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Assessing Deep-Water Culture and Sand-Bed Aquaponics Systems for Lettuce (Lactuca sativa) Yield and Water Consumption

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Abstract

Agriculture and aquaculture play an important role in food security and water withdrawals. Agriculture and aquaculture contribute to over 70% of global water consumption. Aquaponics provides a solution for both sectors to reduce the combined water consumption and pollution and increase food production. The goal of the present study was to assess two aquaponics systems Deep-Water Culture (DWC) and Sand-Bed lettuce (*Lactuca sativa variety capitata* Type Batavia*)* production and water consumption. Each system contained a fish tank, plant bed, collection bed, three replicates per system and a mechanical filter; whereas, DWC contained an additional biological filter after the mechanical filter. The main differences assumed between DWC and Sand-Bed are Sand-Bed uses less water and does not require biological filter as sand media acts like a biological filter. Both systems had similar nitrate and ammonium concentration over 35 days period. PH, temperature, EC, SAR and DO in both systems remained within acceptable ranges compared to literature. Sand-Bed nitrifying bacteria counts were also higher than DWC's bacteria by end of study period. Results showed that the lettuce root length in DWC were nearly two-fold the root length in Sand-Bed which provides larger surface area for nutrients uptake and enhance nitrification rate by bacteria. Calcium, phosphorous, zinc, copper, magnesium and boron concentrations in lettuce samples harvested form the DWC were nearly two-times the concentrations in lettuce samples growing in Sand-Bed. The DWC Lettuce yields per m^2 were 27% higher than the Sand-Bed system's lettuce of 1.42 kg/m^2 in DWC and 1.04 kg/m^2 in Sand-Bed system. However, the daily water consumption in DWC system was higher than Sand-Bed system. Overall, DWC system performed better by producing higher lettuce yield with higher nutritional content while consuming more water compared to Sand bed system. Therefore, the Sand-Bed system requires further research to reach productive yields like the DWC system and benefit from its reduced water consumption and the potential of growing larger variety of crops.

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List of Abbreviations and Acronyms

Acronym/Abbreviation	Description
Al	Aluminum
AOB	Ammonia Oxidizing Bacteria
B	Boron
Ca^{2+}	Calcium ion
Cd	Cadmium
cm	centimeter
$CaCO_3$	Calcium Carbonate
CO_3^{2-}	Carbonate ion
Co	Cobalt
Cr	Chromium
Cu	Cupper
°C	Degrees Celsius
DO	Dissolved Oxygen
dS/m	Deci Siemens per meter
DWC	Deep Water Culture Aquaponics System
EC	Electric Conductivity
g	grams
FCR	Feed Conversion Ratio
Fe	Iron
Fe^{2+}	Iron ion
H	Height
HCO_3^-	Bicarbonate ion
HDPE	High Density Polyethylene
HR	High Resistance
hr	hour
IR	Intermediate Resistance
K	Potassium
Kg	Kilograms
L	Length
m	meter
ml	Milli liters
mg/L	Milligrams per Liter
meq./L	Milliequivalents per Liter
Max	Maximum value
Min	Minimum value

Mg^{2+}	Magnesium ion
Mn	Manganese
Mo	Molybdenum
N	Nitrogen
Na^{+}	Sodium
NH_4^{+}	Ammonia
NO_3^{-}	Nitrate
NOB	Nitrite Oxidizing Bacteria
P	Phosphorous
Pb	Lead
PO_4^{2-}	Phosphate
ppm	Parts per million
Sand-Bed	Sand Media-based Aquaponics System
SAR	Sodium Absorption Rate
SD	Standard deviation of statistical analysis
Si	Silicon
SP	Spontaneous Potential
TDS	Total Dissolved Solids
µg/100g	Micrograms per hundred grams
µg/l	Micrograms per liter
UV	Ultraviolet light
W	Width
WEF	Water Energy Food
Zn	Zinc

**Legend: The acronyms and abbreviations are listed in alphabetical order.*

Chapter 1: Introduction

1.1 Water Stress Problems

On the global level, more than 2 billion people live in countries with high water stress nearly more than 25% of current population (UN-Water, 2018). "Water stress is defined as the ratio of freshwater withdrawn to total renewable freshwater resources" (UN, 2018a). By 2050, global water demand is projected to increase by 55% where more than 40% of global population is forecasted to be living in severe water stress areas (UN-Water, 2018; WWAP and UN-Water, 2014). The world population is projected to reach 9.8 billion by 2050 (UN DESA, 2017). Growth in population imposes increases in water, energy and food demands by 55%, 80% and 70% respectively (Bundschuh, Chen, Chandrasekharam, & Piechocki, 2017). As a priority, the second goal of the 17 UN sustainable development goals (SDG) is SDG 2: "Zero hunger: To end hunger, achieve food security and improved nutrition and promote sustainable agriculture" (UN, 2018a).

Agriculture is essential for food security to meet the global growing population. Agriculture consumes globally over 69-70% of annual water withdrawals and 90% in some dry countries (UN-Water, 2018). The reason for intergovernmental and governmental organizations call for sustainable agriculture is that agriculture is perceived as "the leading cause and victim of water pollution" (UN-Water, 2018). Agriculture drainage waste water are returned to water bodies polluting the natural water resources.

Another source of food security besides agriculture is aquaculture. Aquaculture is a technique to grow aquatic organisms in artificial water tanks by supplying fish feed, adjusting dissolved oxygen levels, pH and temperature (Boyd & Tucker, 1998; Saha, Monroe, & Day, 2016). Aquaculture production is significantly increasing over six-fold from 1990 to 2016 to meet growing global population food demands as fisheries production levels are at a global stable rate of 90.9 million tons in 2016 annually (Ahmed, Thompson, & Glaser, 2019). Global fish stocks are declining from 90% in 1974 to 69% in 2013 leading to direct increase in aquaculture (UN, 2018a). Aquaculture is growing at a rate of 6.2% in 2011 where it contributes for 50% of fish consumption globally (Subasinghe et al., 2009 and FAO, 2013 cited from Eltholth, Fornace, Grace, Rushton, & Häsler, 2018). 1 kg of fish production requires 2.5 - 375 m^3 of water in different aquaculture systems including conventional, semi-intensive and intensive (Goddek et al., 2015; Mohanty, Ambast, Panda, Thakur, & Mohanty, 2017)

Similar to agriculture, aquaculture production pollutes water resources (Ahmed et al., 2019). Fish waste including excretions and unconsumed fish feed accumulate in the system polluting the water. Aquaculture farms dispose the polluted water on daily basis rate of 5–10% per day by adding new fresh water for optimum fish growth and prevention of disease and poisoning (Hu et al., 2015).

Agricultural and aquacultural activities have several negative impacts on water bodies. This includes leaching of pesticides, nitrates, salts, nutrients, manure, fish feed and feces waste contaminating surface and groundwater. Both sectors can provide means for reducing water stress levels by improving water consumption through efficient irrigation schemes, crops and fish selection, treatment and reuse of wastewater and advancement of unconventional agricultural and aquacultural techniques. Countries, business and intergovernmental organizations are implementing measures and techniques to reduce water stress levels, increase water use efficiency and provide alternatives for conventional water resources to meet food demands. 93% of the world's 250 highest revenue generating companies conduct sustainability studies focusing on water resources (UN-Water, 2018; UN, 2018a). Key foci are the efficient and sustainable use of water resources including natural and oceans by combating irrigation inefficiencies, overfishing and wastewater disposal. The solutions under research and implementation include use of non-conventional water resources such as desalination or reuse of wastewater, reducing water consumption in industries such as agriculture, aquaculture and food processing (UN, 2018a).

1.2 Egypt's Water Situation

Northern Africa and Western, Central and Southern Asia region face the highest water stress level above 70 percent in 22 countries including Egypt (FAO, 2018; UN-Water, 2018; UN, 2018b). Countries are considered to be severely water stressed when Water stress level is greater than 70%. Egypt's population is increasing at a rate of 2.38% reaching over 98 million in 2019 (CAPMAS, 2019). Egypt's population is expected to reach 130 million in 2030 at the current rate (EMPMAR, 2018). The ratio of total water consumption in Egypt reached 107%; hence, the Egyptian government works on reducing the ratio to 100% in 2020 and 80% in 2030 (EMPMAR, 2016). In fact, Egypt receives a fixed 55.5 billion m^3/year of water share from the Nile river (EMPMAR, 2018). The total amount of water Egypt receives from all available resources including freshwater and underground water is 59.25 billion m^3/year (EMPMAR, 2018). However, the water consumption in Egypt reached 100 billion m^3/year in 2018 which is met through reuse of drainage water, wastewater and sea water desalination (EMPMAR, 2018).

Water management is considered as a priority for the Egyptian Sustainable Development Strategy's (SDS) environmental pillar objectives set for 2030 aligning with UN Sustainable Development Goals (EMPMAR, 2018). Egyptian Government schemes currently focus on land reclamation in order to cultivate lands and increase food production to cover population needs (Salama, Abd El-ghani, Amro, & Gaafar, 2018). Egypt's SDS 2030 goals can be hindered by the water scarcity challenges that Egypt can face by 2030 (Wahba, 2017). Egypt's estimated agricultural water consumption is 1974 m^3/hectare/year (Abdelkader et al., 2018). In 2017, the agriculture sector consumes 81.6% of Egypt annual water withdrawals and only contributed 12% of the Egypt's annual GDP (CAPMAS, 2019).

Egypt is counted as one of the highest six producers of aquaculture. A recent study assessed the quality of fish in Egyptian's aquaculture as water used in most fish farms have residues of agriculture drainage that contains pesticides, fertilizer, and metals residues (Eltholth et al., 2018). The results showed that fish farm in Al-Gharbiya governorate had their fish livers and gills contaminated with high content of metals such as; lead, zinc, magnesium and cadmium; and hence, the fish were affected by water contamination (Eltholth et al., 2018). Water pollution especially with high metal content can contaminate fish and reduce its economic value (Eltholth et al., 2018 cited from Dahshan et al. 2013; Omar et al. 2015).

The Egyptian government is working on several transformational project to effectively manage water use in agricultural and industrial sectors. Organic farming is a key initiative by several agri-companies as it reduces up to 40% of water consumption in conventional farming techniques (EMPMAR, 2018).

1.3 Agriculture and Aquaculture Solution

Globally, aquaculture industry diverted attention to Recirculating Aquaculture Systems (RAS) as it can produce 500 tons/year using smaller water volume of 4000 m^3 (Bostock et al. 2010; Edwards 2015 cited from N. Ahmed, Thompson, & Glaser, 2019). Recirculating aquaculture systems (RAS) are optimized version of aquaculture where wastewater moves through biological and mechanical filters and recycles back to fish tanks in a continuous closed loop (Tschimer & Kloas, 2017). However, the residual water in the RAS system is highly concentrated with nitrate and phosphorous; this requires further treatment to denitrify the water (Bohl 1977, Kriiner and Rosenthal 1983 cited from (Tschimer & Kloas, 2017)). Plants use those two nutrients (nitrates and phosphorous) for growth. Aquaponics solves the gap by introducing a hydroponics system to RAS benefiting from the high nutrient residual water and producing another cash crop from the integrated system.

Aquaponics is an integrated alternative for food production as it integrates aquaculture and agriculture for fish and plants farming (Buzby and Lin 2014 cited from Pinho et al., 2018). Aquaponics combines aquaculture and soilless agriculture systems (hydroponics) in one integrated cycle for plants and fish farming. Aquaponics reuses wastewater as nutrient-rich solution for plants farming instead of polluting warm bodies. Plants uptake nutrients in waste effluent and purify it back to aquaculture tanks for fish farming. Researchers perceive aquaponics as promising production system for sustainable aquaculture and agriculture (Yina Zou, Hu, Zhang, Xie, Liang, et al., 2016).

Aquaponics technique has sustainable and environmental benefits as it uses less water, less wastewater discharge and higher productivity of input resources such as water and fish feed and output yields of fish and plants compared to conventional techniques (aquaculture and farming) (Pinho, Mello, Fitzsimmons, & Emerenciano, 2018). It also provides economic benefits by increasing the productivity and profitability of aquaculture systems as it decreases waste disposal and water usage and increases valuable by-products (i.e. plants) (Saha et al., 2016; Yina Zou, Hu, Zhang, Xie, Liang, et al., 2016). Aquaponics produces are estimated to yield ten-times more produce compared to conventional agriculture with 85-90% less water consumption over traditional irrigation (Ahmed et al., 2019).

1.4 Aim of Study

The present study was performed to assess small-scale aquaponics Deep Water Culture (DWC) and Sand-Bed system lettuce production yields per one cubic meter of water used in Egypt. Sand-Beds provide an attractive potential due to its similar media to conventional agriculture (soil). Only El Essawy (2018) experimented DWC and Sand-Bed systems assessing the growth of various crops qualitatively showing better qualitative performance in Sand-Bed over DWC. The aim of this study is to answer the following questions: 1) What are the lettuce production yields from DWC and Sand-Bed per cubic meter of water used ? 2) How do the systems affect water and lettuce quality? 3) Can the Sand-Bed system provide better yields over DWC?

Chapter 2: Literature Review

2.1 Aquaponics: An Unconventional Agricultural Technique

A RAS is a closed system for fish production where water is recirculated in the system by maintaining filtration and adding additional water periodically (Mullins, Nerrie, & Sink, 2015). Hydroponics system is an agricultural technique that can be closed or open system for growing crops in dissolved nutrient-based water medium instead of soil and adding artificial nutrients required for crops growth (Diver, 2006; Medina, Jayachandran, Bhat, & Deoraj, 2016; Mullins et al., 2015; Saha et al., 2016). Aquaponics is an unconventional method of agriculture where it uses both aquaculture and hydroponics in an integrated system to sustain water use and grow fish and plants in a closed cycle (Mullins et al., 2015). Fish grow in a water tank (aquaculture) emitting fish wastes that provides necessary nutrients for crops growth (hydroponics). By aquaponics, the bio-integration of RAS with hydroponics replaces the additional nutrients for crops growth and filtrates the water for fish culture (Klinger & Naylor, 2012). Regarding concerns of sustainability of aquaculture and agriculture, aquaponics provides a potentially more sustainable system for growers and consumers (Mullins et al., 2015).

Historically, aquaponics was present in one form or another since 1,000 A.D. in Mayan, Aztec, Asian, South American and Chinese cultures (Mullins et al., 2015; Somerville, Cohen, Pantanella, Stankus, & Lovatelli, 2014). The concept of using fish excrements such as fecal waste to fertilize pants as fish ponds were located next to agricultural lands has existed for millennia. In New Alchemy Institute, and other Northern American and European academic institutions in the late 1970, aquaponics systems started to evolve in modern crops production systems (Somerville et al., 2014). Through further research, small-scale aquaponics systems for crops production developed paving the way for practitioners and crops growers to practice it as means of sustainable food production worldwide commercially and individually (Klinger & Naylor, 2012; Somerville et al., 2014).

Aquaponics uses nutrient-rich water from the fish tanks as fertilizer for the plants. The effluent from the fish tanks is nutrient-rich due to presence of ammonia excreted by fish waste and oxidized into nitrates. Other nutrients such as K, Ca, P are obtained from the fish feed waste disintegrating in the fish water effluent. The nutrient-rich water is then supplied to the plants bed which is like hydroponics (Pinho et al., 2018; Tyson, Treadwell, & Simonne, 2011; Yina Zou, Hu, Zhang, Xie, Liang, et al., 2016). Oxidation of ammonia (NH_4^+) into Nitrates (NO_3^-) occurs by nitrifying bacteria; this process is defined as nitrification process (Pinho et al., 2018; Yina Zou, Hu, Zhang, Xie, Liang, et al., 2016).

2.1.1 Nitrification Process

The nitrification process consists of two sub-processes: 1) ammonia (NH_3) oxidizing into nitrites (NO_2^-) and then NO_2^- oxidizing into nitrates (NO_3^-) (Pinho et al., 2018; Yina Zou, Hu, Zhang, Xie, Liang, et al., 2016). Nitrifying bacteria required in the system consists of two type: ammonia-oxidizing bacteria (AOB) and nitrate-oxidizing bacteria (NOB). The nitrification process occurs via two groups of nitrifying bacteria. The

Nitrosomonas bacteria is the Ammonia-Oxidizing Bacteria (AOB) which converts ammonia to nitrite (Somerville et al., 2014; Yina Zou, Hu, Zhang, Xie, Liang, et al., 2016). The Nitrite-Oxidizing Bacteria (NOB) is the *Nitrobacter* bacteria which consumes nitrite and converts it to nitrate (Somerville et al., 2014; Yina Zou, Hu, Zhang, Xie, Liang, et al., 2016). Both nitrifying bacteria AOB an NOB thrive in soil environments with high surface area, oxygen and water.

2.1.2 Mechanical and Biological Filters

Mechanical and biological filtration units remove pollutants and improve water quality of aquaponics systems (Wongkiew, Hu, Chandran, Lee, & Khanal, 2017). The mechanical filters are usually placed after fish tanks to remove solids in the fish water effluent such uneaten fish feed and feces. The mechanical filtration improved aquaponic systems by 85% (Thorarinsdottir, 2015). Without the mechanical filtration, solid particles accumulate in plant beds which demand more oxygen and clog the plants roots accordingly. Biological filters are usually present after mechanical filters (after solid particles are removed) to enhance nitrification rate in the aquaponic systems. Biofiltration requires high surface area for nitrifying bacteria to grow. Different types of biological filters exist such as sand and bead filters, bio-balls, moving beds bioreactors (MBBR). AOB and NOB bacteria multiply on the biological filters and increase the nitrification rate.

Sikawa & Yakupitiyage (2010) studied the effect of partially filtered and unfiltered nutrient rich catfish pond water on the lettuce (*Lactuca sativa L.*) farming in media-filled beds for 54 days. The study consisted of three different substrates of media: two media based (1) sand (2) gravel and a control in the form of DWC as styrofoam. The lettuce seedlings were transplanted after grown in nursery for twenty-one days. The beds were only irrigated twice daily. Lettuce yields were highest on sand-media, followed by gravel then DWC. Partially filtered water obtained the highest plant yields by 87, 63 and 52% for sand, gravel and DWC, over unfiltered water, respectively

2.1.3 System and Media Types

In aquaponics system types are categorized according to the plant beds growth media. The plant beds (or hydroponic part of the system) have three main types: 1) floating rafts or deep-water culture (DWC), 2) media-filled beds or 3) nutrient film technique (NFT). In the media-filled beds, the media can be sand, gravel, chicken manure and various other media that are still studied under research.

a Use of Biological Filter

In media filled beds, there is no need for biological filter as the media acts as the biological filter due to similar environment to soil in conventional agriculture. Several researchers reported that media-filled beds without biological filter showed higher bacteria growth surface area compared to NFT and DWC with biological filter (Lennard & Leonard, 2006; Yina Zou, Hu, Zhang, Xie, Liang, et al., 2016). Media-filled beds surface area acts as a biological filter converting necessary nutrients to plants roots and enhanced water quality in the aquaponics system (Thorarinsdottir, 2015). However, all types require mechanical filtration as plants' roots can be clogged by fish waste. Higher water replenishment was required in media-beds (gravel) over DWC due to higher water evaporation (Zou et al., 2016). Other research showed different results where media-beds needed less water exchange over DWC systems (5-10%) (Tyson et al. 2011 cited from Zou et al. 2016).

Studies with media-filled beds reported higher plants yield than NFT as there is larger surface area for nitrifying bacteria growth (Li et al., 2019). Sand used as media provided good results in previous studies as it biological filters solid particles efficiently and reduces plant pathogenic risks (Casiano, 1988; McMurtry et al., 1990; Al-Ghawas and Al-Mazidi, 2004 cited from Sikawa & Yakupitiyage, 2010). In an experiment comparing media-beds of sand, rice hull and a mixture of both, sand media-bed had the highest lettuce yield (Thippayarugs et al., 2001)

b Recirculation and Nutrient Removal Rates

Recirculation rate affect the efficiency of aquaponics systems; (Endut, Jusoh, Ali, Wan Nik, & Hassan, 2010) recommended flow rate higher than 100 L/h. The higher the recirculation rate, the higher the waste removal from the system that can be toxic to the fish and plants (Diem, Konnerup, & Brix, 2017; Endut et al., 2010). High water circulation 84-168 L/h showed high growth of Nile tilapia fish (Diem et al., 2017). Higher circulation rates over 300 L/h can impact the aquaponics system negatively as it consumes excessive amount of energy and reduces the time for biofiltration and nitrification to occur.

Several researchers reported low plant yield due to plants' root clogging with algae and other solids particles from fish excreta and fish feed (Sikawa & Yakupitiyage, 2010) when crops were irrigated using water form fish ponds. The authors recommended the use of filtered pond water to reduce accumulation of suspended solids (J E Rakocy, Shultz, Bailey, & Thoman, 2004; Shete et al., 2017; Sikawa & Yakupitiyage, 2010).

Li et al. (2019) measured removal concentrations of total ammonia nitrogen, nitrite, nitrates, total nitrogen and dissolved total phosphorous for media-filled beds and NFT every two hours in one day in two aquaponics systems. The concentrations declined linearly with time in both systems; media-filled beds had greater removal rates over NFT nearly 50% faster. The authors linked the fast removal rate to system type (media-filled beds over NFT) due to the higher biofiltration capacity and porosity improving bacteria growth rate and nutrients conversion (Li et al., 2018, 2017, 2019; Tabassum, Li, Chi, Li, & Zhang, 2018).

2.1.4 Benefits

Aquaponics main benefits are 1) the reuse of aquaculture waste water as nutrient-rich solution for plants farming instead of disposing it in water bodies 2) purification of waste water and reuse in aquaculture system for optimized fish growth. It utilizes nutrients sustainably as plants and fish both benefit and grow commercially (Hu et al., 2015). It also provides a safer environment for plants and fish as it clears toxicity from fish and plants tanks through the nitrification process. Accordingly, plants and fish are less vulnerable to diseases and toxicity.

Aquaponics systems are sustainable recycling systems as they reduce waste economic and environmental costs of aquaculture and agricultural systems by optimizing and purifying the water for fish and plants farming (Li et al., 2019; Piedrahita, 2003; Saha et al., 2016). Aquaponics sustainability does not only address crops and fish production system that reduces water use, improves aquaculture systems productivity and enhances the agriculture process through natural fertilizers but can also be considered as organic means of crops production (Diver, 2006). The United States Department of Agriculture's National Organic Program, NOP, identifies universal standards and guidelines for organic certifications for crops and livestock (fish). AquaRanch is an aquaponic greenhouse in Illinois where it obtained an organic certification for its hydroponic produce through Indiana Certified Organic. Meanwhile, AquaRanch commercializes its tilapia fish as "naturally grown" as there are still some concerns generally from NOP regarding organic aquaculture (Diver, 2006).

2.2 Water Quality

Water quality is important for optimized plant and fish yields and cost-effectiveness (Li et al., 2019). The principal operating conditions in research studies for aquaponics are defined as physicochemical parameters; this includes dissolved oxygen (DO), temperature and pH values (Li et al., 2019; Somerville et al., 2014; Thorarinsdottir, 2015; Yina Zou, Hu, Zhang, Xie, Guimbaud, et al., 2016). Plants and fish require different conditions for optimum growth regarding pH, temperature and DO. Optimum pH values for fish survival is 6.4-9.0, for nitrifying bacteria (7-8.0) and for plants 6-6.5 (Thorarinsdottir, 2015). The pH of 7.0 is good compromise (Li et al., 2019; J E Rakocy et al., 2004; Sikawa & Yakupitiyage, 2010; Somerville et al., 2014). DO concentration should be at least above 5 mg/L for fish growth (Thorarinsdottir, 2015). Nitrates are toxic to fish when concentration level reaches 300 and above (Li et al., 2019).

2.3 Water Use Variables

Data on water consumption and total water use are scare in aquaponics literature. Few studies calculated total water use. Research reported that also crops type influence daily water consumption in aquaponics (Maucieri et al., 2018). In aquaculture industry, water use variables that are widely used for total/daily water use and consumption in aquaculture industry proposed by Boyd, (2005) (Maucieri et al., 2018; Mohanty, Ambast, Panda, Thakur, & Mohanty, 2017; Mohanty, Ambast, Panigrahi, Thakur, & Mandal, 2018). The total water use is defined as the total amount of water applied in an aquaculture system including water added by mechanical means such as pumping and natural processes such as rainfall and run-off (Boyd, 2005). The water consumption use is defined as the amount reduced from total water added due to intentional discharge, evaporation, seepage losses, etc. In aquaculture farms, the daily water consumption can vary from 250% per day for extensive aquaculture and a range between 2 to 10% for intensive aquaculture and less than 1% for recirculating aquaculture systems (RAS) (Hu et al., 2015; Maucieri et al., 2018).

Total water use and water consumption are evaluated similarly for aquaponic systems. Sources of water loss were evaporation, evapotranspiration, spillage, leakage and water exchange (Delaide et al., 2017). Researches assessed like aquaculture industry daily water consumption in aquaponic systems showed a varying range from 0.1 up to 5% in floating systems such as DWC and 1.2 up to 41% in medium-based systems such as gravel beds (Maucieri et al., 2018). The total fresh water use efficiency was expressed in terms of kg plants and fish per m3 of fresh water consumed (kg/m3) (Delaide et al., 2017; Lennard & Leonard, 2005; Suhl et al., 2016). Delaide et al. (2017) reported 0.49 m^3 were consumed to produce 1 kg of vegetable and 0.878 kg of *Nile Tilapia* fish over 30 days. Love, Uhl, & Genello (2015) study showed that a total of 0.40 m^3 of water were consumed to produce 1 kg of crops and 1 kg of *Nile Tilapia Fish*. Conventional agriculture in the Northern Nile Delta (most fertile lands in the Egypt) results ranged from 0.69 to 13.79 kg/m^3 of water consumed for winter field crops, 3.40 to 10.69 kg/m^3 of water consumed for winter vegetables, 0.29 to 6.04 kg/m^3 of water consumed for summer field crops, 2.38 to 7.65 kg/m^3 of water consumed for summer vegetables, 1.00 to 5.38 kg/m^3 of water consumed for autumn season crops (El-Marsafawy, Swelam, & Ghanem, 2018).

2.4 Aquaponics Systems Performance

Performance of aquaponics systems is measured through various indicators regarding fish growth, plant growth, water quality and consumption, nitrification rates based on bacteria abundance and ammonium and nitrates concentration. Li et al. (2019) measured growth of plants in terms of number of plants, height ad fresh weight; and fish plants in terms of feed conversion ratio (FCR) and specific growth rate (SGR). Pinho et al. (2018) defined aquaponics systems productivity in terms of plant performance, water quality and fish performance. Other studies defined the yields increase as the biomass increase for plants and fish. Other researchers measured plant quality via leaf nutrient analysis to assess the nutrient-water effects on the nutrient content of the plants (Delaide, Goddek, Gott, Soyeurt, & Jijakli, 2016; Maucieri et al., 2018). The leaf nutrient analysis included: N, P, K, Mg, Ca, S nutrient content in plants (Maucieri et al., 2018).

On the economic level, the plant yields were measured via plant height, plant fresh weight and dry weight (Saha et al., 2016). Other authors measured the study environment or the physicochemical parameters of water in fish water tanks to assess the optimum conditions for fish and plants growth – this included pH, temperature, DO and nutrient concentration (Goddek & Vermeulen, 2018). Research is mostly focused on improving the productivity of aquaponics systems in terms of fish and cash yields due to initial high capital cost compared to conventional agriculture (Lennard & Leonard, 2006; Sace & Fitzsimmons, 2013)

2.5 Fish Selection and Growth

Fish stocking density, fish feeding rate, and environmental conditions principally affect water quality in aquaponics systems (Li et al., 2019; Thorarinsdottir, 2015). Most of commercial aquaponics systems grow Nile Tilapia (species *Oreochromis niloticus*) due to their high adaptation to changing environment conditions (Bailey & Ferrarezi, 2017; Pinho et al., 2018; Silva, Valdés-Lozano, Escalante, & Gasca-Leyva, 2018). Other commonly used fish in aquaponics is African catfish (species *Clarias gariepinus*) and cray fish (species *Clarias macrocephalu*) (H Effendi, Utomo, & Darmawangsa, 2015; Love, Fry, et al., 2015; Oladimeji, Olufeagba, Ayuba, Sololmon, & Okomoda, 2018; Saha et al., 2016). (Palm, Bissa, & Knaus,

2014) compared Nile Tilapia and African catfish performance in aquaponics systems; where Nile tilapia system produced higher lettuce, basil and cucumber yields.

2.5.1 Fish Feed

The selection of fish feed is "doubly important" as it affects both fish and plants yields (Medina et al., 2016). Fish consume 20-30% of fish feed N content; whereas, the 70-80% are released in water to be disposed as wastes in aquaculture or nutrient effluent for plants in aquaponics (Hargreaves, 1998, Schneider et al. 2005, Krom et al. 1995 cited from Saha et al., 2016). On the economic level, fish feed contributes to 50-70% of aquaculture costs (Siriwardena & Hasan, 2009).

2.5.2 Fish Growth Indicators

Fish yield or biomass increases is the final wet weight of fish minus the initial weight of fish (Baker, 2010; Delaide et al., 2017; Diem et al., 2017; Li et al., 2019). Feed Conversion Ratio (FCR) is the total weight of fish feed used over the study period divided by total fish biomass increase. Fish biomass increase is the final wet weight of fish minus initial wet weight of fish (Hefni Effendi, Wahyuningsih, & Wardiatno, 2017; Fry, Mailloux, Love, Milli, & Cao, 2018; Li et al., 2019; Monsees, Kloas, & Wuertz, 2017; Trejchel et al., 2014). Specific growth rate (SGR) is the ln final wet weight minus ln initial weight of fish x 100 divided by days of study period (Daudpota et al., 2016; Monsees et al., 2017; Trejchel et al., 2014; Yina Zou, Hu, Zhang, Xie, Liang, et al., 2016). (Yina Zou, Hu, Zhang, Xie, Liang, et al., 2016) reported fish biomass increase of 1.5-1.45, SGR of 0.32-0.34 and FCR of 4.3-4.57. Low FCR values reflects better fish feed utilization and higher fish biomass increase (Li et al., 2019). This parameter is widely used in measuring aquaculture production efficiency (Li et al., 2019).

2.6 Plants Selection and Growth

Plants in aquaponic systems are important as they absorb nitrates and other nutrients that are harmful to fish (Carvalho, Bastos, & Souza, 2018). Plants differ in nutrients and nitrate uptake; and therefore, selection of fish and plants combination is important for the nutrient efficiency of the system. Different types of plants can grow in aquaponics including leafy vegetables and vegetable bearing fruits. The selection of plants type depends on fish stocking density and nutrient concentrations in the aqueous solution (Li et al., 2019 cited from Connolly and Trebic, 2010).

Plants have different nitrate requirements during their growth stages depending on roots surface area and plant type. For instance, leafy vegetables require larger amounts of nitrates than fruit bearing vegetables. The larger the roots surface area, the higher the plants uptake of nitrates. Plants optimally absorb nutrients such as P, K, S, Ca and Mg at pH of 6.0-8.0 (Delaide et al., 2016) while other nutrients such as; Fe, Mn, B, Cu and Zn are optimally absorbed at pH below 6.0 (Delaide et al., 2016; Resh, 2016). On the commercial level, use of garnishes of leafy vegetables in restaurants is commonly popular due to their all year availability, quick growth, flavoring and health benefits (Knaus & Palm, 2017; Love, Uhl, et al., 2015). Leafy vegetables used as garnish includes lettuce, basil, parsley and other herbs (Love, Uhl, et al., 2015).

2.7 External Nutrient Additions

To increase nutrient concentration in aquaponics systems, researchers added minerals like calcium hydroxide, potassium hydroxide, iron concentration (Fe^{2+} > 2 mg/L) or other biofertilizers on weekly basis (Hu et al., 2015; James E Rakocy, Masser, & Losordo, 2006). Other reasons for external minerals supplied to water is iron deficiency in fish water effluent or to adjust pH levels by using hydroxides. Nutrient supplementation can enhance plants quality in aquaponics systems and reduce potential risks of nutrient deficiency (Delaide et al., 2017).

2.8 Data Analysis

2.8.1 Water Sampling and Analysis

Most researchers sampled water (100 ml) from each fish tank weekly in the morning. If the samples were not sent immediately to laboratory analysis, the samples are stored at 4° to -20°C before measurement (Hu et al., 2015; Suhl et al., 2016). The water samples are analyzed for chemical, micro and macro-nutrient content analysis. Chemical, or stated in some studies as physical or physicochemical parameters, or environmental conditions include Dissolved Oxygen (DO), pH, water temperature, total dissolved solids (TDS), electric conductivity and sodium absorption rate (SAR). Micro and macro-nutrients analysis include ions concentration of ammonium (NH_4^+), nitrates (NO_3^-), sodium(Na^{2+}), potassium (K^+), calcium (Ca^{2+}), magnesium (Mg) and other minerals such as iron (Fe) and phosphorous (P). Other researchers preferred to collect water samples from the collection tanks or both tanks weekly. Studies showed that tanks have similar physicochemical and nutrient contents within the same system. Due to the dynamics nature of aquaponics systems, DO, pH and temperature sensors are usually equipped to measure the system's changes continuously per day. Studies use the sensors measurements for analyzing the environmental conditions during the examination period (Pinho et al., 2018).

2.8.2 Media Bacterial analysis

Bacteria count at different points during study period represents the growth trend of AOB and NOB and nitrification rates respectively (Hu et al., 2015; Yina Zou et al., 2017; Yuanchun Zou et al., 2018). Water samples, soil samples, and biological filter water or media samples are regularly analyzed to identify AOB and NOB bacteria count and their growth rate and impact on nitrification (AOB and NOB) (Hu et al., 2015; Zou et al., 2017; Zou et al., 2018).

2.8.3 Plants Analysis

Studies examined lettuce yields analysis through several factors either by biomass/growth parameters, leaf nutrient analysis or plant quality index (Estrada-Perez et al., 2018; Goddek & Vermeulen, 2018; Hollmann, 2017; Lennard & Leonard, 2005). A representative sample of lettuce seedlings and final heads at beginning and end of study periods are weighted to obtain their fresh weight, measured for their shoot length, leaf length, leaf width, root length, number of fresh and dry leaves then dried to obtain dry weight. The samples are then sent for laboratory leaf nutrient analysis to measure concentrations of K^+, Na^+, Ca^{2+}, Mg^{2+}, NO_3^-,

Cl^-, SO_4^{2-}, P, Zn^{2+} (Buzby & Lin, 2014; Lennard & Leonard, 2006; Sace & Fitzsimmons, 2013; Silber et al., 2003; Trang, Schierup, & Brix, 2010). To the best of our knowledge, no studies were found in literature measuring vitamins, lipids, ash, protein, moisture and ash matter content; however, the nutritional content of lettuce sold in market contains the aforementioned parameters (Nyathi, Van Halsema, Beletse, Annandale, & Struik, 2018).

2.8.4 Plant Quality Index

Previous studies investigated qualitatively the leaves quality of plants grown in aquaponics through taking several pictures of leaves at time intervals of the study period (either weekly or biweekly). Researchers analyzed the pictures according to their color variation from green to yellow. The authors indexed the color variation using a scale from 1 to 4, 1 being most green and 4 being most yellow and defined it as "plant quality index" (PQI) (Pinho et al., 2018; Pinho, Molinari, de Mello, Fitzsimmons, & Coelho Emerenciano, 2017). Further observations can be concluded from the PQI. Leaf yellowing can be a result of nutrient deficiency due to low nitrate concentrations (J E Rakocy et al., 2004) or due to low nutrient content in fish feed (Pinho et al., 2018).

Chapter 3: Materials and methods:

3.1 Experimental Set-up

The experiment was conducted in a greenhouse setting at the Water Energy Food (WEF) Nexus Lab by the Center for Applied Research on the Environment and Sustainability (CARES). WEF Nexus lab is located next to the Sciences and Engineering building in the American University in Cairo campus. The campus resides along South 90 road in New Cairo, Cairo, Egypt (GPS coordinates 30°01'7.08" N, 31°30'0.74" E). Our experimental set-up is based on protocols described in (Lennard & Leonard, 2006; Oladimeji et al., 2018; James E Rakocy et al., 2006; Resh, 2016; Sikawa & Yakupitiyage, 2010). A pictorial model of the two systems is presented in **Figure 1** and **Figure 2**. The figure was originally drawn by Hisham Elessawy in master's thesis "*Aquaponics as a sustainable alternative to new land reclamation and conventional agriculture in Egypt"* using SketchUp 3D software (El Essawy, 2018). The figures were edited to adjust to the current settings of the experiment.

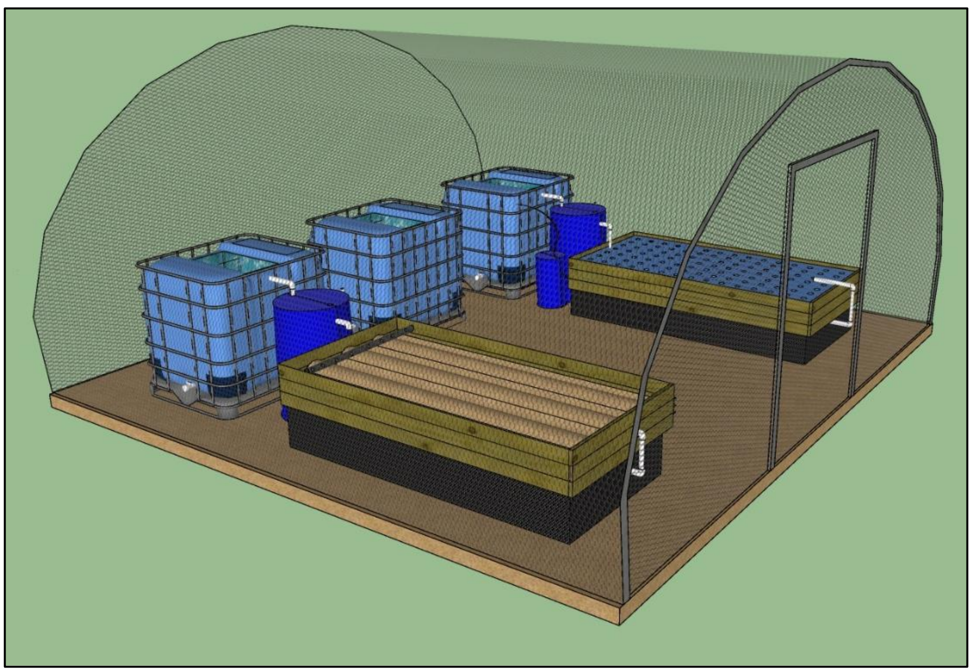


Figure 1: Aquaponics Experimental Design - Front View

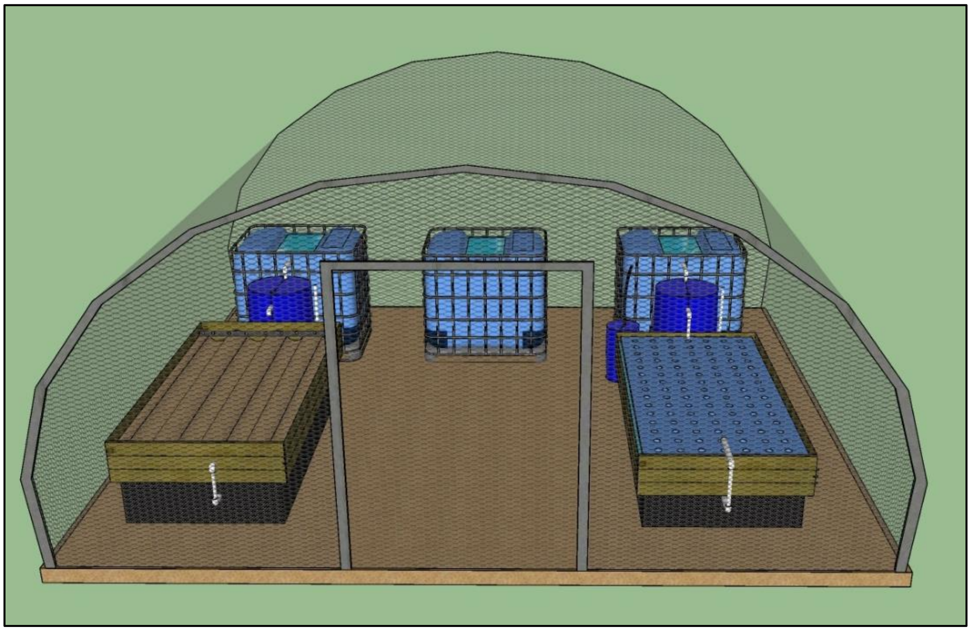


Figure 2: Aquaponics Experimental Design – Side View

The greenhouse is covered with shade cloth with 65% shading density in hot weathers (March to September). In cold weathers, (October to February), High Density Polyethylene (HDPE) treated for ultraviolet light (UV-treated) of thickness 200 microns is used to cover the greenhouse. The fish tanks are made of PVC plastic and placed inside stain-less steel cage to avoid plastic deformation due to water weight. The mechanical and biological filters are also made of PVC plastic. The plant and collection beds were made of yellow pine wood and covered by double waterproof layer of High Density Polyethylene (HDPE) (UV-treated), thickness of 200 microns per each layer.

The water cycle starts from the fish tank into the mechanical filter, then to the biological filter (available only in the DWC system) to the plant bed and ends by the collection tank using gravity. The pumps are placed in the collection tanks to pump the water back to the fish tanks after purified through nutrient and nitrates absorption in the plant beds. The main difference in water flow between DWC and Sand-Bed is the water pumping interval. In both systems, the submersible pump is placed in the collection bed. In the DWC system, the water is continuously pumped through the cycle as all beds are filled with water at a rate of 1750 liters/hr. In the Sand-Bed system, the water pump is linked to a timer that allows water to be pumped for 30 minutes every two hours at a rate of 1750 liters/hr. A top view sketch of both systems' components and water flow direction is presented in **Figure 3**.

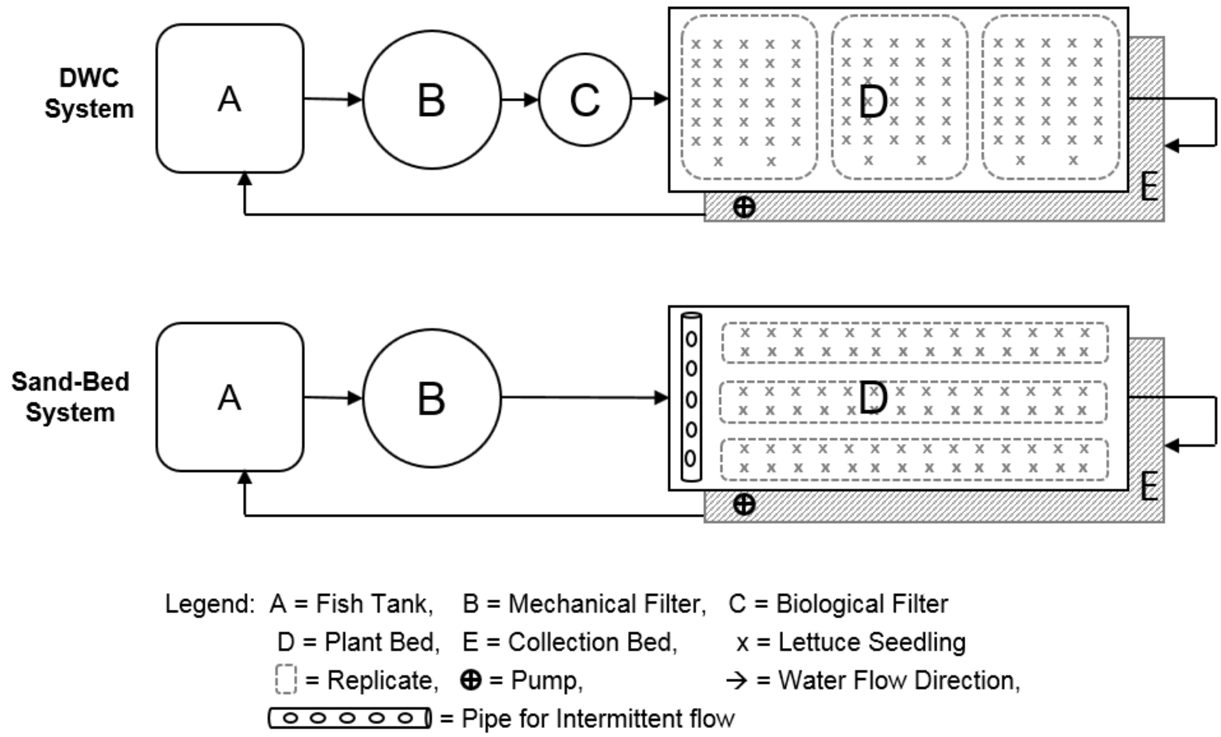


Figure 3: Deep Water Culture (DWC) system and Sand-Bed systems sketch

**Legend: The sketch shows top view of fish tanks, mechanical filters, plant beds, collections beds and direction of water flow; where water is drained via gravity and returns using a submersible pump. In Sand-Bed system, water flow is intermittent and distributed through pipe. In DWC system, water flow is continuous. Each system contains three replicates.*

In DWC plant bed, six styrofoam sheets (thickness 3 cm each) were used to hold the seedlings. Each styrofoam had an opening for planting pots (placed at 10 cm spacings). Seedlings are placed inside planting pots (5-cm diameter) with few coco peat added at the bottom of each pot. In the Sand-Bed plant bed, seedlings are planted directly to the sand-media. Sand media size distribution and texture, chemical, macro- and micro-nutrient and heavy metals laboratory analysis results are presented in **Appendix II: Sand Media Analysis Results.** An eight-inch diameter pipe with several holes was placed along the bottom of the Sand-Bed plant bed. The holed pipe was covered by mesh to trap sands from draining into collection tank. The water is filtered into the collection tank accordingly. The DWC biological filter water was placed manually on weekly basis to the plant bed. The Sand-Bed mechanical filter and DWC biological filter were not part of the initial system's implementation used in (El Essawy, 2018). Aeration was provided using air pumps of 120 liters/min to each fish tank. In the DWC plant bed only, the water has to be aerated similar to hydroponics procedures. An air pump of 80 liters/min aerated the DWC plant bed. The water physical parameters DO, pH and temperature were measured using submerged Nilebot probe sensors for each fish tank. In the DWC plant-bed, pH and temperature were only measured. **Table 1** shows the aquaponics systems dimensions and experimental set-up.

A pilot cycle was conducted over 60 days to asses systems' performance, experiment different crops and identify best practices for managing both systems. The details and results of pilot cycle are available in **Appendix I: Pilot Cycle**.

Table 1: DWC and Sand-Bed Systems Dimensions and Experimental Set-up

System	Item	No.	Description	Density / Dimensions
Greenhouse	Area	1	Greenhouse	L 6.5 m x W 3.4 m x H 2.8 m
	Sensors	2	Humidity Sensor	Operating Temp (0 - 60°C) Operating Relative Humidity (30 - 90%)
		3	Ventilator Air Volume	1000 m^3/hr
		4	Water Sensors	Dissolved Oxygen, pH, Temp
DWC	Tanks Sizes	5	Fish tank	1000 Liters capacity
		6	Mechanical filter tank	200 Liters capacity
		7	Biological filter tank	5 Liters capacity
	Bed sizes	8	Plants bed	L 4.2 m x W 1.2 m x H 0.35 m
		9	Collection bed	L 3.8 m x W 1 m x H 0.4 m
	Biological Filter	10	Bio-ball	2 kg in 5L tank (density 400 kg/m^3)
	Pumps	11	Hydraulic pump rate	1750 liter/hr (25 W)
		12	Air Pump	120 liter/min (90 W)
		13	Air Pump for plant bed	80 liter/min (60 W)
		14	Automatic Fish Feeder	Feeding times: 4 times/day Feed Capacity: 50 g Fish Feed
	Planting Sheets	15	Styrofoam floating sheets	Six styrofoam sheets (each 3 cm thickness)
	Planting pots	16	Pots containing coco peat for holding seedlings	Diameter 5 cm
	Fish Density	17	150 Nile Tilapia fingerlings	2.5 kg/m^3
	Plant Density	18	96 Lettuce seedlings	20 plants/m^2 (32 plants per replicate)
Sand-Bed	Tank Sizes	19	Fish tank	1000 Liters capacity
		20	Mechanical filter tank	200 Liters capacity
	Bed sizes	21	Plants bed	L 4.2 m x W 1.2 m x H 0.35 m
		22	Collection bed	L 3.8 m x W 1 m x H 0.4 m
	Water Drainage	23	Holed pipe covered with mesh	L 4.2 m (Diameter 8 inch)
	Pumps	24	Hydraulic pump rate	1750 liter/hr (25 W)
		25	Air Pump	120 liter/min (90 W)
		26	Automatic Fish Feeder	Feeding times: 4 times/day Feed Capacity: 50 g Fish Feed
	Fish Density	27	150 Nile Tilapia fingerlings	2.5 kg/m^3
	Plant Density	28	96 Lettuce seedlings	20 plants/m^2 (32 plants per replicate)

**Legend: L= length in meters (m), W= width in meters (m), H= height in meters (m), hr=hour, min=minute, W=Watts, g=grams, kg/m^3=kilograms per cubic meters, plants/m^2= plants per squared meters.*

3.2 Fish and Plants selection

According to literature review and pilot cycle results, Nile Tilapia (*Oreochromis niloticus*) and lettuce (*Lactuca sativa variety capitata* Type Batavia *)* were selected to be grown in the experiment. The market name for lettuce is “Batavia” lettuce known for its crispy various and small leaves. Lettuce were selected due to their commercial value, endurance and fast-growing cycle around the year. The Batavia lettuce seeds were bought from Rijk Zwaan company and sent to local seedlings nursery in Alexandria Road. The Batavia Lettuce seeds specifications are High Resistance (HR): Bl:16-27,30-32EU/Nr:0/Pb and Intermediate Resistance (IR): LMV:1. The Batavia seedlings were 35 days old and mature for planting in both systems on April 20th, 2018. The recommended spacing between each consecutive lettuce is 20 cm leading to plant density of 20 lettuce/m^2 per each system (DWC and Sand-Bed). The total seedlings planted were 96 per each system (32 lettuce per replicate). The lettuce was planted on April 20th, 2018 (**Figure 4**) and harvested on May 25th, 2018 for a total growth period of 35 days (**Figure 5**).

Figure 4: Sand-Bed and DWC Systems on April 20th, 2018 (Planting Day)

The Nile Tilapia fingerlings were sourced from local marketplace in Kafr El Sheikh, Egypt. The fingerling’s size ranged from 5 to 15 grams. The fish stocking density per each system was 150 fingerlings/m^3; each fish tank contained 2.5 kg of initial fish biomass by the start of the experiment. The fish was loaded into the tanks more than three months before the start of the experiment on January 5th 2018 to accommodate it to the new environment and conduct the pilot cycle. 25% of the fish biomass were weighed at initial and end of the pilot cycle as commonly measured in literature (Somerville et al., 2014).

The fish was fed commercial feed made of 25% protein six days per week during the 35-days period using manual and automatic feeder. The automatic feeder can only provide 50 g of fish feed during the day. The rest of daily feed portion was provided to each fish tank manually in the morning. The automatic feeder was adjusted to accordingly distribute the rest of the feed around the day up till 5.00 pm. The amount of feed to each system was 2% the estimated fish biomass during the study-period. Each fingerling mass was assumed to increase at a daily rate of 0.5 g; and the amount of feed was estimated accordingly (Medina et al., 2016). Incremental amount of feed given to fish during the study period was recorded. The FCR was calculated accordingly at end of study period.

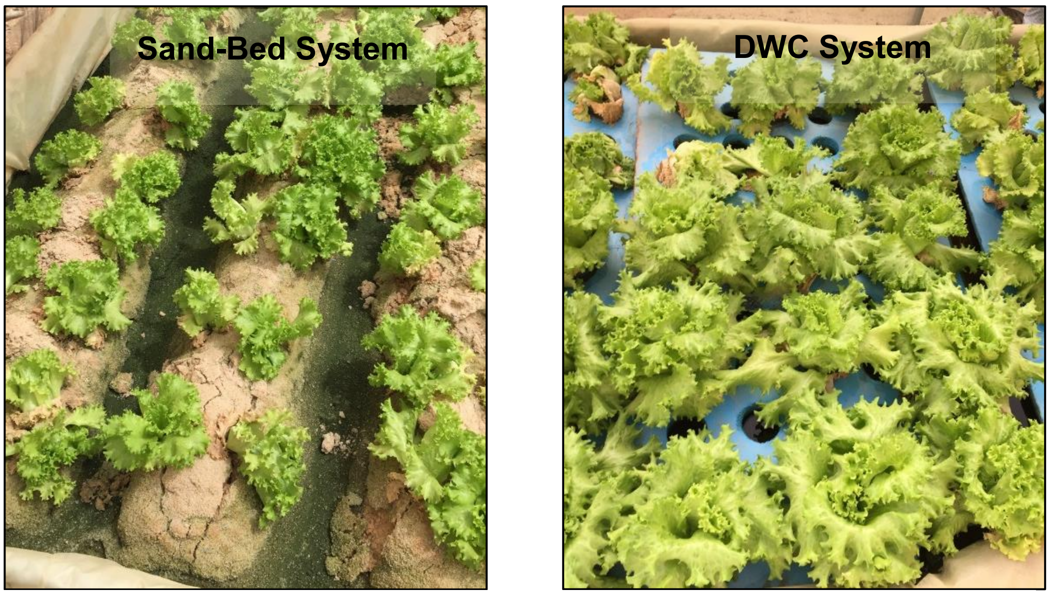


Figure 5: Lettuce grown on Sand-Bed and DWC Systems on May 25th, 2018 (Harvesting Day)

3.3 Data Analysis

3.3.1 Water Quality

Water physicochemical properties DO, pH and temperature were monitored daily using the submerged sensors. Data are sent every 15 minutes on an online platform (Nilebot). DWC and Sand-Bed water samples were collected from fish tanks and collection beds every week and sent for laboratory analysis, at the Soils Water and Environment Research Institute (SWERI) operated by the Ministry of Agriculture and Land Reclamation in Egypt. The samples were analyzed for pH, total dissolved solids (TDS), sodium absorption rate (SAR), electric conductivity (EC), and ion concentrations of ammonium (NH_4^+), nitrate (NO_3^-), sodium (Na^{2+}), potassium (K^+), phosphorous (P), iron (Fe^{2+}), calcium (Ca^{2+}), magnesium (Mg^{2+}), chloride (Cl^-), Zinc (Zn^{2+}), sulphate (SO_4^{2-}), and carbonate (HCO_3^-) using APHA standard methods for the examination of water and wastewater (APHA, 1981).

3.3.2 Water Use Variables

Water level (height) using 1-m ruler was recorded in fish tanks, mechanical filters, biological filter, plant bed and collection beds in DWC and Sand-Bed systems at the beginning of the experiment. The water level was then observed and recorded on weekly basis on all prementioned tanks, filters and beds. The water volume was calculated accordingly by keeping area of tanks, beds, filters constant. Two variables were defined for water use in this study: total water use and total water consumption. The total water use is defined as the water volume used at any selected day in the study period (35 days). Total water consumption is the amount of water lost from total water use due to evaporation and evapotranspiration. 50 liters of tap water were added to replenish water losses in each system; this is a common technique applied in different research (Hu et al. 2015). A total of 250 liters were added to each system over 35 days. Hu et al. 2015 reported that additional 1400 L of fresh water were needed to run a RAS for 139 days 5% daily water exchange (i.e. 70 L per week) which is close to the weekly additional water used in this experiment. We assumed that the weekly water replacement is equal in both systems; and therefore, we neglected the additional value in water use and daily water consumption estimations.

3.3.3 Lettuce Yield

DWC and Sand-Bed systems each consisted of three replicates. A representative sample (n=6) was randomly selected from each replicate (32 lettuce) (Goddek & Vermeulen, 2018). The lettuce leaf length, width, plant/lettuce height and roots length were measured using a ruler. Number of fresh and dry leaves were also counted. The roots were then removed, and the lettuce fresh weight was measured using a scaled balance. The final yield of lettuce was calculated using the mean value of replicates multiplied by total number of lettuce heads per replicate (Sikawa & Yakupitiyage, 2010). The final yield was expressed as kg/m^2 of lettuce where lettuce density in each system were 20 $plants/m^2$.

3.3.4 Lettuce Nutrient Analysis

Samples per each replicate were then sent for lettuce leaf nutrient analysis at the Regional Center for Food & Feed (RCFF) laboratory accredited according to ISO/IEC 17025 from A2LA. The lettuce samples were analyzed for nutrient concentration of calcium, phosphorous, potassium, iron, manganese, zinc, copper, sodium, magnesium, boron and nitrite using AOAC method no. 985.01 (AOAC, 2012). Other parameters were analyzed including percentage content of 1) vitamin B. carotene using method HPLC no. AF 255.1 (Vita, 1997), 2) protein using AOAC Kjeldahl method no. 984.13 (AOAC, 2016), 3) lipid using AOCS official procedure AM 5-04 (AOAC, 2005), 4) crude fiber using AOCS approved procedure Ba 6a-06 (AOAC, 2005), 5) ash, primary, secondary and total moisture using Animal feed official method of analysis 4.1.06 (AOAC, 2012).

3.3.5 Lettuce Plant Quality Index

To further evaluate lettuce quality, we developed a plant quality index (PQI) based on visual characteristics. Additionally, a plant quality index (PQI) was evaluated by grades based upon visual aspects of leaves. The PQI was based on a numerical visual index from 1 to 10. (1) represents yellow leaves and 100%

imperfections in leaves' surface in a lettuce sample; (10) represents very dark green leaves with less than 5% imperfections in leaves' surface. On weekly basis, twelve samples from each replicate were photographed from equal distance using same camera lens 8-megapixel. An example of the qualitative analysis and PQI numerical index are presented in **Appendix III: Lettuce Plant Quality Index**. All images taken for lettuce leaves over weeks 1 till 5 were analyzed at the end of the experiment to avoid biased analysis from evaluator.

3.3.6 Media Bacterial Analysis

Sand samples and bio-ball samples from Sand-Bed and DWC system respectively were collected at the beginning and end of the study period. The samples were analyzed at the SWERI bacteriological lab for nitrifying bacteria (AOB and NOB) content along with to salmonella and shigella bacteria, total and fecal coliform bacteria using DIFCO Manual: Dehydrated Culture Media and Reagents for Microbiology.(DIFCO, 1985).

3.4 Statistical Analysis

All statistical analysis was conducted using SPSS software version 21.0 (IBM, USA). We evaluated all data collected using one-way analysis of variance (ANOVA). Data showed significant differences at level of significance $p<0.05$. We carried out different comparisons within data collected. Within each system (DWC and Sand-Bed), water quality parameters were compared within fish and collection tanks. Further, we compared DWC and Sand-Bed systems water quality parameters. For lettuce growth parameters, three replicates were collected and statistically analyzed within each system. The differences in lettuce growth of the systems were then evaluated. For lettuce quality parameters, each replicate is representative of mean values statistically tested at the laboratory at the Regional Center for Food & Feed (RCFF) laboratory accredited according to ISO/IEC 17025 from A2LA.

Chapter 4: Results

4.1 Water Quality

4.1.1 Chemical, Macro and Micro-Nutrient Analysis

Water quality parameters were measured in fish tanks and collection tanks of both systems DWC and Sand-Bed over the experimental period of 35 days. All measurements were taken every five days. Temperature and Dissolved Oxygen (DO) was monitored daily using NileBot sensors in DWC and Sand-Bed fish tanks. The below tables represent the mean value, standard deviation (SD) , minimum (min) and maximum (max) values over the 35 days duration for all fish tanks. For analysis, four comparisons were made to identify similarities and changes between both systems and within each system: a) DWC and Sand-Bed fish tanks comparison **Table 2** and **Table 3** b) DWC and Sand-Bed collection tanks comparison **Table 4** and **Table 5** c) DWC fish and collection tanks comparison **Table 6** and **Table 7** d) Sand-Bed fish and collection tanks comparison **Table 8** and **Table 9**. Each comparison consists of two tables: 1) comprising of water chemical analysis **tables (2, 4, 6** and **8)** and 2) including water macro and micro nutrient analysis **tables (3,5, 7** and **9)**. All analysis was done using SPSS descriptive statistics and one-way ANOVA for comparing means and testing similarity. Differences between means were considered significant at p value lower than 0.05.

a DWC and Sand-Bed Fish Tanks Comparison

Table 2: Water Chemical Parameters for DWC and Sand-Bed Fish Tanks

		DWC Fish Tank				SAND-BED Fish Tank				
*	Unit	Mean	SD	Min	Max	Mean	SD	Min	Max	Value of p*
Do	mg/L	5.98	0.76	4.99	7.47	4.92	1.32	1.42	7.64	0.00 [a]
Temp	°C	22.07	1.67	18.40	24.4	21.31	1.97	17.4	24.9	0.00 [a]
EC	dS/m	0.67	0.13	0.44	0.79	0.99	0.15	0.82	1.20	0.00 [a]
TDS	ppm	427.67	81.36	279.00	507.00	635.00	101.72	522.00	772.00	0.00 [a]
pH		7.32	0.23	7.00	7.60	7.32	0.33	6.90	7.60	1.00
HCO_3^-	meq./L	1.05	0.21	0.66	1.23	1.57	0.36	1.13	2.17	0.01 [a]
SO_4^{2-}	meq./L	3.89	0.91	2.43	4.82	5.85	1.21	4.40	7.12	0.01 [a]
SAR		1.54	0.38	0.94	1.90	1.62	0.21	1.39	1.94	0.63
NH_4^+	mg/L	1.12	0.71	0.56	2.10	1.70	1.12	0.28	2.80	0.39
NO_3^-	mg/L	8.89	2.75	6.23	12.04	10.63	4.12	7.70	18.90	0.41

**Legend: DO=Dissolved Oxygen, Temp=Temperature, EC=Electric Conductivity, TDS=Total Dissolved Solids, HCO_3^- =Bicarbonate, SO_4^{2-}=Sulfate, SAR=Sodium Absorption Rate, NH_4^+=Ammonia, NO_3^-=Nitrate. All data are analyzed using SPSS one-way ANOVA where mean is the mean value, SD is the standard deviation, min is the minimum value, max is maximum value. Mean values of DWC and Sand-Bed fish tanks are significantly different when value of p < 0.05 noted using superscripts a.*

Water chemical parameters of the water samples in DWC and Sand-Bed fish tanks respectively over 35 days are presented in **Table 2**. Do and temperature differed in both the DWC and Sand-Bed fish tanks ($p<0.05$) with mean values of 5.98 mg/L and 22.07°C in DWC and 4.92 mg/L and 21.31°C in Sand-Bed. DWC and Sand-Bed fish tanks showed similar pH values of 7.32. For the ammonia and nitrate concentrations, the fish tanks in both systems did not differ significantly. NH_4^+ and NO_3^- concentrations were 1.12 and 8.89 mg/L for DWC and 1.7 and 10.63 mg/L for Sand-Bed fish tanks, respectively. The EC and TDS varied significantly ($p<0.05$) with mean values of 0.67 dS/m and 427.67 ppm for DWC and higher mean values of 0.99 dS/m and 635 ppm for Sand-Bed fish tanks. The HCO_3^- and SO_4^{2-} measurements also varied ($p<0.05$) with mean values of 1.04 and 3.89 meq./L for DWC and higher mean values of 1.57 and 5.85 meq./L for Sand-Bed fish tanks respectively. The SAR is similar of 1.54 and 1.62 in DWC and Sand-Bed fish tanks.

Table 3: Water Macro and Micro-Nutrients Parameters for DWC and Sand-Bed Fish Tanks

		DWC Fish Tank				Sand-Bed Fish Tank				
*	Unit	Mean	SD	Min	Max	Mean	SD	Min	Max	Value of p*
Na^+	meq./L	2.36	0.75	1.17	3.14	3.00	0.41	2.37	3.42	0.10
K^+	meq./L	0.23	0.09	0.14	0.33	0.47	0.24	0.21	0.75	0.04 [a]
P	mg/L	15.83	3.32	11.94	21.39	11.20	5.51	5.78	17.68	0.11
Fe	mg/L	0.20	0.25	0.03	0.69	0.76	0.85	0.13	2.11	0.15
Ca^{++}	meq./L	2.13	0.83	1.32	3.68	3.25	0.99	1.58	4.47	0.06
Mg^{++}	meq./L	2.46	0.87	1.52	3.92	3.64	0.87	2.56	4.72	0.04 [a]
Si	mg/L	1.09	0.71	0.50	2.06	1.14	0.60	0.63	2.00	0.89
Cl^-	meq./L	2.23	0.54	1.36	2.71	2.92	0.44	2.54	3.73	0.04 [a]

**Legend: Na^+=Sodium ion, K^+=Potassium ion, P=Phosphorous, Fe=Iron, Ca^{++}=Calcium ion, Mg^{++}=Magnesium ion, Si=Silicon, Cl^-=chlorine ion. All data are analyzed using SPSS one-way ANOVA where mean is the mean value, SD is the standard deviation, min is the minimum value, max is maximum value. Mean values of DWC and Sand-Bed fish tanks are significantly different when value of $p < 0.05$ noted using superscripts a.*

Water macro and micro-nutrient parameters of the water samples in DWC and Sand-Bed fish tanks respectively over 35 days are presented in **Table 3**. The concentrations of Na^+, P, Fe, Ca^{++}, and Si are within similar mean values for 2.36 meq./L, 15.83 mg/L, 0.2, 2.13 meq./ and 1.09 meq./ for DWC and 3 meq./, 11.2. 0.76. 3.25 meq./, and 1.14 meq./ Sand-Bed fish tanks, respectively. K^+, Mg^{++} and Cl^- concentrations varied with mean values of 0.23, 2.46 and 2.23 meq./L for DWC fish tank; whereas the concentrations are slightly higher with mean values of 0.47, 3.64 and 2.92 meq./L for Sand-Bed fish tank, respectively.

b DWC and Sand-Bed Collection Tanks Comparison

Table 4: Water Chemical Parameters for DWC and Sand-Bed Collection Tanks

		DWC Collection Tank				Sand-Bed Collection Tank				
*	Unit	Mean	SD	Min	Max	Mean	SD	Min	Max	Value of p*
EC	dS/m	0.74	0.14	0.55	0.97	1.02	0.17	0.72	1.20	0.01 [a]
TDS	ppm	474.17	85.82	353.00	618.00	655.83	111.52	463.00	772.00	0.01 [a]
PH		7.40	0.18	7.10	7.60	7.25	0.21	7.00	7.50	0.21
HCO_3^-	meq./L	1.25	0.15	1.13	1.51	1.69	0.45	1.23	2.45	0.05
SO_4^{2-}	meq./L	4.15	1.03	2.69	5.61	5.83	1.32	3.29	7.11	0.03 [a]
SAR		1.60	0.23	1.21	1.85	1.56	0.17	1.34	1.80	0.69
NH_4^+	mg/L	1.37	1.07	0.14	2.80	1.53	0.75	0.49	2.80	0.77
NO_3^-	mg/L	10.28	1.56	8.47	12.81	9.36	2.63	4.69	12.46	0.48

**Legend: EC=Electric Conductivity, TDS=Total Dissolved Solids, HCO_3^-=Bicarbonate, SO_4^{2-}=Sulfate, SAR=Sodium Absorption Rate, NH_4^+=Ammonia, NO_3^-=Nitrate. All data are analyzed using SPSS one-way ANOVA where mean is the mean value, SD is the standard deviation, min is the minimum value, max is maximum value. Mean values of DWC and Sand-Bed collection tanks are significantly different when value of $p < 0.05$ noted using superscripts a.*

Water chemical parameters for DWC and Sand-Bed Collection tanks are presented in **Table 4**. EC and TDS showed differences in DWC and Sand-Bed collection tanks of mean values of 0.74 dS/m and 474.17 ppm in DWC and slightly larger mean values of 1.02 dS/m and 655.83 ppm in Sand-Bed. The pH is consistent of 7.4 and 7.0 mean values in the DWC and Sand-Bed collection tanks. The concentrations HCO_3^-, NH_4^+, NO_3^- and SAR did not show significance differences between both systems' collections tanks. The HCO_3^- mean values are 1.25 and 1.69 meq./L for DWC and Sand-Bed collection tanks. The SAR mean values are 1.6 and 1.56 in the DWC and Sand-Bed collection tanks. The NH_4^+ concentration was 1.37 and 1.53 mg/L in DWC and Sand-Bed collection tanks respectively and NO_3^- concentration was 10.28 and 9.36 mg/L in the DWC and Sand-Bed collection tanks respectively. Only SO_4^{2-} mean concentrations varied with mean values of 4.15 meq./L and 5.83 meq./L for DWC and Sand-Bed collection tanks, respectively.

Table 5: Water Macro and Micro-Nutrients Parameters for DWC and Sand-Bed Collection Tanks

		DWC Collection Tank				Sand-Bed Collection Tank				
*	Unit	Mean	SD	Min	Max	Mean	SD	Min	Max	Value of p*
Na^+	meq./L	2.56	0.51	1.66	2.93	2.91	0.49	2.02	3.28	0.25
K^+	meq./L	0.27	0.09	0.14	0.40	0.45	0.16	0.21	0.66	0.04 [a]
P	mg/L	14.81	5.83	4.37	21.73	11.04	3.38	7.17	16.67	0.20
Fe	mg/L	0.48	0.64	0.02	1.60	1.92	2.98	0.10	7.10	0.27
Ca^{++}	meq./L	2.35	0.88	1.32	3.95	3.46	0.69	2.63	4.47	0.04 [a]
Mg^{++}	meq./L	2.71	0.79	1.44	3.68	3.54	1.15	1.89	4.94	0.18
Si	mg/L	1.03	0.62	0.60	2.15	1.51	0.56	0.77	2.05	0.19
Cl^-	meq./L	2.48	0.48	1.69	3.05	2.85	0.38	2.37	3.39	0.17

**Legend: Na^+=Sodium ion, K^+=Potassium ion, P=Phosphorous, Fe=Iron, Ca^{++}=Calcium ion, Mg^{++}=Magnesium ion, Si=Silicon, Cl^-=chlorine ion. All data are analyzed using SPSS one-way ANOVA where mean is the mean value, SD is the standard deviation, min is the minimum value, max is maximum value. Mean values of DWC and Sand-Bed collection tanks are significantly different when value of $p < 0.05$ noted using superscripts a.*

Water macro and micro-nutrient parameters of the water samples in DWC and Sand-Bed collection tanks respectively over 35 days are presented in **Table 5**. In the collection tanks of DWC and Sand-Bed, all macro and micro-nutrients showed no significance except for K^+ and Ca^{++}. The K^+ and Ca^{++} mean concentration is 0.27 and 2.35 meq./L in the DWC collection tank whereas slightly higher of 0.45 and 3.46 meq./L in Sand-Bed collection tank. The mean concentrations of Na+, P, Fe, $Mg^{++,}$ Si and Cl^- were 2.56 meq./L, 14.81 mg/L, 0.48 mg/L, 2.71 meq./L, 1.03 mg/L and 2.48 meq./L in the DWC collection tank and 2.91 meq./L, 11.04 mg/L, 1.92 mg/L, 3.54 meq./L, 1.51 mg/L and 2.85 meq./L in the Sand-Bed collection tank.

c DWC Fish and Collection Tanks Comparison

Table 6: Water Chemical Parameters for DWC Fish and Collection Tanks

		DWC Fish Tank				DWC Collection Tank					DWC
*	Unit	Mean	SD	Min	Max	Mean	SD	Min	Max	Value of p*	Total Mean
EC	dS/m	0.67	0.13	0.44	0.79	0.74	0.14	0.55	0.97	0.35	0.71
TDS	ppm	427.67	81.36	279.00	507.00	474.17	85.82	353.00	618.00	0.36	450.92
pH		7.32	0.23	7.00	7.60	7.40	0.18	7.10	7.60	0.50	7.36
HCO_3^-	meq./L	1.05	0.21	0.66	1.23	1.25	0.15	1.13	1.51	0.09	1.15
SO_4^{2-}	meq./L	3.89	0.91	2.43	4.82	4.15	1.03	2.69	5.61	0.65	4.02
SAR		1.54	0.38	0.94	1.90	1.60	0.23	1.21	1.85	0.71	1.57
NH_4^+	mg/L	1.12	0.71	0.56	2.10	1.37	1.07	0.14	2.80	0.70	1.27
NO_3^-	mg/L	8.89	2.75	6.23	12.04	10.28	1.56	8.47	12.81	0.31	9.59

**Legend: EC=Electric Conductivity, TDS=Total Dissolved Solids, HCO_3^-=Bicarbonate, SO_4^{2-}=Sulfate, SAR=Sodium Absorption Rate, NH_4^+=Ammonia, NO_3^-=Nitrate. All data are analyzed using SPSS one-way ANOVA where mean is the mean value, SD is the standard deviation, min is the minimum value, max is maximum value. Mean values of DWC fish and collection tanks are significantly different when value of $p < 0.05$ noted using superscripts a.*

Water chemical parameters of the water samples in DWC fish and collection tanks respectively over 35 days are presented in **Table 6**. The statistical analysis between the DWC fish and collection tanks showed no significant difference (p>0.05). The total mean values for the Sand-Bed system water chemical parameters are represented in the last column of **Table 6.**

Table 7: Water Macro and Micro-Nutrients Parameters for DWC Fish and Collection Tanks

		DWC Fish Tank				DWC Collection Tank					DWC
*	Unit	Mean	SD	Min	Max	Mean	SD	Min	Max	Value of p*	Total Mean
Na^+	meq./L	2.36	0.75	1.17	3.14	2.56	0.51	1.66	2.93	0.60	2.46
K^+	meq./L	0.23	0.09	0.14	0.33	0.27	0.09	0.14	0.40	0.41	0.25
P	mg/L	15.83	3.32	11.94	21.39	14.81	5.83	4.37	21.73	0.72	15.32
Fe	mg/L	0.20	0.25	0.03	0.69	0.48	0.64	0.02	1.60	0.34	0.34
Ca^{++}	meq./L	2.13	0.83	1.32	3.68	2.35	0.88	1.32	3.95	0.67	2.24
Mg^{++}	meq./L	2.46	0.87	1.52	3.92	2.71	0.79	1.44	3.68	0.60	2.59
Si	mg/L	1.09	0.71	0.50	2.06	1.03	0.62	0.60	2.15	0.89	1.06
Cl^-	meq./L	2.23	0.54	1.36	2.71	2.48	0.48	1.69	3.05	0.41	2.36

**Legend: Na^+=Sodium ion, K^+=Potassium ion, P=Phosphorous, Fe=Iron, Ca^{++}=Calcium ion, Mg^{++}=Magnesium ion, Si=Silicon, Cl^-=chlorine ion. All data are analyzed using SPSS one-way ANOVA where mean is the mean value, SD is the standard deviation, min is the minimum value, max is maximum value. Mean values of DWC fish and collection tanks are significantly different when value of $p < 0.05$.*

Water macro and micro-nutrient parameters of the water samples in DWC fish and collection tanks respectively over 35 days are presented in **Table 7.** Statistical analysis showed that the mean values are within similar range (p>0.05). The total DWC mean value of both tanks is represented in the last column of **Table 7**.

d Sand-Bed Fish and Collection Tanks Comparison

Table 8: Water Chemical Parameters for Sand-Bed Fish and Collection Tanks

		Sand-Bed Fish Tank				Sand-Bed Collection Tank					Sand-Bed
*	Unit	Mean	SD	Min	Max	Mean	SD	Min	Max	Value of p*	Total Mean
EC	dS/m	0.99	0.15	0.82	1.20	1.02	0.17	0.72	1.20	0.75	1.01
TDS	ppm	635.00	101.72	522.00	772.00	655.83	111.52	463.00	772.00	0.74	645.42
pH		7.32	0.33	6.90	7.60	7.25	0.21	7.00	7.50	0.68	7.28
HCO_3^-	meq./L	1.57	0.36	1.13	2.17	1.69	0.45	1.23	2.45	0.64	1.63
SO_4^{2-}	meq./L	5.85	1.21	4.40	7.12	5.83	1.32	3.29	7.11	0.97	5.84
SAR		1.62	0.21	1.39	1.94	1.56	0.17	1.34	1.80	0.56	1.59
NH_4^+	mg/L	1.70	1.12	0.28	2.80	1.53	0.75	0.49	2.80	0.76	1.62
NO_3^-	mg/L	10.63	4.12	7.70	18.90	9.36	2.63	4.69	12.46	0.54	9.99

**Legend: EC=Electric Conductivity, TDS=Total Dissolved Solids, HCO_3^-=Bicarbonate, SO_4^{2-}=Sulfate, SAR=Sodium Absorption Rate, NH_4^+=Ammonia, NO_3^-=Nitrate. All data are analyzed using SPSS one-way ANOVA where mean is the mean value, SD is the standard deviation, min is the minimum value, max is maximum value. Mean values of Sand-Bed fish and collection tanks are significantly different when value of $p < 0.05$.*

In the Sand-Bed system fish and collection, all chemical parameters for water analysis were similar ($p>0.05$) **(Table 8**). Based on the statistical analysis and similarity, the last column in the table represents the total mean for both tanks in Sand-Bed system (**Table 8**).

Table 9: Water Macro and Micro-Nutrient Parameters for Sand-Bed Fish and Collection Tanks

		Sand-Bed Fish Tank				Sand-Bed Collection Tank					Sand-Bed
*	Unit	Mean	SD	Min	Max	Mean	SD	Min	Max	Value of p*	Total Mean
Na^+	meq./L	3.00	0.41	2.37	3.42	2.91	0.49	2.02	3.28	0.74	2.95
K^+	meq./L	0.47	0.24	0.21	0.75	0.45	0.16	0.21	0.66	0.89	0.46
P	mg/l	11.20	5.51	5.78	17.68	11.04	3.38	7.17	16.67	0.95	11.12
Fe	mg/l	0.76	0.85	0.13	2.11	1.92	2.98	0.10	7.10	0.42	1.34
Ca^{++}	meq./L	3.25	0.99	1.58	4.47	3.46	0.69	2.63	4.47	0.67	3.35
Mg^{++}	meq./L	3.64	0.87	2.56	4.72	3.54	1.15	1.89	4.94	0.87	3.59
Si	mg/l	1.14	0.60	0.63	2.00	1.51	0.56	0.77	2.05	0.29	1.32
Cl^-	meq./L	2.92	0.44	2.54	3.73	2.85	0.38	2.37	3.39	0.77	2.89

**Legend: Na^+=Sodium ion, K^+=Potassium ion, P=Phosphorous, Fe=Iron, Ca^{++}=Calcium ion, Mg^{++}=Magnesium ion, Si=Silicon, Cl^-=chlorine ion. All data are analyzed using SPSS one-way ANOVA where mean is the mean value, SD is the standard deviation, min is the minimum value, max is maximum value. Mean values of Sand-Bed fish and collection tanks are significantly different when value of $p < 0.05$.*

Similarly, the nutrient analysis between the Sand-Bed fish and collection tanks showed no significant difference ($p>0.05$) (**Table 9**). Accordingly, the total mean values for the Sand-Bed system macro and micro-nutrient parameters represented in the last column of **Table 9**.

Figures 6, **7** and **8** show variation trends of physicochemical, macro and micro-nutrient parametrs for DWC and Sand-bed fish and collection tanks over 35 days. Samples concentrations were plotted versus days to show levels of increase, decrease or steadiness over time. Variation trends of pH and TDS over 35 days are presented in **Figure 6.** In both systems, pH values had a decreasing rate starting with 7.5 and ending with nearly 7.0. TDS was increasing in Sand-Bed system and nearly steady in DWC system. **Figure 7** shows the variation trends of NH_4^+, NO_3^-, K and P concentrations over 35 days in DWC and Sand-Bed systems. In both systems, NH_4^+ had an increasing rate up till day 27 and started to decrease till day 35 in both systems. For NO_3^- levels, both systems had an increasing rate up till day 20 then a decreasing rate was observed till end of study period. Both K and P concentrations were increasing over the study period. Variation in conentrations of Mg^{2+}, Fe^{2+}, Ca^{2+} and Na^2 over study period are presented in **Figure 8.** Only Fe concentration was observed increasing in both systems by day 27 till day 35. In both systems, Mg^{2+}, Ca^{2+} and Na^2 were nearly steady over study period.

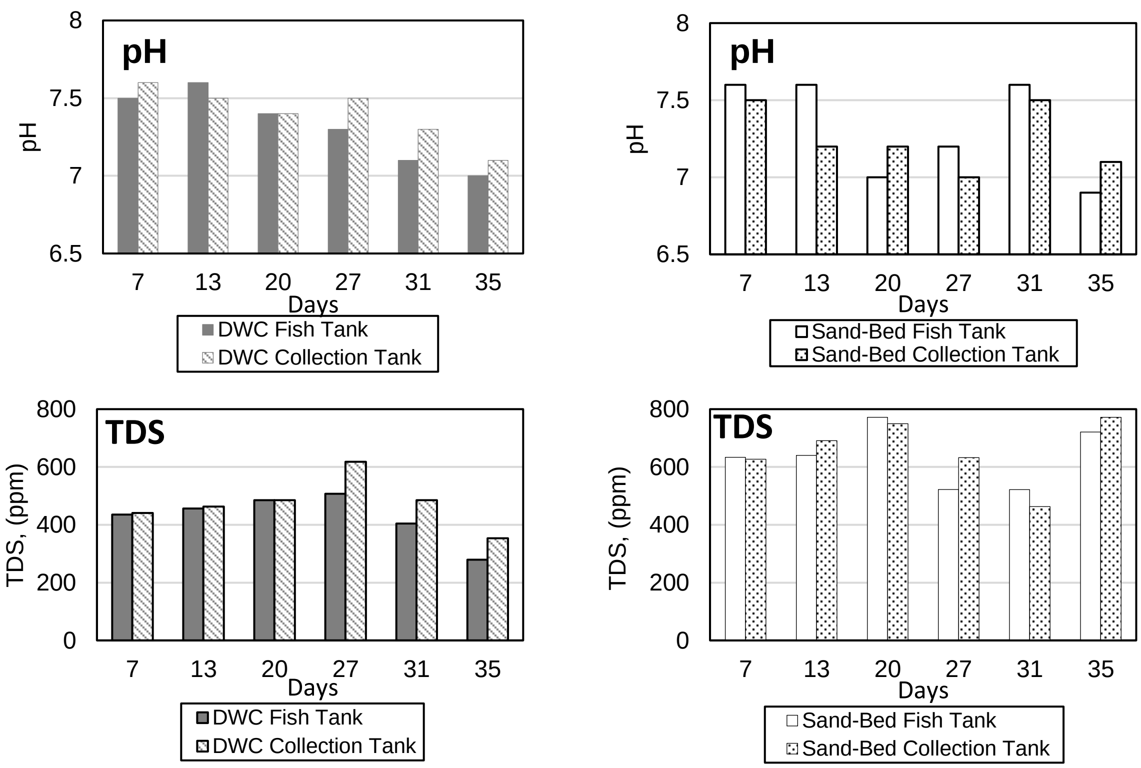


Figure 6: Variation Trends of pH, TDS over time during study period

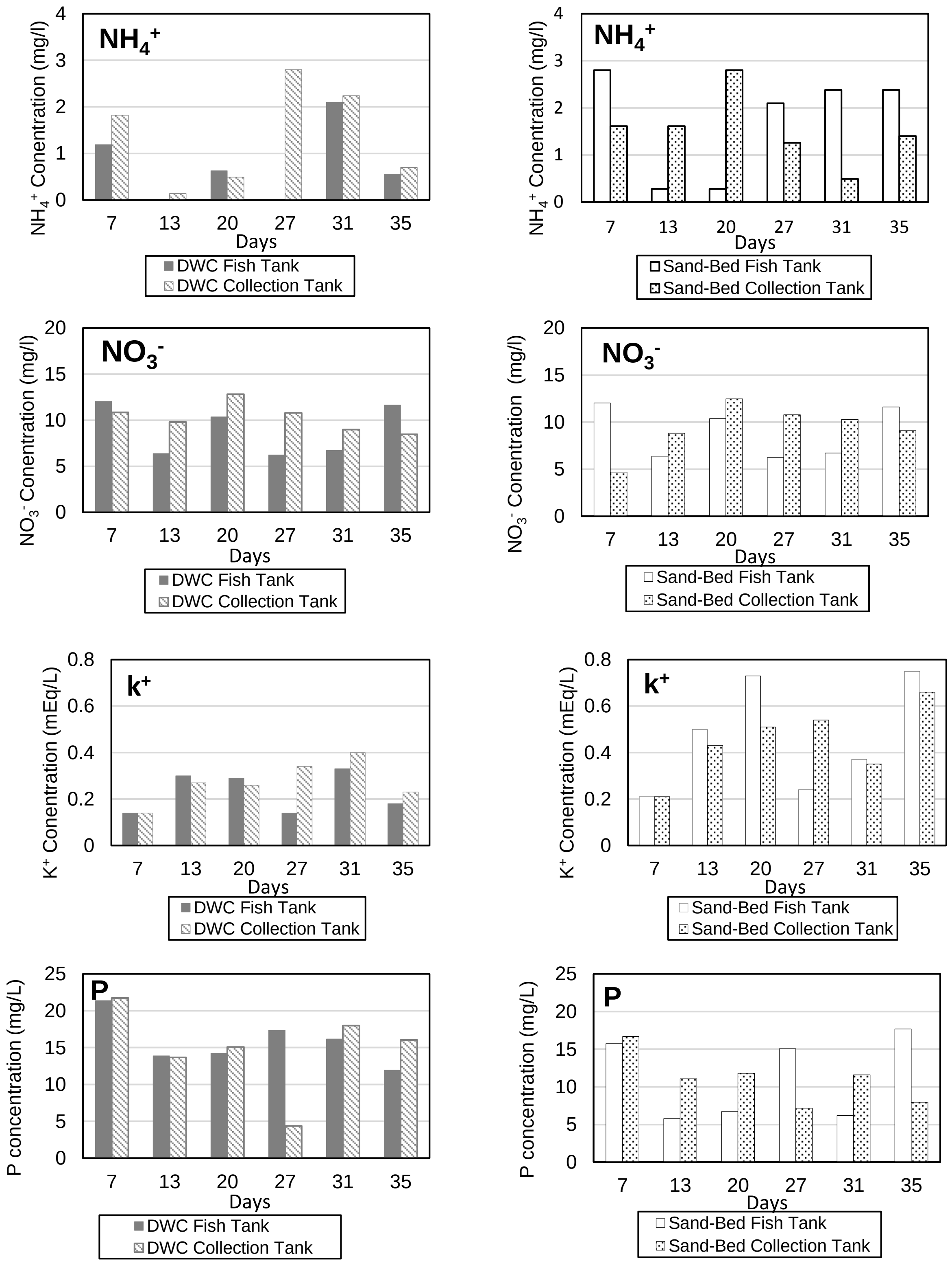


Figure 7: Variation in Concentrations of NH_4^+, NO_3^-, K, and P over time during study period

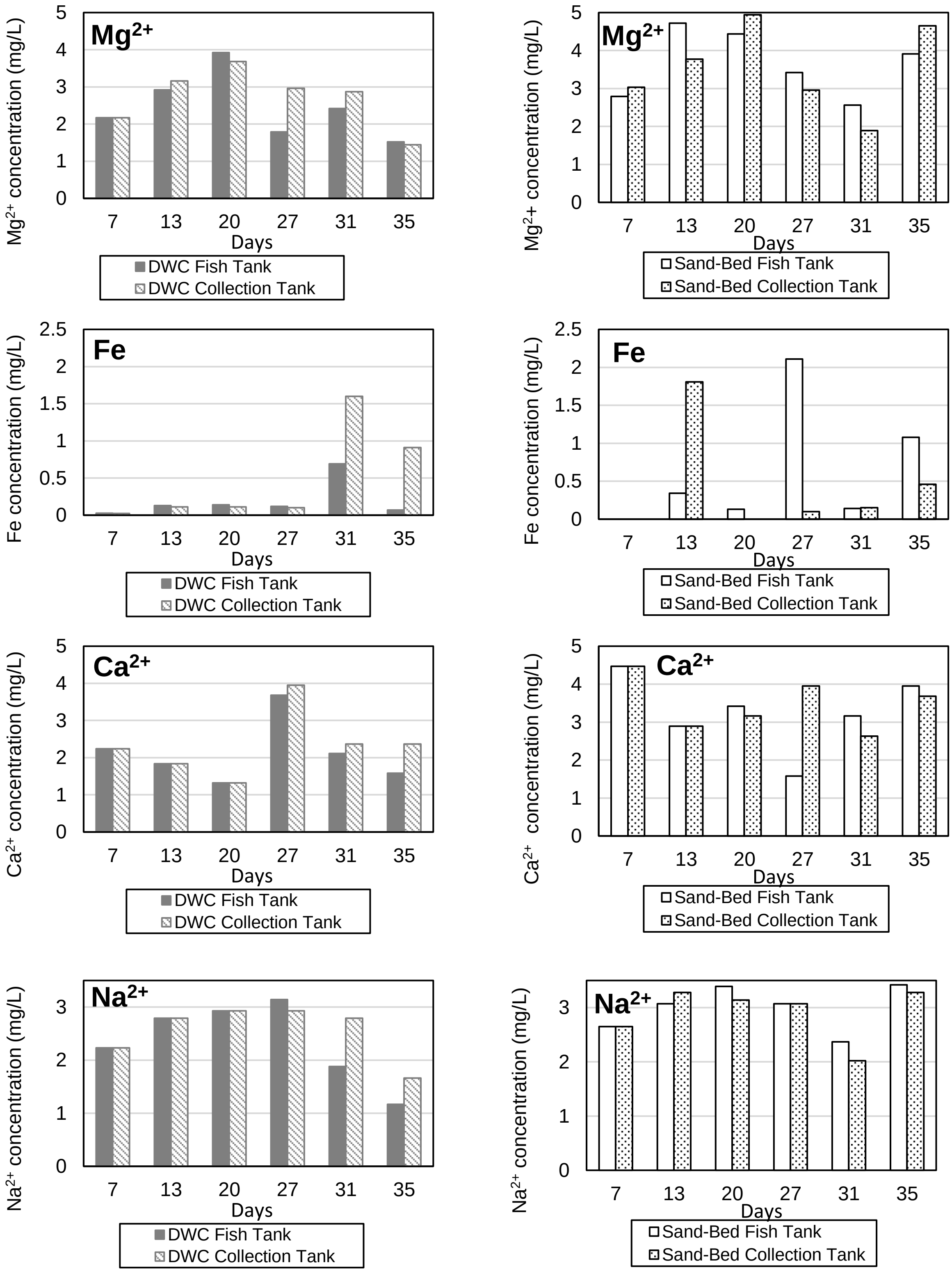


Figure 8: Variation in Concentrations of Mg^{2+}, Fe^{2+}, Ca^{2+} and Na^{2+} over time during study period

e Bacteria Count in DWC and Sand-Bed Systems

Table 10: Bacteria Count in DWC Bioballs Sample

DWC Bioballs Sample		
Bacteria Type	**1 day**	**35 days**
NH_4 Oxidizers* (microbe/L)	3.3×10^4	4.8×10^4
NO_2 Oxidizers* (microbe/L)	3.7×10^4	6.4×10^4
Total Coliform Bacteria (cell/mL)	Nil	Nil
Fecal Coliform Bacteria (cell/mL)	Nil	Nil
Salmonella and Shigella Bacteria (cell/mL)	Nil	Nil

**Legend: NH_4 Oxidizers are ammonia-oxidizing (<u>Nitrosomonas</u> bacteria), NO_2 Oxidizers are nitrite-oxidizing (<u>Nitrobacter</u> bacteria)*

Table 11: Bacteria Count in Sand-Bed Sand Sample

Sand-Bed Sand Sample		
Bacteria Type	**1 day**	**35 days**
NH_4 Oxidizers* (microbe/Kg)	3.2×10^6	1.90×10^5
NO_2 Oxidizers* (microbe/Kg)	3.2×10^6	7.00×10^5
Total Coliform Bacteria (cell/mL)	Nil	13**
Fecal Coliform Bacteria (cell/mL)	Nil	Nil
Salmonella and Shigella Bacteria (cell/mL)	Nil	Nil

**Legend: NH_4 Oxidizers are ammonia-oxidizing bacteria (<u>Nitrosomonas</u> bacteria), NO_2 Oxidizers are nitrite-oxidizing bacteria (<u>Nitrobacter</u> bacteria). **Value is negligible.*

Bioballs sample from the DWC biological filter and sand sample from the SAND-Bed plant bed were collected at the beginning and the end of the cycle. The samples were analyzed for *Nitrosomonas* bacteria (NH_4 Oxidizers), *Nitrobacter* (NO_2 Oxidizers), total and fecal coliform, and Salmonella and Shigella bacteria (**Table 10** and **Table 11**). There is an increase of the *Nitrosomonas* and *Nitrobacter* cell counts in DWC system. DWC system had a count of *Nitrosomonas* and *Nitrobacter* bacteria of 4.8×10^4 and 6.4×10^4 microbes/L respectively. There is a decrease in the *Nitrosomonas* and *Nitrobacter* cell counts in Sand-bed System. Sand-Bed system had a count of *Nitrosomonas* and *Nitrobacter* bacteria of 1.90×10^5 and 7.00×10^5 microbes/kg of sand respectively. There is no pollution occurred as there were insignificant amounts for the total and fecal coliform bacteria and salmonella and shigella bacteria.

4.2 Water Use Variables

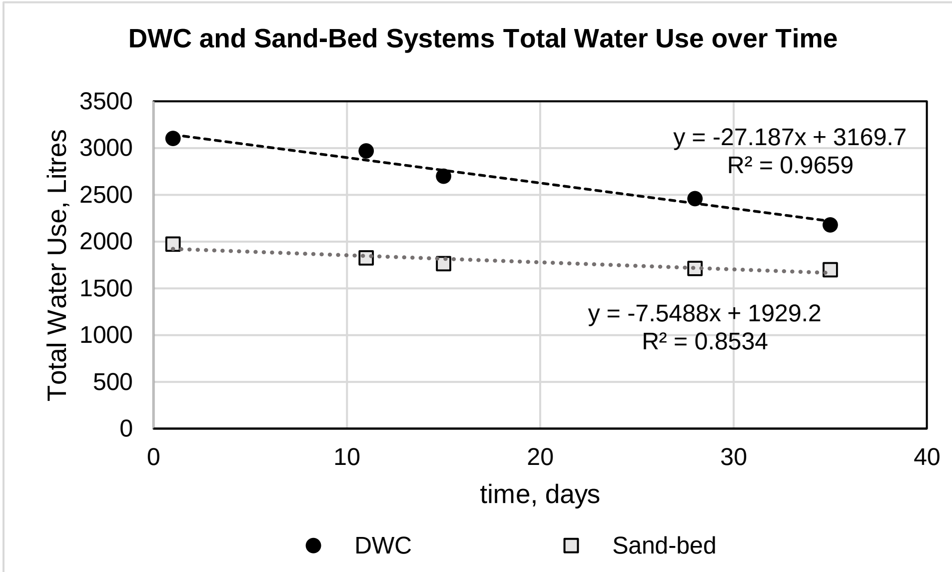


Figure 9: Total Water Use over Time for DWC and Sand-Bed System

**Legend:* *Total water use was set equal to total water volume measured weekly for DWC and Sand-Bed systems. The absolute value of the slope of total water use versus time is the amount of water consumed daily in each system developed using a linear regression.*

Total water use was measured weekly for 35 days. **Figure 9** represents the total water use for both systems DWC and Sand-Bed. The average total water consumption is obtained from the slope of the graph and estimated in **Table 12**. The average total water use in the system in a day was also calculated in **Table 12.** The results show that DWC uses 33% more total water use compared to Sand-Bed. The water consumption of DWC is 1.01% of the total water use whereas Sand-Bed is 0.42% of total water use.

Table 12: Average Total Water Use and Daily Water Consumption in DWC and Sand-Bed Systems Over 35 days

	Unit	DWC	Sand-Bed
Average Total Water Use	Liters	2680.37	1793.30
Average Total Water Consumption	Liters	950.95	264.25
Water Consumption	% day^{-1}	1.01%	0.42%

4.3 Lettuce (*Lactuca sativa*) Nutrient Analysis

Table 13: Summary of Lettuce (Lactuca sativa) Nutrient Parameters in DWC and Sand-Bed Systems

*		DWC				SAND-BED				
Element	**Unit**	**Mean**	**SD**	**Min**	**Max**	**Mean**	**SD**	**Min**	**Max**	**value of p***
Ca	%	0.61	0.10	0.52	0.75	0.31	0.04	0.26	0.35	0.00 [a]
P	%	0.83	0.20	0.60	1.07	0.12	0.03	0.08	0.16	0.00 [a]
K	%	3.87	0.33	3.45	4.22	0.92	0.18	0.74	1.15	0.00 [a]
Fe	mg/Kg	123.73	89.82	0.31	187.29	193.24	23.77	161.22	214.85	0.00 [a]
Mn	mg/Kg	87.48	93.50	21.51	215.97	6.39	0.76	5.45	7.26	0.00 [a]
Zn	mg/Kg	26.34	5.57	20.85	33.70	11.27	1.35	10.18	13.11	0.00 [a]
Cu	mg/Kg	7.90	9.33	1.49	20.72	1.44	0.03	1.42	1.48	0.01 [a]
Na	%	0.69	0.68	0.21	1.62	601.12	313.23	352.85	1029.83	0.00 [a]
Mg	mg/Kg	948.30	33.02	915.79	991.98	351.00	84.11	278.06	465.14	0.00 [a]
B	mg/Kg	10.93	0.97	9.80	12.10	3.17	0.71	2.59	4.14	0.00 [a]
NO_2^-	mg/L	3.07	3.20	0.21	7.40	0.92	0.62	0.42	1.77	0.01 [a]

**Legend: Ca=Calcium ion, P=Phosphorous, K=Potassium, Fe=Iron, Mn=Manganese, Zn=Zinc, Cu=Copper, Na=Sodium, Mg= Magnesium, B=Boron, NO_2^-=Nitrite. All data are analyzed using SPSS one-way ANOVA where mean is the mean value, SD is the standard deviation, min is the minimum value, max is maximum value. Mean values of DWC and Sand-Bed systems are significantly different when value of $p < 0.05$ noted using superscripts a.*

Lettuce (*Lactuca sativa L.*) nutrient content analysis is displayed in **Table 13** for each system. Each System's replicates were collected for analysis. The results were analyzed using SPSS software by a one-way analysis of variance (ANOVA). The mean, standard deviation (SD), minimum (Min) and maximum (Max) values are represented for each measurement per system. All elements were statistically different between both systems. In DWC, mean values of calcium, phosphorous, potassium, manganese, zinc, copper, magnesium, boron and nitrite were higher. Only iron and sodium were higher in the Sand-Bed system.

Table 14: Summary of Lettuce (Lactuca sativa) Chemical Parameters in DWC and Sand-Bed Systems

		DWC				SAND-BED				
	Unit	**Mean**	**SD**	**Min**	**Max**	**Mean**	**SD**	**Min**	**Max**	**value of p***
Vitamin B.Caroten	µg/100g	616.29	341.30	196.07	1006.90	626.78	24.33	594.80	651.21	0.90
Protein	%	17.83	0.68	16.90	18.30	8.20	1.38	6.80	10.00	0.00 [a]
Lipid	%	4.81	0.01	4.79	4.82	2.18	0.41	1.84	2.74	0.00 [a]
Crude fiber	%	12.87	0.66	12.32	13.77	4.35	1.03	3.22	5.66	0.00 [a]
Ash	%	20.65	0.33	20.23	21.00	67.15	5.31	60.35	72.83	0.00 [a]
Primary Moisture	%	93.63	0.53	92.90	94.00	91.17	1.22	90.00	92.80	0.00 [a]
Secondary Moisture	%	8.70	0.58	7.90	9.10	3.93	0.63	3.20	4.70	0.00 [a]
Total Moisture	%	94.23	0.53	93.50	94.60	91.53	1.24	90.40	93.20	0.00 [a]

**All data are analyzed using SPSS one-way ANOVA where mean is the mean value, SD is the standard deviation, min is the minimum value, max is maximum value. Mean values of DWC and Sand-Bed systems are significantly different when value of $p < 0.05$ noted using superscripts a.*

Table 14 represents chemical parameters for Lettuce samples collected from DWC and Sand-Bed Systems. Only vitamin B.Caroten showed similar results for both systems ($p>0.05$) with mean values of 616.29 and 626.78 µg/100g respectively. Protein, Lipid, crude fiber, primary moisture, secondary moisture and total moisture showed differences ($p<0.05$) where mean values where slightly higher in DWC system of 17.83%, 4.81%, 12.87%, 93.63%, 8.7% and 94.23% in DWC system and lower mean values of 8.2%, 2.18%, 4.35%, 67.15%, 91.17%, 3.93% and 91.53% in the Sand-Bed system.

4.4 Lettuce (*Lactuca sativa*) Yield

At the end of the cycle, six samples from each replicate were collected from each systems' three replicates. In the DWC and Sand-Bed Systems, each replicate's samples were collected and measured accordingly. The number of green and dry leaves, weight, shoot length, root length and leaf width and length were recorded. The results were analyzed using SPSS software by a one-way analysis of variance (ANOVA). The mean, standard deviation (SD), minimum (Min) and maximum (Max) values are represented for each measurement per Replicate in each system in **Table 15, Table 16** and **Table 17**.

4.4.1 DWC System

Table 15: Summary of Lettuce (Lactuca sativa) Growth Parameters in DWC System

	Replicate 1				Replicate 2				Replicate 3				
*	Mean	SD	Min	Max	Mean	SD	Min	Max	Mean	SD	Min	Max	value of p*
LL (cm)	14.92	0.50	14.20	15.50	15.65	0.99	14.00	16.60	17.52	1.36	15.00	18.60	0.00[a]
LW (cm)	11.62	1.66	9.00	13.80	11.90	0.62	10.90	12.50	12.03	1.07	10.40	13.50	0.83
PH (cm)	15.57	0.91	14.50	17.00	16.38	0.88	14.80	17.30	18.47	0.96	17.20	20.00	0.00[a]
RL (cm)	36.30	5.23	29.00	41.00	33.27	5.66	27.00	43.00	30.50	-	30.50	30.50	0.50
NFL	25.50	2.66	21.00	28.00	25.83	3.06	24.00	32.00	26.00	2.83	24.00	28.00	0.97
NDL	1.83	2.23	0.00	5.00	0.83	0.98	0.00	2.00	0.50	0.71	0.00	1.00	0.49
FW (g)	60.83	22.00	30.00	95.00	78.33	19.15	55.00	110.00	74.17	12.81	55.00	95.00	0.26

**Legend: LL=Leaf Length, LW=Leaf Width, PH=Plant Height, RL=Root Length, NFL=Number of Fresh Leaves, NDL=Number of Dry Leaves, FW=Fresh Weight. All data are analyzed using SPSS one-way ANOVA where mean is the mean value, SD is the standard deviation, min is the minimum value, max is maximum value. Mean values of DWC replicates are significantly different when value of p < 0.05 noted using superscripts a.*

All parameters for lettuce growth parameters in DWC system were measured among the three replicates' samples (**Table 15**). Only leaf length and plant height showed significant difference ($p<0.05$) between replicates mean values. Leaf length mean values were 14.92, 15.64 and 17.52 cm for replicate 1, 2, and 3 respectively. Plant height mean values were 15.57, 16.38 and 18.47 cm for replicate 1, 2 and 3. This showed that both replicate 3 (farthest from fish tank) had the highest leaf length and plant height.

4.4.2 Sand-Bed System

Table 16: Summary of Lettuce (Lactuca sativa) Growth Parameters in DWC System

	Replicate 1				Replicate 2				Replicate 3				
*	Mean	SD	Min	Max	Mean	SD	Min	Max	Mean	SD	Min	Max	value of p*
LL (cm)	14.43	2.09	12.50	18.50	14.63	1.14	13.50	16.60	14.05	1.08	13.20	16.20	0.80
LW (cm)	11.03	1.42	9.00	13.00	12.18	1.28	10.50	14.40	9.78	0.48	9.00	10.40	0.01[a]
PH (cm)	15.17	2.56	13.00	20.00	15.37	0.81	14.40	16.50	15.05	1.10	14.00	17.00	0.95
RL (cm)	14.67	2.04	11.50	17.00	13.93	2.48	10.00	17.50	15.53	2.17	12.20	18.50	0.48
NFL	17.17	4.12	13.00	24.00	20.00	3.03	16.00	24.00	18.83	0.75	18.00	20.00	0.29
NDL	1.67	3.20	0.00	8.00	1.83	3.13	0.00	8.00	0.33	0.52	0.00	1.00	0.56
FW (g)	41.67	18.62	5.00	55.00	68.33	13.66	55.00	95.00	45.00	15.49	15.00	55.00	0.02[a]

**Legend: LL=Leaf Length, LW=Leaf Width, PH=Plant Height, RL=Root Length, NFL=Number of Fresh Leaves, NDL=Number of Dry Leaves, FW=Fresh Weight. All data are analyzed using SPSS one-way ANOVA where mean is the mean value, SD is the standard deviation, min is the minimum value, max is maximum value. Mean values of Sand-Bed replicates are significantly different when value of $p < 0.05$ noted using superscripts a.*

Replicates 1, 2 and 3 in Sand-Bed system showed similar results except for leaf width and fresh weight mean values (**Table 16**). The mean values for leaf width are 11.03, 12.18 and 9.78 cm for replicates 1, 2 and 3 respectively. The fresh weight mean values were 41.67 grams, 68.33 grams and 45 grams. Both leaf width and fresh weight mean values show highest results in replicate 2 (middle replicate in the Sand-Bed system).

4.4.3 Systems comparison

Table 17: Summary of Lettuce (Lactuca sativa) Growth Parameters in DWC and Sand-Bed System

*	DWC				SAND-BED				
	Mean	**SD**	**Min**	**Max**	**Mean**	**SD**	**Min**	**Max**	**value of p***
LL (cm)	16.03	1.48	14.00	18.60	14.37	1.44	12.50	18.50	0.00 [a]
LW (cm)	11.85	1.14	9.00	13.80	11.00	1.47	9.00	14.40	0.06
PH (cm)	16.81	1.52	14.50	20.00	15.19	1.58	13.00	20.00	0.00 [a]
RL (cm)	34.45	5.33	27.00	43.00	14.71	2.21	10.00	18.50	0.00 [a]
NFL	25.71	2.64	21.00	32.00	18.67	3.05	13.00	24.00	0.00 [a]
NDL	1.21	1.63	0.00	5.00	1.28	2.54	0.00	8.00	0.94
FW (g)	71.11	18.91	30.00	110.00	51.67	19.40	5.00	95.00	0.00 [a]

**Legend: LL=Leaf Length, LW=Leaf Width, PH=Plant Height, RL=Root Length, NFL=Number of Fresh Leaves, NDL=Number of Dry Leaves, FW=Fresh Weight. All data are analyzed using SPSS one-way ANOVA where mean is the mean value, SD Is the standard deviation, min is the minimum value, max is maximum value. Mean values of DWC and Sand-Bed systems are significantly different when value of $p < 0.05$ noted using superscripts a.*

Table 17 represents lettuce growth parameters comparison in DWC and Sand-Bed systems. All lettuce growth parameters showed significant difference across DWC and Sand-Bed systems except for number of dry leaves and leaf width. The DWC leaf length mean value is 16.03 cm; whereas the Sand-Bed leaf length showed lower mean value of 14.37 cm. DWC system plant height showed higher results of mean value 16.81 cm versus Sand-Bed plant height of 15.19 cm. The root length for the DWC system has a higher mean value (34.45 cm) than Sand-Bed system's plant height (14.71 cm). Similarly, number of fresh leaves mean value in DWC system was higher than Sand-Bed system of 25.71 fresh leaves in DWC and 18.67 fresh leaves in Sand-Bed respectively. DWC system lettuce fresh weight mean value was 71.11 g heavier than the Sand-Bed system's lettuce fresh weight mean value of 51.67 g.

4.5 Lettuce Plant Quality Index

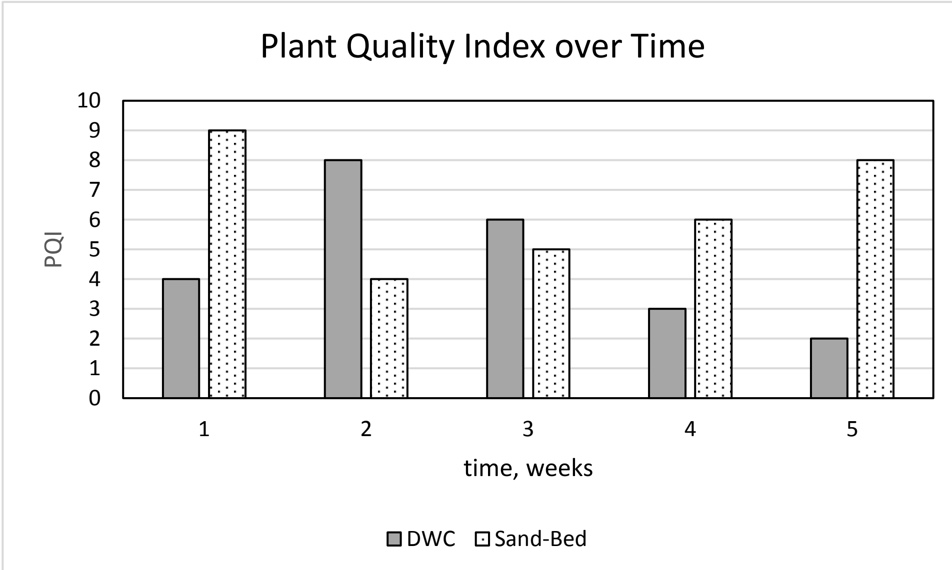


Figure 10: DWC and Sand-Bed systems Lettuce Plant Quality Index over Time

* *Legend: Lettuce Plant Quality index (PQI) is a qualitative measure evaluated by visual numerical index from 1 to 10. (1) represents yellow leaves up to 90% imperfections in leaves' surface in a lettuce sample; (10) represents very dark green leaves without imperfections in leaf surface.*

Plant Quality Index (PQI) was developed by assessing photographs taken for each system's replicates. The results are presented in **Figure 10.** Sand-Bed lettuce leaves were generally greener than DWC lettuce leaves. By end of study period (week 5), DWC lettuce leaves were evaluated with a scale of (2) showing very light yellowish green leaves with up to 80% imperfections on leaf surface. Whereas, Sand-Bed lettuce were evaluated with a scale of (8) showing dark green with up to 10% imperfections on leaf surface by end of study period. Lettuce PQI indicated that lettuce grown in Sand-Bed system had overall better visual quality leaves compared to lettuce grown in DWC system. The PQI results (twelve samples per week for each replicate) were compared using Chi Square test. The results showed that the systems type affects lettuce PQI significantly over weeks 1, 2 and 5.

Chapter 5: Discussion

5.1 Water Parameters related to Lettuce and Nile Tilapia Yield

Water quality parameters are congenial for both tilapia fish and lettuce survival and growth (Saha et al., 2016). There was no death or disease observed on fish or lettuce during the time span of the experiment. PH, temperature, EC, SAR and DO in both systems remained within acceptable ranges compared to literature in both systems regarding fish and plants growth (Boyd & Tucker, 1998; Oladimeji et al., 2018; Pinho et al., 2018, 2017; Saha et al., 2016). The recommended range in aquaponic systems is 0.3 to 0.8 ds/m (Estrada-Perez et al., 2018), only Sand-Bed EC mean value was slightly higher. DO and temperature were higher in DWC compared to Sand-Bed. Still, both systems had reasonable DO concentration above the critical concentration (1.6 mg/L) that can affect fish and lettuce leaf and root growth (Sikawa & Yakupitiyage, 2010; Yoshida, Kitano, & Eguchi, 1997).

The accumulation of TDS and P across both systems is typical for new aquaponic systems used for leafy crops production (Medina et al., 2016; James E Rakocy et al., 2006). Sand-Bed TDS and EC were higher than DWC TDS by 50% due to presence of sand media. Phosphorous concentrations decrease with higher pH availability in the aquaponic solutions; a pH of 5.5-7.2 is optimum for phosphorous nutrient availability and uptake by plants (Cerozi & Fitzsimmons, 2016). The optimum concentration of phosphorus is 11 mg/L at pH ranges of 5.5 to 7.2 (Cerozi & Fitzsimmons, 2016). Accordingly, the mean values of P concentration of DWC and Sand-Bed were optimum of 15.32 and 11.12 mg/L at pH mean values 7.36 and 7.28 respectively (see **Figure 6**). Additionally, the increased P concentration in water did not affect the nutrient content of the plants (Saha et al., 2016).

Among both systems, NH_4^+ levels showed increasing trends; whereas, NO_3^- levels had decreasing trends starting day 20 for both systems (see **Figure 7**). This is common in aquaponic systems as plants' roots absorb nitrates during their growth stage (Zou et al., 2016). At the first two weeks after lettuce transplanting, the lettuce seedlings are small and absorb less nutrients; the nitrate accumulation rate then decreases gradually during growing stage (Zou et al., 2016). Other studies concluded plants uptake nitrates significantly reducing the accumulation of nitrates present in aquaponics solutions (Hu et al., 2015). However, this study's nitrates levels in both systems are at the lower range side reported in literature. The average NO_3^- levels in both systems during the 35 days was around 10 mg/L for DWC and Sand-Bed respectively. In terms of nitrification, both systems had similar mean concentrations for NO_3^- and NH_4^+. At the experiment's nitrates concentration, Lennard and Leonard (2006) and Li et al. (2019) reported optimum production requires additional nitrates in the aquaponic solutions. Compared to the University of the Virgin Islands (UVI) aquaponics system for lettuce growth, our study's water quality for DWC and Sand-Bed had optimal solutions except for nitrates concentration were slightly lower (Bailey & Ferrarezi, 2017). Similarly, Hu et al. (2015) showed that better water quality in tomato aquaponics yielded better fish performance, lower FCR and therefore better feed consumption.

Increase in AOB bacteria has a great effect on nitrite and nitrate concentration as it increases the rate of ammonia oxidation and nitrification process (Martir-Torres & Bruns, 2013). Zou et al. (2016) showed that the highest AOB were present when weekly probiotics capsule containing nitrifying bacteria (nitrifiers addition) was added to the system producing AOB of 1.6 $x10^8$ copies/L during study period of 75 days. Abundance of AOB 6.0 $x10^7$ copies/L were produced in aquaponics system similar to DWC and AOB of 5.0 $x10^7$ copies/L were produced in bed filled with gravel (filler gradation) (Zou et al., 2016). In contrast, the nitrification rate was the highest in filler gradation as gravels or media-based aquaponics provide efficient oxidation environment (Zou et al., 2016). This shows that the highest production of AOB and NOB was in media-bed (gravel). In our study, we measured the density of sand equal to 1986 kg/m^3; and therefore, 1.98 kg of sand is equivalent to 1 liter of water (1 m^3 = 1000 L). Our study showed that Sand-Bed AOB and NOB of 1.9 $x10^5$ and 7.0 $x10^5$ copies/kg of sand respectively were higher than DWC AOB and NOB of 4.8 $x10^4$ and 6.4 $x10^4$ copies/L respectively.

Still, DWC system observed an increase in AOB and NOB count whereas Sand-Bed system observed a decrease in AOB and NOB over 35 days. Zou et al. (2016) reported higher bacteria AOB count in systems; nitrifies addition to the systems played an important factor to increase the nitrifying bacteria biomass and nitrates concentration and plant yield accordingly. The authors mentioned that enhancing bacteria physicochemical conditions such high oxygen enrichment can enhance their growth. The authors reported that gravel-bed system had larger surface area increasing oxygen for bacteria growth. It could be possibly that Sand-Bed system had clogged sands affecting oxygen enrichment in sand media. The previous argument could also be linked to lower DO concentration in Sand-Bed water nutrient content. Other reasons mentioned by Hu et al. (2015), the study evaluated nitrification rate by measuring the abundance of ammonia-oxidizing bacteria (AOB) and nitrite-oxidizing bacteria (NOB) in RAS systems. The water containted 6.85 ± x 10^8 copies AOB 3.26 ± 0.71 x 10^{10} copies and NOB of 3.66 ± 0.65 x 10^{12} and 4.55 ± 0.08x 10^{11} copies for tomato and pak choi-based aquaponics respectively (Hu et al., 2015). The authors inferred that some plants have lower nitrate uptake slower than the formation rate of nitrifying bacteria; in their study, pak-choi plants had lower nitrate uptake (Hu et al., 2015).

5.2 Lettuce Yield

All minerals (Ca, P, K, Mn, Zn, Cu, B, N) concentration for lettuce harvested from DWC were two-fold or higher the minerals' concentration in lettuce harvested from Sand-Bed. Only Fe and Na concentration were significantly higher in the lettuce harvested in Sand-Bed compared to DWC. Linking the nutrient water content, DWC and Sand-Bed fish and collection tanks' nutrient concentration differed only in K, Mg, Ca and Cl^- concentration where Sand-Bed had the higher concentrations amongst the prementioned nutrients (see **Figure 7** and **Figure 8**). In contrast, linking to lettuce nutrient uptake, all three minerals were more 50% higher in DWC lettuce content than Sand-Bed lettuce content. Comparing the water to the lettuce nutrient results both systems, we can deduce that lettuce nutrients uptake in Sand-Bed was lower not solely due to water nutrient concentration but rather due to growth environment. Besides, water nutrient was not sodium-rich in both systems, there was an increased uptake of sodium by the lettuce in Sand-Bed. We deduced that additional sodium could have come from the sand media; and therefore, lettuce grown on Sand-Bed retained more sodium content. Delaide et al. (2016) listed the essential nutrients and optimum external conditions for lettuce nutrient uptake. For instance, minerals like B, S, P, Mg, Ca and K are optimally absorbed at pH values below 6.0 (Delaide et al., 2016). In this study, both systems had the same pH value nearly 7.3. Therefore, Sand-Bed slower nutrient uptake is not linked to pH.

In a similar aquaponics experiment to DWC, the water nutrient quality had similar ammonia concentration as our results (Delaide et al., 2016). The observed lettuce yield was relatively equivalent to values reported at the same study period of 35 days. The authors reported relatively equal or lower lettuce fresh weight to be 80.55 g/plant and 35.72 g/plant in trials 1 and 2 over 36 days (Delaide et al., 2016). However, the concentration of NO_3^-, Ca^{2+}, Fe^{2+}, Mg^{2+} and K^+ were higher in the aquaponic solution used . The leaf nutrient of lettuce *Latuca sativa* (*variety succrine*) had higher Fe, K, P and Ca concentration than the results of this experiment. Remarkable observation was that the Mg reported in this study's lettuce nutrient content was significantly higher than Delaide et al. (2016) results while B and Zn concentration were similar.

Sikawa and Yakupitiyage (2010) conducted an experiment for 54 days using three different growth media: gravel (particle size 2.5 cm), sand (particle size 0.1-0.25 mm) and sytrofoam sheets. The experiment had relatively lower concentrations of DO (0.78 ± 0.53) mg/L, pH (7.2 ± 0.09), NO_3^- (3.06 ± 0.14) mg/L and higher NH_4^+ (2.42 ± 0.08) mg/L (Sikawa & Yakupitiyage, 2010). The nutrient levels reported were also lower than reported in our experiment accordingly for Mg, Zn, Fe (Sikawa & Yakupitiyage, 2010). Only K and Ca were higher 10.09 ±1.35 and 5.21 ± 0.21 mg/L, respectively, compared to our results (Sikawa & Yakupitiyage, 2010). Zou et al. (2016) reported that nitrate concentration ranged from 5-15 mg/L in the first 15 days; however, the nitrification rate had increasing nitrates ranges up to 45 mg/L in day 30 in the filler gradation system over the DWC or water system. The mean wet weight of the lettuce harvested were 34.28 ± 0.8 g/plant for Sand, 22.59 ± 0.3 g/plant for Gravel and for the sytrofoam 18.32 ± 2.43 g/plant over 54 days. The previous results validate that additional nutrient concentration in water enhances the lettuce yield since our experimental results were two-to-three times fold the lettuce yield resulted by Sikawa and Yakupitiyage (2010) and similar to Delaide et al. (2016).

In the current study, lettuce yields in DWC and Sand-Bed systems were 1.42 kg/m^2 and 1.03 kg/m^2 accordingly. Martins et al. (2018) studied lettuce production in NFT aquaponic systems (complemented by biofertilizers) with 100 Tilapia fish strain (*Oreochromis niloticus*) per cubic meter (initial weight 142 g/fish) and 20 lettuce (*Lactuca sativa*) per m^2. After 35 days, the lettuce yield ranged from 20.8 tons per hectare (i.e. 1.89 kg/m^2) to 39.9 tons per hectare (i.e. 3.62 kg/m^2) using different substrates phenolic foam and coconut shell bar respectively (Martins et al., 2018). Although, the nutrient solution used was a mixture of wastewater and biofertilizer (Martins et al., 2018). Similar nutrients and chemical parameters for water quality were reported as the current study; this included P and K. The conventional agriculture systems (with soil), shoot fresh weight reported where 166 g/plant and 90.3 g/plant (Martins et al., 2018).

Similar concentrations of DO and lower NO_3^- values of 0.1 ± 0.1 and 1.3 ± 0.4 mg/L in a study growing Nile Tilapia with initial weight of 70 g and stocking density of 6.4 kg/m^3 and lettuce (*Lactuca sativa*) with initial seedling height of 8.49 ± 0.82 cm and density of 20 lettuce/m^2 (Pinho et al., 2017). The final mean lettuce yield and SGR were 1.0 ± 0.05 kg/m^2 and 16.4±0.1 % day^{-1} respectively; the authors linked linking the low weight due to initial low weight/height of seedlings. In a similar study, Palm et al. (2014) reported lettuce yield of 55.89 ± 4.77 g/plant over 54 days with NO_3^- concentration level 31.06 ± 5.17 mg/L and Nile Tilapia initial stocking density of 5.62 kg/m^3. The authors commented on lettuce low production due to cultivation period and light regime (Palm et al., 2014). Estrada-Perez et al. (2018) deduced that high temperatures and pH caused the slow initial growth of lettuce (*Lactuca sativa*). The study's pH and temperature values were 8.19 ± 0.2 and 27.85 ± 2.64°C (Estrada-Perez et al., 2018). Lettuce growth optimum temperature and PH are 15°C–25°C and pH 6.0–6.5 (Resh, 2016; Yoshida et al., 1997). pH and temperature in both DWC and Sand-Bed were also higher than the optimum conditions for lettuce growth.

For lettuce cultivation in conventional systems with soil, lettuce (*Lactuca sativa*) maximum yield during five crop cycles ranged from 5.75 to 35.8 tons/hectare (0.52 kg/m^2 to 3.25 kg/m^2) (Martins et al., 2018). The authors reported that the higher the number of leaves, the more marketable the crop in the market. The number of leaves reported ranged from 21.8 to 31.3 leaves per lettuce (Martins et al., 2018). In a similar experiment, height of lettuce (*Lactuca sativa*) leaves reported were 15.3 ± 0.44 cm, number of leaves were 14.9 ± 0.4 cm over 21 days (Pinho et al., 2017). In our study, the number of fresh leaves for DWC and Sand-Bed 25.71 and 18.67 leaves per lettuce, respectively, with leaf heights of 16.03 ± 14.48 and 14.37 ± 1.44 cm, respectively, over 35 days. DWC lettuce yield lies within practical lettuce yields for aquaponics and conventional agriculture. Sand-Bed lettuce yield lies within lower range commercial lettuce yields for aquaponics and conventional agriculture. There is potential in both systems to improve the lettuce yields by increasing nitrification rate and providing optimum conditions for lettuce alternatively.

5.3 Media Type

Although, the NO_3^- and NH_4^+ concentrations were similar in both systems DWC and Sand-Bed, Sand-Bed had nearly 30% lower lettuce yield results (1.03 kg/m^3) over DWC (1.42 kg/m^2). Therefore, we believe that the differences could be due to the media environment and its impact on roots' nitrates uptake. Some studies highlighted that sand particles are compacted and can be clogged by solids suspension reducing the ability for roots respiration (Sikawa & Yakupitiyage, 2010). Other experiments showed that media-based aquaponics with a mixture of gravel and sand obtained higher yield than DWC or water-based aquaponics (Oladimeji et al., 2018; Sikawa & Yakupitiyage, 2010; Yina Zou, Hu, Zhang, Xie, Guimbaud, et al., 2016).

In contrast to the results of this experiment, Sikawa & Yakupitiyage (2010) reported that partially filtered fish pond water had higher lettuce (*Lactuca sativa*) yield compared to unfiltered fish pond water by 52% in

Sand media. The results also showed that the sand media produced the highest lettuce yield over gravel and DWC's lettuce production. The lettuce beds were irrigated only twice daily in over 54 days yielding 0.42 ±9.56 kg fresh weight/m^2 and 0.17 ± 12.64 kg fresh weight/m^2 in the sand media-bed and DWC (Sikawa & Yakupitiyage, 2010). Sikawa & Yakupitiyage (2010) observed increase in Ca content for lettuce grown on sand media. P concentration increased in lettuce grown in sands in comparison to DWC and gravel; whereas higher N content on gravel then the other substrates (Sikawa & Yakupitiyage, 2010). This phenomenon was contradicted in this study where lettuce grown on Sand-Bed had lower Ca and P content than DWC; although Ca and P content in water of both systems were similar. Another reason for higher nitrification rate and larger Nitrobacter bacteria growth is larger surface area such as root surfaces area (Hu et al., 2015). For example, tomato root surface area was 6.3 times higher than that of pak-choi resulting 4.4 times abudnance of NOB in tomato than that of oak choi based aquaponics. Likewise, DWC provides larger surface area for roots growth over Sand-Bed surface area provided for roots growth. The lettuce root length in DWC (34.45 ± 5.33 cm) were almost two-folds the root length in Sand-Bed (14.71 ± 2.21 cm).

Research shows potential in using gravel as media where Lennard and Leonard (2006) and Sikawa & Yakupitiyage (2010) reported efficient nitrification in gravel media-based aquaponics due to availability of air space for roots nutrient uptake. Moreover, (Li et al., 2019) experimented adding immobilized biofilm and fiber in media beds to enhance media-beds nitrification performance. The results showed that the media-bed systems with biofilm and fiber enhanced the systems' efficiencies for total ammonia nitrogen removal by 71.4% (Li et al., 2019).

5.4 Production Parameters based on Aquaponics System

Table 18: Water Consumption and Production Yields for DWC and Sand-Bed Systems Over 35 days

Parameters	Density	DWC	Sand-Bed
Average Total Water Use (m^3)	-	2.68	1.79
Average Daily Water Consumption (% day^{-1})		1.01%	0.42%
Production Yields			
Lettuce *(Lactuca sativa)* (kg/m^2)	20 lettuce/m^2	1.42	1.03
Nile Tilapia *(Oreochromis niloticus)* (kg/m^3)	150 fish/m^3	7.20	13.80
FCR (%)	-	1.32	0.69

Legend: Production Yield for lettuce and Nile Tilapia is final wet weight gain (kg) minus initial wet weight gain (kg). FCR (Food Conversion Ratio) = Total feed given (g) to fish divided by total weight gain (g) of fish.* DWC system produced 7.2 kg/m^3 of Nile Tilapia and 1.42 kg/m^2 of lettuce using 2.68 m^3 of water over 35 days. Sand-Bed system produced 13.8 Kg/m^3 of Nile Tilapia and 1.03 kg/m^2 of lettuce using 1.97 m^3 of water over 35 days. The daily water consumption of DWC system was estimated to be 1.01% day^{-1} of total water use whereas Sand-Bed system total daily water consumption was 0.42% day^{-1} of total water use. DWC and Sand-bed production parameters are presented in **Table 18. Delaide et al., (2017) reported that total water of 2673 L were used in a DWC system with a daily water consumption of 3.6%. Whereas other studies reported daily water consumption of a range between 0.5 – 10% of total water use observed in aquaponic systems (Love, Uhl, et al., 2015; Rakocy et al., 2006) and a daily water consumption averaged 2.65% of total water use in gravel-based system (Lennard & Leonard, 2005). The researchers concluded that daily water loss is mainly influenced by the plant bed/fish tank volume ratio and crops type grown (Lennard & Leonard, 2005). There was no significant difference linked to type of system used or type of flow (constant or intermittent). Danaher, Shultz, Rakocy, Bailey, & Knight, (2011) reported total daily water consumption to be 1.6% of total water use in the system in a floating system similar to DWC. Lennard & Leonard (2004) reported 2.86% and 2.43% of total water used for constant and reciprocating flow in gravel-based system growing lettuce over 21 days. The results obtained for both DWC and Sand-Bed system daily water consumption is at the lower range of literature. However, the potential of water conservation in both systems is validated when compared to daily water consumption in aquaculture systems of 1.4% of daily water consumption, 10-15% daily water in conventional aquaculture, semi-intensive 5% and intensive 10% (Al-Hafedh et al., 2008; Mohanty et al., 2018 from krummeneauer et al., 2006 and Boyd et al., 2007). Increasing production yields of plants in parallel to fish production in aquaponics systems improves daily water consumption efficiency.

Hypothesis testing of the fish productivity response between the two systems was not designed in this experiment due to the short duration of study period (35 days) relative to typical fish grow-out period (Medina et al., 2016). However, measurements including the fish biomass per system and daily feed rate were recorded during the study period. There was no death observed for fish in both systems during the study period. The high survival rate was expected as most literature reported rates above 90% (Cerozi & Fitzsimmons, 2017; Lam, Ma, Jusoh, & Ambak, 2015; Pinho et al., 2017; J E Rakocy et al., 2004). The results also showed increased fish biomass in Sand-Bed over DWC of 13.8 kg/m^3 and 7.2 kg/m^3 respectively. The initial biomass for both systems was 2.5 kg/m^3 (7.5 grams per fingerling of Nile Tilapia). Kamal (2006) reported initial biomass of 1.05 kg/m^3 (10.5 grams per fingerling of Nile Tilapia) in density of 15 plants per m^2 yielding 17.95 kg/m^3 of fish biomass over 180 days. Medina et al. (2016) reported an increase of 347.5 ± 91.2 g and 217.6 ± 23.5 g for tilapia fish with starting weight of 67.3 ± 3.7 g over study period of 60 days. The differences in fish biomass increase between studies could be related to differences in initial weigh, initial biomass and modifying the growth rates (Pinho et al., 2018).

Estrada-Perez et al. (2018) studied different stocking for Nile Tilapia at 30, 60 and 90 fish/m^3 and growing lettuce (*Lactuca sativa*) FCR 1.36 ± 0.04 ranged from to 0.84 ± 0.03.The results showed that FCR, water, EC and nutrients expect for P increases at the lowest stocking rates. In comparison to 150 fish/m^3 used in this experiment, the FCR of DWC and Sand-Bed systems are commercial of FCR 0.69 and 1.32 respectively. According to literature, the FCR for Nile Tilapia is productive in ranges from 0.82 to 0.98 up to 1.2 to 1.5 (Li et al., 2019; Thorarinsdottir, 2015). High FCR greater than 1.5 could be linked to lack of experience of fish feeding through overdosing (Li et al., 2019). In a similar study set-up over 135 days, the total increase of tilapia fish was 15.56 kg consuming fish feed of 33.54 kg where the FCR was 2.16 (Li et al., 2019). Comparing to Pinho et al. (2018) results, FCR for tilapia fish was 2.0 over 35 days, initial and final biomass were 31.56 ± 1.50 g and 70.13 ± 3.98 g respectively yielding 7.15 kg/m3 and survival rate 98% in 500 Liters tank. The previous results were obtained at similar temperature, DO and pH concentration as our system except lower NO_3^- and NH_4^+ levels. Our system's tilapia production showed more productive results.

Other studies like Suhl et al. (2016) reported yearly production of 1.55 kg Tilapia and 46.1 kg tomato fruit per one m^3 water in DWC system. Suhl et al. (2016) reported lower fish production than literature due to changing environmental conditions during study period and lower fish stocking density (initial stocking density was 2.5 kg/m^3). Similar experiment setup had low NO_3^- concentration of an average of 14.6 mg/and low nutrient levels referring that the low nutrient levels could be caused by the low fish stocking density compared to studies containing N content of 127.7 mg/L (Kloas et al., 2015; Suhl et al., 2016). The optimization of fish production can deliver higher nutrient content for plant production and increase fertilizer savings compared to conventional agriculture (Kloas et al., 2015; Suhl et al., 2016). During this study period, there was a sand storm that changed weather conditions over 10 days. Although the production of fish and lettuce compared to literature is efficient in both systems; both yields can further increase. Optimizing environmental conditions in the greenhouse and increasing fish stocking density are potential factors for increasing fish and lettuce yields in both systems.

Chapter 6: Conclusion and Recommendations

DWC and Sand-Bed systems were tested to assess the systems' lettuce (*Lactuca sativa* Type Batavia) production and water consumption over 35 days. The main differences between DWC and Sand-Bed systems were: 1) Water Use, 2) media for nitrifying bacteria (Nitrobacter and Nitrosomonas) growth and 3) Water flow. DWC system used an average of 2.68 m^3, had continuous water flow and incorporated a biological filter tank with high surface area bioballs to enhance nitrifying bacteria growth. Sand-Bed system used less water an average of 1.79 m^3, had intermittent water flow and sand media acted as a biological filter due to its high surface area. Over 35 days, the main physicochemical parameters including DO, pH, temperature, EC, SAR and TDS were within acceptable range for aquaponic systems. Overall, *Nile Tilapia* fish, nitrifying bacteria and lettuce all had an observed growth and did not show signs of diseases or death. The systems had similar nitrates and ammonium concentration; however, the nitrifying bacteria count was higher in Sand-Bed system due to sand larger surface area. This was further shown in higher water nutrient content in Sand-Bed system. However, lettuce nutrient content in DWC system was nearly two-times higher than the nutrient content of lettuce grown on Sand-Bed system. The study showed that high nutrient uptake by lettuce in DWC could be linked to longer roots compared to Sand-Bed lettuce roots length. DWC plant bed allowed larger area for roots growth. Lettuce yields in DWC system (1.42 kg/m^2) were also higher by 27% more than lettuce yields in Sand-Bed system (1.03 kg/m^2). The results were further validated by the higher lettuce growth and nutrient parameters including plant height, leaf length, number of fresh leaves and nutrients content in lettuce grown on DWC system. On the contrary, DWC lettuce were visually nearly yellow with up to 80% imperfections using PQI developed in this study. Sand-Bed system had higher PQI with dark green lettuce yields with up to 10% imperfections.

In terms of water use, DWC used and consumed daily over 30% more water compared to Sand-Bed. As previously mentioned in other studies, system type and water flow do not affect water consumption and losses in aquaponics systems. Crops type and plant bed to fish tank volume ratio affected water losses in aquaponics systems. It was possibly that higher daily water consumption in DWC system is caused by larger water volume used in plant bed. Overall, the performance of systems in terms of water use is more efficient than aquaculture and conventional agriculture water use as both systems demonstrated productive yields for lettuce and fish production. Sand-Bed system shows potential due to its minimal water use, less equipment required and similar media characteristics to soil that helped bacterial growth and enhanced lettuce PQI. Still, Sand-Bed lettuce yields are considered on the lower range compared to conventional agriculture. DWC system's performance demonstrated higher productive yields and higher water consumption with lower lettuce PQI. Further enhancements to DWC can be achieved by additional nutrient supplementation to improve lettuce PQI. For Sand-Bed system, further research is recommended to tackle roots length growth and enhance nutrient uptake to reach productive yields like the DWC system and benefit from its reduced water consumption and the potential of growing larger variety of crops.

Besides the focus on increasing fish feed and stocking density for improving the water nutrient content, few scientific studies approached a new technique known as new double recirculating aquaponic system yet still under optimization (DRAPS) (Kloas et al., 2015; Suhl et al., 2016). The aquaponics systems (DWC and Sand-Bed) used in this study integrate both fish and plant cycles through a single recirculating cycle. DRAPS separates the fish and plant cycle where each cycle can have its optimal water parameters such as pH, temperature, nutrients for growth (Suhl et al., 2016). Moreover, nutrient uptake by plants increased in sand-media beds by using DRAPS technique (Li et al., 2019; Suhl et al., 2016). Other scientists study the effect of adding biofloc systems increasing the potential of aquaponics yield (Pinho et al., 2017 cited from Crab et al. 2012, Avnimelech, 2007; Wambach, 2013; Luo et al., 2014). Pinho et al. (2017) showed an increase of 40% in lettuce yield from biofloc systems over clean water systems of 1.4 kg/m^2 and 1.0 kg/m^2 respectively due to higher microbial activity provided by biofloc technology (BFT) systems.

Further enhancements to Sand-Bed system can be achieved by adding gravel to the system for higher porosity and better oxygen enrichment for nitrification. Zou et al. (2016) demonstrated that filler gradation (adding different sizes gravel and pearlite to sand media) improved the nitrogen utilization efficiency process by 8.8% and 16.0 % respectively. The authors concluded that filler gradation had the highest nitrification activity as graded filler enhance oxygen in the beds and in parallel increase nitrification activity due to higher porous media less anerobic microenvironment. The results also showed that filler gradation had the highest nitrous oxide conversion ratio 1.7% which increase its contribution to global warming. Higher nitrogen accumulation in plants biomass is linked to profitable nitrogen recovery warming. Additional nutrient supplementation to aquaponics can also lead to a 39% increase in the lettuce production compared to without supplementation (Delaide et al., 2016; Pinho et al., 2018; Zou et al., 2016).

References

Abdelkader, A., Elshorbagy, A., Tuninetti, M., Laio, F., Ridolfi, L., Fahmy, H., & Hoekstra, A. Y. (2018). National water, food, and trade modeling framework: The case of Egypt. *Science of the Total Environment*, *639*, 485–496. https://doi.org/10.1016/j.scitotenv.2018.05.197

Ahmed, N., Thompson, S., & Glaser, M. (2019). Global aquaculture productivity, environmental sustainability, and climate change adaptability. *Environmental Management*, *63*(2), 159–172. https://doi.org/10.1007/s00267-018-1117-3

Al-Hafedh, Y. S., Alam, A., & Beltagi, M. S. (2008). Food production and water conservation in a recirculating aquaponic system in Saudi Arabia at different ratios of fish feed to plants. *Journal of the World Aquaculture Society*, *39*(4), 510–520. https://doi.org/10.1111/j.1749-7345.2008.00181.x

AOAC. (2005). AOCS Official Procedure AM 5-04. *Official methods of analysis of AOAC International.Vol.1 (18th ed.)* Gaithersburg, Md.: Association of official analytical chemists.

AOAC. (2012). *Official methods of analysis of AOAC International*. *Vol.1 (19th ed.).* Washington, D.C.: Association of official analytical chemists.

AOAC. (2016). *Official methods of analysis of AOAC International. Vol. 1 (20th ed.).* Washington, D.C.: Association of official Analytical Chemists (AOAC).

American Public Health Association (APHA) (1981). *Standard Methods for the Examination of Water and Wastewater. (15th ed.). Washington, D.C: American Public Health Association.*

Bailey, D. S., & Ferrarezi, R. S. (2017). Valuation of vegetable crops produced in the UVI commercial aquaponic system. *Aquaculture Reports*, *7*, 77–82. https://doi.org/10.1016/j.aqrep.2017.06.002

Baker, A. (2010). Preliminary development and evaluation of an aquaponic system for the american insular pacific. Honolulu: University of Hawaii at Manoa Retrieved from http://hdl.handle.net/10125/101649

Boyd, C. E., & Tucker, C. S. (1998). Water quality requirements. In *Pond Aquaculture Water Quality Management* (pp. 87–153). Boston, MA: Springer US. https://doi.org/10.1007/978-1-4615-5407-3_3

Bundschuh, J., Chen, G., Chandrasekharam, D., & Piechocki, J. (2017). Solar, wind and geothermal energy applications in agriculture: back to the future? *Geothermal, wind and solar energy applications in agriculture and aquaculture Vol. 13*, 3:5. Netherlands: Crc Press-Taylor & Francis Group. Retrieved from https://doi.org/10.1201/9781315158969

Buzby, K. M., & Lin, L.-S. (2014). Scaling aquaponic systems: Balancing plant uptake with fish output. *Aquacultural Engineering*, *63*, 39–44. https://doi.org/10.1016/j.aquaeng.2014.09.002

CAPMAS. (2019). Egypt Statistics - CAPMAS. Retrieved March 12, 2019, fromhttps://www.capmas.gov.eg/Pages/ShowPDF.aspx?page_id=https://capmastat.capmas.gov.eg/

devinfo/libraries/aspx/Home.aspx

Carvalho, R. da S. C., Bastos, R. G., & Souza, C. F. (2018). Influence of the use of wastewater on nutrient absorption and production of lettuce grown in a hydroponic system. *Agricultural Water Management*, *203*, 311–321. https://doi.org/10.1016/j.agwat.2018.03.028

Cerozi, B. da S., & Fitzsimmons, K. (2016). The effect of pH on phosphorus availability and speciation in an aquaponics nutrient solution. *Bioresource Technology*, *219*, 778–781. https://doi.org/10.1016/j.biortech.2016.08.079

Cerozi, B. da S., & Fitzsimmons, K. (2017). Effect of dietary phytase on phosphorus use efficiency and dynamics in aquaponics. *Aquaculture International*, *25*(3), 1227–1238. https://doi.org/10.1007/s10499-016-0109-7

Daudpota, A. M., Abbas, G., Kalhoro, H., Shah, S. A., Ferrando, S., Gallus, L., … Hafeez-ur-Rehman, M. (2016). Comparison of growth, feed conversion and body composition of juvenile hybrid Red Tilapia (*Oreochromis niloticus x O. mossambicus*) and Nile Tilapia reared in concrete tanks. *Pakistan Journal of Zoology*, *48*(3), 809–816.

Delaide, B., Delhaye, G., Dermience, M., Gott, J., Soyeurt, H., & Jijakli, M. H. (2017). Plant and fish production performance, nutrient mass balances, energy and water use of the PAFF Box, a small-scale aquaponic system. *Aquacultural Engineering*, *78*, 130–139. https://doi.org/10.1016/j.aquaeng.2017.06.002

Delaide, B., Goddek, S., Gott, J., Soyeurt, H., & Jijakli, M. H. (2016). Lettuce (*Lactuca sativa L. var. sucrine*) growth performance in complemented aquaponic solution outperforms hydroponics. *Water*, *8*(10), 1–11. https://doi.org/10.3390/w8100467

Diem, N. T. T., Konnerup, D., & Brix, H. (2017). Effects of recirculation rates on water quality and *Oreochromis niloticus* growth in aquaponic systems. *Aquacultural Engineering*, *78*, 95–104. https://doi.org/10.1016/j.aquaeng.2017.05.002

DIFCO. (1985). *DIFCO manual : dehydrated culture media and reagents for microbiology*. Detroit, Mich.: DIFCO Laboratories.

Diver, S. (2006). Aquaponics - Integration of Hydroponics with Aquaculture. *National Center for Appropriate Technology*. Retrieved from https://attra.ncat.org/attra-pub/summaries/summary.php?pub=56

Effendi, H, Utomo, B. A., & Darmawangsa, G. M. (2015). Phytoremediation of freshwater crayfish (*Cherax quadricarinatus*) culture wastewater with spinach (*Ipomoea aquatica*) in aquaponic system. *AACL Bioflux*, *8*(3), 421–430. Retrieved from http://www.bioflux.com.ro/docs/2015.421-430.pdf

Effendi, Hefni, Wahyuningsih, S., & Wardiatno, Y. (2017). The use of nile tilapia (*Oreochromis niloticus*) cultivation wastewater for the production of romaine lettuce (*Lactuca sativa L. var. longifolia*) in water recirculation system. *Applied Water Science*, *7*(6), 3055–3063. https://doi.org/10.1007/s13201-016-0418-z

Egyptian Ministry of Planning Monitoring and Adminstrative Reform (EMPMAR). (2016). *Sustainable Development Strategy: Egypt 2030 Vision*. Cairo. Retrieved from http://sdsegypt2030.com/wp-content/uploads/2016/10/10.-Environment-Pillar.pdf

Egyptian Ministry of Planning Monitoring and Adminstrative Reform (EMPMAR). (2018). Egypt′s voluntary national review 2018. Cairo. Retrieved fromhttps://sustainabledevelopment.un.org/content/documents/20269EGY_VNR_2018_final_with_Hyperlink_9720185b45d.pdf

El-Marsafawy, S. M., Swelam, A., & Ghanem, A. (2018). Evolution of crop water productivity in the Nile Delta over three decades (1985–2015). *Water, 10*(9), 1168. https://doi.org/10.3390/w10091168

El Essawy, H. (2018). Aquaponics as a sustainable alternative to new land reclamation and conventional agriculture in egypt. The American University in Cairo. Retrieved from http://dar.aucegypt.edu/handle/10526/5281

Eltholth, M., Fornace, K., Grace, D., Rushton, J., & Häsler, B. (2018). Assessing the chemical and microbiological quality of farmed tilapia in Egyptian fresh fish markets. *Global Food Security*, *17*, 14–20. https://doi.org/10.1016/j.gfs.2018.03.003

Endut, A., Jusoh, A., Ali, N., Wan Nik, W. B., & Hassan, A. (2010). A study on the optimal hydraulic loading rate and plant ratios in recirculation aquaponic system. *Bioresource Technology*, *101*(5), 1511–1517. https://doi.org/10.1016/j.biortech.2009.09.040

Estrada-Perez, N., Hernandez-Llamas, A., M. J. Ruiz-Velazco, J., Zavala-Leal, I., Romero-Bañuelos, C. A., Cruz-Crespo, E., … Campos-Mendoza, A. (2018). Stochastic modelling of aquaponic production of tilapia (*Oreochromis niloticus*) with lettuce (*Lactuca sativa*) and cucumber (*Cucumis sativus*). *Aquaculture Research*, *49*(12), 3723–3734. https://doi.org/10.1111/are.13840

FAO (2018). Progress on level of water stress - Global baseline for SDG 6 Indicator 6.4.2 2018. Rome. FAO/UN-Water. 58 pp. License: CC BYNC-SA 3.0 IGO

Fry, J. P., Mailloux, N. A., Love, D. C., Milli, M. C., & Cao, L. (2018). Feed conversion efficiency in aquaculture: Do we measure it correctly? *Environmental Research Letters*, *13*(2). https://doi.org/10.1088/1748-9326/aaa273

Goddek, S., Delaide, B., Mankasingh, U., Ragnarsdottir, K. V., Jijakli, H., & Thorarinsdottir, R. (2015). Challenges of sustainable and commercial aquaponics. *Sustainability, 7*(4), 4199–4224. https://doi.org/10.3390/su7044199

Goddek, S., & Vermeulen, T. (2018). Comparison of *Lactuca sativa* growth performance in conventional and RAS-based hydroponic systems. *Aquaculture International*, *26*(6), 1377–1386. https://doi.org/10.1007/s10499-018-0293-8

Hollmann, R. E. (2017). An aquaponics life cycle assessment: Evaluating an innovative method for growing local fish and lettuce. Colorado: University of Colorado at Denver, United States. Retrieved from http://digital.auraria.edu/content/AA/00/00/60/47/00001/Hollmann_ucdenver_0765N_10813.pdf

Hu, Z., Lee, J. W., Chandran, K., Kim, S., Brotto, A. C., & Khanal, S. K. (2015). Effect of plant species on nitrogen recovery in aquaponics. *Bioresource Technology*, *188*, 92–98. https://doi.org/10.1016/j.biortech.2015.01.013

J. Danaher, J., R. Shultz, C., E. Rakocy, J., Bailey, D., & Knight, L. (2011). Effect of a parabolic screen filter on water quality and production of Nile Tilapia (*Oreochromis niloticus*) and water spinach (*Ipomoea aquatica*) in a recirculating raft aquaponic system. *International Journal of Recirculating Aquaculture Vol. 12*. https://doi.org/10.21061/ijra.v12i1.1353

Kamal, S. M. (2006). Aquaponic production of nile tilapia (*Oreochromis niloticus*) and bell pepper (*Capsicum annuuml.*) In recirculating water system. *Egyptian Journal of Aquatic Biology and Fisheries*, *10*(3), 79–85. https://doi.org/10.21608/ejabf.2006.1864

Klinger, D., & Naylor, R. (2012). Searching for solutions in aquaculture: Charting a sustainable course. *Annual Review of Environment and Resources*, *37*(1), 247–276. https://doi.org/10.1146/annurev-environ-021111-161531

Kloas, W., Groß, R., Baganz, D., Graupner, J., Monsees, H., Schmidt, U., … Rennert, B. (2015). A new concept for aquaponic systems to improve sustainability, increase productivity, and reduce environmental impacts. *Aquaculture Environment Interactions*, *7*(2). https://doi.org/10.3354/aei00146

Knaus, U., & Palm, H. W. (2017). Effects of the fish species choice on vegetables in aquaponics under spring-summer conditions in northern Germany (Mecklenburg Western Pomerania). *Aquaculture*, *473*, 62–73. https://doi.org/10.1016/j.aquaculture.2017.01.020

Lam, S. S., Ma, N. L., Jusoh, A., & Ambak, M. A. (2015). Biological nutrient removal by recirculating aquaponic system: Optimization of the dimension ratio between the hydroponic & rearing tank components. *International Biodeterioration & Biodegradation*, *102*, 107–115. https://doi.org/10.1016/j.ibiod.2015.03.012

Lennard, W. A., & Leonard, B. V. (2004). A comparison of reciprocating flow versus constant flow in an integrated, gravel bed, aquaponic test system. *Aquaculture International*, *12*(6), 539–553. https://doi.org/10.1007/s10499-004-8528-2

Lennard, W. A., & Leonard, B. V. (2005). A comparison of reciprocating flow versus constant flow in an integrated, gravel bed, aquaponic test system. *Aquaculture International*, *12*(6), 539–553. https://doi.org/10.1007/s10499-005-8528-x

Lennard, W. A., & Leonard, B. V. (2006). A comparison of three different hydroponic sub-systems (gravel bed, floating and nutrient film technique) in an aquaponic test system. *Aquaculture International*, *14*(6), 539–550. https://doi.org/10.1007/s10499-006-9053-2

Li, C., Yu, H., Tabassum, S., Li, L., Mu, Y., Wu, D., … Xu, P. (2018). Effect of calcium silicate hydrates coupled with *Myriophyllum spicatum* on phosphorus release and immobilization in shallow lake sediment. *Chemical Engineering Journal*, *331*, 462–470. https://doi.org/https://doi.org/10.1016/j.cej.2017.08.134

Li, C., Yu, H., Tabassum, S., Li, L., Wu, D., Zhang, Z., … Xu, P. (2017). Effect of calcium silicate hydrates (CSH) on phosphorus immobilization and speciation in shallow lake sediment. *Chemical Engineering Journal*, *317*, 844–853. https://doi.org/https://doi.org/10.1016/j.cej.2017.02.117

Li, C., Zhang, B., Luo, P., Shi, H., Li, L., Gao, Y., … Wu, W.-M. (2019). Performance of a pilot-scale aquaponics system using hydroponics and immobilized biofilm treatment for water quality control. *Journal of Cleaner Production*, *208*, 274–284. https://doi.org/10.1016/J.JCLEPRO.2018.10.170

Love, D. C., Fry, J. P., Li, X., Hill, E. S., Genello, L., Semmens, K., & Thompson, R. E. (2015). Commercial aquaponics production and profitability: Findings from an international survey. *Aquaculture*, *435*, 67–74. https://doi.org/10.1016/j.aquaculture.2014.09.023

Love, D. C., Uhl, M. S., & Genello, L. (2015). Energy and water use of a small-scale raft aquaponics system in Baltimore, Maryland, United States. *Aquacultural Engineering*, *68*, 19–27. https://doi.org/10.1016/j.aquaeng.2015.07.003

Martins, E. A. S., Geisenhoff, L. O., Ribeiro, E. F., Oliveira, F. C. de, & Jordan, R. A. (2018). Yield of lettuce grown in hydroponic and aquaponic systems using different substrates. *Revista Brasileira de Engenharia Agrícola e Ambiental*, *22*(8), 525–529. https://doi.org/10.1590/1807-1929/agriambi.v22n8p525-529

Martir-Torres, M. C., & Bruns, M. A. (2013). Comparative diversity and abundance of ammonia monooxygenase genes in mulched and vegetated soils. *Soil Biology and Biochemistry*, *57*, 758–768. https://doi.org/10.1016/J.SOILBIO.2012.10.016

Maucieri, C., Forchino, A. A., Nicoletto, C., Junge, R., Pastres, R., Sambo, P., & Borin, M. (2018). Life cycle assessment of a micro aquaponic system for educational purposes built using recovered material. *Journal of Cleaner Production*, *172*, 3119–3127. https://doi.org/10.1016/j.jclepro.2017.11.097

Medina, M., Jayachandran, K., Bhat, M. G., & Deoraj, A. (2016). Assessing plant growth, water quality and economic effects from application of a plant-based aquafeed in a recirculating aquaponic system.

Aquaculture International, *24*(1), 415–427. https://doi.org/10.1007/s10499-015-9934-3

Mohanty, R. K., Ambast, S. K., Panda, D. K., Thakur, A. K., & Mohanty, S. (2017). Density-dependent water use in carp polyculture: Impacts on production performance and water productivity. *Aquaculture*, *470*, 32–39. https://doi.org/10.1016/j.aquaculture.2016.12.007

Mohanty, R. K., Ambast, S. K., Panigrahi, P., & Mandal, K. G. (2018). Water quality suitability and water use indices: Useful management tools in coastal aquaculture of Litopenaeus vannamei. *Aquaculture*, *485*, 210–219. https://doi.org/10.1016/j.aquaculture.2017.11.048

Monsees, H., Kloas, W., & Wuertz, S. (2017). Decoupled systems on trial: Eliminating bottlenecks to improve aquaponic processes. *PLOS ONE*, *12*(9), 1–18. https://doi.org/10.1371/journal.pone.0183056

Mullins, C., Nerrie, B., & Sink, T. D. (2015). *Principles of Small-Scale Aquaponics. Southern Regional Aquaculture Center*, *5007*, 8. Retrieved from http://files/714/Mullins and Nerrie - Principles of Small-Scale Aquaponics.pdf

Nyathi, M. K., Van Halsema, G. E., Beletse, Y. G., Annandale, J. G., & Struik, P. C. (2018). Nutritional water productivity of selected leafy vegetables. *Agricultural Water Management*, *209*, 111–122. https://doi.org/10.1016/j.agwat.2018.07.025

Oladimeji, A. S., Olufeagba, S. O., Ayuba, V. O., Sololmon, S. G., & Okomoda, V. T. (2018). Effects of different growth media on water quality and plant yield in a catfish-pumpkin aquaponics system. *Journal of King Saud University - Science*. https://doi.org/10.1016/j.jksus.2018.02.001

Palm, H., Bissa, K., & Knaus, U. (2014). Significant factors affecting the economic sustainability of closed aquaponic systems. Part II: fish and plant growth. *Aquaculture, Aquarium, Conservation & Legislation*, *7*(3), 162–175.

Piedrahita, R. H. (2003). Reducing the potential environmental impact of tank aquaculture effluents through intensification and recirculation. *Aquaculture*, *226*(1), 35–44. https://doi.org/10.1016/S0044-8486(03)00465-4

Pinho, S. M., Mello, G. L. de, Fitzsimmons, K. M., & Emerenciano, M. G. C. (2018). Integrated production of fish *(*p*acu Piaractus mesopotamicus* and Red Tilapia *Oreochromis sp.*) with two varieties of garnish (scallion and parsley) in aquaponics system. *Aquaculture International*, *26*(1), 99–112. https://doi.org/10.1007/s10499-017-0198-y

Pinho, S. M., Molinari, D., de Mello, G. L., Fitzsimmons, K. M., & Coelho Emerenciano, M. G. (2017). Effluent from a biofloc technology (BFT) tilapia culture on the aquaponics production of different lettuce varieties. *Ecological Engineering*, *103*, 146–153. https://doi.org/10.1016/j.ecoleng.2017.03.009

Rakocy, J E, Shultz, R. C., Bailey, D., & Thoman, E. S. (2004). Aquaponic production of tilapia and basil: Comparing a batch and staggered cropping system. *Acta Horticulturae* (Vol. 648). https://doi.org/10.17660/ActaHortic.2004.648.8

Rakocy, James E, Masser, M. P., & Losordo, T. M. (2006). Recirculating Aquaculture Tank Production Systems: Aquaponics—Integrating Fish and Plant Culture. *Southern Regional Aquaculture Center (SRAC)*, *SRAC Publication ID: 454* . Retrieved from https://www.ncrac.org/files/biblio/SRAC0454.pdf

Resh, H. M. (2016). *Hydroponic food production : a definitive guidebook for the advanced home gardener and the commercial hydroponic grower* (7th ed.). Baton Rouge: CRC Press.

Sace, C. F., & Fitzsimmons, K. M. (2013). Vegetable production in a recirculating aquaponic system using Nile tilapia (*Oreochromis niloticus*) with and without freshwater prawn (*Macrobrachium rosenbergii*). *Academia Journal of Agricultural Research*, *1*(12), 236–250. Retrieved from https://www.semanticscholar.org/paper/Vegetable-production-in-a-recirculating-aquaponic-(-Sace-Fitzsimmons/c3a2c2500f5cbe42e6b5a5e06622fffac9a68ff4

Saha, S., Monroe, A., & Day, M. R. (2016). Growth, yield, plant quality and nutrition of basil (*Ocimum basilicum L.*) under soilless agricultural systems. *Annals of Agricultural Sciences*, *61*(2), 181–186. https://doi.org/10.1016/j.aoas.2016.10.001

Salama, F. M., Abd El-ghani, M. M., Amro, A. A. E. R., & Gaafar, A. E. S. (2018). Vegetation dynamics and species diversity in a Saharan Oasis, Egypt. *Notulae Scientia Biologicae*, *10*(3). https://doi.org/10.15835/nsb10310296

Shete, A. P., Verma, A. K., Chadha, N. K., Prakash, C., Chandrakant, M. H., & Nuwansi, K. K. T. (2017). Evaluation of different hydroponic media for mint (*Mentha arvensis*) with common carp (*Cyprinus carpio*) juveniles in an aquaponic system. *Aquaculture International*, *25*(3), 1291–1301. https://doi.org/10.1007/s10499-017-0114-5

Sikawa, D. C., & Yakupitiyage, A. (2010). The hydroponic production of lettuce (*Lactuca sativa L*) by using hybrid catfish (*Clarias macrocephalus* × *C. gariepinus*) pond water: Potentials and constraints. *Agricultural Water Management*, *97*(9), 1317–1325. https://doi.org/10.1016/J.AGWAT.2010.03.013

Silber, A., Xu, G., Levkovitch, I., Soriano, S., Bilu, A., & Wallach, R. (2003). High fertigation frequency: the effects on uptake of nutrients, water and plant growth. *Plant and Soil*, *253*(2), 467–477. https://doi.org/10.1023/A:1024857814743

Silva, L., Valdés-Lozano, D., Escalante, E., & Gasca-Leyva, E. (2018). Dynamic root floating technique: An option to reduce electric power consumption in aquaponic systems. *Journal of Cleaner Production*, *183*, 132–142. https://doi.org/10.1016/j.jclepro.2018.02.086

Siriwardena, S., & Hasan, M. R. (2009). *Impact of rising feed ingredient prices on aquafeeds and* *aquaculture production*. Rome: Food and Agriculture Organization of the United Nations.

Somerville, C., Cohen, M., Pantanella, E., Stankus, A., & Lovatelli, A. (2014). *Small-scale aquaponic food production : integrated fish and plant farming*. FAO Fisheries and Aquaculture Technical Paper No.589. Rome: Food and Agriculture Organization of the United Nations. 262 pp.

Suhl, J., Dannehl, D., Kloas, W., Baganz, D., Jobs, S., Scheibe, G., & Schmidt, U. (2016). Advanced aquaponics: Evaluation of intensive tomato production in aquaponics vs. conventional hydroponics. *Agricultural Water Management*, *178*, 335–344. https://doi.org/10.1016/j.agwat.2016.10.013

Tabassum, S., Li, Y., Chi, L., Li, C., & Zhang, Z. (2018). Efficient nitrification treatment of comprehensive industrial wastewater by using Novel Mass Bio System. *Journal of Cleaner Production*, *172*, 368–384. https://doi.org/https://doi.org/10.1016/j.jclepro.2017.10.022

Thippayarugs, S., Suzuki, K., Katsuta, Y., Yoshida, A., Matsumoto, N., Kabaki, N., & Wongwiwatchai, C. (January 01, 2001). Vegetable Production Using Energy-Saving Hydroponics Systems in Northeast Thailand. Jircas Journal for Scientific Papers, 33-38.

Thorarinsdottir, R. I. (2015). *Aquaponics guidelines*. Brusseles: Eco-innovation Initiative of the European Union. Retrieved from http://hdl.handle.net/1946/23343

Trang, N. T. D., Schierup, H.-H., & Brix, H. (2010). Leaf vegetables for use in integrated hydroponics and aquaculture systems: Effects of root flooding on growth, mineral composition and nutrient uptake. *African Journal of Biotechnology*, *9*(27), 4186–4196. Retrieved from https://www.ajol.info/index.php/ajb/article/view/82623

Trejchel, K., Żarski, D., Palińska-Żarska, K., Krejszeff, S., Dryl, B., Dakowski, K., & Kucharczyk, D. (2014). Determination of the optimal feeding rate and light regime conditions in juvenile burbot, *Lota lota (L.)*, under intensive aquaculture. *Aquaculture International*, *22*(1), 195–203. https://doi.org/10.1007/s10499-013-9670-5

Tschimer, M., & Kloas, W. (2017). Increasing the sustainability of aquaculture systems: Insects as alternative protein source for fish diets. *GAIA: Ecological Perspectives for Science & Society*, *26*(4), 332–340. Retrieved from http:doi.org//10.0.56.176/gaia.26.4.10

Tyson, R. V, Treadwell, D. D., & Simonne, E. H. (2011). Opportunities and Challenges to Sustainability in Aquaponic Systems. *HortTechnology*, *21*(1), 6–13. https://doi.org/10.21273/HORTTECH.21.1.6.

UN-Water (2018). Water, Food and Energy. *UN-Water*. Retrieved from http://www.unwater.org/water-facts/water-food-and-energy/

United Nations (UN) (2018a). *Sustainable Development Goals : Final Report 2018*. New York: United Nations.

United Nations (UN) (2018b). Sustainable Development Goal 6 Synthesis Report 2018 on Water and Sanitation. New York.

UN DESA. (2017). *World Population Prospects: The 2017 Revision, Key Findings and Advance Tables* (No. ESA/P/WP/248).

Vita. (1997). HPLC method No. Af 255.1. *Danish food monitoring programme* (3rd ed.). National Food Agency of Denmark.

Wahba, M. A. S. (2017). Assessment of options for the sustainable use of agricultural drainage water for irrigation in Egypt by simulation modelling. *Irrigation and Drainage*, *66*(1), 118–128. https://doi.org/10.1002/ird.2029

Wongkiew, S., Hu, Z., Chandran, K., Lee, J. W., & Khanal, S. K. (2017). Nitrogen transformations in aquaponic systems: A review. *Aquacultural Engineering*, *76*, 9–19. https://doi.org/10.1016/j.aquaeng.2017.01.004

World Water Assessment Programme (WWAP) and UN-Water. (2014). *The United Nations world water development report 2014*. Paris: United Nations Educational, Scientific and Cultural Organization.

Yoshida, S., Kitano, M., & Eguchi, H. (1997). Growth of lettuce plants (Lactuca sativa L.) under control of dissolved O2 concentration in hydroponics. Biotronics, 26, 39–45. Retrieved from http://www.scopus.com/inward/record.url?scp=0031447804&partnerID=8YFLogxK

Zou, Yina, Hu, Z., Zhang, J., Fang, Y., Li, M., & Zhang, J. (2017). Mitigation of N_2O emission from aquaponics by optimizing the nitrogen transformation process: Aeration management and exogenous carbon (*PLA*) addition. *Journal of Agricultural and Food Chemistry*, *65*(40), 8806–8812. https://doi.org/10.1021/acs.jafc.7b03211

Zou, Yina, Hu, Z., Zhang, J., Xie, H., Guimbaud, C., & Fang, Y. (2016). Effects of pH on nitrogen transformations in media-based aquaponics. *Bioresource Technology*, *210*, 81–87. https://doi.org/10.1016/j.biortech.2015.12.079

Zou, Yina, Hu, Z., Zhang, J., Xie, H., Liang, S., Wang, J., & Yan, R. (2016). Attempts to improve nitrogen utilization efficiency of aquaponics through nitrifies addition and filler gradation. *Environmental Science and Pollution Research*, *23*(7), 6671–6679. https://doi.org/10.1007/s11356-015-5898-0

Zou, Yuanchun, Duan, X., Xue, Z., E, M., Sun, M., Lu, X., … Yu, X. (2018). Water use conflict between wetland and agriculture. *Journal of Environmental Management*, *224*, 140–146. https://doi.org/10.1016/j.jenvman.2018.07.052

Appendices

Appendix I: Pilot Cycle

A. Purpose of Pilot cycle

The purpose of the pilot cycle was to test the system dynamics in terms of water flow, water quality, and crops and fish growth response. The tested fish and crops were Nile tilapia fish (*Oreochromis niloticus*) and different varieties of lettuce (*Lactuca sativa variety capitata* Type Batavia and Type Butterhead) and Spinach (*Spinacia oleracea* Type Baby Spinach). The systems' setup was similar to the one used in the study's experiment (**Figure 1** and **Figure 2**)

B. Experimental Set-Up

On 20 November 2017, all DWC and Sand-Bed systems' tanks and beds (including fish tanks, mechanical and biological filters, plant and collection beds) were filled with tap water, aerated and left for 10 days to remove any chlorine and prepare the environment for fish loading. On December 20th 2017, seeds (Batavia lettuce and Butterhead Lettuce) were bought from Rijk Zwaan company and sent to local seedlings nursery in Alexandria Road. The Batavia seeds specifications are OTHILIE RZ (80-11) with High Resistance (HR): Bl:16-32,34EU/Nr:0/Pb and Intermediate Resistance (IR): LMV:1/Fol:1. The Butterhead seeds specifications are HR: Bl:16-22,24,25,29-35EU/Nr:0/Pb IR: LMV:1. Baby Spinach seeds specifications are PYTHON RZ F1 (51-106) with HR: CMV/Pfs:1-7,9,11,13,15,16. For Baby Spinach, the seeds will be planted immediately into the DWC and Sand-Bed systems. On 25 December 2017, 150 Nile Tilapia fish were loaded into each fish tank for DWC and Sand-Bed systems. The fish were loaded into tanks and fed on daily basis for two months to adapt for the new environment.

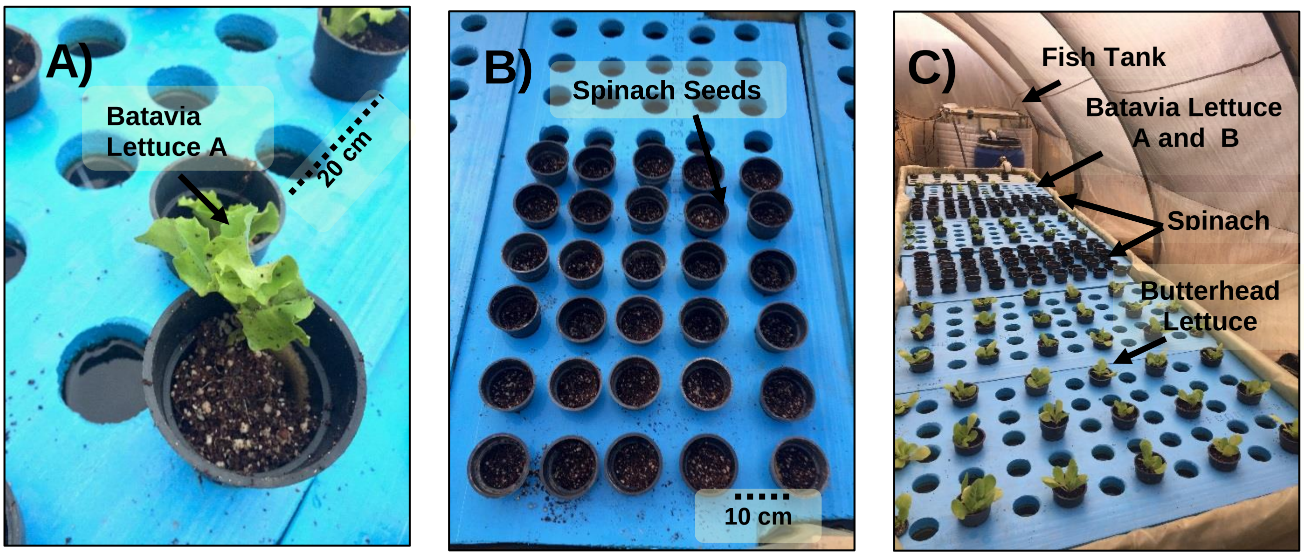
On January 25th 2018, seedlings were 35 days old and mature for planting in both systems. Number of seedlings for Batavia Lettuce was not enough for both systems designed sample. Other seedlings for Batavia Lettuce were brought to offset the number. The seedlings were not identical and had two Batavia types. For this reason, initial Batavia lettuce type is named Batavia Lettuce 1 and second Batavia lettuce type is named Batavia Lettuce 2. The objective of the pilot was to identify the optimum crops for cycle 2, potential threats, maximum productivity from system i.e. maximum crops per square meters, and knowledge for running aquaponics systems.

For the pilot cycle, the growth period was during the winter season for two months; crops were planted on January 25th, 2018 and harvested on March 25th, 2018. Hence, the design was based on using same water without additional water during the cycle to assess water use and quality.

Pilot Cycle started with 150 fish fingerlings of an average weight of 5 grams in each system. The feeding frequency was set using an automatic feeder to two times per day. The fish feeding rate is 2% of the total fish weight. By end of pilot cycle, 25% of the total number of fish was collected as a sample representative of weight.

a DWC

In the DWC system, the plant bed was initially divided into three batches (**Figure 11**). First batch near the fish tank for Batavia lettuce divided 20 cm apart, second batch for baby spinach divided 10 cm apart, and third batch for Butterhead Lettuce divided 20 cm apart. The initial design consisted of 36 Batavia Lettuce, 36 butterhead and 100 Spinach. The actual implementation consisted of 16 Batavia Lettuce 2 in the first batch after the mechanical filter, the second batch consisted of 20 Batavia Lettuce 2, third batch of 100 baby spinach and then fourth batch of 36 Butterhead Lettuce. No added minerals were sprayed over the crops as of the initial design to investigate optimum crop for the study.

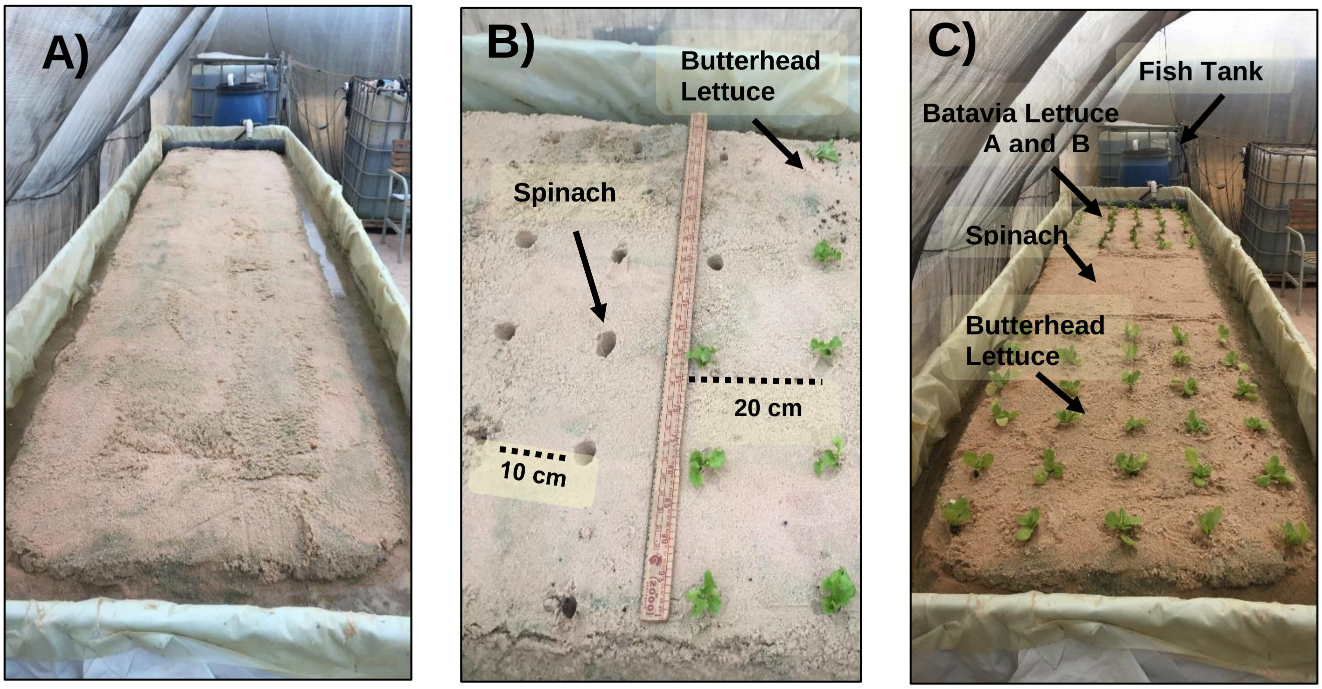


**Legend: A) An example of pot used for Batavia lettuce planting. B) Spinach seeds placed in pots in DWC styrofoam C) DWC system three batches layout, Starting the Fish tank side: Batavia Lettuce A and B, followed by Spinach, and finally by butterhead lettuce seedlings.*

Figure 11: Pilot Cycle DWC System Lettuce and Spinach Layout

b Sand-Bed

In the Sand-Bed system, the plant bed was initially divided into three batches (**Figure 12**). The first batch is near the fish tank designed for 30 Batavia lettuce at 20 cm apart, second batch for 90 Baby spinach at 10 cm apart and third batch for 30 Butterhead lettuce at 20 cm apart. The actual design consisted of 20 Batavia Lettuce A near the fish tank, then 12 Batavia Lettuce B, followed by 90 Baby Spinach and 33 Butterhead.



**Legend: A) Sand-Bed layout. B) Lettuce seedlings (green plants) and spinach seeds (holes in sands) spaced at 20 and 10 cm respectively using a ruler. C) Sand-Bed system three batches layout, Starting the Fish tank side: Batavia Lettuce A and B, followed by Spinach, and finally by butterhead lettuce seedlings.*

Figure 12: Pilot Cycle DWC System Lettuce and Spinach Layout

c Data Measurement

Examples of how plants' measurements were taken throughout cycle duration, instantaneous water physicochemical measurements and water volumes are presented in figures (**Figure 13, Figure 14, Figure 15, Figure 16, Figure 17** and **Figure 18**).

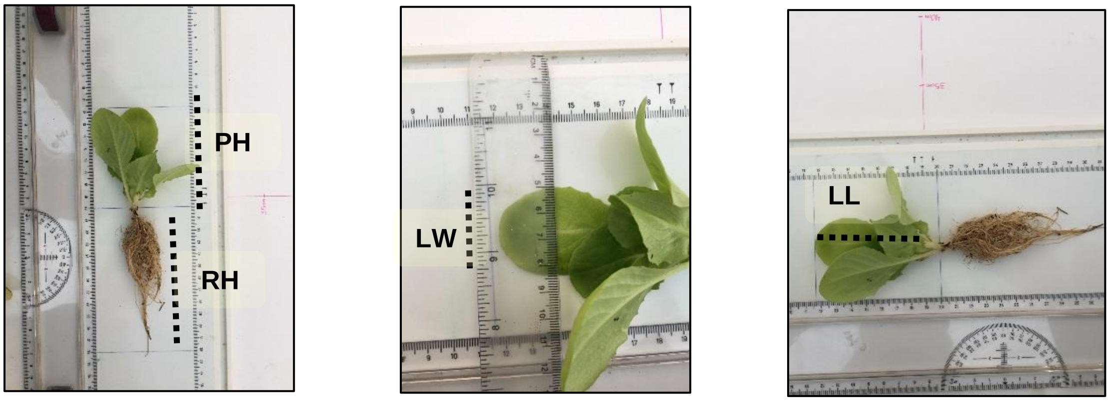


Figure 13: Example of Measuring Lettuce Seedlings Plant Height (PH), Root Length (RL), Leaf Width (LW) and Leaf Length (LL)

Figure 14: Temperature Sensor, Water Test Kit and Water submerged sensors

**Legend: a) Temperature, b) Water Test Kit for pH, NO_2^- NO_3^- and NH_4^+ and c) pH, submerged in Sand-Bed fish tank, DWC fish tank and DWC plant bed*

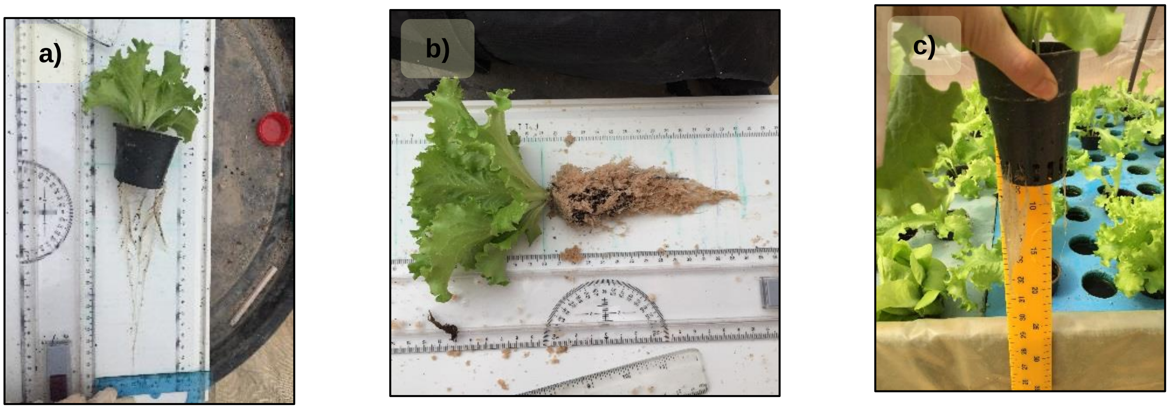


Figure 15: Example of measuring root length (RL) during third and fifth weeks

**Legend: a) DWC Batavia lettuce root length (RL) measurement during week 3. b) Sand-Bed Batavia lettuce root length (RL) measurement during week 3. c) DWC lettuce root length (RL) measurement during week 5.*

By third week, we stopped measuring Sand-Bed lettuce roots lengths as removing the lettuce back and forth from the sand media can affect its growth. For DWC lettuce, we only measured the root length form the pod and assumed halfway through the pods as the roots starting point.

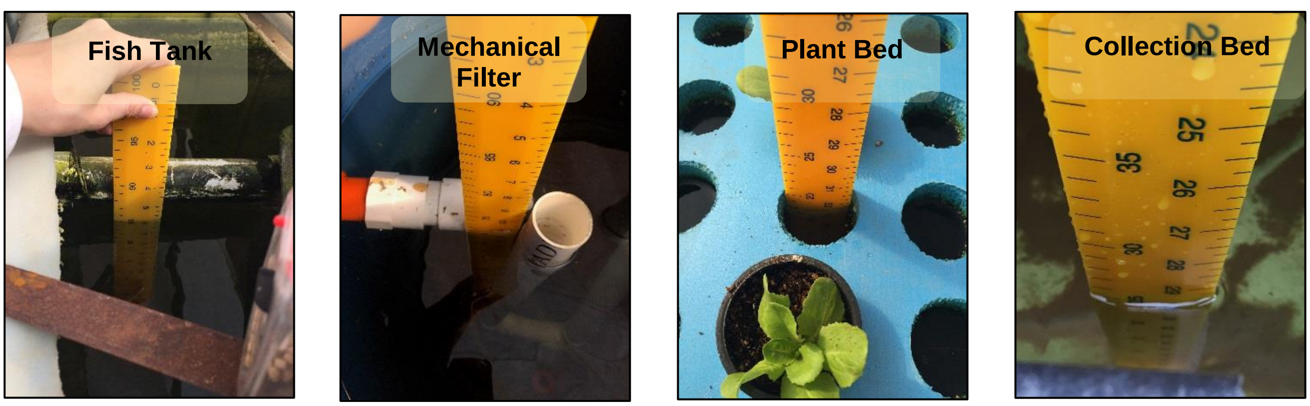


Figure 16: Example of measuring water heights in fish tanks, mechanical filters and plant and collection beds to estimate water volumes

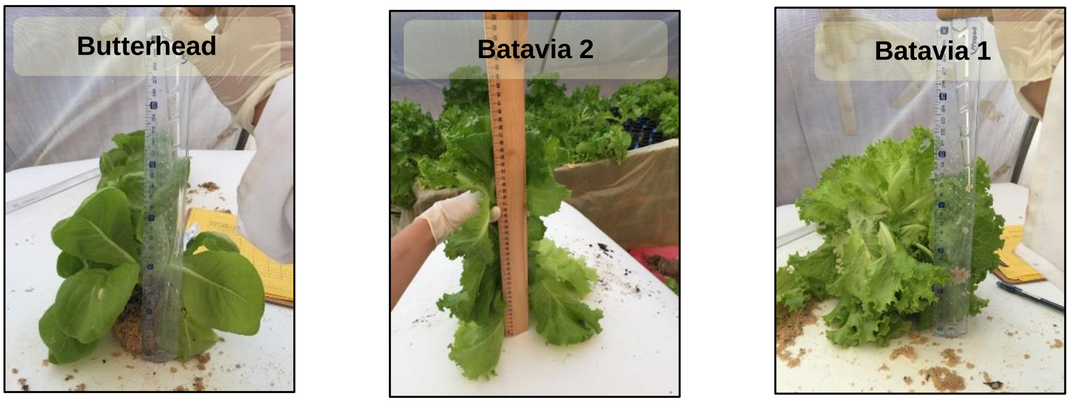


Figure 17: Example of lettuce plant height (PH) measurement at end of pilot cycle

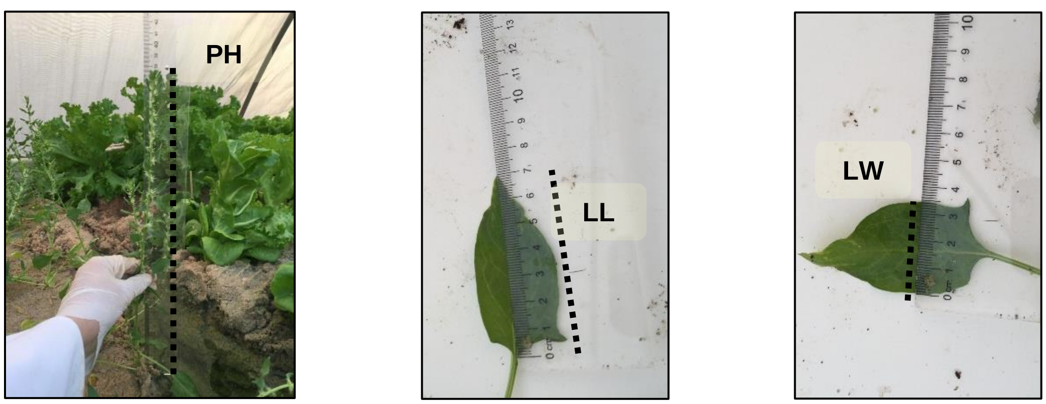


Figure 18: Example of Measuring Spinach Plant Height (PH), Leaf Width (LW) and Leaf Length (LL) at end of pilot cycle (Day 60)

C. Results

a. Water Chemical, Macro and Micro-nutrient Analysis

i. DWC and Sand-Bed Fish Tanks Comparison

Table 19: Pilot Cycle Water Chemical Parameters for DWC and Sand-Bed Fish Tanks

		DWC Fish Tank				Sand-Bed Fish Tank				
*	Unit	Mean	SD	Min	Max	Mean	SD	Min	Max	Value of p*
EC	dS/m	0.63[a]	0.05	0.58	0.70	0.96[a]	0.10	0.85	1.13	0.00
TDS	ppm	402.36[a]	31.81	368.00	449.00	612.44[a]	65.23	546.00	721.00	0.00
PH		7.91	0.27	7.50	8.20	7.82	0.11	7.70	7.90	0.51
HCO_3^-	meq./L	1.21	0.57	0.20	1.60	1.27	0.47	0.50	1.79	0.86
SO_4^{2-}	meq./L	2.42	1.05	0.69	3.36	5.02	2.42	0.90	7.18	0.06
SAR		1.47[a]	0.32	1.19	2.00	1.94[a]	1.24	1.20	4.15	0.44
NH_4^+	mg/L	0.92	0.80	0.00	2.10	1.15	0.49	0.35	1.61	0.61
NO_3^-	mg/L	9.02[a]	5.23	2.73	16.24	11.34[a]	3.91	5.60	16.52	0.45

** Legend: EC=Electric Conductivity, TDS=Total Dissolved Solids, HCO_3^-=Bicarbonate, SO_4^{2-}=Sulfate, SAR=Sodium Absorption Rate, NH_4^+=Ammonia, NO_3^-=Nitrate. All data are analyzed using SPSS one-way ANOVA where mean is the mean value, SD is the standard deviation, min is the minimum value, max is maximum value. Mean values of DWC and Sand-Bed fish tanks are significantly different when value of $p < 0.05$ noted using superscripts a.*

Table 20: Pilot Cycle Water Macro and Micro-Nutrients Parameters for DWC and Sand-Bed Fish Tanks

		DWC Fish Tank				Sand-Bed Fish Tank				
Nutrient	Unit	Mean	SD	Min	Max	Mean	SD	Min	Max	Value of p*
Na^+	meq./L	2.10	0.27	1.74	2.35	3.21	1.25	2.30	5.40	0.09
K^+	meq./L	0.22	0.12	0.07	0.35	0.24	0.08	0.11	0.32	0.75
P	mg/l	2.10	2.91	0.08	7.13	0.87	1.60	0.05	3.27	0.47
Fe	mg/l	0.22	0.21	0.04	0.55	0.23	0.17	0.06	0.46	0.91
Ca^{++}	meq./L	2.31[a]	0.61	1.58	2.89	4.85[a]	1.56	2.40	6.58	0.01
Mg^{++}	meq./L	1.94	0.92	0.95	3.11	1.88	0.82	1.00	3.18	0.91
Cl^-	meq./L	2.93	0.80	2.37	4.31	3.89	2.14	2.54	7.60	0.37

** Legend: Na^+=Sodium ion, K^+=Potassium ion, P=Phosphorous, Fe=Iron, Ca^{++}=Calcium ion, Mg^{++}=Magnesium ion, Cl^- =chlorine ion. All data are analyzed using SPSS one-way ANOVA where mean is the mean value, SD is the standard deviation, min is the minimum value, max is maximum value. Mean values of DWC and Sand-Bed fish tanks are significantly different when value of $p < 0.05$ noted using superscripts a.*

ii. Pilot Cycle DWC and Sand-Bed Collection Tanks Comparison

Table 21: Pilot Cycle Water Chemical Parameters for DWC and Sand-Bed Collection Tanks

		DWC Collection Tank				Sand-Bed Collection Tank				
*	Unit	Mean	SD	Min	Max	Mean	SD	Min	Max	Value of p*
EC	dS/m	0.64[a]	0.05	0.58	0.69	0.97[a]	0.11	0.89	1.13	0.00
TDS	ppm	406.45[a]	30.03	368.00	441.00	621.45[a]	68.76	566.00	721.00	0.00
PH		7.93	0.22	7.70	8.20	7.81	0.12	7.70	7.93	0.39
HCO_3^-	meq./L	1.51	0.16	1.32	1.70	1.44	0.24	1.32	1.79	0.63
SO_4^{2-}	meq./L	2.80[a]	0.35	2.33	3.12	6.05[a]	0.83	5.20	7.18	0.00
SAR		1.36	0.16	1.19	1.52	1.37	0.14	1.20	1.53	0.91
NH_4^+	mg/L	0.82	0.48	0.28	1.40	1.23	0.20	1.05	1.40	0.17
NO_3^-	mg/L	9.66	5.51	2.12	14.20	14.13	8.99	5.81	26.11	0.43

** Legend: EC=Electric Conductivity, TDS=Total Dissolved Solids, HCO_3^-=Bicarbonate, SO_4^{2-}=Sulfate, SAR=Sodium Absorption Rate, NH_4^+=Ammonia, NO_3^-=Nitrate. All data are analyzed using SPSS one-way ANOVA where mean is the mean value, SD is the standard deviation, min is the minimum value, max is maximum value. Mean values of DWC and Sand-Bed collection tanks are significantly different when value of $p < 0.05$ noted using superscripts a.*

Table 22: Pilot Cycle Water Macro and Micro-Nutrients Parameters for DWC and Sand-Bed Collection Tanks

		DWC Fish Collection Tank				Sand-Bed Collection Tank				
Nutrient	Unit	Mean	SD	Min	Max	Mean	SD	Min	Max	Value of p*
Na^+	meq./L	2.08[a]	0.32	1.74	2.37	2.65[a]	0.31	2.30	3.00	0.04
K^+	meq./L	0.25	0.13	0.06	0.35	0.24	0.09	0.11	0.32	0.93
P	mg/l	2.45[a]	3.62	0.08	7.74	0.84[s]	1.55	0.02	3.17	0.45
Fe	mg/l	0.25	0.21	0.03	0.54	0.13	0.08	0.04	0.20	0.32
Ca^{++}	meq./L	2.30[a]	0.45	1.84	2.89	5.40[a]	0.90	4.47	6.58	0.00
Mg^{++}	meq./L	2.25	0.70	1.51	3.13	2.18	0.68	1.67	3.18	0.88
Cl^-	meq./L	2.54	0.57	1.86	3.22	2.97	0.63	2.54	3.90	0.36

** Legend: Na^+=Sodium ion, K^+=Potassium ion, P=Phosphorous, Fe=Iron, Ca^{++}=Calcium ion, Mg^{++}=Magnesium ion, Cl^-=chlorine ion. All data are analyzed using SPSS one-way ANOVA where mean is the mean value, SD is the standard deviation, min is the minimum value, max is maximum value. Mean values of DWC and Sand-Bed collection tanks are significantly different when value of $p < 0.05$ noted using superscripts a.*

iii. Pilot Cycle DWC Fish and Collection Tanks Comparison

Table 23: Pilot Cycle Water Chemical Parameters for DWC Fish and Collection Tanks

		DWC Fish Tank				DWC Collection Tank					DWC
*	Unit	Mean	SD	Min	Max	Mean	SD	Min	Max	Value of p*	Total Mean
EC	dS/m	0.63	0.05	0.58	0.70	0.64	0.05	0.58	0.69	0.83	0.63
TDS	ppm	402.36	31.81	368.00	449.00	406.45	30.03	368.00	441.00	0.85	404.18
PH		7.91	0.27	7.50	8.20	7.93	0.22	7.70	8.20	0.91	7.91
HCO_3^-	meq./L	1.21	0.57	0.20	1.60	1.51	0.16	1.32	1.70	0.35	1.34
SO_4^{2-}	meq./L	2.42	1.05	0.69	3.36	2.80	0.35	2.33	3.12	0.52	2.59
SAR		1.47	0.32	1.19	2.00	1.36	0.16	1.19	1.52	0.54	1.42
NH_4^+	mg/L	0.92	0.80	0.00	2.10	0.82	0.48	0.28	1.40	0.83	0.88
NO_3^-	mg/L	9.02	5.23	2.73	16.24	9.66	5.51	2.12	14.20	0.87	9.30

** Legend: EC=Electric Conductivity, TDS=Total Dissolved Solids, HCO_3^-=Bicarbonate, SO_4^{2-}=Sulfate, SAR=Sodium Absorption Rate, NH_4^+=Ammonia, NO_3^-=Nitrate. All data are analyzed using SPSS one-way ANOVA where mean is the mean value, SD is the standard deviation, min is the minimum value, max is maximum value. Mean values of DWC fish and collection tanks are significantly different when value of $p < 0.05$ noted using superscripts a.*

Table 24: Pilot Cycle Water Macro and Micro-Nutrients Parameters for DWC Fish and Collection Tanks

		DWC Fish Tank				DWC Collection Tank					DWC
*	Unit	Mean	SD	Min	Max	Mean	SD	Min	Max	Value of p*	Total Mean
Na^+	meq./L	2.10	0.27	1.74	2.35	2.08	0.32	1.74	2.37	0.92	2.09
K^+	meq./L	0.22	0.12	0.07	0.35	0.25	0.13	0.06	0.35	0.74	0.23
P	mg/l	2.10	2.91	0.08	7.13	2.45	3.62	0.08	7.74	0.88	2.26
Fe	mg/l	0.22	0.21	0.04	0.55	0.25	0.21	0.03	0.54	0.81	0.23
Ca^{++}	meq./L	2.31	0.61	1.58	2.89	2.30	0.45	1.84	2.89	0.99	2.30
Mg^{++}	meq./L	1.94	0.92	0.95	3.11	2.25	0.70	1.51	3.13	0.59	2.08
Cl^-	meq./L	2.93	0.80	2.37	4.31	2.54	0.57	1.86	3.22	0.44	2.76

** Legend: Na^+=Sodium ion, K^+=Potassium ion, P=Phosphorous, Fe=Iron, Ca^{++}=Calcium ion, Mg^{++}=Magnesium ion, Cl^-=chlorine ion. All data are analyzed using SPSS one-way ANOVA where mean is the mean value, SD is the standard deviation, min is the minimum value, max is maximum value. Mean values of DWC fish and collection tanks are significantly different when value of $p < 0.05$ noted using superscripts a.*

iv. Pilot Cycle Sand-Bed Fish and Collection Tanks Comparison

Table 25: Pilot Cycle Water Chemical Parameters for Sand-Bed Fish and Collection Tanks

		Sand-Bed Fish Tank				Sand-Bed Collection Tank					Sand-Bed
*	Unit	Mean	SD	Min	Max	Mean	SD	Min	Max	Value of p*	Total Mean
EC	dS/m	0.96	0.10	0.85	1.13	0.97	0.11	0.89	1.13	0.82	0.96
TDS	ppm	612.44	65.23	546.00	721.00	621.45	68.76	566.00	721.00	0.85	616.44
PH		7.82	0.11	7.70	7.90	7.81	0.12	7.70	7.93	0.91	7.81
HCO_3^-	meq./L	1.27	0.47	0.50	1.79	1.44	0.24	1.32	1.79	0.54	1.34
SO_4^{2-}	meq./L	5.02	2.42	0.90	7.18	6.05	0.83	5.20	7.18	0.44	5.48
SAR		1.94	1.24	1.20	4.15	1.37	0.14	1.20	1.53	0.40	1.68
NH_4^+	mg/L	1.15	0.49	0.35	1.61	1.23	0.20	1.05	1.40	0.78	1.18
NO_3^-	mg/L	11.34	3.91	5.60	16.52	14.13	8.99	5.81	26.11	0.55	12.58

** Legend: EC=Electric Conductivity, TDS=Total Dissolved Solids, HCO_3^-=Bicarbonate, SO_4^{2-}=Sulfate, SAR=Sodium Absorption Rate, NH_4^+=Ammonia, NO_3^-=Nitrate. All data are analyzed using SPSS one-way ANOVA where mean is the mean value, SD Is the standard deviation, min is the minimum value, max is maximum value. Mean values of Sand-Bed fish and collection tanks are significantly different when value of $p < 0.05$ noted using superscripts a.*

Table 26: Pilot Cycle Water Macro and Micro-Nutrients Parameters for Sand-Bed Fish and Collection Tanks

		Sand-Bed Fish Tank				Sand-Bed Collection Tank					Sand-Bed
*	Unit	Mean	SD	Min	Max	Mean	SD	Min	Max	Value of p*	Total Mean
Na^+	meq./L	3.21	1.25	2.3	5.4	2.65	0.31	2.3	3	0.41	2.96
K^+	meq./L	0.24	0.08	0.11	0.32	0.24	0.09	0.11	0.32	0.99	0.24
P	mg/l	0.87	1.6	0.05	3.27	0.84	1.55	0.02	3.17	0.98	0.86
Fe	mg/l	0.23	0.17	0.06	0.46	0.13	0.08	0.04	0.2	0.31	0.19
Ca^{++}	meq./L	4.85	1.56	2.4	6.58	5.4	0.9	4.47	6.58	0.56	5.09
Mg^{++}	meq./L	1.88	0.82	1	3.18	2.18	0.68	1.67	3.18	0.58	2.01
Cl^-	meq./L	3.89	2.14	2.54	7.6	2.97	0.63	2.54	3.9	0.44	3.48

** Legend: Na^+=Sodium ion, K^+=Potassium ion, P=Phosphorous, Fe=Iron, Ca^{++}=Calcium ion, Mg^{++}=Magnesium ion, Cl^- =chlorine ion. All data are analyzed using SPSS one-way ANOVA where mean is the mean value, SD is the standard deviation, min is the minimum value, max is maximum value. Mean values of Sand-Bed fish and collection tanks are significantly different when value of $p < 0.05$ noted using superscripts a.*

b. Crops Nutrient Content

A representative sample from each crop was sent to the FEED for nutrient and chemical analysis. *Baby Spinach* was only analyzed for nutrients analysis (Ca, P, K, Fe and Mn) in the Sand-Bed system as the harvest collected was not enough to undergo all lab tests.

Table 27: Summary of Pilot Cycle Crops Nutrient Parameters in DWC and Sand-Bed Systems

System		**DWC**			**Sand-Bed**			
Nutrient (Crop)	Unit	Butterhead Lettuce	Batavia Lettuce 1	Batavia Lettuce 2	Butterhead Lettuce	Batavia Lettuce 1	Batavia Lettuce 2	Baby Spinach
Ca	%	1.74	2.9	2.05	1.9	1.46	2	2.6
P	%	0.95	1.1	1.14	0.7	0.58	0.67	0.5
K	%	8.2	7.3	8.1	6.4	1.8	6.6	6.4
Fe	ppm	249.7	520.3	401.3	357	415.3	252	266
Mn	ppm	46.6	79.2	133.9	26.4	7.5	40.1	1.12
Zn	ppm	44.8	96.4	366.8	62.9	3.6	1.5	**
Cu	ppm	6.3	10.3	16.3	6.2	2.1	8.5	**
Na	%	0.69	0.61	0.21	0.59	0.38	0.38	**
Mg	%	0.58	1.3	0.73	0.44	0.38	0.43	**
B	ppm	40.7	47.5	43.2	28.9	30.8	30.9	**
NO_2^-	mg/L	**	13.71	**	**	13.97	**	**

**Legend: Ca=Calcium concentration in %, P=Phosphorous concentration in %, K=Potassium concentration in %, Fe=iron concentration in ppm, Mn=Manganese concentration in ppm, Zn=Zinc concentration in ppm, Cu=Cupper concentration in ppm, Na=Sodium concentration in ppm, Mg=Magnesium concentration in %, B=Boron concentration in ppm, NO_2^- in mg/L. **Sample size was only sufficient to conduct some of the nutrient analysis.*

Table 28: Summary of Pilot Cycle Crops Chemical Parameters in DWC and Sand-Bed Systems

System		**DWC**			**Sand-Bed**		
Element (Crop)	Unit	Butterhead Lettuce	Batavia Lettuce	Batavia Lettuce 2	Butterhead Lettuce	Batavia Lettuce 1	Batavia Lettuce 2
Protein	%	19.3	21.2	18.7	16.9	17.5	17.8
Lipid	%	5.22	5.23	6.1	5.79	5.26	4.64
Crude Fiber	%	15.73	12.52	17.51	10.95	10.34	12.76
Ash	%	18.8	20.2	20.2	21.7	28.7	26.2
Primary Moisture	%	94.4	95.6	95.1	94.1	94.8	93.8
Secondary Moisture	%	6.6	4.5	5.7	6.2	5.4	5.3
Total Moisture	%	94.7	95.8	95.4	94.5	95	94.1
B.Caroten	ug/100g	**	288.75±14.43	**	**	318.7±15.93	**

***Sample size was only sufficient to conduct some of the nutrient analysis.*

c. Crops Yield Analysis

Plant height was measured on weekly basis to assess the growth of crops on weekly basis.

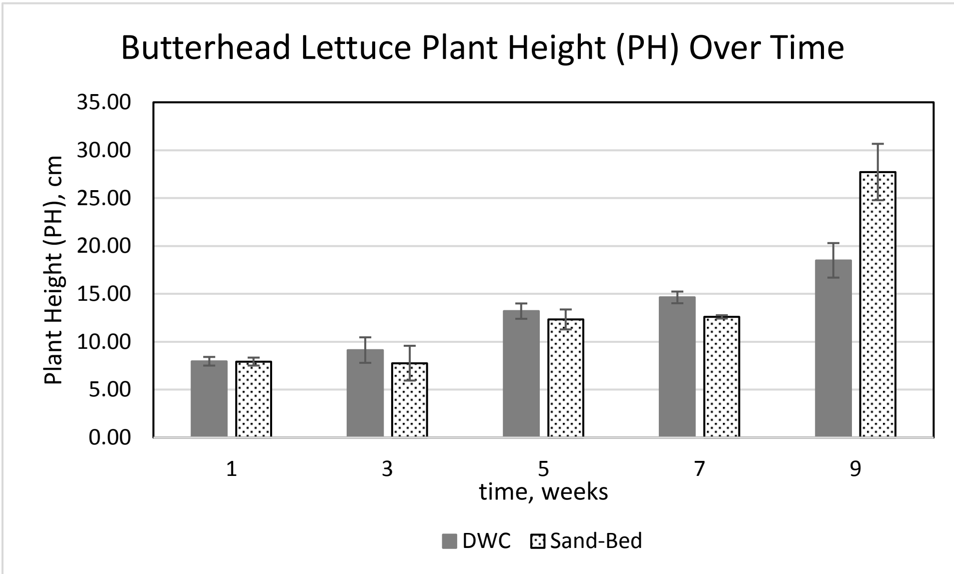


Figure 19: Pilot Cycle Butterhead Plant Height Over Time in DWC and Sand-Bed Systems

**Legend: Plant height (cm) in cm represents the mean value of 5 samples measured weekly. Error bars represent standard deviation.*

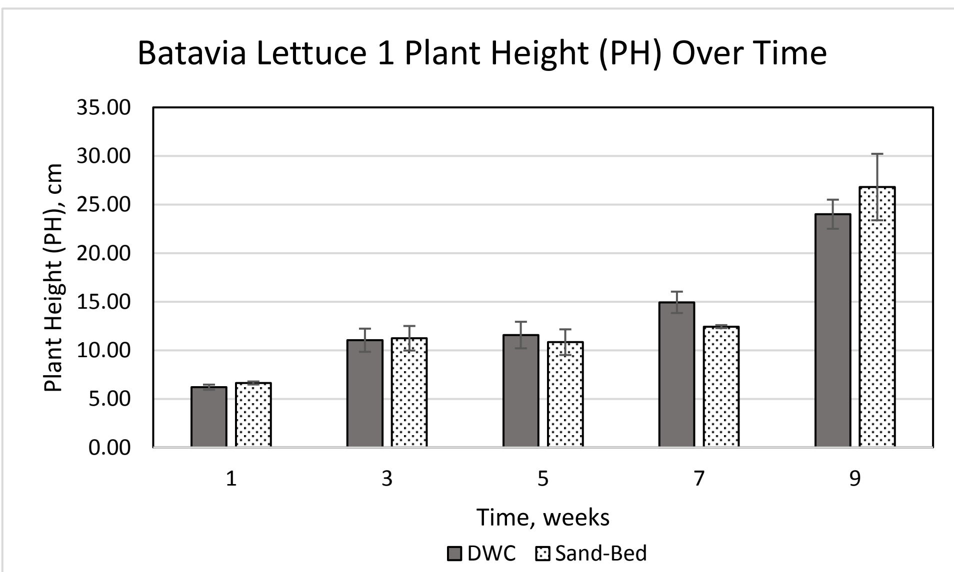


Figure 20: Pilot Cycle Batavia Lettuce 1 Plant Height Over Time in DWC and Sand-Bed Systems

**Legend: Plant height (cm) in cm represents the mean value of 5 samples measured weekly. Error bars represent standard deviation.*

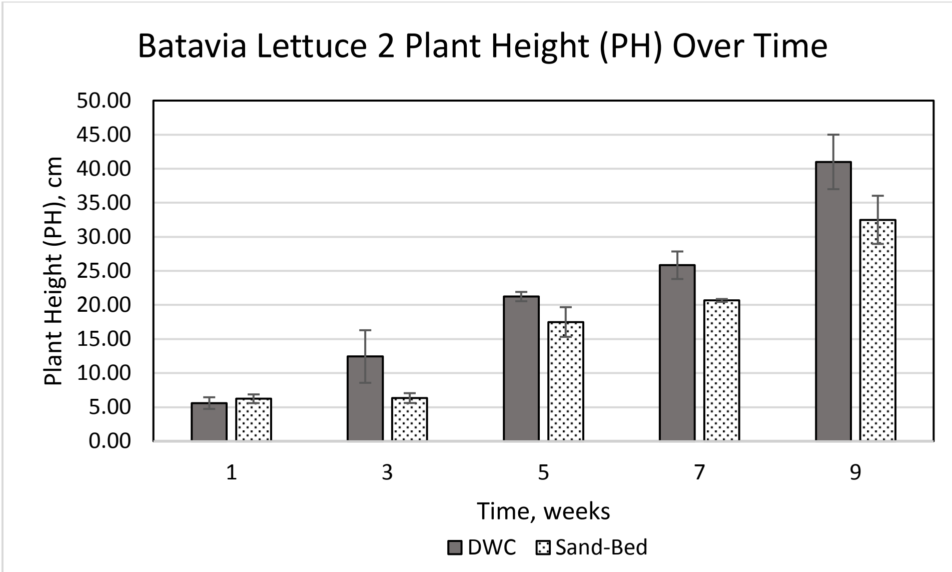


Figure 21: Pilot Cycle Batavia Lettuce 2 Plant Height Over Time in DWC and Sand-Bed Systems
**Legend: Plant height (cm) in cm represents the mean value of 5 samples measured weekly. Error bars represent standard deviation.*

Table 29: Summary of Pilot Cycle Butterhead Lettuce Growth Parameters in DWC and Sand-Bed Systems

System / Crop / **Parameter**	Unit	**DWC *Butterhead Lettuce*** *Mean*	*SD*	**Sand-Bed *Butterhead Lettuce*** *Mean*	*SD*
PH	cm	18.50[a]	1.80	27.72[a]	2.94
RL	cm	61.20[a]	13.79	15.60[a]	1.56
LL	cm	15.48	1.37	14.00	1.50
LW	cm	10.72	1.03	9.58	1.62
NFL	cm	55.00[a]	6.78	38.4[a]	6.9498
FW	g	156.00	26.08	244.00	103.34

* *Legend: PH=Plant Height in cm, RL=Root Length in cm, LL=Leaf Length in centimeters (cm), LW=Leaf Width in cm, NFL=Number of Fresh Leaves, FW=Fresh Weight in grams (g). All data are analyzed using SPSS one-way ANOVA where mean is the mean value, SD is the standard deviation. Mean values of DWC and Sand-Bed systems are significantly different when value of $p < 0.05$ noted using superscripts a.*

Table 30: Summary of Pilot Cycle Batavia Lettuce 1 Growth Parameters in DWC and Sand-Bed Systems

System Crop		**DWC Batavia Lettuce 1**		**Sand-Bed Batavia Lettuce 1**	
Nutrient	Unit	*Mean*	*SD*	*Mean*	*SD*
PH	cm	24.00	1.50	26.80	3.42
RL	cm	48.60[a]	8.18	12.40[a]	1.47
LL	cm	16.60	2.90	16.52	0.80
LW	cm	13.88[a]	2.04	11.20[a]	0.45
NFL	cm	55.40[a]	4.56	39.40[a]	4.62
FW	g	226.00	44.50	278.00	83.19

* *Legend: PH=Plant Height in cm, RL=Root Length in cm, LL=Leaf Length in centimeters (cm), LW=Leaf Width in cm, NFL=Number of Fresh Leaves, FW=Fresh Weight in grams (g). All data are analyzed using SPSS one-way ANOVA where mean is the mean value, SD is the standard deviation. Mean values of DWC and Sand-Bed systems are significantly different when value of $p < 0.05$ noted using superscripts a.*

Table 31: Summary of Pilot Cycle Batavia Lettuce 2 Growth Parameters in DWC and Sand-Bed Systems

System Crop		**DWC Batavia Lettuce 2**		**Sand-Bed Batavia Lettuce 2**	
Nutrient	Unit	*Mean*	*SD*	*Mean*	*SD*
PH	cm	41.00	4.00	32.50	3.54
RL	cm	46.80[a]	9.60	11.36[a]	1.59
LL	cm	22.50[a]	0.87	19.92[a]	0.56
LW	cm	15.56	2.32	14.92	1.49
NFL	cm	26.80	6.46	22.00	3.67
FW	g	376.00[a]	149.93	176.00[a]	34.35

* *Legend: PH=Plant Height in cm, RL=Root Length in cm, LL=Leaf Length in centimeters (cm), LW=Leaf Width in cm, NFL=Number of Fresh Leaves, FW=Fresh Weight in grams (g). All data are analyzed using SPSS one-way ANOVA where mean is the mean value, SD is the standard deviation. Mean values of DWC and Sand-Bed systems are significantly different when value of $p < 0.05$ noted using superscripts a.*

Baby Spinach did not grow in the DWC system; hence, no results were recorded. In the Sand-Bed system, Baby Spinach 75 out of 90 seeds grew over the 60 days period. The weight of the overall Baby spinach harvested was recorded due to the deficient growth.

d. Water Use Variables

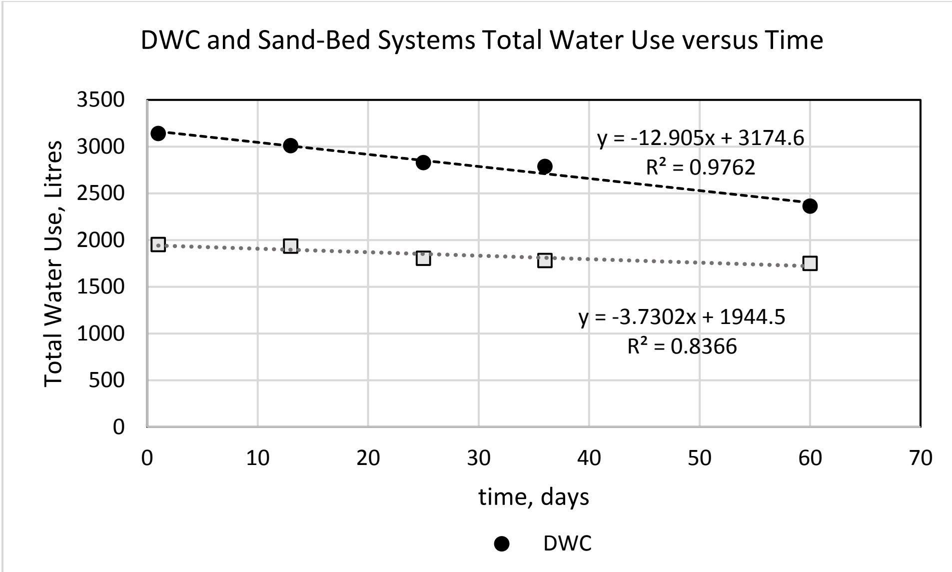


Figure 22: Pilot Cycle DWC and Sand-Bed Systems Total Water Use versus Time

Table 32: Pilot Cycle Average Total Water Use and Average Total Water Consumption in DWC and Sand-Bed Systems Over 60 days

	Unit	DWC	Sand-Bed
Average Total Water Use	Liters	2826	1844
Average Daily Water Consumption	Liters	774.30	223.80

e. Production Parameters based on Aquaponics System

Table 33: Pilot Cycle Water Consumption and Production Yields for DWC and Sand-Bed Systems over 60 days

Parameters	Density	DWC	Sand-Bed
Average Total Water Use (m^3)	-	2.83	1.84
Average Water Consumption (m^3)	-	774.3	223.80
Production Yields			
Butterhead Lettuce (kg/m^2)	24 lettuce/m^2	3.74	5.86
Batavia Lettuce 1 (kg/m^2)	15 lettuce/m^2	3.39	4.17
Batavia Lettuce 2 (Kg/m^2)	15 lettuce/m^2	5.64	2.64
Nile Tilapia *(Oreochromis niloticus)* (kg/m^3)	150 fish/m^3	2.5	2.5
FCR (%)	-	2.37	2.37

**Production Yield for lettuce and Nile Tilapia is final wet weight gain (kg) minus initial wet weight gain (kg). FCR (Food Conversion Ratio) = Total feed given (g) to fish divided by total weight gain (g) of fish.*

Appendix II: Sand Media Analysis Results

Sand sample was analyzed for mechanical on the start of pilot (January 25^{th}, 2018 – pilot cycle day 1) and by end day of main cycle (May 25^{th}, 2018 – main cycle day 35). Sand sample was also analyzed for chemical, macro, micro-nutrient and heavy metals analysis on the start of the pilot cycle experiment on January 25^{th}, 2018 (pilot cycle day 1), February 24^{th}, 2018 (pilot cycle day 30), and March 25^{th}, 2018 (pilot cycle day 60) and by end of main cycle on May 25^{th}, 2018 (main cycle day 35). All laboratory analysis was performed at the Soils Water and Environment Research Institute (SWERI) operated by the Ministry of Agriculture and Land Reclamation in Egypt. The below tables represent all sand media analysis results (tables 34 – 42).

A. Mechanical Analysis

Table 34: Sand Media Sample Size Distribution and Texture on January 25^{th}, 2018 – Pilot Cycle Day 1

	Mechanical Analysis				
Day 1	Coarse Sand	Fine Sand	Silt	Clay	Texture
	42.2%	45.8%	10.0%	2.0%	Sand

**Legend: Analysis was done on 3 kg sand sample for mechanical analysis.*

Table 35: Sand Media Sample Size Distribution and Texture on May 25^{th}, 2018 – Main Cycle day 35

	Mechanical Analysis				
Day 35	Coarse Sand	Fine Sand	Silt	Clay	Texture
	56.6%	33.2%	6.1%	4.1%	Sand

**Legend: Analysis was done on 3 kg sand sample for mechanical analysis.*

B. Chemical Analysis

Table 36: Sand Media Sample Chemical Analysis on January 25th, 2018 – Pilot Cycle Day 1

Day 1	Chemical Analysis											
	$CaCO_3$ (%)	Cations (meq./L)				Anions (meq./L)				SP	EC (dS/m)	PH
		k^+	Na^+	Mg^{++}	Ca^{++}	SO_4^{2-}	Cl^-	HCO_3^-	CO_3^{2-}		dS/m	
	2.8	0.5	28.6	10.2	18.7	4.9	50.9	2.2	-	25	5.99	7.88

**Legend: Analysis was done on 3 kg sand sample for chemical analysis. $CaCO_3$ = Calcium Carbonate in %. Cations concentrations in (meq./L) included K^+ = potassium ion, Na^+ = sodium ion, Mg^{++} = Magnesium ion, and Ca^{++} = Calcium ion. Anions concentrations in (meq./L) included SO_4^{2-} = Sulphate ion, Cl^- = Chloride ion, HCO_3^- = Bicarbonate ion,* and *CO_3^{2-} = Carbonate ion. SP = Spontaneous Potential, EC = Electric Conductivity in dS/m.*

Table 37: Sand Media Sample Chemical Analysis on February 24th, 2018 – Pilot Cycle Day 30

Day 30	Chemical Analysis											
	$CaCO_3$ (%)	Cations (meq./L)				Anions (meq./L)				SP	EC (dS/m)	PH
		k^+	Na^+	Mg^{++}	Ca^{++}	SO_4^{2-}	Cl^-	HCO_3^-	CO_3^{2-}		dS/m	
	**	0.25	1.17	2.69	3.29	1.52	4.24	1.65	-	24	0.64	7.67

**Legend: Analysis was done on 3 kg sand sample for chemical analysis. $CaCo_3$ = Calcium Carbonate in %. Cations concentrations in (meq./L) included K^+ = potassium ion, Na^+ = sodium ion, Mg^{++} = Magnesium ion, and Ca^{++} = Calcium ion. Anions concentrations in (meq./L) included SO_4^{2-} = Sulphate ion, Cl^- = Chloride ion, HCO_3^- = Bicarbonate ion,* and *CO_3^{2-} = Carbonate ion. SP = Spontaneous Potential, EC = Electric Conductivity in dS/m.*
***$CaCO_3$ was not measured in this analysis.*

Table 38: Sand Media Sample Chemical Analysis on March 25th, 2018 – Pilot Cycle Day 60

Day 60	Chemical Analysis											
	$CaCO_3$ (%)	Cations (meq./L)				Anions (meq./L)				SP	EC (dS/m)	PH
		k^+	Na^+	Mg^{++}	Ca^{++}	SO_4^{2-}	Cl^-	HCO_3^-	CO_3^{2-}		dS/m	
	**	0.34	1.52	1.69	5.92	1.18	5.93	2.63	-	21	0.85	7.8

**Legend: Analysis was done on 3 kg sand sample for chemical analysis. $CaCo_3$ = Calcium Carbonate in %. Cations concentrations in (meq./L) included K^+ = potassium ion, Na^+ = sodium ion, Mg^{++} = Magnesium ion, and Ca^{++} = Calcium ion. Anions concentrations in (meq./L) included SO_4^{2-} = Sulphate ion, Cl^- = Chloride ion, HCO_3^- = Bicarbonate ion,* and *CO_3^{2-} = Carbonate ion. SP = Spontaneous Potential, EC = Electric Conductivity in dS/m.*
***$CaCO_3$ was not measured in this analysis.*

Table 39: Sand Media Sample Chemical Analysis on May 25th, 2018 – Main Cycle Day 35

Day 35	Chemical Analysis											
	$CaCO_3$ (%)	Cations (meq./L)				Anions (meq./L)				SP	EC (dS/m)	PH
		k^+	Na^+	Mg^{++}	Ca^{++}	SO_4^{2-}	Cl^-	HCO_3^-	CO_3^{2-}		dS/m	
	**	0.2	10.6	2.2	6	1	17.44	0.56	-	2.5	1.99	7.93

**Legend: Analysis was done on 3 kg sand sample for chemical analysis. $CaCo_3$ = Calcium Carbonate in %. Cations concentrations in (meq./L) included K^+ = potassium ion, Na^+ = sodium ion, Mg^{++} = Magnesium ion, and Ca^{++} = Calcium ion. Anions concentrations in (meq./L) included SO_4^{2-} = Sulphate ion, Cl^- = Chloride ion, HCO_3^- = Bicarbonate ion,* and *CO_3^{2-} = Carbonate ion. SP = Spontaneous Potential, EC = Electric Conductivity in dS/m.*
***$CaCO_3$ was not measured in this analysis.*

C. Macro, Micro-Nutrients, and Heavy Metals Analysis

Table 40: Sand Media Sample Macro, Micro-Nutrients and Heavy Metals Analysis on February 24th, 2018 – Pilot Cycle Day 30

Day 30	**Total macro, micronutrients and heavy metals content of the soil sample (mg/kg)**													
Co	Mo	Al	Si	Pb	Cd	Cr	Ni	Cu	Fe	Zn	Mn	K	P	N (%)
-	-	-	257.3	-	1	-	54.4	2	19.7	59.5	16.5	81.6	295	0.04

**Legend: Analysis was done on 3 kg sand sample for available macro, micronutrients and heavy metals content. Concentrations in mg/Kg for Co = Cobalt, Mo = Molybdenum, Al = Aluminum, Si = Silicon, Pb = Lead, Cd = Cadmium, Cr = Chromium, Ni = Nickel, Cu = Cupper, Fe = Iron, Zn = Zinc, Mn = Manganese, K = Potassium, P = Phosphorous. N = Nitrogen Content in %.*

Table 41: Sand Media Sample Macro, Micro-Nutrients and Heavy Metals Analysis on March 25th, 2018 – Pilot Cycle Day 60

Day60	**Total macro, micronutrients and heavy metals content of the soil sample (mg/kg)**													
Co	Mo	Al	Si	Pb	Cd	Cr	Ni	Cu	Fe	Zn	Mn	K	P	N (%)
**<0.2	0.518	1.696	0.42	**<1.5	**<0.1	0.12	0.002	0.014	6.712	0.714	0.872	15.88	15.7	197

**Legend: Analysis was done on 3 kg sand sample for available macro, micronutrients and heavy metals content. Concentrations in mg/Kg for Co = Cobalt, Mo = Molybdenum, Al = Aluminum, Si = Silicon, Pb = Lead, Cd = Cadmium, Cr = Chromium, Ni = Nickel, Cu = Cupper, Fe = Iron, Zn = Zinc, Mn = Manganese, K = Potassium, P = Phosphorous. N = Nitrogen Content in %. **Detection limit is in µg/l.*

Table 42: Sand Media Sample Macro, Micro-Nutrients and Heavy Metals Analysis on May 25th, 2018 – Main Cycle Day 35

Day 35	**Total macro, micronutrients and heavy metals content of the soil sample (mg/kg)**													
Co	Mo	Al	Si	Pb	Cd	Cr	Ni	Cu	Fe	Zn	Mn	K	P	N (%)
**	**	**	**	**	**	**	**	0.2	7.18	1.22	0.75	21.21	13.35	44

Legend: Analysis was done on 3 kg sand sample for available macro, micronutrients and heavy metals content. Concentrations in mg/Kg for Co = Cobalt, Mo = Molybdenum, Al = Aluminum, Si = Silicon, Pb = Lead, Cd = Cadmium, Cr = Chromium, Ni = Nickel, Cu = Cupper, Fe = Iron, Zn = Zinc, Mn = Manganese, K = Potassium, P = Phosphorous. N = Nitrogen Content in %. Heavy metals analysis was not measured.*

Appendix III: Lettuce Plant Quality Index

An example of how lettuce plant quality index (PQI) was developed in the main cycle is represented below on pages **75 - 77**. 12 samples were photographed from each system's replicate (3 replicates per system) weekly. Camera lens used was 8 megapixels and all pictures were taken at equal distance by placing a ruler for scaling. All pictures collected were analyzed by same evaluator at end of experiment to avoid biased evaluation. The index was developed numerically shown in **Figure 23**; where (1) represents yellow leaves with up to 90% imperfections on leaf surface, (2) represents very light yellowish green leaves with up to 80% imperfections on leaf surface (3) light yellowish green leaves with up to 70% imperfections on leaf surface (4) yellowish green with up to 60% imperfections on leaf surface (5) green with up to 50% imperfections on leaf surface (6) green up to with 40% imperfections on leaf surface (7) green with up to 30% imperfections on leaf surface (8) dark green with up to 20% imperfections on leaf surface (9) dark green with up to 10% imperfections on leaf surface (10) very dark green without imperfections on leaf surface. Each picture was assigned a numerical value according to PQI developed. The mode value was then computed to represent each systems' replicates per week. The lettuce PQI is represented for each system over 35 days (week 1, week 2, week 3, week 4 and week 5) in **Figure 10**.

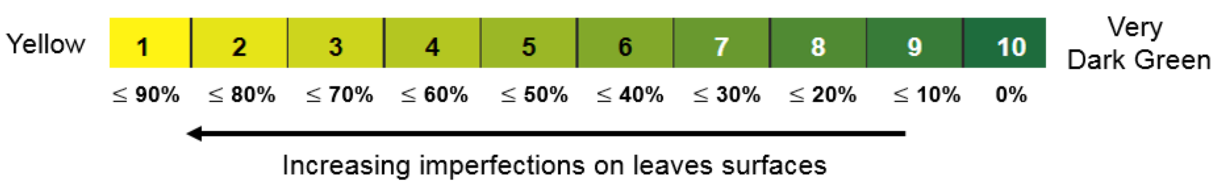
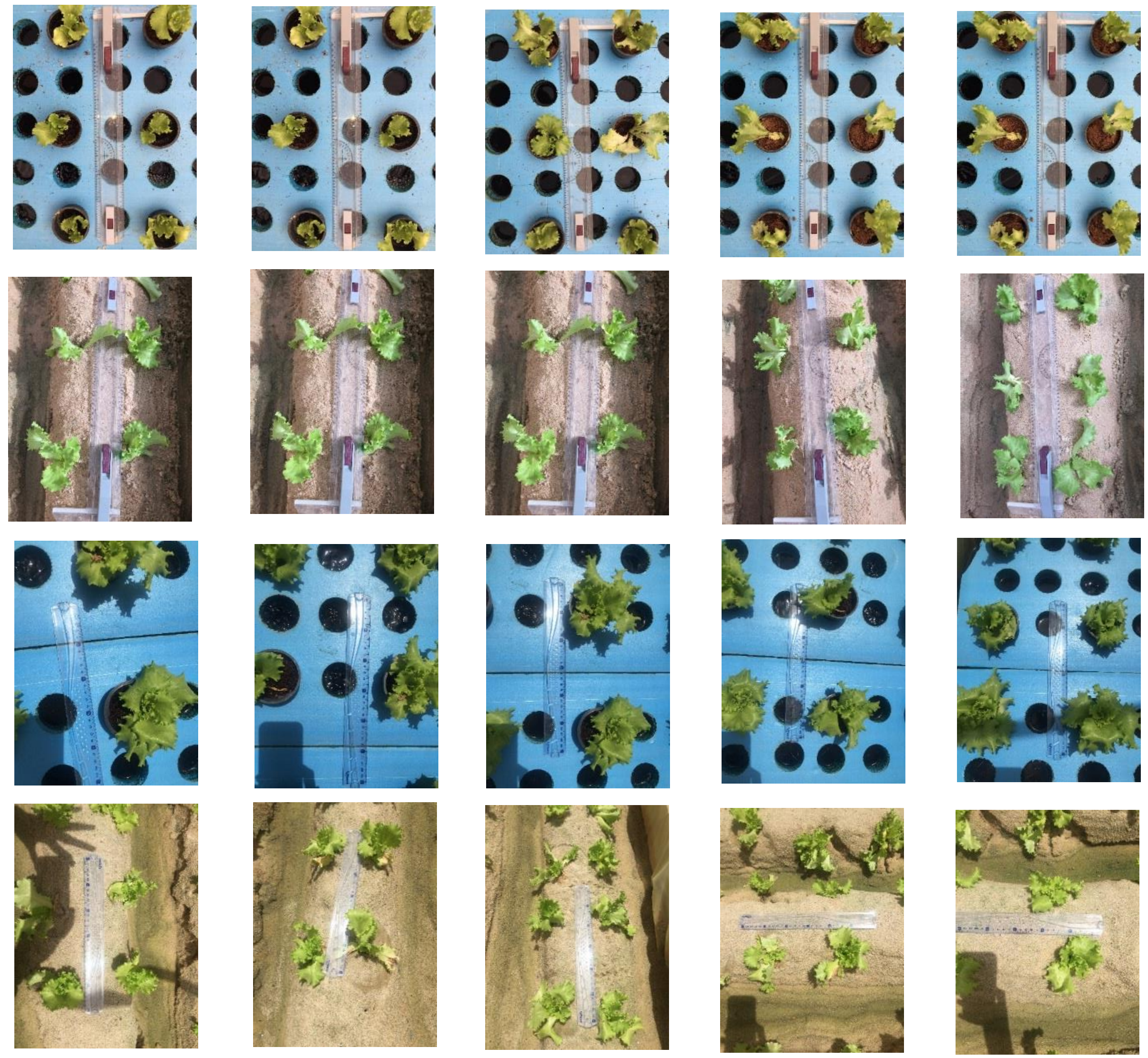
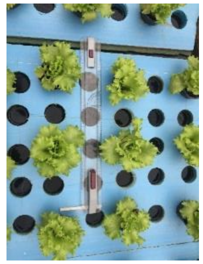
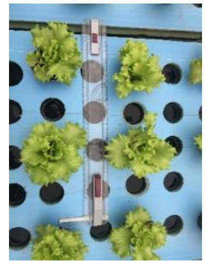
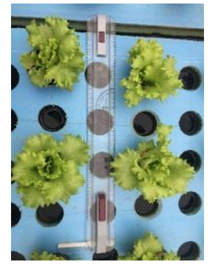
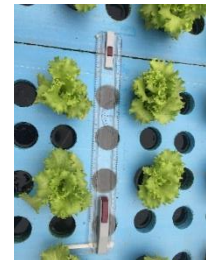
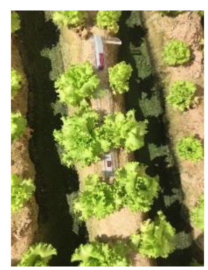
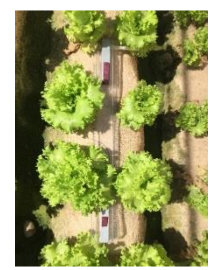


Figure 23: Plant Quality Index numerical scale

Weeks/System	PQI Mode
Week 1 (DWC)	**4**
Week 1 (Sand-Bed)	**9**
Week 2 (DWC)	**8**
Week 2 (Sand-Bed)	**4**



Week 3 (DWC)	**6**	    
Week 3 (Sand-Bed)	**5**	    
Week 4 (DWC)	**3**	    
Week 4 (Sand-Bed)	**6**	    

Week 5 (DWC)	**2**
Week 5 (Sand-Bed)	**8**