839-5852.
- Glovco O., Kumar V., Fedorova G., Randak T., Grabic R., 2014, *Seasonal changes in an antibiotics, antidepressants/psychiatric drugs, antihistamines and lipid regulators in a wastewater treatment plant*, Chemosphere, 111:418-426.

- Govan F., (2011), *'Killer cucumbers' row between Spain and Germany*, The Telegraph, newspaper, published on 30 May 2011, available at: http://www.telegraph.co.uk/news/worldnews/europe/germany/8546207/Killer-cucumbers-row-between-Spain-and-Germany.html last accessed 09 Sep 2015.
- Gründemann C., Garcia-Käufer M., Lamy E., Hanschen F.S., Huber R., (2015), *4-Methylthiobutyl isothiocyanate (Erucin) from rocket plant dichotomously affects the activity of human immunocompetent cells*, Phytomedicine, 22:369–378.
- Hua H.,Zhang L., Wang Y.P., (2016), *Crop development based assessment framework for guiding the conjunctive use of fresh water and sewage water for cropping practice—A case study*, Agricultural Water Management, 169:98–105.
- Hunter L.,Falen C., and Moore A., (2014), *Cover Crops for High-Desert Farming Systems in Idaho*, University of Idaho Bulletin, Published April 2014, http://www.cals.uidaho.edu/edcomm/pdf/BUL/BUL889.pdf.
- IndexMundi, (2016), *Qatar*, available at:
  http://www.indexmundi.com/qatar/#Economy, last accessed 10 Dec 2016.
- Kamizoulis G., Bahri A., Brissaud F., Angelakis AN., (2010), *Wastewater Recycling and Reuse Practice in Mediterranean Region: Recommended Guidelines*, available at:
  http://www.a-angelakis.gr/files/pubrep/recycling_med.pdf, last accessed 14 Dec 2016.

- Kamran M.A., Syed J. H., Eqani S. A., Munis M. F., Hassan Javed Chaudhary H. J., (2015), *Effect of plant growth-promoting rhizobacteria inoculation on cadmium (Cd) uptake by Eruca sativa*, Environ Sci Pollut Res, 22:9275–9283.
- Karnjanapiboonwong A., Suski J.G., Shah A. A., Cai Q.S., Morse A.N., Anderson T.A., 2011, *Occurance of PPCPs at a wastewater treatment plant and in soil and groundwater at a land application site*, Water Air Soil Pollution, 216:257-273.
- KAUST-website, (2016), *Research Projects*, King Abdullah University of Science and Technology, available at:

  https://cda.kaust.edu.sa/Pages/ResearchProjects.aspx, last accessed 12 Dec 2016.
- Kayikcioglu H. H., (2012), *Short-term effects of irrigation with treated domestic wastewater on microbiological activity of a Vertic xerofluvent soil under Mediterranean conditions*, Journal of Environmental Management, 102:108-114.
- Khalil R.K.S., Gomaa M.A.E, Khalil M.I.M., (2015), *Detection of shiga-toxin producing E. coli (STEC) in leafy greens sold at local retail markets in Alexandria, Egypt*, International Journal of Food Microbiology, 197:58–64.
- Latté, K.P., Appel, K.-E., Lampen, A., (2011), *Health benefits and possible risks of broccoli – an overview*, Food Chem. Toxicol. Int. J. Publ. Br. Ind. Biol. Res. Assoc., 49: 3287–3309.
- Lech, J.J., Lewis, S.K., Ren, L., (1996), *In vivo estrogenic activity of nonylphenol in rainbow trout*, Fund. Appl. Toxicol., 30: 229–232.

- Lonigro A., Rubino P., Lacasella V., Montemurro N., (2016), *Faecal pollution on vegetables and soil drip irrigated with treated municipal wastewaters*, Agricultural Water Management, (2016), available at:
    - http://dx.doi.org/10.1016/j.agwat.2016.02.001, last accessed 12 Dec 2016
- Magesan, G.N., Williamson, J.C., Sparling, G.P., Schipper, L.A., Lloyd-Jones, A.R., (1999), *Hydraulic conductivity in soils irrigated with wastewaters of differing strengths: field and laboratory studies*, Australian Journal of Soil Research, 37: 391–402.
- Marsoni M., De Mattia F., Labra M., Bruno A., Bracale M., Vannini C., (2014), *Uptake and effects of a mixture of widely used therapeutic drugs in Eruca sativa L. and Zea mays L. plants*, Ecotoxicology and Environmental Safety, 108:52–57.
- Mendes R., Paolina Garbeva P., Raaijmakers J. M., (2013), *The rhizosphere microbiome: significance of plant beneficial, plant pathogenic, and human pathogenic microorganisms*, FEMS Microbiol Rev., 37:634–663.
- Ministry of Development Planning and Statistics, (2013), *Water Statistics*, available at:

    http://www.mdps.gov.qa/en/statistics/Statistical%20Releases/Environmental/Water/2013/EnglishLayout.pdf, last accessed 12 Dec 2016.
- Ministry of Development Planning and Statistics, (2014), *Agricultural Statistics*, available at: http://www.mdps.gov.qa/en/statistics/Statistical%20Releases/Environmental/Agriculture/2014/1_Agricultural2014.pdf, Last accessed 10 Dec 2016.

- Ministry of Development Planning and Statistics, (2014), *Environmental Statistics*, available at:

  http://www.mdps.gov.qa/en/statistics/Statistical%20Releases/Environmental/EnvironmentalStatistics/Environment_MDPS_AE_2014.pdf, last accessed 12 Dec 2016.
- Morales M., and Janick J., (2002), *Trends in new crops and new uses*, J. Janick and A. Whipkey (eds.). ASHS Press, Alexandria, VA. available at: https://www.hort.purdue.edu/newcrop/ncnu02/pdf/morales.pdf, last accessed 12 Dec 2016.
- Müller, A.K., Westergaard, K., Christensen, S., Sorensen, S.J., (2002), *The diversity and function of soil microbial communities exposed to different disturbances*, Microbial Ecology, 44: 49–58.
- Oki T, Kanae S, (2004), *Virtual water trade and world water resources*, Water Science Environment 2004, 49:203-209.
- Penny R Hirsch P.R. and Mauchline T. H., (2012), *Figure 1: Separating out root-associated microbiomes for metagenomic analyses*, Nature Biotechnology, 30:961–962.
- Powell J.T., Chatziefthimiou A.D., Banack S.A., Cox P. A., Metcalf J.S., (2015), *Desert crust microorganisms, their environment, and human health*, Journal of Arid Environments, 112 :127-133.
- Prosser R.S., Sibley P.K, 2015, *Review: Human health risk assessment of pharmaceuticals and personal care products in plant tissue due to biosolids and*

manure amendments, and wastewater irrigation, Environment International, 75:223-233.

- Qadir M., B.R. Sharma, A. Bruggeman , R. Choukr-Allah , F. Karajeh, (2007), *Non-conventional water resources and opportunities for water augmentation to achieve food security in water scarce countries*, Agricultural Water Management, 87:2-22.
- Qatar Electricity and Water Company, (2015), *2015 Annual Report*, available at: https://www.qewc.com/qewc/en/index.php/reports/category/19-2015, last accessed 12 Dec 2016.
- Qatar Meteorological Department, (2016), *Climatological Normals*, available at: http://qweather.gov.qa/ClimateNormals.aspx, last accessed 10 Dec 2016.
- Qatar Statistical Authority; 2011; *Qatar in Figures 2011*; Qatar Statistical Authority; 26th issue December 2011.
- QNFSP, (2013), *Master Plan*, Qatar National Food Security Programme.
- Rashida M. I., Mujawar L. H., Shahzade T., Almeelbi T., Ismail I. M. I., Oves M., (2016), *Bacteria and fungi can contribute to nutrients bioavailability and aggregate formation in degraded soils*, Microbiological Research, 183 :26–41.
- Richer R., Anchassi D., El-Assaad I., El-Matbouly M., Ali F., Makki I., Metcalf J.S., (2012), *Short communication: Variation in the coverage of biological soil crusts in the State of Qatar*, Journal of Arid Environments, 78:187-190.
- Rio Carillio A.M.,Frei C.,(2009), *Water: a key resource in energy production*, Energy Policy, 37:4303-4312.

- Rusan M.J. M., Hinnawi S., Rousan L., (2007), *Long term effect of wastewater irrigation of forage crops on soil and plant quality parameters*, Desalination, 215:143–152.
- SAIC, Al-Sulaiteen Agricultural & Industrial Complex, Research & Development Overview, website, available at: http://saic.com.qa/saic/index.php?option=com_content&view=article&id=69&Itemid=2, last accessed 13 Dec 2016.
- Scott C, Pierce S., Pasqualetti M., Jones A., Montz B., and Hoover J.,(2011), *Policy and institutional dimensions of the water-energy nexus*, Energy Policy, 39:6622-6630.
- Shomar B., (2015), *Geochemistry of soil and groundwater in arid regions: Qatar as a case study*, Groundwater for Sustainable Development, 1:33–40.
- Shomar B., Darwish M.,Rowell C, (2014), *What does Integrated Water Resources Management from Local to Global Perspective Mean? Qatar as a Case Study, the Very Rich Country with No Water*, Water Resource Manage.,28:2781.
- Shomar B. and Dare A., *Ten key research issues for integrated and sustainable wastewater reuse in the Middle East*, Environmental Science Pollution Res, 22:5699–5710.
- Shuler K.D., Nie S.J, Shuler P.N., (2004), *Growing and Marketing Arugula at Local Green Markets in Southeast Florida*, Proceedings of the Florida State Horticultural Society available at: http://fshs.org/proceedings-o/2004-vol-117/267-274.pdf, last accessed 12 Dec 2016.

- Siddiqi A, Anadon L.D, (2011), *The water energy nexus in Middle East and North Africa*, Energy Policy, Doi: 10.1016/j.enpol,2011, 04-023.
- Thapliyal, A., Vasudevan, P., Dastidar, M.G., Tandon, M., Mishra, S., (2013) *Effects of irrigation with domestic wastewater on productivity of green chili and soil status*, Communications in Soil Science and Plant Analysis, 44(15): 2327–2343.
- The Cooperation Council for the Arab States of the Gulf-Secretariat General; (2012); website; available at: http://www.gcc-sg.org/eng/, Last accessed 10 Oct 2016.
- USDA, SR-28, (2016), Full Report (All Nutrients): 11959, *Arugula, raw*, The National Agricultural Library, available at:
    - https://ndb.nal.usda.gov/ndb/foods/show/3569?fgcd=&man=&lfacet=&count=&max=35&sort=&qlookup=arugula&offset=&format=Full&new=&measureby=, last accessed 3 May 2016.
- Xu E.G.B, Liu S., Ying G., Zheng G.J.S., Lee J.H.W., Leung K.M.Y, (2014), *The occurrence and ecological risks of endocrine disrupting chemicals in sewage effluents from three different sewage treatment plants, and in natural seawater from a marine reserve of Hong Kong*, Marine Pollution Bulletin, 85:352–362.
- Yang Y., Bing Li B., Zou S., Fang H.H.P., ZhangT., (2014), *Fate of antibiotic resistance genes in sewage treatment plant revealed by metagenomic approach*, Water Research, 62:97-106.