HYDROPONICS SYSTEM FOR WASTEWATER TREATMENT AND REUSE IN HORTICULTURE

By

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DECLARATION

I declare that this thesis is my own account of my research and contains as its main content work, which has not previously been submitted for a degree at any tertiary education institution.

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LIST OF PULICATIONS

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ABSTRACT

As human population increases, the need for water increases in domestic, agricultural, industrial and urban sectors. Wastewater reuse after treatment is gaining acceptance world wide, as availability of fresh water sources decreases. However, it is also important to point out social and cultural differences that still exist in different pars of the world including those where reuse of wastewater for food production or any domestic use is not yet acceptable. The major concerns with effluent reuse are primarily its impact on human health and environmental risk. As a result, effluent reuse should be undertaken with caution after careful consideration of the potential impacts and risks.

This thesis examined the potential to use the hydroponics nutrient film technique to grow commercially important crops using secondary-treated domestic wastewater. The crops chosen were a fruit crop (*Lycopersicon esculantum* - tomato), a leafy crop (*Beta vulgaris ssp. cicla* - silver beet) and a flower crop (*Dianthus caryophyllus* - carnation). Secondary-treated domestic wastewater was chosen because of the reduced risk of pathogen and heavy metal contamination in the crops and due to the guideline requirements for use of treated effluent for food crops. The possibility of using the effluent after the hydroponics treatment for further irrigation was also studied.

The ability of secondary-treated effluent to supply adequate nutrients to the crops was assessed relative to a commercially available hydroponics solution (Chapter 3). The amount of time the solution was left in the system (nutrient solution retention time) was dependant on the plant uptake of the solution. The results obtained showed that the nutrients in secondary treated effluent was adequate for the carnations, but not for

the food crops. The food crops from both treatments were compared to the produce purchased from a supermarket. The food crops showed signs of nutrient deficiency, particularly nitrogen.

Based on the findings of the first experiment, the nutrient solution retention time was amended to 14 days. The carnations were not tested with the shorter nutrient solution retention time (NSRT) because they performed well in the previous trial with the longer nutrient solution retention time. The edible food crops performed better and did not show signs of nutrient deficiency when the nutrient solution retention time was reduced to 14 days.

Further statistical analysis was conducted with the data from Chapters 3 and 4. Nutrient and water balances were calculated and the possible reason that the plants grown in the 14-day nutrient solution retention time took up more water, was a result of increased nutrients and better growth. A simple model was constructed to calculate height of the plants using multiple regression. The model was validated against the data collected from this study.

The experiment conducted in Chapter 6 determined the nutritional quality of the food crops. The harvests from the wastewater and commercially available hydroponics solution were compared to produce purchased from a supermarket and tested for total caroteniods, total soluble solids and ascorbic acid concentrations. The nutritional quality of the wastewater grown produce was comparable to those grown in the hydroponic solution and those purchased.

The risk of pathogen contamination to food crops and the die-off of pathogens in the hydroponic channels were studied in Chapter 7. This was tested by spiking the

commercial hydroponic medium with *Escherichia coli* and *Salmonella typhimurium* and monitoring bacterial pathogen die-off in the secondary treated domestic wastewater. The pathogen quality of the crop was tested in all treatments as well as on organically grown produce found at a local supermarket. The results of this experiment did not show any contamination on the surface of the food crops or within the food crops.

This study demonstrated that growing tomatoes, silver beet and carnations using secondary-treated domestic wastewater was successful when the nutrient solution retention time was adjusted to the optimum level. In arid, developing and remote communities, this system is ideal as it conserves and reuses water for commercially important crops without compromising the health of the environment or of human beings. It can also be implemented in urban areas, as the system can be scaled according to the availability of space. In addition to this, the effluent after going through this system can be used for open irrigation as it meets the World Health Organisation guidelines.

However, a number of additional concerns need further investigation. They include the transmission risk of other types of pathogen, which depends on the source of wastewater, and the effects of hormones and antibiotics on food crops and their effect on human health.

ABBREVIATIONS

BOD	biological oxygen demand
CM	control medium (commercial hydroponics medium)
CMS	control medium spiked (commercial hydroponics medium spiked)
DO	dissolved oxygen
EC	electrical conductivity
ETC	Environmental Technology Centre
MAD	Maurice Alan Derrick
NATA	National Association of Testing Authorities
NFT	nutrient film technique
NH_4^+-N	ammonium-nitrogen
NO_3^--N	nitrate-nitrogen
NSRT	nutrient solution retention time
O	organically grown produce
PO_4^{3-}-P	phosphate-phosphorus
se	standard error
TKN	total kheldjal nitrogen
TN	total nitrogen
TP	total phosphorus
TSS	total soluble solids
WC	water culture
WHO	World Health Organisation
WW	secondary-treated domestic wastewater

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CHAPTER 1

INTRODUCTION

1.1 NEED FOR REUSE OF TREATED WASTEWATER

As the human population increases, there is greater competition from domestic, industrial and agricultural users for fresh water supplies. The WHO (2006a) estimated that over 40% of the world's population will live in areas where water is scarce in the next 50 years. Arid and semi arid regions, like the Mediterranean Basin, are struggling to keep up with the demand for fresh water (Oron *et al.,* 2007). Oron *et al.* (2007) recognised that this shortage was linked to agriculture, changes in the environment and climate. In some cities, like Sydney, Australia water demand has reached the capacity of water supply (Anderson, 2006). Approximately 70% of the population in Europe live in water-stressed regions (Hochstrat and Wingtens, 2003). According to the United Nations Millennium Declaration (United Nations General Assembly., 2000), one of their goals was to, by the year 2015, halving the number of people who have inadequate access to drinking water as well has halve the number of people suffering from hunger. They also resolved to increase equitable and sustainable access to water supplies.

Most countries discharge their treated effluent into the ocean or other water bodies (Menegaki *et al.,* 2007). In Korea, 6.1 billion cubic metres of treated effluent is discharged annually (Yim *et al.,* 2007). Between 2001 and 2002, the Australian Bureau of Statistics reported that approximately 62,455GL of treated effluent was discharged, and only about 425GL was reused (Trewin, 2006).

As a result, the need to assess the potential of reusing treated wastewater is becoming inevitable to meet the supply and demand. Wastewater reuse was common in the past, but due to health concerns, this practice was curtailed. In some countries, effluent reuse occurred without treatment, which undoubtably caused health and environmental problems. The main concern with water recycling is the uncertainty of its quality (Colebatch, 2006) and the potential to health problems and cause environmental harm. To reduce the risks, effluent should undergo treatment before reuse (WHO, 2006a). The level of treatment depends on the source of the wastewater and also on the type of reuse planned (Toze, 2006b).

According to Michel (2004) the Hague, Bonn, Kyoto conferences and the Johannesburg World Summit on Sustainable Development, recognised the need for better water management. One of the methods of managing water efficiently is to recycle water, which can reduce the pressure on both the supply and disposal aspects of water management (Colebatch, 2006). Over the past 20 years, the increased water stress has led to a wider acceptance of water reuse in Europe (Ghermandi *et al.,* 2007). Countries in Europe carry out wastewater reuse under their own national and regional guidelines (Hochstrat *et al.,* 2006). While it is becoming increasingly necessary to reuse wastewater, however, this has not been put into practice in many developed and developing countries (Yang and Abbaspour, 2007).

In Australia, the driest inhabited continent in the world, wastewater reuse is especially necessary. Decline of rainfall, population growth, the need for water supply for environmental flows and lack of planning for the long term, have revealed the requirement for innovative water management strategies (von Huben and Cho, 2006). The Council of Australian Governments recognised the need for national management of water resources and released a document called the Intergovernmental Agreement on a National Water Initiative. One of the outcomes addressed was to promote reuse and recycling of wastewater if it was economical (Council of Australian Governments., 2004).

There have been many studies conducted to inject aquifers directly with treated wastewater (Cooper, 1991) and to irrigate large public open spaces (Oron *et al.,* 1999) as a method of effluent reuse. Integrating decentralised wastewater treatment and reuse systems is becoming more accepted (Miller, 2006). Moreover when large volumes of reclaimed water are available it can be a reliable water source for agricultural irrigation (Toze, 2006a). By 2040, treated wastewater will be the main source of irrigation water in Israel as given in the 1995 Israel Water Commission Report (Haruvy *et al.,* 2000).

The increasing need for fresh food production has created more demand for freshwater for irrigation. However by using treated effluent for irrigation for fresh food production, the nutrients available in the treated effluent can be put to beneficial use for plant growth (Menegaki *et al.,* 2007).

1.2 CONCERNS WITH WASTEWATER REUSE IN HORTICULTURE

There have been reports of higher crop yields when using treated effluent as well as a reduction in the use of fertilisers compared to the use of irrigation water together with fertilisers (Hussain *et al.,* 2002). Toze (2006a) recognised the potential of nutrients present in treated effluent as a fertiliser source in agriculture, and Darwish *et al.* (1999) found that it was profitable to grow crops using secondary treated effluent. However, the main barriers for its wide acceptance are the concerns related to environmental and human health risks due to the presence of pathogens, heavy metals, chemicals and for aesthetic reasons.

Public acceptance is highly important for reusing wastewater for irrigation, because without it, it can be a barrier to its use in society (Schäfer and Beder, 2006; Menegaki *et al.*, 2007). Crops that humans consume either raw or processed could accumulate contaminants from the effluent and as a result, lead to negative impacts on human health. This is why the risks with using effluent should be studied before using it to grow edible food crops. Selecting plants of other commercial value such as in cut flowers and other industrial application could be advantageous to address this risk (Darwish *et al.,* 2007). Using appropriate culture systems and management strategies may reduce the risks and can handle the aesthetic barriers of using treated effluent for growing plants of commercial value.

1.3 WASTEWATER REUSE USING THE HYDROPONICS SYSTEM

To address the risk of pathogen contamination on the crops, and health risk to workers, using secondary-treated domestic effluent in a hydroponics set-up would be beneficial (Oyama *et al.,* 2008). Hydroponics is a method of growing plants in a soil-less environment. The nutrient source is provided directly to the plant roots in solution. Therefore it can be expected that the risk of physical contact with the effluent by agricultural workers, is minimal, as a result of the containment of the effluent.

There has been extensive research carried out on hydroponics using chemical fertilisers, however, integrating this system with wastewater reuse is a developing area. Some studies have examined the possibility of using treated effluent for plant growth in a hydroponics system. Most have found that it is possible to grow crops using effluent, however, the studies have mainly concentrated on using this system for wastewater treatment. Wastewater hydroponics is a potential system to treat and reuse wastewater especially integrated with decentralised treatment plants.

However, there are few studies on the nutrient availability in secondary treated effluent for optimum plant growth, pathogen contamination in edible parts of the plant and nutritional quality of vegetables grown in treated effluent using the hydroponics system. One of the most important aspects of fresh food is its nutritional quality (Prasanna *et al.,* 2007), however, it is not clear whether secondary treated domestic wastewater effluent is able to provide necessary nutrients to food crops as well as adequate nutritional quality of the produce.

1.4 SCOPE OF THIS RESEARCH

The aim of this thesis is to investigate:

- The feasibility of utilising secondary-treated domestic wastewater as a nutrient source for horticulture in a hydroponics set-up.

The objectives of this study are to determine:

- The growth performance of food crops and a flower crop using secondary-treated domestic wastewater as the nutrient source in a hydroponic system;
- The die-off of pathogens in the hydroponics system;
- The risk of pathogen contamination in the food crops;
- The nutritional quality of the harvest grown in secondary treated effluent as a growth medium, and;
- The nutrient removal capacity of the system.

The above objectives have been studied through four major experiments and are elaborated in the following chapters.

- Chapter 2 is a review of the current literature, determining the need for wastewater treatment and reuse using the hydroponic system.
- Chapter 3 is the preliminary experiment carried out to determine whether secondary treated domestic wastewater contained adequate nutrients for plant growth.
- Chapter 4 questions whether reducing the nutrient solution retention time to increase nutrient availability in the system improved the growth and production of the plants.
- Chapter 5 analyses the data from Chapters 3 and 4 to calculate water and nutrient balances for the different plants. The relationship between different parameters and plant growth were ascertained through multiple regression analyses.

- Chapter 6 tests the nutritional quality of the edible crops (tomatoes and silver beet) grown in the secondary treated domestic effluent compared to those in a commercial hydroponics medium and to market produce.
- Chapter 7 discusses the analysis of pathogen die-off rate in the hydroponics channels and the risk of pathogen contamination in the edible crops. It compared the pathogen die-off rate in the solutions of two different types of hydroponic systems.
- Chapter 8 is a general discussion on the significance of using secondary treated domestic effluent on crops and flowers in hydroponics, and its limitations.
- Chapter 9 contains the conclusions and recommendations for further research based on the findings of this research.

CHAPTER 2

LITERATURE REVIEW

2.1 INTRODUCTION

The United Nations General Assembly (2000) adopted a declaration in values and principles; peace, security and disarmament; development and poverty eradication; protection of common environment; human rights, democracy and good governance; protecting the vulnerable, meeting the special needs of Africa and; strengthening the United Nations. As a result of this declaration, eight Millennium Development Goals were developed (United Nations., 2007), of these the first goal was to eradicate extreme poverty and hunger, and the seventh goal was to ensure environmental sustainability.

With population growth, concerns over providing adequate sanitation and food supply for people will also increase. As sources for fresh water supplies are depleting, wastewater reuse is becoming necessary for domestic purposes, industries, agriculture and horticulture. The agricultural industry cannot cope with the increase in demand for food. The mobilisation of land and water resources for urban development is increasing and water has become the most limiting factor for increasing agricultural production (Angelakis *et al.,* 1999). In Greece, approximately 84% of fresh water is used for agricultural irrigation, while 14% is used for domestic purposes and 2% for industrial and other usage (Maloupa *et al.,* 1999). As most of the accessible water resources are committed, identifying alternative water resources for irrigation purposes is essential (Angelakis *et al.,* 1999). Use of treated wastewater for irrigation is the most sustainable method of saving water (Capra and Scicolone, 2007).

In countries with large populations, such as China, fresh water resources are not distributed evenly amongst the people (Chu *et al.,* 2004). This is also the case in most developing countries, where the infrastructure as well as environmental reasons limit the access of many people to reliable water supply (Jie *et al.,* 2007). In some developed countries like Australia, the infrastructure is efficient, however potable water has been unwisely used. Treatment of wastewater is often considered to be the duty of state governments and not individual households (Colebatch, 2006), this will need to change and some ownership to be given to the people for responsible use and management of freshwater sources for efficient use of water resources (Radcliffe, 2006).

Water management should include reuse and recycling of effluent. This is currently being practised in sectors like industry and agriculture. As land availability for agriculture is reducing, other methods for intensive food production are required. A method of intensive food production is hydroponics, where plants can be grown in limited space using a nutrient solution fed directly to the roots. Hydroponics can be used as a vehicle for treated wastewater reuse as it contains nutrients required for plant growth. The main concerns with using wastewater are: nutrient availability for adequate growth; pathogen contamination and; nutritional quality of food crops. This chapter will review the problems associated with, and the need for wastewater hydroponics, as a feasible and safe option for effluent reuse systems.

2.2 WATER SUPPLY AND SANITATION

Presently, one of the world's major concerns is to supply its population with adequate fresh water and sanitation. In the year 2000, there were approximately 1 billion people who lacked safe drinking water and about 3 billion people that lacked adequate sanitation (WHO/UNICEF, 2000) and a majority of people in developing countries lived without adequate sanitation (Ho, 2002). In order to meet the first goal of the Millennium Development Goals, adequate sanitation needs to be provided to approximately 1.6 billion people between 2005 and 2015 (United Nations., 2007). In conjunction with this, population growth and industrialisation has increased the demand for water (Riper and Geselbracht, 1997; Ali, 2002). As a result, the search for alternative sources of fresh water for agriculture and industrial needs has also increased (Oron *et al.,* 1999).

Most communities that suffer inadequate sanitation are usually located in relatively high population density areas where water supply may be inadequate in quantity and/or quality (Ho, 2002). In Africa due to the demand for safe water supply by a rapidly growing urban population and poverty increase, the requirement for waste treatment and management services are much higher than their current supply (Binns *et al.,* 2003). In areas like this, the number of people living on less than a dollar a day has increased (United Nations., 2007). Of most concern is that approximately 1.5 million children under the age of five die each year from diarrhoeal diseases (United Nations., 2007).

According to USEPA (2004), within the next 20 years about 60% of the word's populations will live in cities. In 2005, one in three people living in urban areas in developing countries lacked adequate sanitation and access to potable water (United Nations., 2007). As this occurs, either planning and implementation for better sewage collection and treatment is required, or a large amount of the wastewater generated will be disposed off without treatment into rivers and natural water systems. Not to mention the fact that the concept of wastewater is actually unknown in many slum areas.

Many countries have tried to meet the increasing demand for water by tapping into groundwater reservoirs. Most of the groundwater reserves used are over extracted, which can cause environmental degradation of other natural systems dependant on the groundwater. Countries that have an adequate supply of fresh water currently will have to look at alternative methods of water supply in order to have a sustainable supply for future generations. Wastewater reuse is an option to water conservation (Ali, 2002). Increase in urbanisation increases domestic water use, as a result, supplying wastewater for non-potable purposes like agriculture can become economically viable option (Haruvy, 1997).

Arid and semi arid regions suffer more from the shortage of fresh water supply, as the rate of recharge is lower than in wet regions. In dry countries like Australia, population increase coupled with reduced rainfall has seen the need for water conservation and reuse (Anderson and Davis, 2006). Currently in urban areas there is awareness that water conservation and reuse is necessary. The situation is more heightened in rural communities where centralised water supplies may not be regular or it is difficult to supply them with fresh water.

2.3 WASTEWATER AND ITS REUSE POTENTIAL

Wastewater is generated from different sources like industries, agriculture and domestic sources. Industrial wastewater may contain high concentrations of chemicals and heavy metals. Agricultural wastewater, usually obtained from crop farms, livestock production and also processing is usually rich in nutrients necessary for plant growth and therefore high biochemical oxygen demand (BOD). Domestic wastewater can be classified into three main categories, grey water, yellow water and black water. Grey water is the wastewater from kitchen and bathroom sinks, laundry areas and showers; yellow water is mainly urine or urine with minimal flush water and; black water consists of both urine and faecal material from the toilet. These effluents can contain some chemicals and heavy metals from cleaning agents.

The main concerns with treatment and reuse of wastewater are; nutrients (mainly nitrogen and phosphorus) from organic domestic and agricultural wastes; fertilisers and chemicals from agricultural areas; heavy metals from industry; pathogens from humans and; pharmaceuticals and pathogens from hospital wastes (Toze, 2006a; Capra and Scicolone, 2007). Prior treatment of wastewater is therefore essential before discharge due to environmental and human health risks it can cause.

Conventional wastewater treatment in urban areas has been in central treatment plants that are usually far away from the origin of the wastewater (Jantrania and Gross, 2006) such as households, hospitals and industries. For example, as urbanization increased in Europe, the increased generation of wastewater has put pressure on the piped sewerage systems (Shuval *et al.,* 1997) and therefore, the recent understanding is that onsite or decentralized treatment of wastewater is the sustainable option.

Decentralised systems help to treat different types of wastes separately, at the source or close to the source (Jantrania and Gross, 2006). Decentralised systems are gaining popularity due to their increased efficiency and potential to save costs (Ujang and Henze, 2006) while offering sustainable alternatives. The main advantage of this option is that the effluent after treatment can be reused onsite (Jantrania and Gross, 2006) thereby conserving water and meeting local water demand.

Wastewater treatment

In wealthy countries wastewater is usually treated to a certain national or international standard before disposal. The treatment process normally goes through primary and secondary treatment (depending on the contaminants in the wastewater and the quality required of the treated effluent).

The first step in primary treatment is screening to remove any floating material that may interfere with treatment or could damage equipment. The wastewater flow is reduced to allow the sand and grit to settle, these are landfilled (Sturman *et al.*, 2004; Water Corporation., 2006). After this stage, the rest of the solids are allowed to settle out and are removed through a pipe. The liquid flows to a primary clarifier. If there are any more solids present, they settle in the primary clarifier and are removed as raw sludge (Pierce *et al.,* 1998).

The focus in secondary treatment is the removal of BOD, which is done by different methods, the most popular being activated sludge process. Subiaco wastewater treatment plant, in Western Australia, uses the conventional activated sludge process with biological nutrient removal (Water Corporation., 2006). Air is provided through mechanical blowers to encourage microbial activity and the excess activated sludge is removed. The overflow effluent from the sedimentation tanks will be of suitable quality that can be discharged into the ocean (Water Corporation., 2006).

2.4 GUIDELINES FOR REUSING WASTEWATER

The World Health Organization has published guidelines for the safe disposal/reuse of wastewater (WHO, 1989). The criteria were slightly different for developed and developing countries, meaning the guidelines were not very stringent for developing countries. The main reason was that higher treatment requirements, cost of treatment and technology requirements were not attainable for a developing country. As a result the WHO recommended guidelines that these countries may be able to implement without ignoring the health risks to people as well as the environment (Table 2.1). Reuse policies have been established in many countries followed by the application of projects for reusing wastewater, according to the location and resources available to those countries (Al Salem, 1996).

The USEPA (2004) suggested guidelines for water quality for reuse in agriculture for food crops that do not undergo commercial processing are; secondary treatment; filtration and disinfection where pH level is 6.9, BOD ≤ 10mg/L, no detectable fecal coliform and; residual chlorine should be <1mg/L (Table 2.1). Where water is reused for agricultural non food crops, treatment should be secondary and disinfected, BOD ≤30mg/L, TSS ≤30mg/L, <200 fecal coliform/100mL and minimal residual chlorine should be 1mg/L.

In Australia, the guidelines recommend that effluent for irrigation of food crops that are not in direct contact with the effluent has to be treated to a secondary level and must have pathogen reduction (ARMC (Australia) *et al.,* 2000). The specific parameters (Table 2.1) are pH 6.5 to 8.5 and thermotolerant coliform <1000 cfu/100mL (ARMC (Australia) *et al.,* 2000).

Table 2.1: Guidelines for wastewater reuse for food crops not in direct contact with water

Guideline	(EPP, 1995)	(ARMC (Australia) *et al.,* 2000)	(USEPA, 2004)	(WHO, 1989)
Wastewater reuse quality for food crops	Thermotolerant coliforms – median value of <1000cfu/100mL pH 6.5 – 8.5 EC 0.8mS/cm	Thermotolerant coliforms - <1000cfu/100mL pH 6.5 – 8.5	No detectable fecal coliform - <200 cfu/100mL pH 6.9	Fecal coliforms ≤1000cfu/100mL Nitrate 5-30mg/L pH 6.5- 8.5 EC 0.7-3dS/m

There are legal limitations for wastewater reclamation and reuse which need to be addressed for any particular region or country (Salgot and Pascual, 1996). It is essential that wastewater reuse in agriculture should guarantee and safeguard hygienic standards and should not have any adverse effects on the environment as a whole due to the major pollutants, pathogens, heavy metals, nitrogen and phosphorus (Al Salem, 1996).

2.5 WASTEWATER REUSE IN AGRICULTURE

The need for wastewater reuse in agriculture is great, however sustainable practices are required in this area. Reuse of wastewater in agriculture can be considered a form of wastewater management that recycles nutrients to land, which prevents pollution of surface waters (Viessman and Hammer, 2005). Reclaimed wastewater has been used for agriculture for centuries (Al Salem, 1996; Asano and Levine, 1996). Today, wastewater irrigation is practiced in almost all arid areas around the world and agriculture is the largest user of water (USEPA, 2004). Wastewater reuse was commonly integrated with agricultural use (Avnimelech, 1993; Radcliffe, 2004; Winblad and Simpson-Hebert, 2004).

In countries like Israel, Jordan and Tunisia, treated effluent is the greatest source of irrigation water. Israel is the leader in effluent reuse, with over 70% of its wastewater reused for agriculture (Kanarek and Michail, 1996; Haruvy, 1997). In California, about 63% of effluent reuse was in agricultural irrigation and 13% in landscape irrigation (Radcliffe, 2004). The amount of wastewater reused in 2000 was approximately 600GL used over about 4800 locations from 234 wastewater treatment plants (Radcliffe, 2004). In Australia, between 2001-2002 approximately 9% (166.5GL) of wastewater was reused (Radcliffe, 2004).

Wastewater irrigation is able to supply plants most of the nitrogen, phosphorus and micronutrients required (Boyden and Rababah, 1996; Haruvy, 1997; Scott *et al.*, 2000) allowing farmers to purchase less fertiliser (Sadovski *et al.,* 1978). Use of urine separating toilets and using the urine directly on land to grow crops is practised in some regions (Winblad and Simpson-Hebert, 2004). Approximately 80% of nitrogen and 50% of phosphorus in domestic wastewater comes from human urine, which has a volume of about one to one and a half litres per person per day (Adamsson, 2000). Urine separation and use of yellow water as liquid nitrogen is becoming widely recognised as an efficient use of nitrogen.

Most countries in North Africa, the Middle East and Southern Europe need to reuse wastewater on a large scale in order to sustain their population and economic growth (Shelef and Azov, 1996). In many developing countries, approximately 11 million people die attributed to malnutrition and hunger (Food and Agriculture Organisation., 2005). As a means of rectifying this, sustainable agricultural practices are important (Food and Agriculture Organisation., 2005).

The driving force for reuse of treated wastewater in Israel is the severe water crisis (Shelef and Azov, 1996). A few decades ago, the USA and many countries did not see the need to reuse wastewater, as there was no economic benefit to do so (Shuval, 1991). However, in the arid developing/under-developed countries, the economic motivation to use wastewater was higher for farmers, as freshwater was unaffordable or unavailable (Shuval, 1991). It had been decided by most countries in this region that the reuse of treated wastewater was a significant component of the sound administration and economy of water resources (Al Salem, 1996). In addition to the standards, socio-economic and hydrological situations of the countries need to be considered (Salgot and Pascual, 1996). Regulations for wastewater reuse for irrigation purposes are usually based on biological quality of the water (Salgot and Pascual, 1996) and not chemical quality.

Lubello *et al.,* (2004) found that using tertiary treated effluent, as an irrigation source, had no major limitations for plant nurseries as the nutrient content was able to maintain plant growth. According to Al Salem (1996), no adverse effects on the environment and health have been reported, if the wastewater was properly treated.

However, one of the main problems with conventional agriculture is its susceptibility to natural disasters (Food and Agriculture Organisation., 2005). The effects of this are extremely negative on remote or developing communities as their main source of income and/or nutrition can be destroyed (Food and Agriculture Organisation., 2005). Conventional agriculture has increased food availability, however, the negative environmental effects, like land clearing and pollution, has been disruptive to natural systems (Foley *et al.,* 2005).

Wastewater reuse in urban areas

Wastewater reuse in urban areas is also increasing, especially in large cities, coastal and tourist areas. The approximate quantity that is recycled in Japan is 150GL every year (JSWA, 2002). Of that, urban wastewater reuse is about 8% of the total reclaimed water (USEPA, 2004) and is reused for irrigation of public lawns, water features, car washing, cleaning streets and firefighting and in some cases, for toilet flushing as well.

In Australia, water use efficiency is low (James, 1994) and it is one of the driest continents in the world, wastewater reuse projects are essential to curb inefficiency and to meet the growing water demands (Boyden and Rababah, 1996). Since the 1980s, water availability and water quality for consumptive uses have risen to be significant Australian political issues (Taylor and Dalton, 2003). Reduction in irrigation supplies to areas like the Murray-Darling basin and water restrictions in areas like Melbourne and Perth (Macdonald *et al.,* 2005) highlight the need to reuse treated effluent.

Nutrients

Nutrients in wastewater, such as nitrogen and phosphorus, are the most problematic to the environment and can also cause health problems if discharged to the environment. Drinking water or food that has a nitrite-nitrogen concentration higher than 10mg/L is harmful to humans and about 30mg/L is harmful to animals (EPP, 1995). Levels of nitrate in potable water that exceed 4.5mg/L to 9mg/L are considered hazardous and can cause methemoglobinemia in infants (Haruvy, 1997). The drinking water quality standard for nitrogen is 10mg/L for nitrate and nitrite combined (Viessman and Hammer, 2005). Phosphorus in domestic wastewater mainly comes from human excreta and some detergents. A discharge phosphorus concentration of about 10mg/L is advantageous, if it is used for agricultural land, dependent on the soil type (EPP, 1995).

The accumulation of nitrogen and phosphorus in natural waters will result in eutrophication, this then stimulates the growth of primary producers, algae and plants (Forster, 2003). The quality of the substrate should be monitored so as to avoid accumulation of nutrients or other contaminants, which may cause groundwater contamination. However, these nutrients along with other micronutrients present in wastewater are beneficial if it is reused in agriculture.

Pathogens

Pathogens are the main health concern due to the potential to infect human beings, animals and the environment. After the British and European epidemics of the 1850s, disease transmission mechanisms and risks were recognised (Radcliffe, 2004). Wastewater streams which, contain a wide range of potentially infectious micro-organisms such as viruses, bacteria, protozoa and helminths, are a major human risk. From various studies, it has been suggested that the use of raw sewage in agriculture has led to the spread of some parasitic diseases (Al Salem, 1996). Brenner *et al.* (1988) found that some organisms like *Giardia* and *Cryptosporidium* are a major problem in wastewater and could be a concern in reuse systems.

Virus from human faeces can be present in wastewater in high numbers, up to 100,000 infectious particles per litre. They have been known to survive in water systems for long periods. As they can have a significant effect on public health, their reduction or removal is important. However, detection and enumeration of viruses in wastewater require considerable time and specialised equipment (Gersberg *et al.,* 1987; Gersberg *et al.,* 2006; Lambertini *et al.,* 2008).

Al Salem (1996) found that wastewater needed treatment especially where parasitic diseases spread easily, like those caused by intestinal parasites. Infective protozoan forms can be present in wastewater in the form of cysts and can cause disease in humans (Rose, 1997). School-aged children affected by intestinal worms can show reduced growth and cognitive functions (United Nations., 2007).

There have been cases of protozoan infections caused by the use of contaminated water on vegetables (Froese and Kindzierski, 1998) and as a result, effective treatment and monitoring is required. Human faeces and urine have been known to transmit infections by helminths (WHO, 2006b). Significant research in this area has shown that wastewater treatment including filtration and disinfection remove, destroy and inactivate parasites to low or undetectable levels (Asano and Levine, 1996; Crook, 1997; Adin and Asano, 1998). Direct contact with untreated wastewater can increase helminth infections in children more than in adult workers (WHO, 2006b). This transmission route is especially common in developing countries.

In order to avoid microbial contamination, the plants can be grown in such a way that their edible parts are not in contact with the treated wastewater, or reuse options should consider reducing the risk of contact between humans and wastewater. Processing the produce, washing and cleaning the produce can also reduce the level of pathogens on the edible parts of the plants (Abdul-Raouf *et al.,* 1993).

There is a wide spectrum of infectious diseases that may be involved and monitoring even some of them is not practical because it is both time consuming and expensive (Blumenthal *et al.,* 2000). As a result, thermotolerant coliforms are used as indicator bacteria for the faecal contamination and thus the possible presence of associated pathogens (Cooper, 1991). There is a concern that faecal coliforms and other micro-organisms can contaminate water and the soil if wastewater is used in agriculture (Furlani, 1999).

Pharmaceuticals and personal care products

There are many pharmaceuticals available for human and animal use. Many of these are not fully metabolised, thus are more likely to be found in wastewater (Jones *et al.,* 2005). Predictions about the fate of pharmaceuticals can be made based on the physical and chemical properties of compounds. However, it is difficult to determine their fate and behaviour during and after wastewater treatment (Jones *et al.,* 2005). During the wastewater treatment process they can remain unaltered, or be modified partially or completely (Xia *et al.,* 2005).

Braga *et al*. (2005) studied the fate of steroid estrogens in marine ecosystems and concluded that further investigation was required to determine whether the presence of estrogens in small concentrations affected marine ecosystems. Pharmaceuticals have been identified in municipal wastewater however, there have been no documented adverse human health effects that have been found from exposure in wastewater agriculture (WHO, 2006b).

Heavy metals

Household and industry use of toxic chemicals have increased and this has led to growing concern of the health risks associated with wastewater reuse (WHO, 2006b). Accumulation of heavy metals in plants through wastewater irrigation and application of sewage sludge is a controversial issue (Scott *et al.,* 2000).

Heavy metal contamination is an issue in centralised treatment plants where there is usually a mix of agricultural, industrial and domestic wastewater. Some countries like Scandinavia and Netherlands have stringent guidelines when discharging effluent that contains heavy metals (Scott *et al.,* 2000), while Sweden has a zero accumulation of metals policy (Witter, 1996). In Australia, there is concern over heavy metal accumulation from secondary treated wastewater irrigation (Smith *et al.,* 1996).

Heavy metal accumulation and uptake by plants depends on the plant species (Mattioni *et al.,* 1997). Visible symptoms of strong plant metal toxicity are root system reduction and retarded growth of above ground plant parts (Wilkins, 1978). As a result of these factors, it is important to know the effect of heavy metals and their uptake into plants if it is present in treated effluent. The risks are lower with reusing only domestic wastewater. There is little concern when reusing treated effluents for crop irrigation because heavy metals can be removed during common treatment processes (Sheikh *et al.,* 1987).

Farms where town refuse ash was used as a fertiliser had concentrations of lead and cadmium above recommended levels, which could be a human health concern (Pasquini, 2006). It was speculated that plants might be able to accumulate contaminants directly from the ash when it was deposited on the plant leaves. This may encourage the accumulation of heavy metals to toxic concentrations (Pasquini, 2006). Muchweti *et al*. (2006) found that vegetables grown in sludge amended soils containing high levels of heavy metals had concentrations of cadmium, copper, lead and zinc above recommended levels.

Kachenko and Singh (2006) found that accumulation of cadmium and lead could occur if crops were grown in soils where smelters were present. According to them, international guidelines were not as stringent as the Australian guidelines for cadmium and lead in vegetables. In water medium, Rai *et al.* (2002) found that *E. ferox* grown in water bodies polluted with heavy metals like chromium (0.135 μgcm^{-3}), cadmium (0.052 μgcm^{-3}), lead (0.912 μgcm^{-3}) and copper (1.59μgcm^{-3}) could lead to health problems in humans via bioaccumulation in the edible parts of the plant.

A study using silver beet in a hydroponics system examined the heavy metal concentration in the vegetables using secondary treated domestic effluent (Nair *et al.,* 2008). They found that the secondary treated effluent might not have contained adequate macro-nutrients for plant growth and as a result the silver beet could have taken up excess Zn and Cu to compensate for this deficiency. It is important that there are adequate nutrients in the treated effluent so as to minimise accumulation of unwanted elements.

2.6 WASTEWATER HYDROPONICS

The conversion of forests to agricultural land has been reported to be about 13 million hectares per annum, usually occurring in developing countries (United Nations., 2007). In order to reduce the number of people that have inadequate nutrition and sanitation, sustainable methods for food production need to be employed (Food and Agriculture Organisation., 2005). Hydroponics can help reduce the pressure on agricultural land and produce food crops where land availability is low.

Hydroponics is a method of growing plants in an artificial nutrient medium with controlled physical and chemical condition (Van Os, 1999; Oyama *et al.*, 2004; Gómez-López *et al.*, 2006). Problems from traditional agricultural and horticultural industries, like; discharge of nutrients; excess use of pesticides and; limited land availability has seen the increased use of hydroponics (Van Os, 1999; Silberbush and Ben-Asher, 2001; Mason, 2003).

Advantages of soil-less systems include higher production per unit area; energy saving, a better control of growth and independence from the quality of soil (Van Os, 1999). Another advantage of horticultural production in hydroponics systems compared to growing in soils is that external properties like temperature and pH do not limit nutrient uptake (Schwarz *et al.,* 1996). As it is possible to control temperature and amount of nutrients in the solution, crops meeting particular market standards are more likely to be produced.

Closed systems save up to 30% of water and up to 40% of nutrient (Van Os, 1999). Closed growing systems prevent the leaching of chemicals into ground and surface water. A disadvantage of hydroponics systems is the risk for rapid dispersal of diseases over the nursery (Van Os, 1999). The recirculating nutrient solution must be disinfected for long-term crop production to avoid an outbreak of root-borne disease (Van Os, 1999).

The main production system in hydroponics is the nutrient film technique (NFT) (Furlani, 1999). NFT is where there is a constant supply of nutrient solution, without a bed, for the plant roots. It is important to supply the right amount of water and nutrients in hydroponics systems (Lizarraga *et al.,* 2003).

Closed soil-less growing systems can be utilised as an environmentally friendly system and eventually to a sustainable horticulture sector (Van Os, 1999). Wastewater hydroponics, which uses wastewater as the nutrient source, is gaining attention as a bio-integrated food production system, as it helps meet the goals of sustainability by following certain principles; the waste products of one system are recycled as food for a second biological system; increasing diversity and; enhancing the system stability by regulating nutrient levels naturally (Diver, 2000).

Ohtani *et al.* (2000) saw the importance of reusing the drainage nutrient solution from traditional hydroponics systems, in order to prevent environmental pollution. This system can be taken further and used to reuse wastewater to grow plants that are able to utilise the different nutrients in the water. Various studies have been conducted in this area where the hydroponics system is used to treat different types of effluent. Rahman (1996) conducted a study in Singapore, where plants were grown in a hydroponics system using fish tank water to see whether the plants were capable of using the excess nutrients generated from the fish pond. The study was successful as both the fish and plants increased in weight and treated the fish tank water. Pinto (1996) successfully used pig slurry in the NFT system to grow crops such as tobacco, tomato and celery.

The most common type of plants used in wastewater hydroponics (for further treatment) are typically macrophytes (Osem *et al.,* 2007). Swedish research combined conventional biological treatment with hydroponics and grew micro-algae. They demonstrated that this type of treatment can be used to produce valuable plants while treating the wastewater (Norstrom *et al.,* 2003).

Mavrogianopoulos *et al.* (2002), found that wastewater as a nutrient solution in a closed gravel hydroponics culture of giant reed, provided the plants with sufficient nutrients for good growth and biomass. It was also found that plants grown in a hydroponics system absorbed most of the available nutrients from primary settled municipal effluent and more than 80% of nitrogen and 77% of total phosphorus were removed by applying it to the roots of the plants (Boyden and Rababah, 1996).

Valliant *et al.* (2004) used a combination of raw stormwater-sewage wastewater in a hydroponics system to grow woolly digitalis and foxglove and established that the NFT system significantly reduced the total organic load in the wastewaters. This system also reduced the amount of ammonium (NH_4^+) in the wastewater but the removal of total phosphorus and total nitrogen was not sufficient to allow discharge into eutrophically sensitive areas (Valliant *et al.,* 2004). Adamsson (2000) was able to grow tomatoes hydroponically using separated human urine (0.5%) and supplemented with 10mL/L of an EDTA-Fe solution.

Primary municipal sewage was successfully utilised for production of lettuce using the hydroponics system (Rababah and Ashbolt, 2000). They also recognised the need for adding nutrients like potassium for better commercial crop yield. The secondary effluent may be a good source of nitrogen and phosphorus concentration essential for food production (Furikawa and Fujita, 1993). Ayaz (1996) was successful in growing three types of macrophytes as a tertiary treatment system and observed that hydroponics can be used in limited space. The hydroponics technique was also found to be capable of degrading surfactants without large reductions in plant growth when plants such as wheat were used (Garland *et al.,* 2004).

Aquatic plants are able to accumulate metals (Crowder, 1991) and have been used in constructed wetlands to remove trace metals (Gallon *et al.,* 2004). Similarly in hydroponics, Castaldi and Melis (2004) studied the effects that different growing media (substrates); pumice; a compost containing *Posidonia oceanica* leaves found at the beach and; a combination of pruning wastes with sewage sludge, have on heavy metal concentrations in tomatoes that were hydroponically grown in a greenhouse. They found that the metal concentration in the fruits and leaves was about the same in all the plants and concluded that the substrates were suitable for use, as they did not cause metal accumulation in the tomato fruits and plants. These effects may differ slightly in liquid medium. A similar concept to wastewater hydroponics is used in the Vertical Farm concept, where plants are grown in multi-story buildings. This system treats and reuses wastewater onsite in urban areas where there is a shortage of space (Despommier, 2004).

Nitrogen and phosphorus

In hydroponics systems, the nitrogen and phosphorus dynamics are different to soil systems. The nutrient composition of most hydroponic nutrient solutions is determined by mimicking the plant shoot rather than the soil (Bot and Adamowicz, 2006). One of the main reasons for this is that the nutrient concentrations are not buffered as well as in soil media due to plant growth (Bot and Adamowicz, 2006). Plants require nitrogen in large concentrations compared to the other nutrients (Gorska *et al.*, 2008). Phosphorus availability has been known to decrease with high pH as it precipitates with calcium (Bot and Adamowicz, 2006).

Plant models

Models are a useful tool in understanding developmental mechanisms; understanding the interrelationship between various aspects of development and; determining areas where further empirical research needs to be conducted (Prusinkiewicz, 2004). This tool has been used in traditional hydroponics where numerous studies have been conducted on evaluating the required concentration of nutrients in traditional hydroponics solutions. As a result, predictions can be made on the required concentrations of nutrients for different plant types and the estimated quality and quantity of the produce. In wastewater hydroponics, it is important to first determine the suitability of the wastewater to provide the required nutrients for crop production (Lopez and Vurro, 2008). Rababah and Ashbolt (2000), recognised the need for further investigation in mathematical models for a variety of plants, particularly in nutrient uptake in wastewater hydroponics.

2.7 CONCLUSION

Utilising hydroponic techniques to tertiary treat domestic wastewater as well as to grow commercially important plants is an option that needs further investigation. There are risks when reusing wastewater. The way to utilise this valuable resource is to minimise the risks involved, such as avoiding contact of edible parts of the plant with the effluent. From the literature, it can be seen that there have been a number of studies conducted on the ability of wastewater to grow plants.

However, when combining wastewater and hydroponic techniques, there is lack of information on the pathogen contamination level, if any, on the edible parts of the plants. Another area that needs investigation is the concentration of accumulated heavy metals in different parts of the plant. Nutritional value of the edible parts of the plants is also an area that needs to be examined, when it comes to the reuse of wastewater.

If this technique is to be implemented there is a requirement to understand whether the secondary treated domestic wastewater will provide the plants with sufficient nutrients required for plant growth. These areas mentioned need to be researched further to improve the efficiency and applicability of reusing wastewater through hydroponics technique for edible or valuable crop production.

CHAPTER 3

NUTRIENT AVAILABILITY IN SECONDARY TREATED DOMESTIC WASTEWATER FOR PLANT GROWTH

3.1 INTRODUCTION

Domestic wastewater typically contains nutrients (relatively high concentrations of nitrogen, phosphorus and potassium) which are essential for plant growth and potentially toxic ions such as sodium and chloride (Unkovich *et al.,* 2004). The nutrients in reclaimed domestic wastewater can be utilised to grow plants. When using wastewater as a resource for growing plants, to be economical and efficient, it is important to assess the nutrient composition of wastewater, the efficiency of the nutrients to support optimum plant growth and the quality effects on plants due to heavy metals and pathogens contained in wastewater.

Due to the health concerns linked with using untreated effluent (Ayres and Mara, 1996), it is recommended to treat the domestic wastewater to a secondary level before using the effluent in agriculture (Oyama *et al.,* 2004). Usually wastewater reuse in developed countries for irrigation is after secondary treatment, however, in many other parts of the world, raw wastewater has been used in agriculture (Mara *et al.,* 1993). Secondary treatment typically reduces the nitrogen concentration for safe disposal and reduces the pathogen level (Unkovich *et al.,* 2004). Secondary treated wastewater still contains the nutrients that can be recovered for plant growth (Oyama *et al.,* 2004), although the concentrations and proportions of the nutrients may not be optimal for growth.

Tomatoes are an important fruit all over the world, eaten raw or cooked; in the US, tomatoes are one of the highest produced and consumed fruits (Wilcox *et al.,* 2003). Cheng *et al.* (2004) found that it was possible to grow tomatoes as part of a swine wastewater treatment and nutrient recovery process. Leafy plants could be good for stripping off nitrogen from wastewater, however its growth can be impaired if sufficient nitrogen is not available (Chen *et al.,* 2004).

Floriculture while using nutrients in wastewater reduces the risk of transferring contaminants like heavy metals and pathogens to consumers and workers from treated wastewater. Carnations are one of the most important cut flowers grown and used around the world (Reid, 2000) and hence are a good target crop for the present study. Using the hydroponics system for carnation production reduces the risk of wilt, which can be a problem when growing carnations in soil (Department of Health and Ageing Office of the Gene Technology Regulation., 2006).

This chapter investigated the potential for secondary treated domestic wastewater to provide the essential nutrients to support adequate growth of three types of commercially important plants, a leafy vegetable (silver beet), a fruit crop (tomato) and a cut-flower crop (carnation).

3.2 MATERIALS AND METHODS

Wastewater and control medium

The secondary treated domestic wastewater (WW) was collected from a domestic wastewater treatment plant (Perth, Western Australia) in 200L drums for the experiments. The wastewater was collected and used within 24 hours. The control medium used was a commercially available hydroponics nutrient solution (Ag-grow by Aquaponics WA) for fruits and vegetables. This nutrient solution was chosen for this study as it was readily available and it was the most popular in the store. The control medium (CM) was prepared as per the specified ratio of 5mL of hydroponics medium to 1L of water, recommended for vegetables, fruits and flowers. The chemical quality of secondary treated domestic effluent and the prepared control medium were analysed.

Plant selection

Common edible food crops chosen for this study were silver beet (*Beta vulgaris*), tomato (*Lycopersicon esculentum*) and a cut flower, carnation (*Dianthus caryophyllus*). The seedlings were obtained from a local nursery.

Hydroponics design

The experiment was conducted in a greenhouse (Figure 3.1) to provide uniform conditions throughout the growth phase. Secondary treated wastewater was pumped from a 42L reservoir to the channel where plants were grown. The 295cm x 12cm x 12cm channels were connected by an inlet and outlet to the reservoir. The volume of solution in one channel at any one time was approximately 7L. The effluent was drained by gravity flow back into the reservoir. The flow rate was 200L/hr. Each tray was set up as shown in Figure 3.2. Uniform-sized seedlings were purchased from a commercial nursery and planted in a pot containing expanded clay balls, that were commercially available and inserted into the eight planting slots of each channel. The planting slots of the channels were 8cm x 9cm.

Figure 3.1: Greenhouse and hydroponics set-up

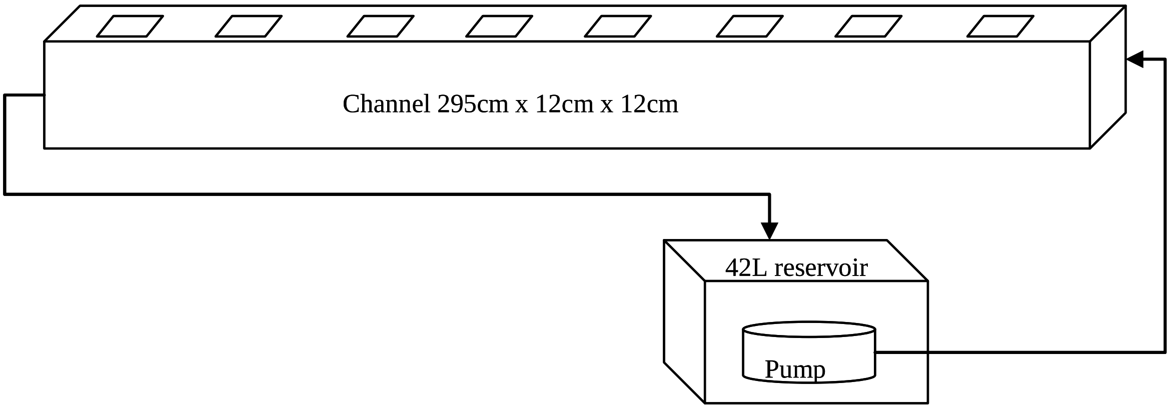


Figure 3.2: Hydroponics design

The experiment was conducted in triplicate (for both wastewater and control medium (commercial hydroponics medium)) with 8 seedlings of a species in each tray. Pumping from the reservoir into the channels and recirculation of effluent were considered to provide adequate aeration. The medium was changed every 4-5 weeks depending on the usage of the medium. Growth was monitored until the plants were ready to be harvested.

Chemical analysis

Chemical analyses of the media and growth measurement of plants were conducted every fortnight. Triplicate samples were tested for each parameter. The wastewater and control medium effluent were sampled from the reservoir and analysed for pH, electrical conductivity (EC) and dissolved oxygen (DO). The nutrient analysis for ammonium-nitrogen (NH_4^+-N), nitrate-nitrogen (NO_3^--N) and phosphate-phosphorus (PO_4^{3-}-P) were conducted using the Hach (2002) methods, which are American Public Health Association (APHA) approved. An initial composition (chlorine, phosphorus, potassium, sulfur, sodium, calcium, magnesium, copper, zinc, manganese, iron and boron) of the secondary treated wastewater and the control medium, was determined by inductively coupled plasma optical emission spectroscopy (ICP-OES) at a NATA accredited laboratory.

Plant growth measurements

Plant growth was measured by noting the stem length (centimetres) (Adamsson, 2000) and any change in appearance (observations and photographs). The number of buds, flowers and fruits were also counted every fortnight. Once the silver beet and tomatoes were harvested, the total weight of the plants were taken according to the methods described by Adamsson (2000). After harvest, the edible plant parts, that is leaves of silver beet and fruit from tomato plants and organically grown samples bought from the market were oven dried and analysed for micro and macro-nutrients (total nitrogen, phosphorus, potassium, sulfur, sodium, calcium, magnesium, copper, zinc, manganese, iron and boron). Nitrogen was analysed according to methods followed by Sweeny and Rexroad (1987); phosphorus, potassium, sulfur, sodium, calcium, magnesium, copper, zinc, manganese, iron and boron were analysed according to Zall *et al.* (1959) at a NATA accredited laboratory. The micro and macro-nutrients of samples from this experiment were compared against organically grown samples bought from the market.

For carnations, the shelf life as a cut flower was studied. On day 168, the flowers were harvested from the carnation plants. They were then put in a vase with water that was changed every 7 days. The flower and bud survival was monitored over 21 days. The number of flowers and buds alive was counted every 7 days.

Nutrient solution change

The solution was completely changed with fresh solution when there was less than 5L in the storage containers. The solution for all the silver beet channels was changed on the 35th day. The tomato solution was changed on days 35, 57 and 81. The carnation solution was changed on days 35, 57, 81, and 140.

Statistics

To determine whether there was a significant difference between the media samples and the plant samples, results of the experiments were analysed using Independent-Samples T Test and One-Way ANOVA.

3.3 RESULTS

Plant growth and production

Silver beet

The silver beet attained full growth and was ready to harvest at 56 days. Plants that showing signs of stress in the first week were replaced with healthy ones at 7 days. By day 28, the plants in the control medium (CM) were growing better than those in the wastewater (WW) (Figure 3.3). By day 42, yellowing of leaves was noted in about 58% of the WW plants and in about 25% of the CM plants (Figure 3.4). Overall the height of CM silver beet was significantly greater than that of WW (Figure 3.5). The total weight (g) of silver beet produced from 3 channels in WW was 981g and in CM was 4051g from 24 plants each. Those grown in CM had an approximate 75% increase.

Figure 3.3: Growth of silver beet grown in wastewater (WW) (foreground) and control medium (CM) (background).

Figure 3.4: Yellowing of silver beet grown in wastewater (WW).

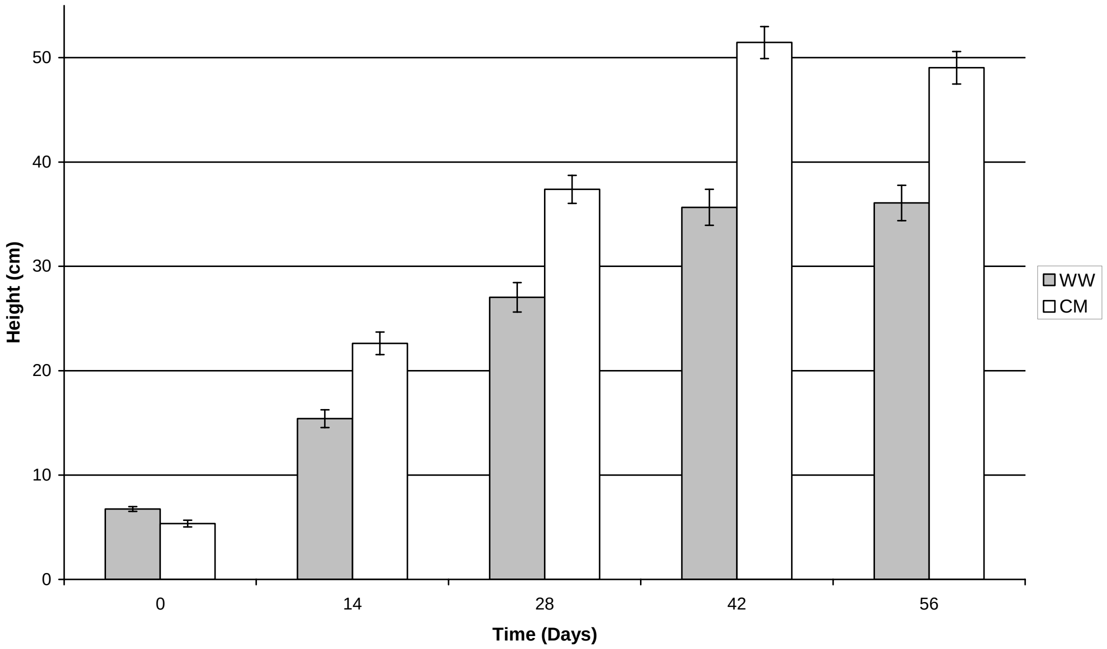


Figure 3.5: Comparison of height (cm) of silver beet grown in wastewater (WW) and control medium (CM) (mean of 3 replicates ± standard error (se)).

Tomatoes

Tomato fruits were ready for harvest within 96 days when the experiment was concluded. Some tomato seedlings also showed stress during the first week and were replaced by healthier ones. By day 56, the plants were showing some nutritional stress, three tomato plants in the CM had yellow leaves and there was grey mould on the leaves and stems of the CM tomato plants. By day 70, the death rate of tomato plants was 4.2% in the WW channels and 8.3% in the CM. The plants in the CM had more grey mould forming than WW. By day 84, the older leaves in some of the tomato plants in the WW had begun turning yellow and purple (Figure 3.6). The affected leaves on these plants were removed. The plant size (biomass) was greater in CM than in WW.

There were symptoms resembling cracking in some tomato fruits grown in WW (Figure 3.7). Affected fruit had a brownish lesion encircling it (Figure 3.7). The tomato fruit size was bigger in CM (Figures 3.8 and 3.9) than those from WW. Like the silver beet, CM tomato plants were taller ($p<0.05$) than WW tomato plants (Figure 3.10). However, there was no statistical significant difference in average diameter or average weight between the tomato fruits grown in WW and CM ($p>0.05$) (Table 3.1). The total weight of WW tomatoes harvested was 1562g and CM tomatoes 3227g. There were more tomatoes in CM treatment than in WW (Table 3.1).

Figure 3.6: Purple and yellow tomato leaves from wastewater (WW) treatment.

Figure 3.7: Cracking in wastewater (WW) tomato

Figure 3.8: Tomatoes harvested from control medium (CM) (left), wastewater (WW) (right).

Figure 3.9: Size of whole tomatoes grown in control medium (CM) (top) and wastewater (WW) (middle) compared to organic produce (O) from market (bottom).

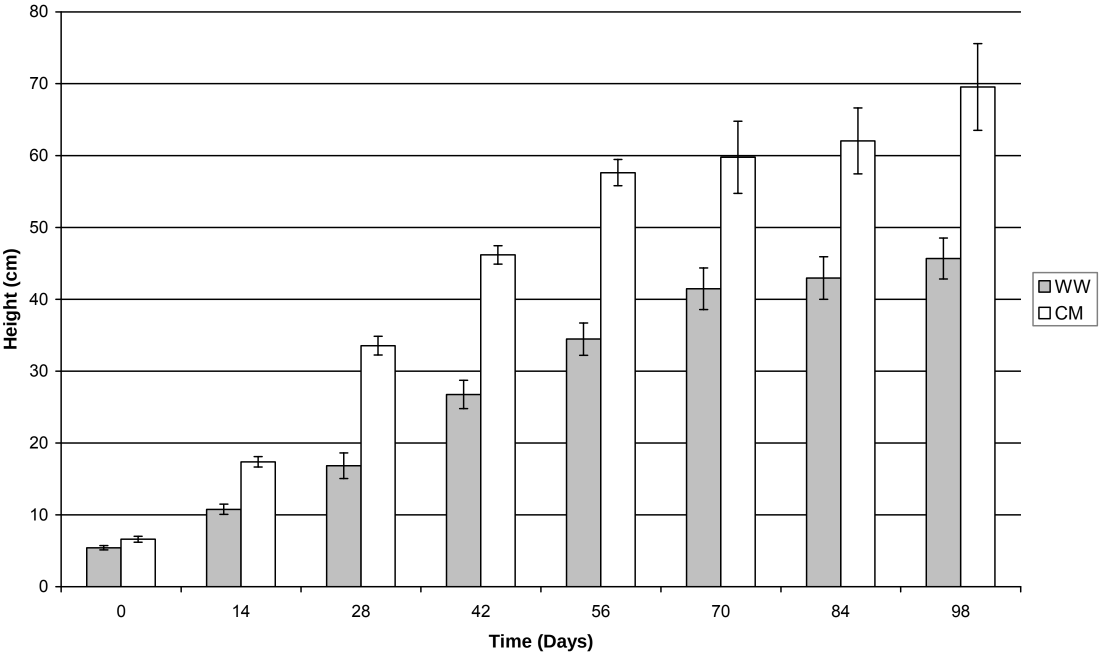


Figure 3.10: Comparison of height (cm) of tomato plants grown in wastewater (WW) and control medium (CM) (mean of 3 replicates ± se).

Table 3.1: Total number of tomato fruits, average diameter ± se and average weight ± se in wastewater (WW) and control medium (CM).

	WW	**CM**
Total number of fruit	56	109
Diameter (cm ± se)	11 ± 1	14 ± 1
Weight (g± se)	31 ± 6	49 ± 4

Carnations

Overall, carnations took the longest to grow (168 days) compared to tomatoes (98 days) and silver beet (56 days). The carnations grown in WW produced more flowers than those in CM (Figure 3.11). The WW carnations had approximately four times more buds than CM carnations (Table 3.2, Figure 3.12).

Figure 3.11: Carnations grown in wastewater (WW) (left) and control medium (CM) (right).

Table 3.2: Mean number of carnation buds and flowers (± se) grown in wastewater (WW) and control medium (CM) per plant

Day	**WW**		**CM**	
	No of buds	**No of flowers**	**No of buds**	**No of flowers**
84	0.04 ± 0	0	0	0
98	0.13 ± 0.01	0	0.2 ± 0.1	0
112	1 ± 0.2	0	0.2 ± 0.1	0
126	2 ± 0.5	0.04 ± 0	0.2 ± 0.1	0
140	3 ± 0.7	0.04 ± 0	0.6 ± 0.2	0.13 ± 0.06
154	4 ± 0.9	0.1 ± 0.01	0.7 ± 0.2	0
168	8 ± 1	0.4 ± 0.1	2 ± 0.4	0

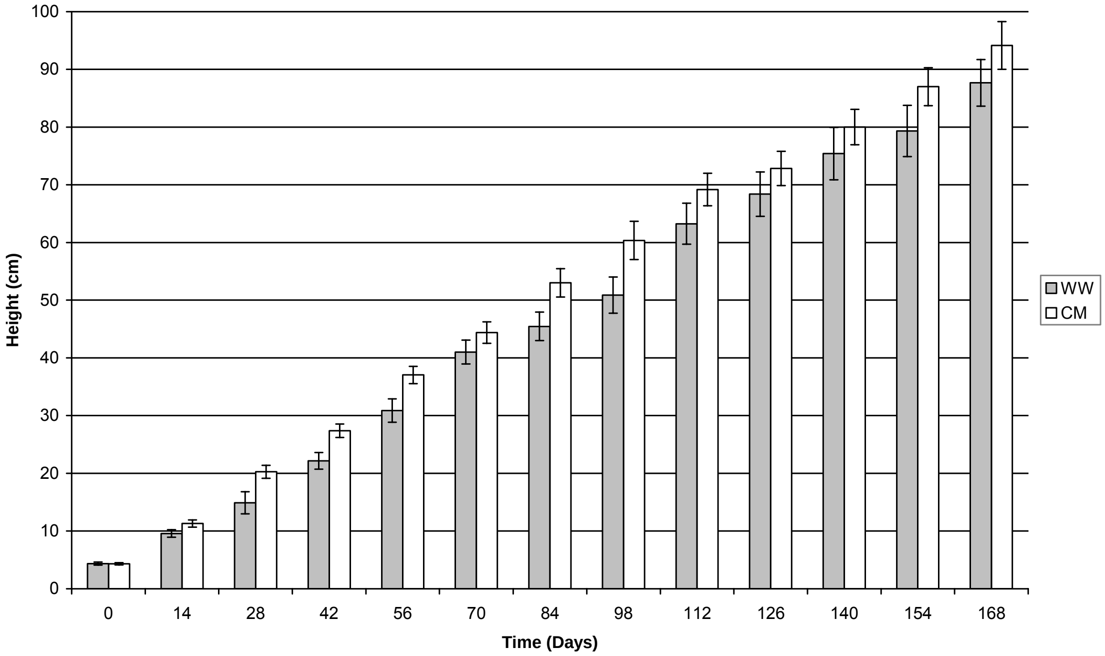


Figure 3.12: Comparison of height (cm) of carnation plant grown in wastewater (WW) and control medium (CM) (mean of 3 replicates ± se).

Water treatment efficiency

The initial chemical composition of secondary treated WW and prepared CM solution is shown in Table 3.3. Most of the elements in the CM had a higher concentration except chlorine (85.4mg/L in CM; 94.8mg/L in WW) and sodium

(55.4mg/L in CM; 75.8mg/L in WW) (Table 3.3). The potassium and iron concentration in the CM (140mg/L and 1.78mg/L respectively) was about 14 times and 18 times more than in the WW (11.4mg/L and 0.05mg/L respectively).

Table 3.2: Chemical composition of the secondary treated domestic wastewater (WW) and formulated control medium (CM) (mg/L).

Element	**Secondary treated WW (mg/L)**	**Prepared solution of CM (mg/L)**
Chlorine	95	85
Sodium	76	55
Nitrogen	44	125
Calcium	20	80
Sulfur	12	55
Potassium	11	140
Phosphorus	6	24
Magnesium	4	43
Copper	0.1	0.2
Zinc	0.1	0.4
Manganese	0.1	0.4
Iron	0.1	2
Boron	0.1	0.2

Silver beet

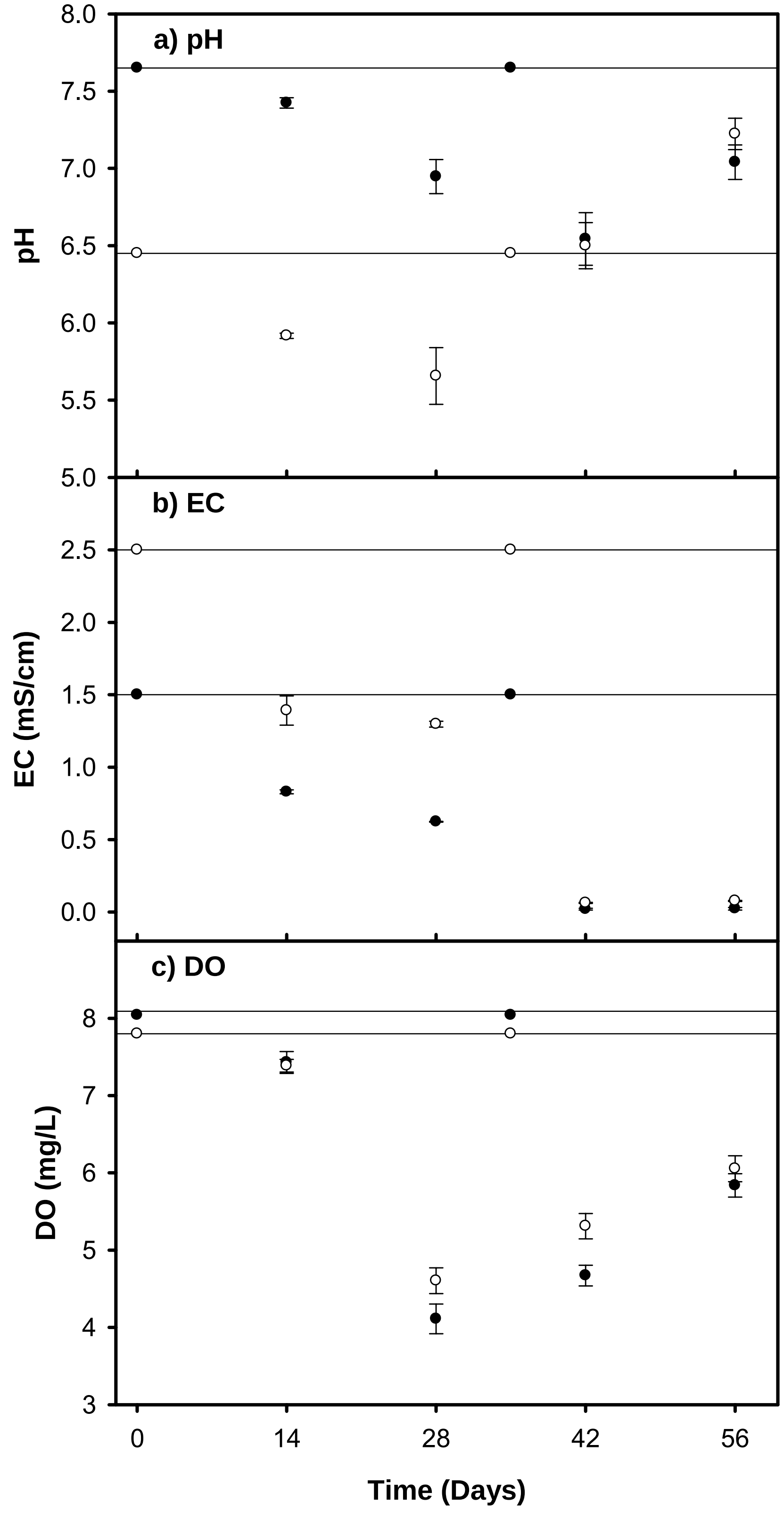
The pH, EC and DO in WW and CM treatments growing silver beet generally decreased after solution change (days 0 to 4), however, increased between days 42 to 56 (Figures 3.13 a, b and c). The final wastewater effluent after the treatments using silver beet had a pH 7.04, DO 5.85mg/L and EC 0.07mS/cm, while the final effluent of CM had pH 7.3, DO 6mg/L and EC 0.08mS/cm (Table 3.7).

The NO_3^--N concentration was about fifty times higher (range) in CM than WW (Figure 3.14b). In the CM treatments, the NO_3^--N concentration decreased after every solution change. WW contained four times higher NH_4^+-N than CM (Figure 3.14a). The NH_4^+-N concentration decreased for both WW and CM treatments after every solution change. CM contained two and half times higher (range) PO_4^{3-}-P concentration in fresh solutions than WW and the levels were higher (range) throughout (Figure 3.14c). There was a general decrease of PO_4^{3-}-P in both treatments except between days 14–28 ($p<0.05$) where the concentration increased (WW, 1mg/L - 1.2mg/L and CM, 6.1mg/L - 7.1mg/L).

The day 56 NH_4^+-N, NO_3^--N and PO_4^{3-}-P concentration decreased in all treatments (Figure 3.13 and Table 3.7), and there was no significant difference between the WW and CM effluent (Table 3.4).

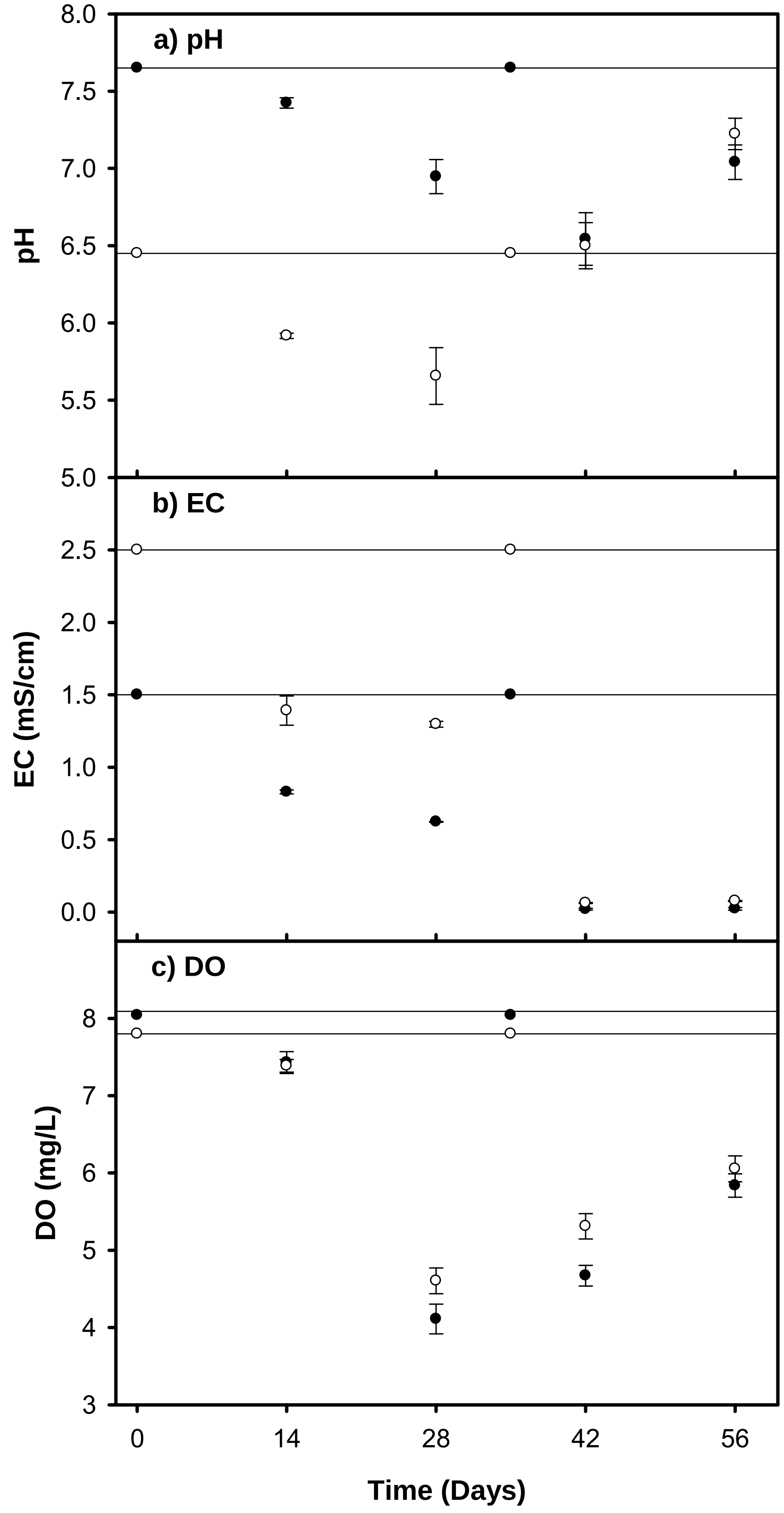
Table 3.4: p values from ANOVA between the control medium (CM) and wastewater (WW) silver beet solutions.

Day	**pH**	**EC**	**DO**	**NO_3^--N**	**NH_4^+-N**	**PO_4^{3-}-P**
14	0.000	0.804	0.005	0.008	0.009	0.001
28	0.004	0.125	0.000	0.001	0.499	0.001
42	0.858	0.039	0.003	0.032	0.084	0.001
56	0.29	0.392	0.005	0.209	0.483	0.099



○ Control medium ● Wastewater

Figure 3.13: Changes in pH (a), EC (b) and DO (c) in silver beet channels. Vertical bars where visible indicate standard errors. Horizontal lines indicate initial solution concentration. Solution change on day 35.



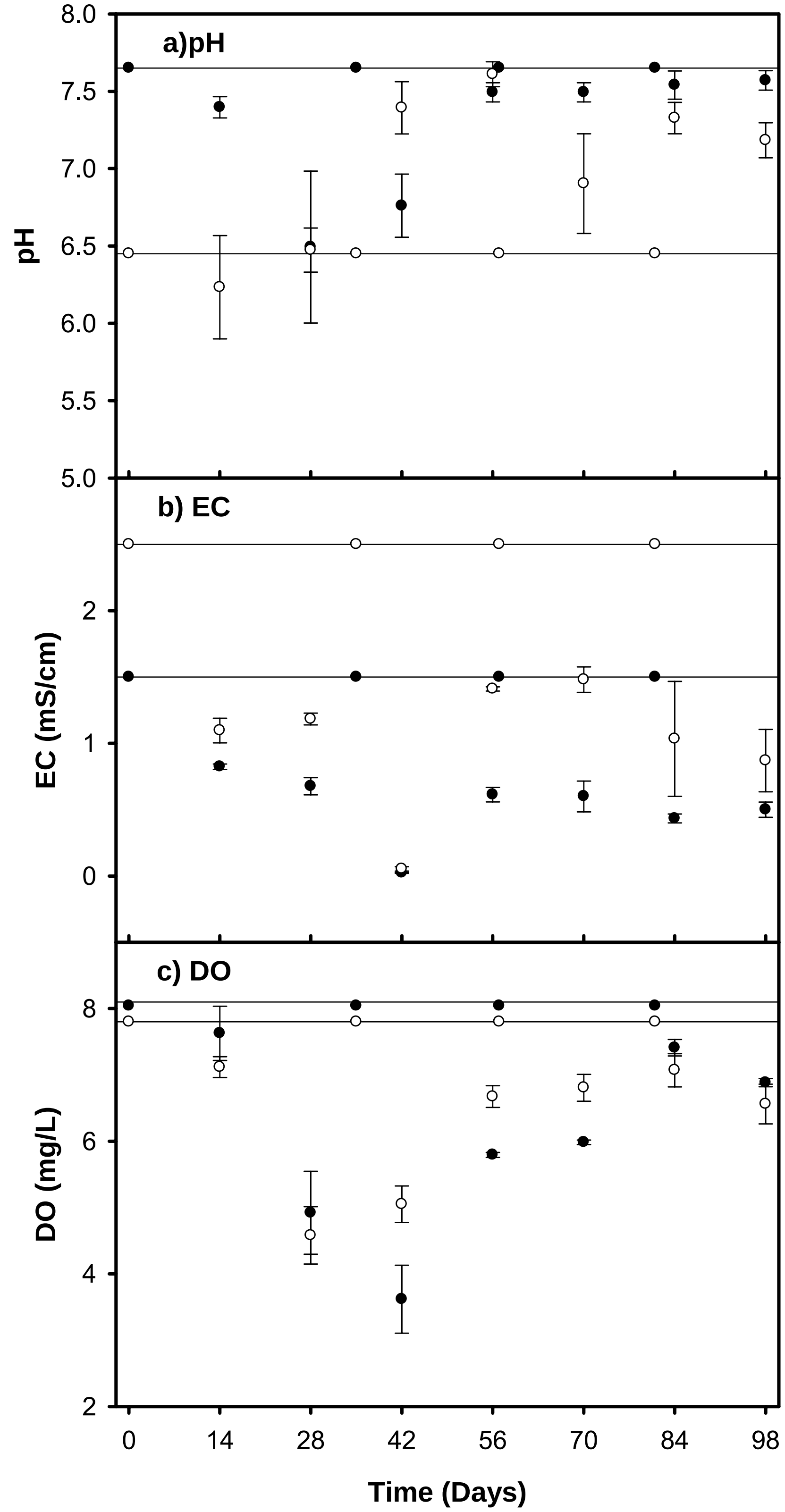
○ Control medium ● Wastewater

Figure 3.14: NO_3^--N (a), NH_4^+-N (b) and PO_4^{3-}-P (c) in silver beet channels. Vertical bars where visible indicate standard errors. Horizontal lines indicate initial solution concentrations. Solution change on day 35.

Tomatoes

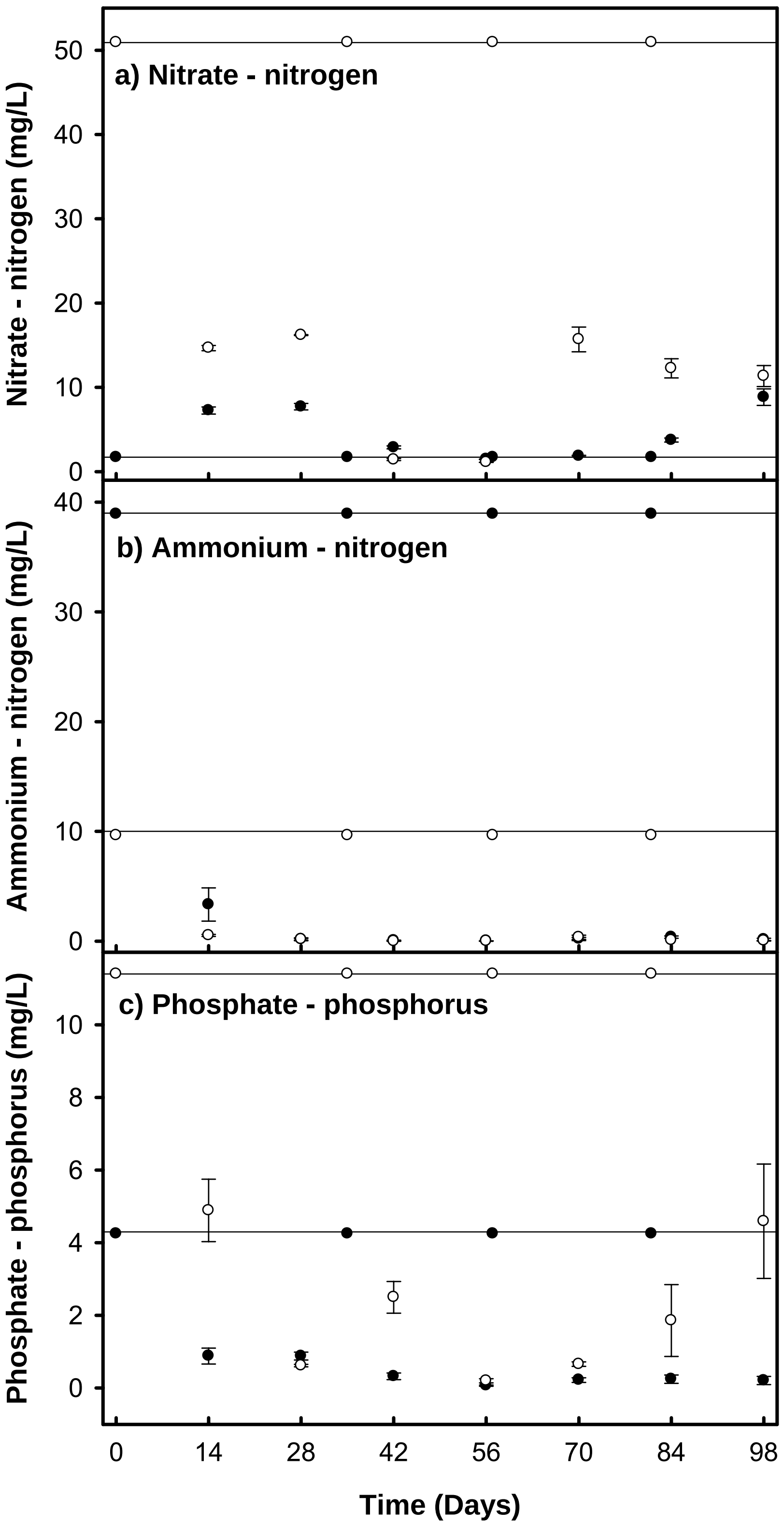
In both treatments, pH and EC levels generally decreased and the DO concentration increased between days 42 to 56. However between days 14 to 28, the levels increased in CM (6.45 - 7.61) (Figure 3.15a). During this time WW pH decreased from 7.4 to 6.5 (Figure 3.15a). Between days 42 to 56 the EC concentration increased in WW (0.02mS/cm - 0.6mS/cm) and CM (0.05mS/cm - 1.4mS/cm) (Figure 3.15b). After solution change, the EC concentration increased in WW (0.4mS/cm - 0.5mS/cm) and decreased in CM (1mS/cm - 0.9mS/cm) (Figure 3.15b). The DO concentration increased between days 42 and 56 in both treatments (WW 3.6mg/L to 5.8mg/L and CM 5.05mg/L to 6.67mg/L) (Figure 3.15c). The final WW effluent after the hydroponics treatment had pH 7.6, EC 0.6mS/cm, DO 5.8mg/L and for CM had pH 7.3, EC 1.4mS/cm, DO 6.67mg/L (Table 3.7).

The NO_3^--N concentration in WW increased after solution change (Figure 3,15a). There was an increase in concentration between days 14 and 28 in CM solution (14.67mg/L to 16.21mg/L) (Figure 3.15a). NH_4^+-N concentration (Figure 3.15b) in both WW and CM dropped to zero between solution changes. The lowest NH_4^+-N concentration for WW was 0.03mg/L (day 56) and 0.16mg/L (day 98), the lowest concentration for CM was 0.13mg/L (day 42) and 0.18mg/L (day 98). The rate of decline was faster than when the plants were larger after the first growth phase (Table 3.7). There was a general decrease in PO_4^{3-}-P (Figure 3.15c), however there was an increase in concentration in CM between days 84 and 98 (1.86mg/L to 4.59mg/L).



○ Control medium ● Wastewater

Figure 3.15: Changes in pH (a), EC (b) and DO (c) in tomato channels. Vertical bars where visible indicate standard errors. Horizontal lines indicate initial solution concentrations. Solution change on days 35, 57 and 81.



○ Control medium ● Wastewater

Figure 3.16: NO_3^--N (a), NH_4^+-N (b) and PO_4^{3-}-P (c) concentration in tomato channels. Vertical bars where visible indicate standard errors. Horizontal lines indicate initial solution concentrations. Solution change on days 35, 57 and 81.

Table 3.5: Tomato solution p (0.05) values from ANOVA between in wastewater (WW) and control medium (CM) treatments.

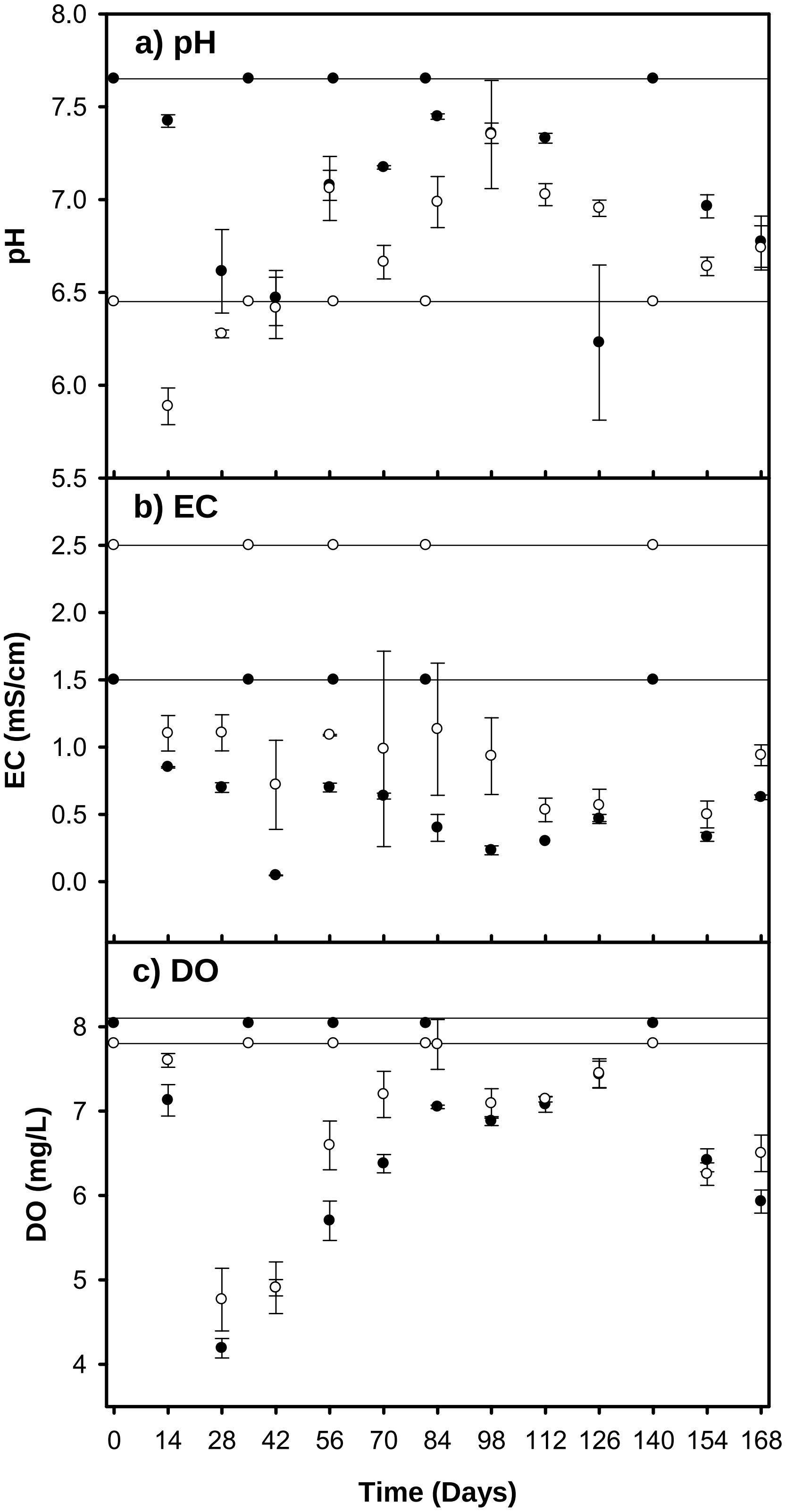
Day	pH	EC	DO	NO_3^--N	NH_4^+-N	PO_4^{3-}-P
14	0.027	0.046	0.309	0.035	0.139	0.011
28	0.971	0.003	0.678	0.008	0.951	0.086
42	0.075	0.152	0.07	0.195	0.21	0.008
56	0.315	0.000	0.007	0.039	0.089	0.092
70	0.147	0.004	0.016	0.098	0.596	0.008
84	0.193	0.289	0.24	0.17	0.07	0.181
98	0.041	0.351	0.201	0.737	0.369	0.049

Carnations

The final WW effluent after the hydroponics treatment had pH 6.8, EC 0.03mS/cm, DO 6.4mg/L and CM had pH 6.6, EC 0.5mS/cm, DO 6.3mg/L (Table 3.7). The only time that the pH in CM decreased was between days 0 to 14 (6.45 - 5.89) and days 98 to 126 (7.35 - 6.95) (Figure 3.17a). The WW pH at the same period decreased from 7.7 - 7.4 between days 0 to 14 and 7.4 - 6.2 between days 98 to 126 (Figure 3.17a). EC increased in WW from day 98 (0.23mS/cm) to day 126 (0.47mS/cm), while in the CM; it increased from day (0.23mS/cm) to day 126 (0.57mS/cm) (Figure 3.17b). The DO concentration increased for both channels from day 42 (4.91mg/L in WW and 4.91mg/L in CM) to day 56 (5.7mg/L in WW and 6.59mg/L in CM) and 98 (6.88mg/L in WW and 7.09mg/L in CM) to 126 (7.43mg/L in WW and 7.45mg/L in CM) (Figure 3.17c).

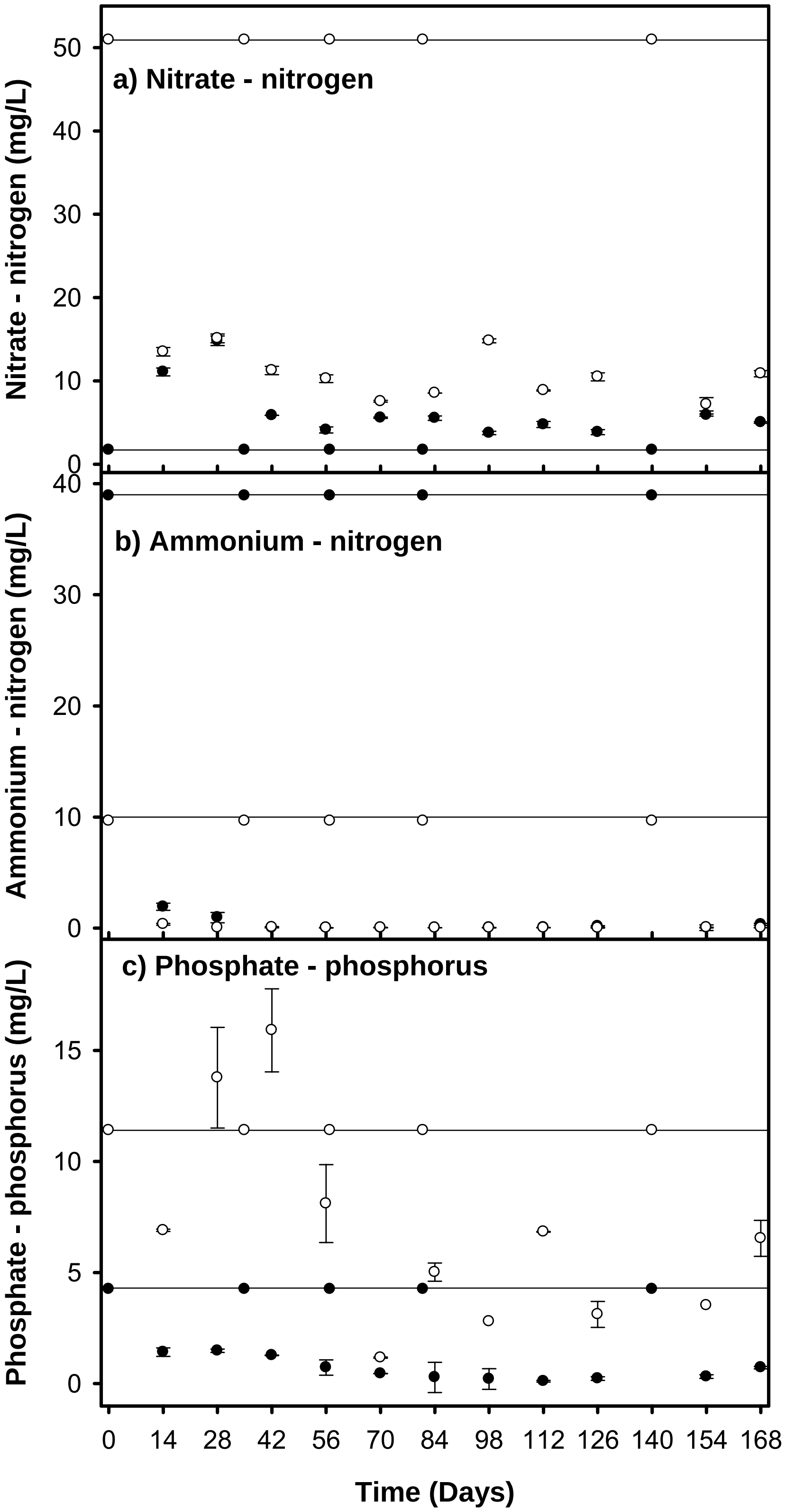
There was a similar nutrient (NO_3^--N, NH_4^+-N and PO_4^{3-}-P) trend in carnation solutions to silver beet and tomatoes. The NH_4^+-N concentration was higher in the WW treatment than the CM. The NO_3^--N and PO_4^{3-}-P concentration was higher in the CM treatment than the WW.

There was an increase in NO_3^--N concentration in CM between days 14 to 28, 84 to 98, 112 to 126 and 154 to 168 (Figure 3.18a). The NO_3^--N concentration increased in WW from day 0 to 28 (1.7mg/L to 14.8mg/L), 35 to 56 (1.7mg/L to 4.1mg/L) and 57 to 70 (1.7mg/L to 5.6mg/L) (Figure 3.18a). The lowest concentration NH_4^+-N was recorded on day 56 for WW (0.02mg/L) and day 126 for CM (0.01mg/L) (Figure 3.18b). In the CM channel, the NH_4^+-N concentration increased between days 84 to 98 (7.2mg/L to 10.9mg/L) and between days 154 to 168 (8.5mg/L to 14.8mg/L) (Figure 3.18b). There was a general decrease in PO_4^{3-}-P in WW, but the concentration fluctuated in CM (Figure 3.18c). The PO_4^{3-}-P concentration in the CM decreased from days 81 to 98 (11.4mg/L to 2.8mg/L). In the WW channel, the PO_4^{3-}-P concentration dropped after the solution was replaced, with the exception of an increase between days 154 to 168 (0.2mg/L to 0.7mg/L) (Table 3.7).



○ Control medium ● Wastewater

Figure 3.17: Comparison of pH (a), EC (b) and DO (c) changes in carnation channels. Vertical bars where visible indicate standard errors. Horizontal lines indicate initial solution concentrations. Solution change on days 35, 57, 81 and 140.



○ Control medium ● Wastewater

Figure 3.18: NO_3^--N (a), NH_4^+-N (b) and PO_4^{3-}-P (c) in carnation channels. Vertical bars where visible indicate standard errors. Horizontal lines indicate initial solution concentrations. Solution change on days 35, 57, 81 and 140.

Table 3.6: Carnation solution p values from ANOVA between wastewater (WW) and control medium (CM) treatments.

Day	pH	EC	DO	NO_3^--N	NH_4^+-N	PO_4^{3-}-P
14	0.000	0.128	0.079	0.476	0.009	0.000
28	0.34	0.043	0.212	0.935	0.121	0.006
42	0.822	0.112	1	0.7	0.418	0.001
56	0.935	0.000	0.0704	0.079	0.938	0.014
70	0.005	0.01	0.049	0.033	0.262	0.000
84	0.029	.0217	0.067	0.088	0.622	0.000
98	0.009	0.057	0.551	0.064	0.251	0.000
112	0.160	0.468	0.957	0.057	0.061	0.000
154	0.015	0.189	0.439	0.738	0.726	0.000
168	0.864	0.017	0.089	0.026	0.036	0.002

Table 3.7: Quality of effluent disposed after hydroponics treatment of silver beet, tomatoes and carnations

	Days of effluent disposal													
	Wastewater							**Control medium**						
	0	**35**	**56**	**81**	**98**	**140**	**168**	**0**	**35**	**56**	**81**	**98**	**140**	**168**
							Silver beet							
pH	7.7	7	7					6.5	6.5	7.2				
EC (mS/cm)	1.5	0.6	0.02					2.5	1.3	0.08				
DO (mg/L)	8	4	6					8	5	6				
NO_3^--N (mg/L)	1.72	1.3	0.3					50.9	19	4.2				
NH_4^+-N (mg/L)	39	0.1	0.05					10	0.2	0.04				
PO_4^{3-}-P (mg/L)	4.3	1.2	0.04					11	7.1	2.4				
							Tomatoes							
pH	7.7	6.5	7.5	7.7	7.6			6.5	6.5	7.6	6.5	7.2		
EC (mS/cm)	1.5	0.7	0.6	0.6	0.5			2.5	1.2	1.4	1.5	0.9		
DO (mg/L)	8	4.9	5.8	8	6.9			8	4.6	6.7	7.8	6.6		
NO_3^--N (mg/L)	1.72	7.7	1.5	1.8	8.8			50.9	16	1.1	16	11.3		
NH_4^+-N (mg/L)	39	0.2	0.03	0.2	0.2			10	0.2	0.02	0.4	0.05		
PO_4^{3-}-P (mg/L)	4.3	0.9	0.07	0.2	0.2			11	0.6	0.2	0.7	4.6		
							Carnations							
pH	7.7	6.7	7.1	7.2		6.2	6.8	6.5	4.2	5.7	6.4		7.4	6
EC (mS/cm)	1.5	0.7	0.7	0.6		0.5	0.6	2.5	1	1.1	1		0.6	0.9
DO (mg/L)	8	4	5.7	6.4		7.4	6	8	4.8	6.6	7.2		7.4	6.5
NO_3^--N (mg/L)	1.72	15	4	5.6		3.9	5	50.9	15	10	7.6		10.5	10.9
NH_4^+-N (mg/L)	39	1	0.03	0.03		0.2	0.3	10	0.05	0.03	0.5		0.01	0.04
PO_4^{3-}-P (mg/L)	4.3	1.5	0.7	0.5		0.2	0.7	11	13.8	8.1	1.2		3	6.5

Nutrient concentration in silver beet and tomatoes

Table 3.8 shows the nutrient concentration in silver beet leaves and tomato fruits. The copper concentration was similar in all silver beet samples. In the leaves, sodium and zinc are the only elements that have a higher concentration in WW (44737mg/kg and 888mg/kg respectively) than the CM (17933mg/kg and 338mg/kg respectively) and the organically grown (34690mg/kg and 79mg/kg respectively) silver beet.

Total nitrogen, sulphur and sodium concentrations in the organically grown (46300mg/kg, 37400mg/kg and 34690mg/kg respectively) silver beet were higher than the CM (45133mg/kg, 3393mg/kg and 17933mg/kg respectively). The iron concentration in the organic produce was approximately (223mg/kg in O; 85mg/kg in WW; 113mg/kg in CM) that in the WW and CM silver beet.

Phosphorus, potassium, calcium and magnesium concentrations were higher in the CM silver beet (6163mg/kg, 40510mg/kg, 12880mg/kg and 10690mg/kg respectively) than in the organically grown (4220mg/kg, 18700mg/kg, 12270mg/kg and 6740mg/kg respectively) and WW (3177mg/kg, 14867mg/kg, 9237mg/kg and 4193mg/kg respectively). The boron concentration was higher in both the CM (53mg/kg) and WW (58mg/kg) than O grown silver beet.

The organically grown tomatoes had the highest concentration of the remaining trace elements. Phosphorus concentration in the CM tomatoes (5947mg/L) was higher than the rest (WW 2897mg/L, O 5350mg/L). The CM (63mg/L) tomatoes had the highest concentration of zinc (WW 33mg/L, O 28mg/L). The sodium concentration in the WW grown tomatoes (3520mg/L) was higher than in those grown in the CM (1407mg/L) and the organic produce (1080mg/L).

Table 3.8: Comparison of chemical concentration (mg/kg) in silver beet leaves, tomato fruits grown in wastewater (WW), control medium (CM) and organically (O).

Chemical	**Silver beet ± se (mg/kg)**			**Tomatoes ± se (mg/kg)**		
	WW	**CM**	**O**	**WW**	**CM**	**O**
Total nitrogen	24767 ± 2663	45133 ± 2577	46300	16200 ± 12474	23933 ± 723	38900
Phosphorus	3177 ± 292	6163 ± 1010	4220	2897 ± 304	5947 ± 1115	5350
Potassium	14867 ± 2481	40510 ± 6118	18700	21657 ± 1779	45833 ± 6110	63950
Sulfur	368 ± 11	3393 ± 211	37400	1607 ± 196	2570 ± 301	3020
Sodium	44373 ± 3625	17933 ± 2137	34690	3520 ± 332	1407 ± 803	1080
Calcium	9237 ± 1085	12880 ± 704	12270	1203 ± 337	1377 ± 659	1530
Magnesium	4193 ± 798	10690 ± 1630	6740	1047 ± 55	2150 ± 160	3210
Copper	12 ± 2	14.89 ± 0	12	8 ± 1	11 ± 1	33
Zinc	888 ± 84	338 ± 125	79	33 ± 3	63 ± 23	28
Manganese	33 ± 6	198 ± 19	19	15 ± 1	25 ± 3	27
Iron	85 ± 12	113 ± 13	223	34 ± 2	40 ± 2	71
Boron	58 ± 3	53 ± 5	27	13 ± 2	12 ± 1	20

Carnations

Survival of buds and flowers in a vase

The survival of the buds and flowers was similar for both WW and CM when placed in tap water.

Table 3.9: Survival of number of buds and flowers in a vase. Values are means of 24 plants ± standard errors.

	WW		**CM**	
Day	**Buds**	**Flowers**	**Buds**	**Flowers**
0	2 ± 0.3	5 ± 0.4	2 ± 0.7	6 ± 0.8
7	1 ± 0.2	3 ± 0.5	2 ± 0.5	3 ± 1
14	1 ± 0.2	2 ± 0.5	2 ± 0.6	2 ± 1

3.4 DISCUSSION

Effluent quality

The pH and EC levels of the final effluent after growing silver beet, tomatoes and carnations met the disposal limits set by EPP (1995), which are pH between 6.5 to 8.5 and EC<0.8mS/cm. After every solution change, the amount of NH_4^+-N declined and the concentration of NO_3^--N increased in the WW solutions (Table 3.7) before discharge. This may be due to the aeration from the recirculating system encouraging nitrification in the system (Kelly *et al.,* 2006). According to the EPP (1995), the acceptable concentration for nitrate for irrigation is <10mg/L to 15mg/L and all wastewater crop solutions were below 15mg/L. The PO_4^{3-}-P effluent concentrations after going through the hydroponic system were all below 1.5mg/L in the WW treatment. The EPP (1995) guidelines recommend phosphorus concentration of treated effluent to be <10mg/L, however, this depends on the phosphorus sorption capacity of the soil.

Crop production and nutrient quality

The leafy vegetable silver beet (Swiss chard, *Beta vulgaris* type cycla) is known for its nutritional properties and year round supply (Roura *et al.,* 2000). It is similar to spinach however in some places silver beet is preferred due to its low price. Oyama *et al.* (2005) found that bok choy (a small leafy vegetable) did not grow successfully in secondary treated domestic wastewater when grown in an aerated hydroponic system.

Secondary treated domestic wastewater for silver beet production meets the EC threshold concentration, which is <2mS/cm and for tomato solution is 2.3mS/cm (Jarwal *et al.,* 2006). In this experiment, tomato WW solution had a maximum conductivity concentration of 1.5mS/cm, by contrast with the CM that had an initial concentration around 2.5mS/cm (Figure 3.15b).

From observations (Figures 3.3, 3.4 and 3.6) the silver beet and tomatoes had similar symptoms (stunted growth and yellow leaves) in WW treatment and according to symptoms described by Wier and Cresswell (1993) as well as the leaf analysis conducted (Table 3.8), the most likely nutrient that is deficient would be nitrogen and/or sulfur. This can also be seen in Table 3.8 where the concentration of total nitrogen in the tomatoes grown in wastewater was much lower than those from the other treatment. In order to have sufficient nutrients for the crops' growth, more nutrients would need to be provided.

It can be clearly seen that the silver beet and tomatoes grown in CM had much better height than those grown in the WW (Figures 3.5 and 3.10). The CM produced a higher number of tomatoes (Table 3.1) than the WW and this could also be attributed to the concentration of nutrients in the commercial solution. Increasing the concentration of only nitrogen or phosphorus may result in a deficiency of another nutrient (Marschner, 1995). From this it could be deduced that the best solution to this problem could be to reduce the nutrient solution retention time in the channels and replace with fresh medium, which could increase the supply of nutrients. The cuticle cracking on the WW tomatoes (Figure 3.7) could be a result of the infrequent nutrient solution retention time, as infrequent watering can be a cause (Dorais *et al.,* 2004).

Michitsch *et al*. (2007) found that turfgrass species grown in anaerobically treated diluted wastewater (1:19) had poor growth compared to other treatments (nutrient solutions and commercial fertiliser + wastewater). Cost of chemical fertilisers can be high (Parveen *et al.,* 2006) and in order to reduce this cost, Michitsch *et al*. (2007) suggested reducing the nutrient solution retention time of the wastewater to two weeks so that more nutrients would be available for plants.

The most ideal pH for carnation growth is around 6.5 to 7.5 (Reid, 2000). However, pH concentration in carnation media has been known to drop to around 4.5 (Dantas *et al.,* 2001). The carnation media in this study had a pH level of around 5.8 to 7.3 in CM and 6.3 to 7.7 in WW (Figure 3.17a). Carnations prefer water that has an EC between 0.7mS/cm to 1.2mS/cm (Reid, 2000). Baas *et al.* (1995) found that when salinity was 4.8dS/m, carnation production decreased. They also found that even though the plant was under stress due to high EC, there were no visible symptoms present. However, in this study, the EC was between 1.5mS/cm to 0.3mS/cm in WW and 2.5mS/cm to 0.5mS/cm in CM, which was well within the threshold level.

There was no significant difference between the carnations grown in WW and CM (Figure 3.12) in terms of the height of the plants. However, the number of buds and flowers produced from WW-fed carnations was significantly higher than CM-fed carnations (Table 3.2). Carnations are normally harvested when the bud has fully opened, or they could also be harvested when the petals expand above the calyx (Salinger, 1987). The survival of the flowers after harvest is extremely important, this also affects their sale price (Heo *et al.,* 2004). There was no significant difference in the survival of buds and flowers grown in WW and CM (Table 3.9). The buds and flowered carnations survived in water for approximately 18 days in both treatments from the time they were picked.

Based on the results, it seems that the carnations had adequate supply of nutrients (NO_3^--N, NH_4^+-N and PO_4^{3-}-P) for their growth as the final WW effluent still contained nutrients. By contrast, the silver beet had a higher demand for nutrients, particularly nitrogen resulting in very low nutrient concentrations within 35 and 20 days of the solution change (Table 3.7).

The results of this chapter have shown that cut flowers can be successfully grown using the NFT technique with secondary treated domestic wastewater, which can generate income in rural/developing communities as well as reduce environmental degradation, both of which are Millennium Development Goals (United Nations., 2007).

3.5 CONCLUSION

The carnation plants grown in wastewater seem to receive adequate nutrients from secondary treated domestic effluent even with three weeks of medium retention in the channels. From the results it can be seen that secondary treated domestic wastewater contains the essential nutrients for plant growth, however it has been noticed that the silver beet and tomato plants' growth and production in WW was not comparable to CM, which could be due to inadequate supply of nutrients, particularly nitrogen. It is therefore important to investigate the frequency of media change instead of adding chemical fertilisers to the existing media. It is also important to find a balance of when it is safe to discharge the effluent and not affect the plant growth greatly.

CHAPTER 4

EFFECT OF INCREASED NUTRIENT AVAILABILITY ON THE PLANTS

4.1 INTRODUCTION

Chapter 3 revealed that the edible food crops (tomatoes and silver beet) grown in secondary treated domestic WW did not receive sufficient nutrient with approximately three weeks retention time of solutions. As a result, stunted growth and reduced production was obtained. For this reason, in this study, reduced media retention time was tested to improve the nutrient availability to plants.

As the carnations grown in the previous study (Chapter 3) showed satisfactory growth in WW and the plants grown in WW were of better quality than those grown in the control medium, it was decided not to include carnations in this trial.

The aim of this study was to examine whether a nutrient solution retention time of 14 days using secondary treated domestic effluent would provide sufficient nutrients to tomatoes and silver beet to achieve comparable production as obtained by the commercial medium.

4.2 MATERIALS AND METHODS

The materials and methods used were the same as those described in section 3.2. There were two major differences in this trial. One was that the nutrient solution retention time was reduced to 14 days and only the silver beet and tomatoes were tested. The other was that Total Keldjahl Nitrogen (TKN) and Total Phosphorus (TP) was measured in the WW and CM treatments according to the Hach (2002) methods. The conductivity of the tap water was also measured. The data were statistically analysed using the same methods described in chapter three.

4.3 RESULTS

Initial concentration of the solutions after each solution change was the same as day 0 followed by the effluent quality before solution change.

Plant growth and production in silver beet and tomatoes

The physical appearance of the silver beet (leaves) grown in WW was comparable to those grown in CM (Figures 4.1 and 4.2) and better than those grown in WW in Chapter 3 (Figure 3.2). The tomato fruit grown in WW was also close in size to those grown in CM (Figure 4.3). The growth of the silver beet in both media followed a similar trend of increasing with time and then the growth slowed down during the last seven days. The tomato growth followed a similar trend to the silver beet (Figure 4.4). The growth of tomato plants in CM was overall more than those in WW (Figure 4.4), however, the quality of the fruits grown in WW was comparable to those in CM (Figure 4.3). From the 84th day, the number of buds, flowers and fruits of tomato in CM were much higher than in WW (Table 4.1).

The total weight of the silver beet leaves was higher in the WW (2000g) than the CM (1180g). The average diameter and weight of tomato fruits were slightly higher in CM grown tomatoes (Table 4.2).

Figure 4.1: Silver beet growth WW (right), CM (left) on day 56.

Figure 4.2: Size of silver beet leaves WW (left) and CM (right) after 56 days.

Figure 4.3: Quality of tomatoes grown in different media.

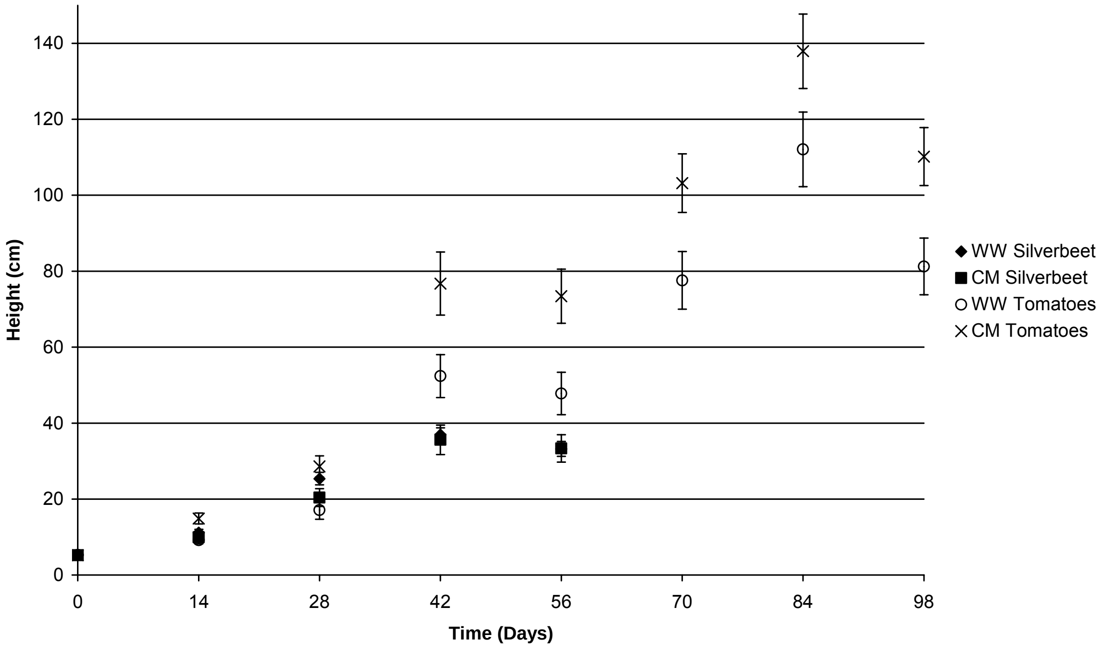


Figure 4.4: Height of silver beet and tomatoes in wastewater (WW) and control medium (CM) (mean of 3 replicates ± se).

Table 4.1: Average number of buds ± se, flowers ± se and fruits ± se per plant in tomato plants grown in wastewater (WW) and control medium (CM).

Day	Average No. Buds		Average No. Flowers		Average No. Fruits	
	WW	**CM**	**WW**	**CM**	**WW**	**CM**
28	1 ± 0.3	2 ± 0.4	0	0	0	0
42	5 ± 0.5	6 ± 1	3 ± 0.6	3 ± 0.6	0	0
56	2 ± 0.3	4 ± 0.5	2 ± 0.5	4 ± 0.8	0	0
70	8 ± 1	8 ± 1	4 ± 1	6 ± 1	1 ± 0	2 ± 0
84	12 ± 1	18 ± 3	6 ± 1	7 ± 1	2 ± 0.4	4 ± 1
98	5 ± 0.7	17 ± 3	7 ± 1	15 ± 3	4 ± 0.5	7 ± 1

Table 4.2: Total number of fruits on tomato plants, average diameter and average weight of tomato fruit in the two media.

Media	Total number of fruit	Average diameter (cm)	Average weight (g)
WW	93	6 ± 0.5	10 ± 2
CM	166	8 ± 0.04	17 ± 2

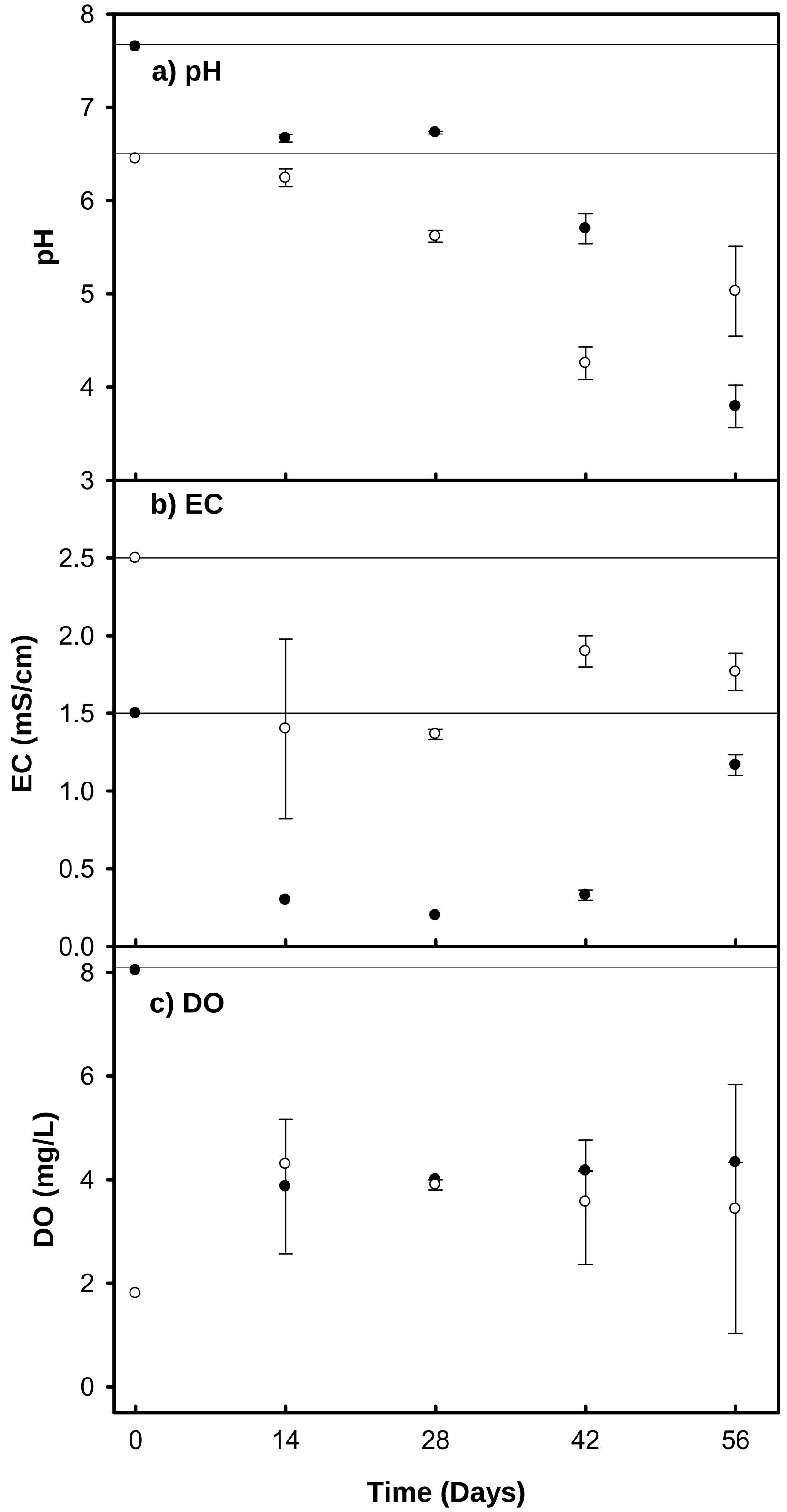
Water treatment efficiency

Silver beet

There was a general decrease in the pH of silver beet medium from day 0 to day 14 when the solution was changed. At subsequent changes on 28, 48 and 56 days, the pH decrease was greater and the pH in WW channels was <4 and in CM channels was 5 by day 56 (Figure 4.5a and Table 4.3). The EC in the silver beet solutions decreased from day 0 to 14, and over subsequent 14-day periods experienced a similar decline except at day 42 in WW and day 56 in CM (Figure 4.5b). The lowest EC concentration (effluent) in WW channels was 0.2mS/cm and in CM channels 1.9mS/cm (Table 4.3). The DO concentration declined in WW to about 4mg/L after each 14-day period in the hydroponics system (Figure 4.5c).

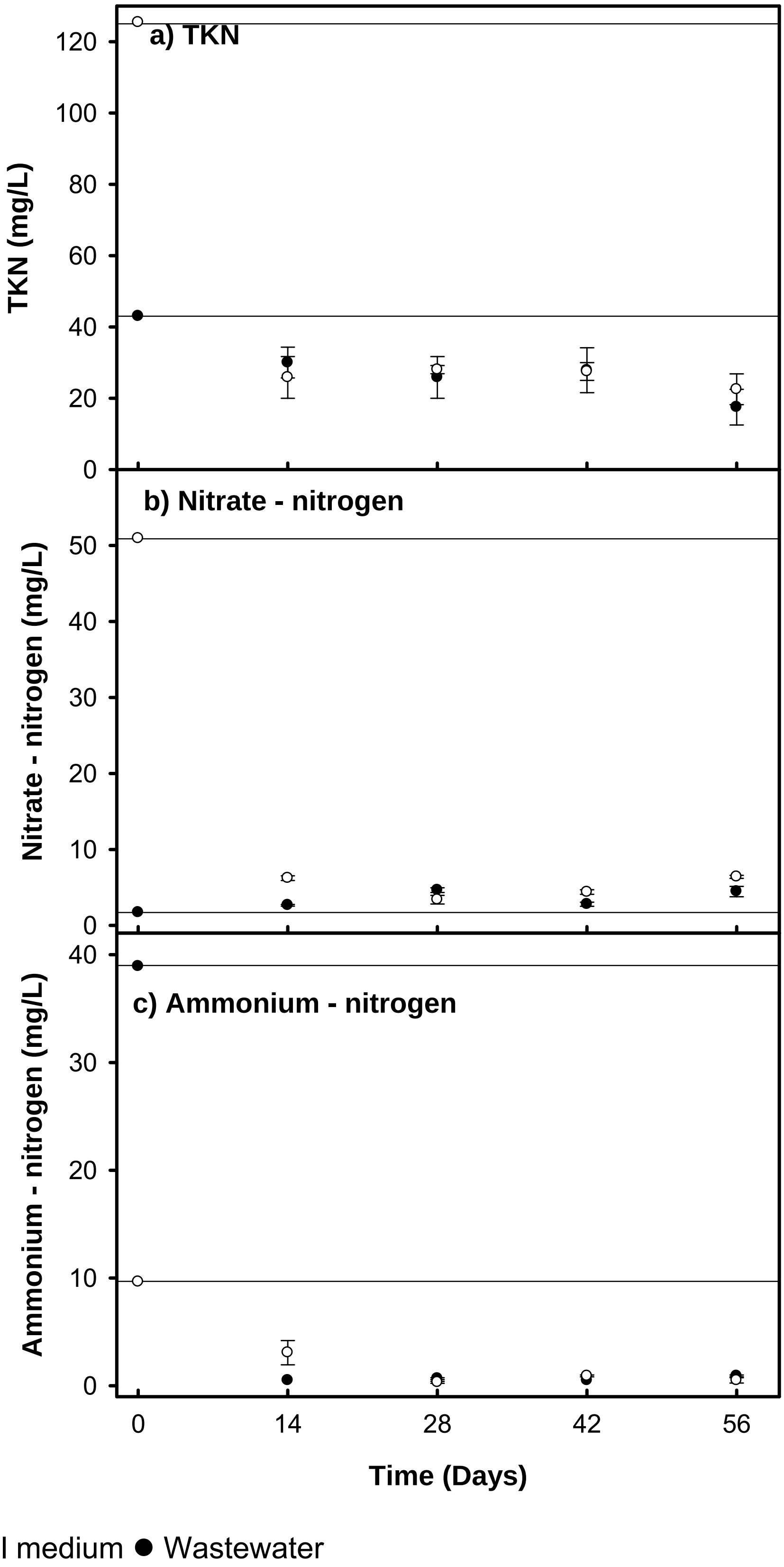
The TKN concentrations decreased in all the treatments after each 14-day period especially in CM (Figure 4.6a). The effluent TKN concentration, at the end of each 14-day period, ranged between 18mg/L - 30mg/L in WW channels and 23mg/L - 28mg/L in CM channels (Table 4.3). NO_3^--N concentration increased after the addition of fresh solution in WW channels. By contrast, the CM solutions decreased in concentration (Figure 4.6b). The NO_3^--N effluent concentration range in WW channels at the end of each 14-day period was 2.7mg/L - 4.7mg/L and in CM channels was 3.4mg/L - 6.4mg/L (Table 4.3). The

NH_4^+-N concentration decreased after each 14-day period with silver beet WW solution ranging between 0.5mg/L - 0.9mg/L and CM solution ranging between 0.3mg/L - 3mg/L (Figure 4.6c). TP concentration gradually decreased after every 14-day period (Figure 4.7a) ranging between 1.2mg/L - 4.5mg/L in WW channels and 1.7mg/L - 24mg/L in CM channels (Table 4.3). PO_4^{3-}-P concentration also gradually decreased after every 14-day period (Figure 4.7b). The effluent ranged between 0.3mg/L - 1.4mg/L in WW channels and 0.4mg/L - 8.4mg/L in CM channels (Table 4.3).



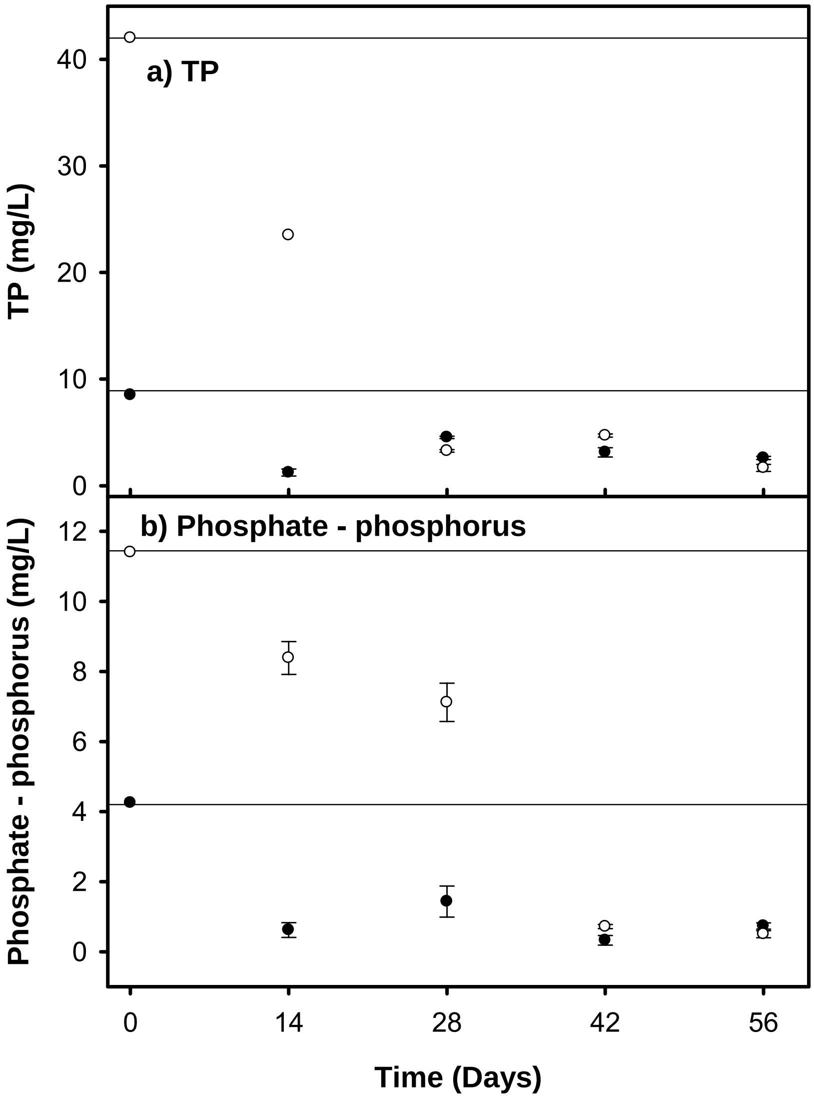
○ Control medium ● Wastewater

Figure 4.5: pH (a), EC (b) and DO (c) in silver beet channels. Vertical bars where visible indicate standard errors. Horizontal lines indicate initial solution concentrations. Values at day 14, 28, 42 and 56 were taken immediately before a solution change.



○ Control medium ● Wastewater

Figure 4.6: TKN (a), NO_3^--N (b) and NH_4^+-N (c) concentrations in silver beet channels. Vertical bars where visible indicate standard errors. Horizontal lines indicate initial solution concentration. Values at day 14, 28, 42 and 56 were taken immediately before a solution change.



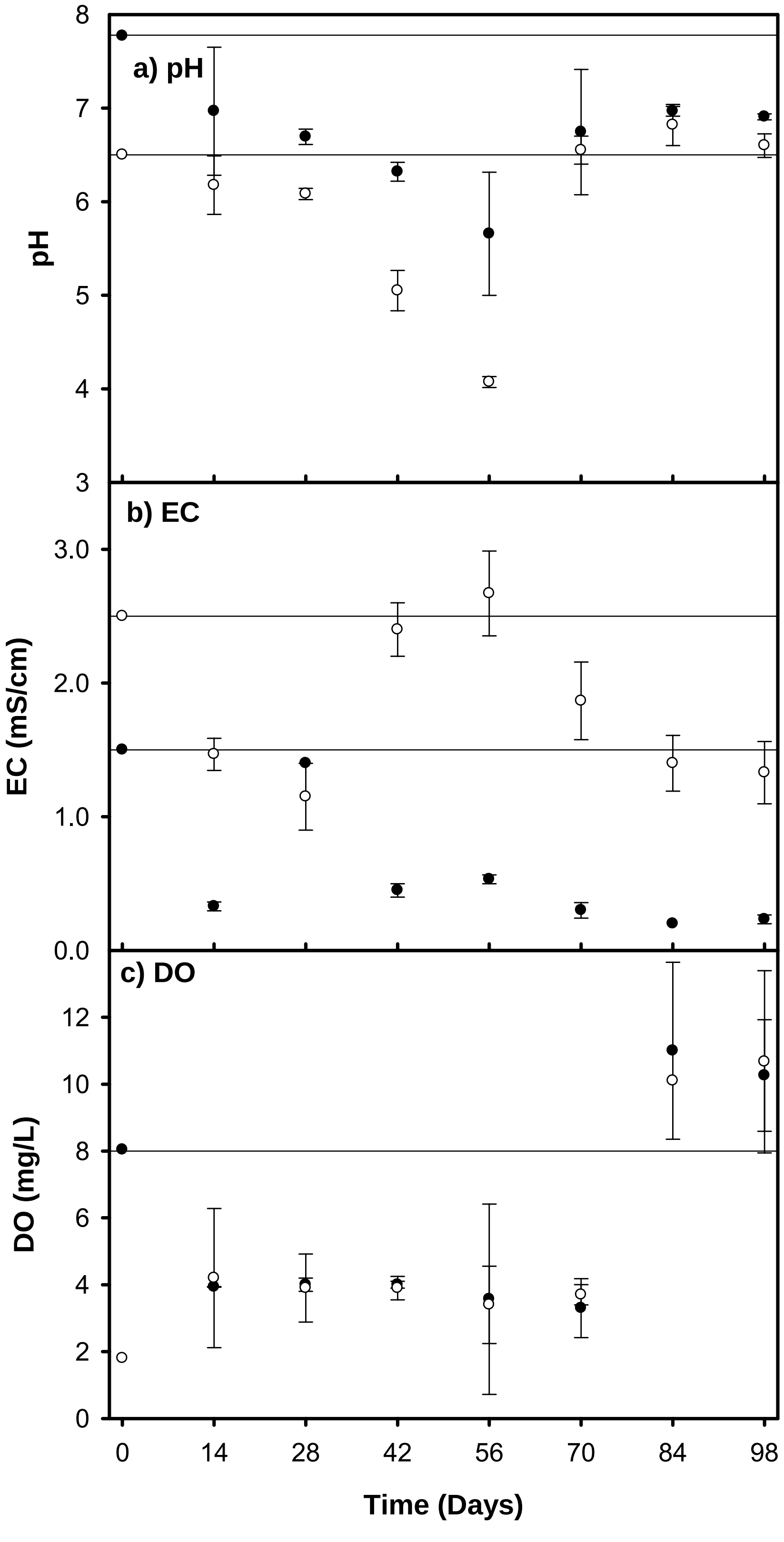
○ Control medium ● Wastewater
Figure 4.7: TP (a) and PO_4^{3-}-P (b) concentrations in silver beet channels. Vertical bars where visible indicate standard errors. Horizontal lines indicates initial solution concentrations. Values at day 14, 28, 42 and 56 were taken immediately before a solution change.

Tomatoes

There was a general decrease in pH of the tomato solution after every solution change in WW treatment. However, there was an increase in pH on day 70, 84 and 98 in CM treatments (Figure 4.8a). The EC declined at the end of each 14-day period in WW channels (Figure 4.8b). This trend was similar in the CM channels except on day 56 where the EC increased. The lowest EC concentration in WW channels was 0.2mS/cm and 2.4mS/cm (Table 4.3). DO concentrations generally decreased to 4mg/L, however in the last two 14-day periods, the concentration was similar in both treatments to the initial values of fresh solutions (Figure 4.8c and Table 4.3).

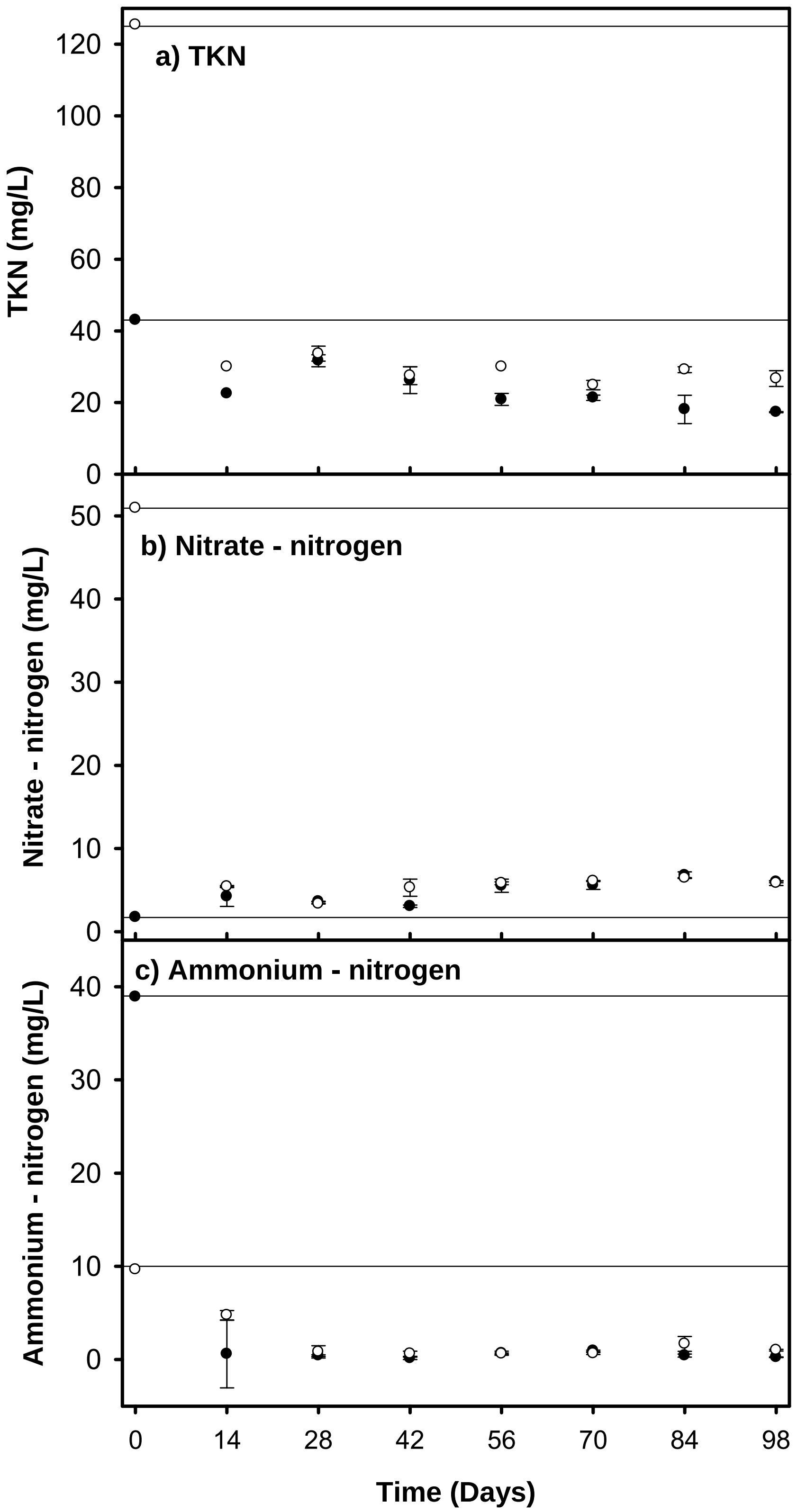
TKN concentration followed a similar pattern to the silver beet treatments, where they decreased over each 14-day period (Figure 4.9a). The effluent ranged between 17mg/L - 32mg/L in WW channels and 25mg/L - 34mg/L in CM channels (Table 4.3). NO_3^--N concentration also increased in WW channels as they did after silver beet growth, while the in the CM channels, the concentration decreased (Figure 4.9b). The NO_3^--N concentration in the effluent ranged between 3.1mg/L - 6.8mg/L in WW channels and 3.4mg/L - 6.9mg/L in CM channels (Table 4.3). In both treatments the NH_4^+-N concentration decreased (Figure 4.9c). The NH_4^+-N concentration in tomato WW solution ranged between 0.1mg/L - 0.9mg/L and CM ranged between 0.6mg/L - 4.8mg/L (Figure 4.9c and Table 4.3). Table 4.3 shows that the reduction of TKN in WW (43mg/L to 17mg/L - 18mg/L) is comparably lower than CM (125mg/L to 23mg/L - 26mg/L).

Like the silver beet treatments, with tomato treatments' TP concentration decreased after every 14-day period especially in CM (Figure 4.10a). The effluent ranged between 1.1mg/L to 2.3mg/L in WW channels and 5.2mg/L to 23.5mg/L in CM channels at the end of 14-day periods of growth (Table 4.3). PO_4^{3-}-P concentration also gradually decreased after every 14-day period (Figure 4.10b). The effluent ranged between 0.1mg/L to 1.2mg/L in WW channels and 0.8mg/L to 7.8mg/L in CM channels (Table 4.3).



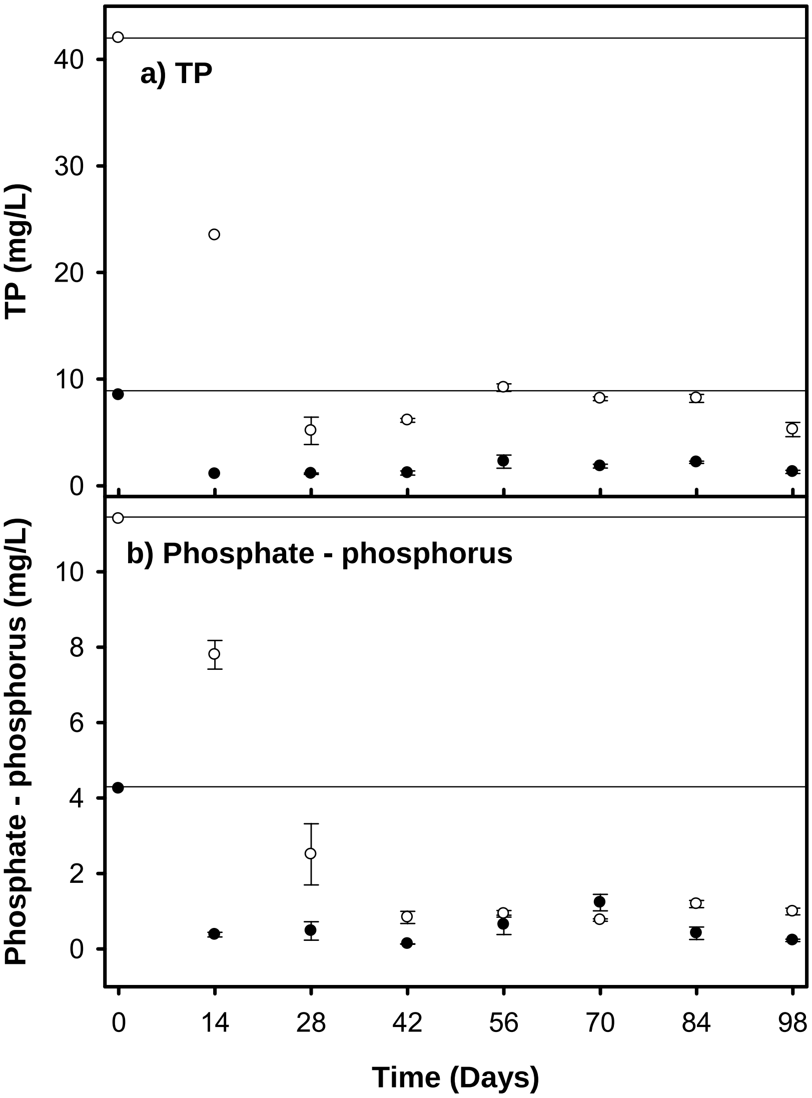
○ Control medium ● Wastewater

Figure 4.8: pH (a), EC (b) and DO (c) in tomato channels. Vertical bars where visible indicate standard errors. Horizontal lines indicate initial solution concentrations. Values at day 14, 28, 42, 56, 70, 84 and 98 were taken immediately before a solution change.



○ Control medium ● Wastewater

Figure 4.9: TKN (a), NO_3^--N (b) and NH_4^+-N (c) concentration in tomato channels. Vertical bars where visible indicate standard errors. Horizontal lines indicate initial solution concentrations. Values at day 14, 28, 42, 56, 70, 84 and 98 were taken immediately before a solution change.



○ Control medium ● Wastewater

Figure 4.10: TP (a) and PO_4^{3-}-P (b) concentration in tomato channels. Vertical bars where visible indicate standard errors. Horizontal lines indicate initial solution concentrations. Values at day 14, 28, 42, 56, 70, 84 and 98 were taken immediately before a solution change.

Table 4.3: Quality of effluent disposed after hydroponics treatment of silver beet and tomatoes.

	Days of effluent disposal															
	Wastewater								**Control medium**							
	0	**14**	**28**	**42**	**56**	**70**	**84**	**98**	**0**	**14**	**28**	**42**	**56**	**70**	**84**	**98**
								Silver beet								
pH	7.7	6.7	6.7	5.7	4				6.5	6	5.6	4.3	5			
EC (mS/cm)	1.5	0.3	0.2	0.3	1.2				2.5	1.4	1.4	1.9	1.8			
DO (mg/L)	8	4	4	4	4				7.8	4	4	4	3			
TKN (mg/L)	43	30	26	28	18				125	26	28	28	23			
NO_3^--N (mg/L)	2	2.7	4.7	2.8	4.5				51	6.2	3.4	4.4	6.4			
NH_4^+-N (mg/L)	39	0.5	0.7	0.5	0.9				10	3.1	0.3	0.9	0.5			
TN (mg/L)	45	33	31	31	22				176	32	31	32	29			
TP (mg/L)	9	1.2	4.5	3.1	2.6				42	24	3.3	4.7	1.7			
PO_4^{3-}-P (mg/L)	4	0.6	1.4	0.3	0.7				11	8.4	7.1	0.7	0.5			
								Tomatoes								
pH	7.7	7	6.7	6.3	5.7	6.7	7	6.9	6.5	6.2	6.1	5.1	4.1	6.6	6.8	6.6
EC (mS/cm)	1.5	0.3	1.4	0.5	0.5	0.3	0.2	0.2	2.5	1.5	1.2	2.4	2.7	1.9	1.4	1.3
DO (mg/L)	8	4	4	4	4	3	11	10	7.8	4	4	4	3	4	10	11
TKN (mg/L)	43	23	32	26	21	21	18	17	125	30	34	28	30	25	29	27
NO_3^--N (mg/L)	2	4.2	3.6	3.1	5.5	5.6	6.8	6.0	51	5.4	3.4	5.3	5.8	6.1	6.5	5.9
NH_4^+-N (mg/L)	39	0.6	0.4	0.1	0.7	0.9	0.4	0.2	10	4.8	0.8	0.6	0.6	0.6	1.7	1
TN (mg/L)	45	27	35	29	27	27	25	23	176	35	37	33	36	31	36	33
TP (mg/L)	9	1.1	1.1	1.2	2.3	1.8	2.2	1.3	42	24	5.2	6.1	9.2	8.2	8.2	5.3
PO_4^{3-}-P (mg/L)	4	0.4	0.5	0.1	0.6	1.2	0.4	0.2	11	7.8	2.5	0.8	0.9	0.8	1.2	1

Table 4.4: Silver beet and tomato solution p values from ANOVA for wastewater (WW) and control medium (CM) treatments.

	Days							
	0	**14**	**28**	**42**	**56**	**70**	**84**	**98**
Silver beet								
pH	-	0.015	0.000	0.004	0.081			
EC	-	0.000	0.000	0.000	0.426			
DO	-	0.031	0.374	0.016	0.017			
TKN	-	0.594	0.734	0.963	0.492			
NO_3^--N	-	0.000	0.017	0.015	0.05			
NH_4^+-N	-	0.083	0.047	0.007	0.189			
TP	-	0.000	0.002	0.028	0.061			
PO_4^{3-}-P	-	0.000	0.001	0.06	0.976			
Tomatoes								
pH	-	0.069	0.002	0.012	0.075	0.305	0.551	0.077
EC	-	0.001	0.437	0.005	0.003	0.006	0.004	0.61
DO	-	0.304	0.721	0.842	0.616	0.313	0.022	0.279
TKN	-	-	0.522	0.789	0.005	0.017	0.051	0.013
NO_3^--N	-	0.000	0.001	0.175	0.509	0.593	0.453	0.467
NH_4^+-N	-	0.003	0.629	0.264	0.84	0.119	0.199	0.373
TP	-	-	0.046	0.000	0.001	0.000	0.001	0.004
PO_4^{3-}-P	-	0.000	0.093	0.042	0.345	0.102	0.016	0.001

Nutrient composition of produce

The mineral nutrient concentrations in the CM silver beet leaves were higher than in the WW silver beet. By contrast, the sodium in both tomatoes and silver beet was higher in WW than CM (Table 4.5).

The nitrogen, sulfur, calcium, magnesium and iron concentrations were higher in the organic silver beet than the WW silver beet. However, the zinc and boron concentrations were higher in WW grown silver beet than in the organic produce. With the tomatoes, the potassium, magnesium, copper and iron concentrations were higher in the organic produce than the WW and CM. Sodium was higher in the CM and WW tomatoes than in the organic tomatoes.

Table 4.5: Concentration of nutrients in silver beet leaves and tomato fruits.

Element	Silver beet Leaves (mg/kg)			Tomatoes (mg/kg)		
	WW	**CM**	**O**	**WW**	**CM**	**O**
Total Nitrogen	26800 ± 1630	48200 ± 956	46300	24000 ± 880	28000 ± 790	38900
Phosphorus	3870 ± 180	7920 ± 160	4220	6320 ± 205	5860 ± 320	5350
Potassium	17403 ± 240	75000 ± 2220	18700	26200 ± 430	34100 ± 760	63950
Sulfur	2870 ± 280	3600 ± 480	37400	2220 ± 28	2250 ± 127	3020
Sodium	34606 ± 1700	14500 ± 630	34690	8190 ± 32	1060 ± 23	1080
Calcium	8250 ± 220	10760 ± 206	12270	1280 ± 96	540 ± 46	1530
Magnesium	3300 ± 320	7430 ± 470	6740	1710 ± 39	1740 ± 32	3210
Copper	8 ± 0.2	11 ± 0.4	12	11 ± 0.2	9 ± 0.2	33
Zinc	1065 ± 21	1186 ± 13	79	33 ± 0.5	70 ± 1	28
Manganese	29 ± 1	136 ± 2	19	10 ± 1	20 ± 0.5	27
Iron	75 ± 3	98 ± 1	223	39 ± 0.5	46 ± 0.4	71
Boron	50 ±1	43 ± 1	27	16 ± 1	13 ± 1	20

4.4 DISCUSSION

Water treatment through hydroponics

When considering the final disposal quality of the effluent after hydroponics, it was noted that both systems (silver beet and tomatoes) met the recommended EC concentration for effluent to be used for irrigation which is 0.8mS/cm (EPP, 1995).

PO_4^{3-}-P concentrations after each 14-day period in this experiment were lower than in the experiment carried out in chapter three, which demonstrated that the effluent could be reused after going through the hydroponics system. In this study the TP and PO_4^{3-}-P effluent concentration decreased in all treatments (Figures 4.8 and 4.11; Table 4.3) and met the EPP (1995) guidelines for open irrigation, depending on the phosphorus sorption capacity of the soils.

The TKN concentration of the final effluent were within the recommended guidelines for irrigation (EPP, 1995). The effluent after both studies (Chapters 3 and 4) could be used for further irrigation.

Crop production and nutrient quality

Most plants thrive in pH between 5 to 7 (Mason, 2003), the ideal pH levels for silver beet growth is between 6 to 7 (Burt, 2000). It is reported that at very high or very low pH, availability of nutrients can be reduced (Bugbee, 2004). Cation-anion ratios in nutrients absorbed would also affect pH in solution. The drop in pH in the WW and CM during plant growth (Figures 4.6a and 4.9a; Table 4.3) could be due to nitrification as the release of hydrogen ions occurs during both processes lowers the pH (Pierce *et al.,* 1998; Savvas *et al.,* 2003). Nitrate and ammonium concentrations have also been known to affect pH balance (Singha *et al.,* 1987). Uptake of ammonium-nitrogen by plants makes solutions acidic, while nitrate-nitrogen uptake makes is alkaline, this may be the cause of pH changes (Imas *et al.,* 1997). The aeration from pumping may have encouraged nitrification. The major concern with low pH is its ability to affect metal solubility and hence affect plant growth (Asano *et al.,* 1985).

Low pH (4 to 5) can affect solubility of metals in soil (Khai, 2007), the low pH measured in this experiment was at the end of the 14-day period, which may have affected plant growth. This may have been the reason that the height of silver beet in Chapter 3 (Figure 3.5) was more than that of this study (Figure 4.4), particularly in the CM treatment.

Salinity in the media can limit plant growth (Greenway and Munns 1980 in (Baas *et al.,* 1995). An increase in conductivity of the media can be an indication of harmful effects of ions like sodium chloride in some vegetables (Baas *et al.,* 1995). An increase in conductivity, may also imply a reduced uptake of nutrients (Baas *et al.,* 1995). There is concern over the use of treated effluent due to the risk of high salinity (Unkovich *et al.,* 2004), which may cause stress to the plants**.** In terms of suitability of the media for silver beet growth, the threshold concentration is 2mS/cm (Jarwal *et al.,* 2006), the conductivity in the silver beet channels met this level (Figure 4.6b and Table 4.3).

The conductivity threshold for the medium to grown tomatoes is 2.3mS/cm (Jarwal *et al.,* 2006). The medium in the WW tomato channels had a maximum EC concentration of 1.5mS/cm, however the solution in the CM tomato channels had an initial concentration around 2.5mS/cm (Figure 4.9b and Table 4.3). Absorption of sodium can have a negative effect on potassium uptake in tomato crops (Magán *et al.,* 2005). However, this did not seem to be the case in this experiment (Table 4.4), where there was an increase in potassium and sodium when compared to the previous experiment (Table 3.5). When tomato crops were grown in medium with various EC concentrations (between, 2.5mS/cm, 4mS/cm, 5.5mS/cm, 7mS/cm, 8.5mS/cm), sodium, chlorine and potassium uptake was not affected (Magán *et al.,* 2005).

Use of treated wastewater may be advantageous as it is common to find high nutrient concentrations of nitrogen, phosphorus, potassium, calcium and marginal concentrations of salt and boron (Unkovich *et al.,* 2004) even after secondary treatment. However, from Table 3.1, chlorine and sodium were the only elements whose concentrations were higher in WW than CM. This may be harmful to the plants as sodium accumulation can reduce the uptake of potassium (Silberbush *et al.,* 2005). There was a reduced uptake of sodium and the potassium concentration increased in both silver beet and tomatoes grown in secondary treated domestic wastewater. Hessini *et al*. (2005) found that wild Swiss chard could tolerate up to 200mM sodium chloride and it seemed that the sodium concentration in the organically grown silver beet was higher than those grown hydroponically (Table 4.4).

The DO concentration generally decreased over time in the hydroponics system in both studies (Chapters 3 and 4), which may be due to microbial and root respiration (Pierce *et al.,* 1998). The microbes growing on the root systems could also have contributed to oxygen uptake (Sutton *et al.,* 2006). However, towards the end of the experiment, the dissolved oxygen concentrations in both tomato treatments increased (Figure 4.9c), this could be due to reduced microbial activity (Holtman *et al.,* 2005).

The NH_4^+-N concentration (Figures 4.7c and 4.10c; Table 4.3) decreased in the system, which could be due to both plant uptake and nitrification. Britto and Kronzucker (2002), found that high concentrations (10mM) of ammonium could cause leaf chlorosis and stunted growth in roots and shoots, and this can be a problem when reusing treated effluent. Excessive amounts of ammonium can impair photosynthesis (Savvas *et al.,* 2003). According to Siddiqi *et al.* (2002) when ammonium is the sole nitrogen source, it reduces potassium, calcium and magnesium concentration in tomatoes while reducing the growth of the plant.

The presence of nitrate in the solution medium alleviated the harmful effects of ammonium uptake and could improve the growth rate (Siddiqi *et al.,* 2002).

When the nutrient solution retention time was reduced, the amount of nitrogen remaining at each solution change increased and can be the cause of improved growth noticed in Chapter 4 compared to that in Chapter 3. The decrease in concentration of TKN was much higher in CM than WW effluent after each 14-day period. NO_3^--N is readily mobile, is in the storage form and does not require to be assimilated in the roots (Marschner, 1995) and in the CM solution, this is the most abundant form of nitrogen .

The yellowish discolouration of bok choy leaves grown in wastewater was attributed to iron or nitrogen deficiencies (Oyama *et al.,* 2005). This yellowish discolouration was also seen in the previous experiment (Chapter 3) can be due to lack in nitrogen (Wier and Cresswell, 1993). High rates of nitrogen are important for the production of good quality vegetables. Nitrogen deficiency reduces growth of plants, size of leaves and changes colour of leaves from pale green to yellow with the older leaves affected first (McPharlin and Phillips, 1989). The plants grown in this experiment did not exhibit these deficiency symptoms as compared to those in Chapter 3 (Tables 4.3 and 3.5) revealing that the 14-day retention time provided sufficient nitrogen for silver beet from secondary treated domestic wastewater. Leaf analysis of nitrogen present in silver beet also demonstrates that there was more nitrogen available for the plants (Tables 3.8 and 4.4).

It can also be seen in Chapter 3 (Table 3.1) in the WW grown silver beet, nitrogen was low, potassium and sulphur are marginal, magnesium, phosphorus and boron seemed adequate. Sodium, zinc and boron were high in the plants possibly because nitrogen and sulphur deficiencies so the plants accumulated sodium, zinc and boron in excess.

Zinc deficient plants are able to accumulate phosphorus to high or toxic levels (Knight *et al.,* 2004). Zinc and copper can be toxic to plants if the concentration in the growth medium is high enough (Moreno *et al.,* 2002). This does not seem to be a problem in this study as the concentrations were acceptable, 0.1mg/L and 0.35mg/L zinc available in WW and CM respectively; 0.06mg/L and 0.2mg/L copper was available in WW and CM respectively. Zinc concentrations (Tables 3.1, 3.5 and 4.4) in the hydroponically grown vegetables were higher than that of the organically grown ones. The leaf and fruit analysis shows that the phosphorus concentration was similar between the WW and O treatments (Table 4.4). A study conducted by Nair *et al.* (2008) found that high concentrations of zinc in silver beet could be an indication that other nutrients were deficient in the growth medium.

If the nitrogen was the only deficient nutrient then increasing nitrogen concentration could increase plant yield significantly (Tyson *et al.,* 2007). Increasing the nitrogen concentration alone in the secondary treated domestic wastewater only may have caused problems with manganese, sulfur and phosphorus uptake (Price, 2006) because they were at low concentrations. A possible solution could be to increase nitrogen, potassium, phosphorus, sulfur and manganese to match the concentration of the control medium solution.

Reducing the retention time improved the concentration of nitrogen, phosphorus and sulfur in this study (Tables 3.8 and 4.4).

de Carmello and Anti (2006) found that tomatoes absorbed potassium in large concentrations followed by nitrogen and calcium while, magnesium, phosphorus and sulphur were absorbed in smaller amounts. A study conducted by Kawashima and Valente Soares (2003) using produce bought from local markets, found lower concentrations of potassium, sodium, calcium, magnesium, copper, zinc, manganese and iron in their kale and spinach than in this study. In this study (Table 4.4) iron and magnesium had lower concentrations in the WW grown silver beet than the CM and organic silver beet.

The WW tomato fruit showed cuticle cracking, as there was a problem with the tomato skin (Chapter 3). This could have been due to the nutrient solution retention time in Chapter 3 or the availability of boron and calcium (Dorais *et al.,* 2004). Boron is an important component for the cell wall, however in large concentrations, it can interfere with cell wall synthesis (Reid *et al.,* 2004). Siddiqi *et al*. (2002) showed that in the absence of nitrate, there was a reduction in calcium concentration or uptake in fruit and leaf tissue. However in this experiment (Chapter 4), the tomatoes grown in secondary treated domestic wastewater did not exhibit this cuticle cracking (Figure 4.4) as compared to the previous experiment (Figure 3.6), when the nutrient solution retention time was consistent (14 days). The fruit analysis shows that the concentration of boron and calcium increased slightly over that of the Chapter 3 experiment (Tables 3.8 and 4.4).

The 14-day nutrient solution retention time in this experiment provided more nutrients for both silver beet and tomatoes, however, the nitrogen was still low. There was an increase in the production of both silver beet (51%) and tomatoes (49%) in the experiments as discussed in Chapter 4 than in Chapter 3. The study showed that secondary treated domestic effluent can be effectively used in hydroponics system to grow silver beet and tomatoes. However, the study also shows that the retention times needed to be adjusted so as to provide adequate nutrient availability.

4.5 CONCLUSION

The plants grown in this study were more productive than those grown in Chapter 3. There was an increase in the amount of fruit and dry weight of vegetable leaves, and the general appearance of the wastewater grown plants had improved. The vegetables grown in Chapter 3 exhibited nutrient deficiency, particularly nitrogen. The deficiency was caused by the long nutrient retention time of the solution thereby reducing the nutrient supply relative to what was required by the plants. This study showed that a nutrient solution retention time of 14 days improved nutrient availability to silver beet and tomatoes while the effluent after the hydroponics process met the World Health Organisation (1989) discharge guidelines. While still shorter retention times (<14-days) may further improve plant growth, they could also increase the risk that the effluent did not meet discharge standards.

CHAPTER 5

MODELLING, WATER AND NUTRIENT BALANCES

5.1 INTRODUCTION

The production of quality food crops in all aspects of agriculture depends on various factors such as nutrients, soil condition and availability of water. In the NFT hydroponics systems, the nutrient composition of the solution is a major factor to consider. Most commercial hydroponic solutions are prepared according to the plant nutrient requirement.

By contrast, when using wastewater as a nutrient solution, the adequacy of the solution for plant requirements needs to be established, particularly with commercially important crops (Lopez and Vurro, 2008). Water uptake is an important factor that should be considered when designing a hydroponic system (Salas *et al.,* 2000). For this system to be used, the community/user would need to determine how much wastewater is required, to generate a certain amount of crops.

It is for this reason that water and nutrient balance analyses are required and to develop a general model of what parameters are important to produce a good quality crop. This Chapter examines the data collected in Chapters 3 and 4 in order to analyse a water and nutrient balance for silver beet, tomatoes and carnations. Basic models will also be determined from the existing data.

5.2 MATERIALS AND METHODS

The data collected from Chapters 3 and 4 were subjected to further statistical analysis.

Total and daily water and nutrient balances were determined for the nutrient solutions of silver beet, tomatoes and carnations. Nutrient balances were determined by multiplying the concentration of nutrients by the volume of water in the influent and effluent. Nitrogen and phosphorus balances for WW and CM grown silver beet leaves and tomato fruits was calculated by multiplying their concentration in the edible parts by the total weight (biomass) of the leaves and fruits.

Statistical analysis was conducted for WW treatments using the SPSS 11 for Mac OSX computer software package. Multiple regression was used to calculate coefficients for the model equations and Pearson's correlation was used to find the significant individual factors affecting silver beet, tomato and carnation growth. The model equations were validated against existing data collected according to the method described by Pardossi *et al.* (2008). The parameters that affected plant growth used were; days; pH; EC; DO; NO_3^--N; NH_4^+-N; PO_4^{3-}-P in both varied and 14-day NSRT. The concentrations used in the model were obtained during sampling time.

5.3 RESULTS

Water and nutrient balances in the solutions

The volume of water treated increased with the 14-day NSRT (Table 5.1). The daily water uptake increased by about 2L/day in the 14-day NSRT treatments. The final nutrient concentrations in the varied NSRT were lower than the 14-day NSRT (Table 5.2). There was an increase in NO_3^--N concentration in the wastewater solutions except the silver beet grown in varied NSRT (Table 5.2) indicating better nutrient stripping in the varied NSRT.

Table 5.1: Total water (L) balance for different nutrient solution retention times (NSRTs).

Total water (L)	**Varied NSRT**						**14-day NSRT**			
	Silver beet (56 days)		**Tomatoes (98 days)**		**Carnations (168 days)**		**Silver beet (56 days)**		**Tomatoes (98 days)**	
	WW	**CM**	**WW**	**CM**	**WW**	**CM**	**WW**	**CM**	**WW**	**CM**
Influent (L)	240	240	480	480	720	720	480	480	840	840
Effluent (L)	30	30	60	60	90	90	150	150	225	225
Loss (L)	210	210	420	420	630	630	330	330	615	615
Daily water uptake (L/day)	3.8	3.8	4.3	4.3	3.8	3.8	5.9	5.9	6.3	6.3

Table 5.2: Total nutrient (mg) balance for different nutrient solution retention times (NSRTs) in the solutions from all the channels

Nutrient (mg)	**Varied NSRT**						**14-day NSRT**			
	Silver beet		**Tomatoes**		**Carnations**		**Silver beet**		**Tomatoes**	
	WW	**CM**	**WW**	**CM**	**WW**	**CM**	**WW**	**CM**	**WW**	**CM**
Total load in the influent										
NO_3^--N	413	12216	960	24480	1032	30600	960	24480	1680	42840
NH_4^+-N	9360	2400	18720	4800	35400	6000	18720	4800	32760	8400
PO_4^{3-}-P	1008	2640	1920	5280	2580	6600	1920	5280	3360	9240
Total load in the effluent										
NO_3^--N	48	696	1188	2664	3015	4860	2205	3060	7830	8640
NH_4^+-N	5	7	38	40	140	57	390	720	743	2273
PO_4^{3-}-P	37	285	82	366	324	2934	450	2505	765	3375
Total loss										
NO_3^--N	365	11520	+228	21816	+1983	25740	+1245	21420	+6150	34200
NH_4^+-N	9355	2393	18682	4760	35260	5943	18330	4080	32017	6127
PO_4^{3-}-P	971	2355	1832	4914	2256	3666	1470	2775	2595	5865
Total percentage loss/gain (%)										
NO_3^--N	88.4	94	124	89	192	84	130.6	87.5	366	79.8
NH_4^+-N	99.9	99.7	99.8	99.2	99.6	99	97.6	85	97.7	72.9
PO_4^{3-}-P	96	89	95	93	93	55.5	76.5	52.5	77	63
Daily nutrient uptake (mg/day)										
NO_3^--N	7	203	+4	223	+12	153	+22	383	+63	349
NH_4^+-N	167	43	191	49	210	35	327	73	327	63
PO_4^{3-}-P	17	42	33	50	13	22	26	50	26	60
PO_4^{3-}-P	17	42	33	50	13	22	26	50	26	60

+ an increase in NO_3^-–N from nitrification of NH_4^+-N

Nitrogen and phosphorus balance in silver beet and tomatoes

The amount of P in CM tomatoes (varied NSRT) reduced (by 14%), as did the total N (69%) and P (63%) in CM silver beet (Table 5.3). The amount of total N in WW tomatoes was lower in the varied NSRT experiment than the 14-day NSRT experiment (Table 5.3).

Table 5.3: Total nitrogen and phosphorus in the edible parts of the plant

Nutrient (mg)	**Varied NSRT**				**14-day NSRT**			
	Silver beet		**Tomatoes**		**Silver beet**		**Tomatoes**	
	WW	**CM**	**WW**	**CM**	**WW**	**CM**	**WW**	**CM**
Total N (mg)	24300	182830	25290	77220	53600	56880	22320	79020
P (mg)	3120	24970	4520	19190	7740	9350	5880	16540

Models

The model equations for the different solution retention times obtained from the multiple regression are;

Silver beet (varied NSRT)

Growth = 4.027 + (0.04521 x day) + (6.106 x pH) – (2.172 x DO) – (23.231 x EC) – (0.214 x NO_3^-) – (0.137 x NH_4^+) + (3.297 x PO_4^{3-})

Tomatoes (varied NSRT)

Growth = -27.985 + (0.466 x day) + (5.914 x pH) – (1.251 x DO) + (0.117 x EC) – (0.707 x NO_3^-) – (0.653 x NH_4^+) + (0.576 x PO_4^{3-})

Carnations (varied NSRT)

Growth = 11.579 + (0.503 x day) - (2.075 x pH) + (0.944 x DO) + (2.139 x EC) – (0.0314 x NO_3^-) + (0.06744 x NH_4^+) - (1.036 x PO_4^{3-})

Silver beet (14-day NSRT)

Growth = 45.805 + (1.113 x day) + (4.999 x pH) - (18.275 x DO) - (11.471 x EC) - (0.688 x NO_3^-) + (1.996 x NH_4^+) + (2.089 x PO_4^{3-})

Tomatoes (14-day NSRT)

Growth = -46.248 + (0.646 x day) + (4.694 x pH) + (0.401 x DO) - (12.821 x EC) + (1.999 x NO_3^-) - (1.039 x NH_4^+) - (12.983 x PO_4^{3-})

The concentrations used in the equations were obtained during sampling time.

Varied NSRT correlation

From the Pearson's correlation analysis of the silver beet growth data (Table 5.4), it can be seen that NO_3^--N was not a significant parameter ($p>0.05$). In the tomatoes, NO_3^--N, pH and EC were not significant individual parameters when compared to growth (Table 5.4). In the carnations only days, NO_3^--N and PO_4^{3-}-P were significant individual factors (Table 5.4).

Table 5.4: Multiple regressions of WW silver beet growth vs different parameters in the varied nutrient solution retention time (NSRT).

	Days	**pH**	**DO**	**EC**	**NO_3^--N**	**NH_4^+-N**	**PO_4^{3-}-P**
Silver beet growth	0.925	-0.758	0.773	-0.931	-0.346*	-0.748	-0.821
Tomato growth	0.968	0.292*	-0.086*	0.579	-0.079*	-0.601	-0.689
Carnation growth	0.968	-0.144*	-0.217*	-0.193*	-0.361	-0.107*	-0.282

* Correlations are not significant at the 0.05 level

14-day NSRT

With the 14-day NSRT of silver beet, all the parameters were significant for growth (Table 5.5). The individual parameter that was not significant in the tomato growth was pH (Table 5.5).

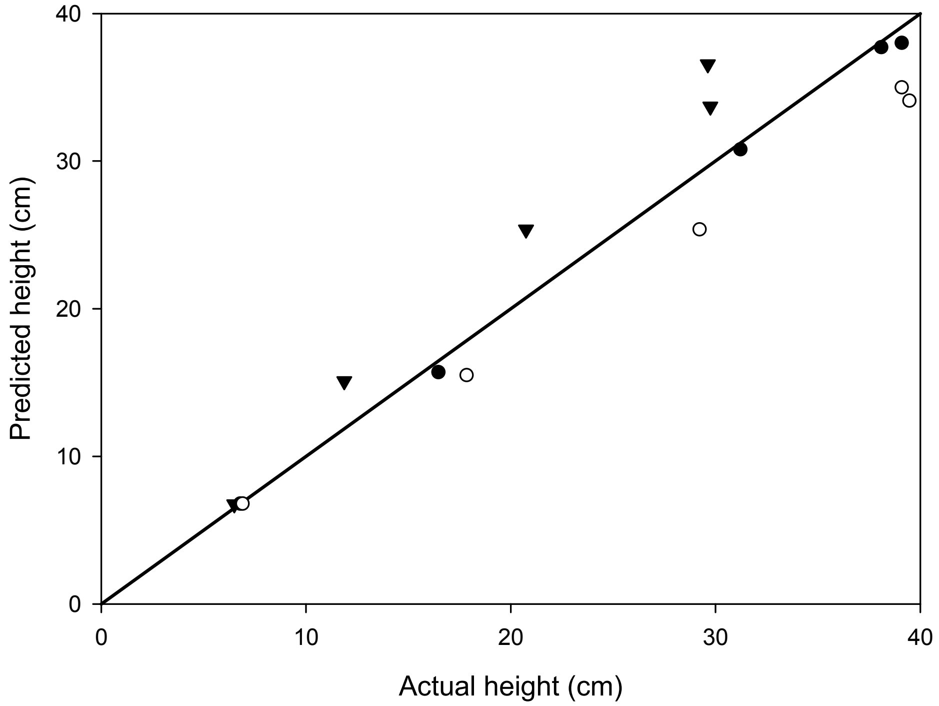
Table 5.5: Correlation coefficients between growth and parameters in the 14-day nutrient solution retention time (NSRT).

	Days	**pH**	**DO**	**EC**	**NO_3^--N**	**NH_4^+-N**	**PO_4^{3-}-P**
Silver beet growth	0.928	-0.723	-0.658	-0.626	0.634	-0.685	-0.68
Tomato growth	0.903	-0.061*	0.487	-0.668	0.855	-0.434	-0.415

* Correlations are not significant at the 0.05 level

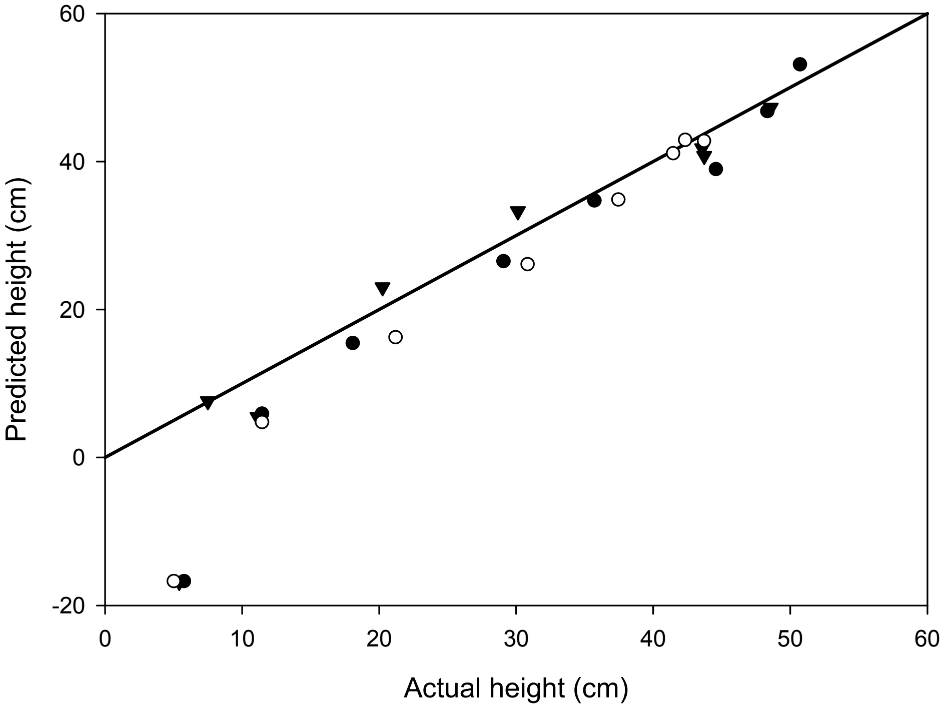
Model validation

The model equations obtained from the multiple regression, for the varied NSRT and 14-day NSRT appeared to predict the growth of the plants (Figures 5.1, 5.2, 5.3, 5.4 and 5.4).



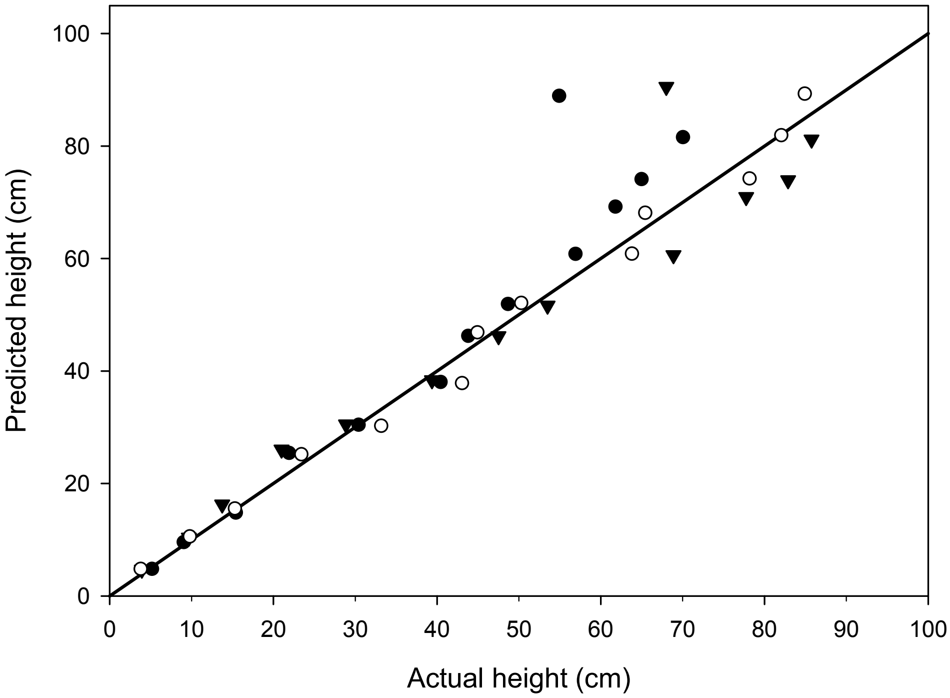
○ Channel 1 ● Channel 2 ▼Channel 3

Figure 5.1: Predicted height vs actual height of silver beet grown in the wastewater varied nutrient solution retention time.

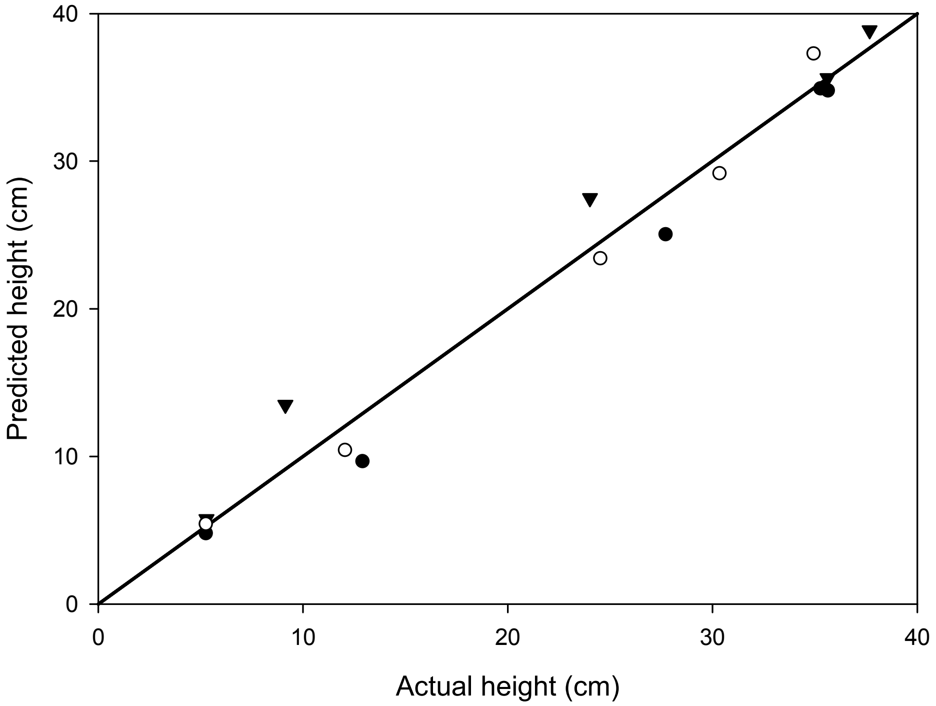


○ Channel 1 ● Channel 2 ▼Channel 3

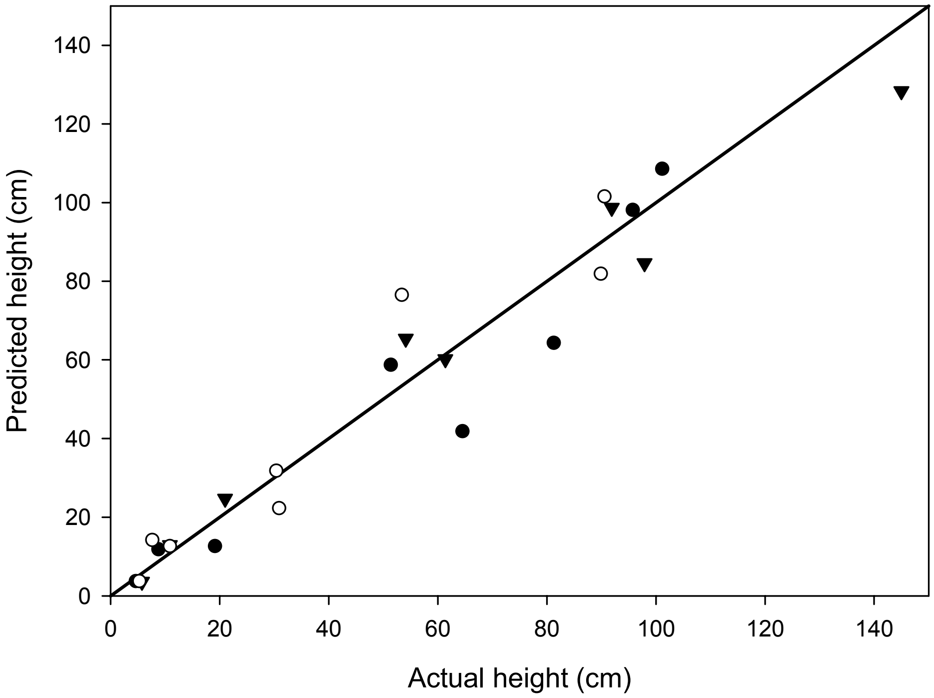
Figure 5.2: Predicted height vs actual height of tomatoes grown in the wastewater varied nutrient solution retention time.



○ Channel 1 ● Channel 2 ▼Channel 3
Figure 5.3: Predicted height vs actual height of carnations grown in the wastewater varied nutrient solution retention time.



○ Channel 1 ● Channel 2 ▼Channel 3
Figure 5.4: Predicted height vs actual height of silver beet grown in the wastewater 14-day nutrient solution retention time.



○ Channel 1 ● Channel 2 ▼Channel 3
Figure 5.5: Predicted height vs actual height of tomatoes grown in the wastewater 14-day nutrient solution retention time.

5.4 DISCUSSION

Water and nutrient (N and P) balances

Plants have varying nutrient and water requirements, usually dependant on the type of plant. Most water loss in soil-less cultures are as a result of plant uptake as well as water leaching to the substrate (Ondrašek *et al.,* 2007). In hydroponics systems, nutrient and water uptake are linearly related (Pardossi *et al.,* 2008). Instead of using fresh water resources to grow commercially important crops, wastewater effluent is a viable option as a nutrient source. In this experiment the volume of water used increased in the 14-day NSRT (Table 5.1), this may be due to the requirement of higher nutrient concentration for plant growth in the 14-day NSRT (Table 5.2). However, the total weight of plants only increased in the silver beet grown in the 14-day NSRT (Tables 3.1 and 4.2).

Kelly *et al.* (2006) recognised that the largest amount of nutrient required for good plant growth is usually nitrogen. In the varied NSRT, the amount of nitrate-nitrogen and phosphate-phosphorus in the silver beet WW effluent was lower than in the CM effluent (Table 5.2). This could be the cause of small concentrations of nitrogen in the WW plants that were grown in the varied NSRT (Table 5.3). Zhang *et al.* (2008) recognised that the mutual relationship between nitrogen and phosphorus uptake may not be clearly seen when using wastewater as a nutrient solution, one of the reasons is because the concentration of ammonium-nitrogen is higher than nitrate-nitrogen. In Table 5.2, the conversion of ammonium-nitrogen to nitrate-nitrogen was probably the reason for the increase in nitrate-nitrogen in WW treatments.

When the nutrient solution retention time was reduced, the amount of nitrogen and phosphorus generally increased in the silver beet leaves, which increased its potential for increasing the nutrition for plant production (Tables 5.2 and 5.3). This was not the case for the nitrogen concentration in WW tomato fruits, however, the phosphorus concentration increased (Table 5.3).

Multiple regression

The use of models is increasing particularly in problem solving and decision-making (Sargent, 1998). Most models have been developed for specific situations and require validation and verification (Sargent, 1998). In order to increase the accuracy of a model, it requires field data to test the model and compare the model results to the actual collected data (Prusinkiewicz, 2004).

The model equation obtained from the multiple regression showed that the predicted values and the actual values for all of the NSRT treatments were close to observed data (Figures 5.1, 5.2, 5.3, 5.4 and 5.5). The parameters that were not significant individually to the height (Table 5.4 and 5.5) of the plants could be because in wastewater, nutrients like nitrate-nitrogen require conversion from ammonium-nitrogen (Wang and Li, 2004), which may influence pH, EC, DO and other nutrients.

A method to rectify this would be to identify the links between the different parameters and growth in hydroponic systems as Silberbush *et al.* (2005) conducted. That model took into account the water loss through transpiration, salinity build-up in the system and salinity interaction with plant growth including nutrient uptake. It would also be ideal to create a model specifically for hydroponic systems utilising secondary treated domestic water similar to that conducted by Rababah and Ashbolt (2000) for primary municipal sewage. That study investigated the effects of consuming lettuce grown in the NFT system on human health as well as the phosphorus removal efficiency. However, this study focused on using hydroponics systems as a tertiary treatment option as well as the nutritional quality of the plants. Rababah and Ashbolt (2000) recognised that further investigations were required to obtain a complete nutrient removal model. In order to predict plant production and maintain treatment efficiency without reducing the output, further research is required to obtain more accurate models.

Mattson *et al.* (2006) developed a model for hydroponically grown cut flower roses. In their study, the shoots, base leaves, base stems and roots were analysed to determine their model. In this study, only the nutrient solution was analysed to determine the carnation model (Figure 5.3), which seems adequate, however, further investigations are required to validate the model.

Factors such as temperature, solar radiation are required for a more accurate model as these factors influence plant growth. The models generated in this study are the first step in determining whether the nutrient concentration of secondary treated domestic wastewater could have been suitable for silver beet, tomato and carnation production.

5.5 CONCLUSION

Modelling as well as water and nutrient balances are important for further development of wastewater hydroponics. This is because this information could assist communities by allowing them to plan ahead using this system. If the model is accurate, then appropriate decisions over the type of plant, amount of effluent, concentration of nutrients in solution and nutrients required by plant could be considered before implementing this system. This chapter has given an indication of the volume of wastewater required to utilise the various concentrations of nutrients present if growing silver beet, tomatoes and carnations. It has also demonstrated the ability of using a model to predict growth. However, this model requires further validation to increase the quality of prediction in different environments.

CHAPTER 6

NUTRITIONAL QUALITY OF SILVER BEET AND TOMATOES GROWN IN SECONDARY TREATED WASTEWATER

6.1 INTRODUCTION

There have been numerous studies that have been conducted on the potential of treated domestic wastewater to provide essential nutrients for plant growth, however there are less studies on the nutritional quality of the edible food crops grown in secondary treated domestic wastewater and using a hydroponics setup. Nutritional quality of food crops is also an important factor to consider, especially when using treated effluent as the main nutrient source for food crops. Carotenoids, ascorbic acid (vitamin C) and total soluble solids (TSS) are some of the important nutritional parameters used to assess fruit and vegetable quality (Bourne and Prescott, 2002).

Carotenoids levels are an important quality aspect in fruit and vegetables for human consumption (Thane and Reddy, 1997). One of the most important forms of carotenoids in tomatoes is lycopene, which was reported to aid in preventing cancer (Abushita *et al.,* 2000; Fröhlich *et al.,* 2006). The measure of TSS is done in % Brix, which is a refractive index used to indicate the total soluble solids present (Baxter *et al.,* 2005). The higher the TSS in fruits, the better the taste of fruits (Fridman *et al.,* 2000). Spinach and tomatoes are one of the best sources of ascorbic acid (vitamin C) (Michaelsen *et al.,* 2003). Vitamin C is important for prevention of scurvy and also encourages healing of wounds (Michaelsen *et al.,* 2003). Therefore the levels of carotenoids, TSS and ascorbic acid are important elements determining the nutritional quality of the vegetables and fruits.

The aim of this study was to compare the nutritional quality of tomatoes and silver beet grown in different hydroponics media (WW and CM), with those bought in local supermarkets but were organically grown (O).

6.2 MATERIALS AND METHODS

The edible parts of silver beet (leaves) and tomato plants (fruits) were collected from the experiment described in Chapter 4 and used for the quality analysis.

The edible parts of the plants were separated from the plant and washed with distilled water and the wet weight was analysed for total soluble solids (TSS), total carotenoids and ascorbic acid (vitamin C). Samples were analysed in triplicate. TSS was measured using an Atago digital pocket refractometer. Total carotenoids and ascorbic acid were estimated according to methods followed by Lalel (2002). The statistical analysis was conducted in the same way as described in Chapter 3.

6.3 RESULTS

The results given in Table 6.1 shows that there were no significant difference between the nutritional quality of crops in all samples except the total carotenoid between tomatoes grown in WW, CM and O. The total carotenoid concentration was significantly lower in the purchased organically grown tomatoes than those grown in WW and CM. Hydroponically grown wastewater medium crops had similar values as those grown in commercial hydroponics medium and the organic produce (Table 6.1).

Table 6.1: Nutritional quality of silver beet and tomatoes grown in wastewater (WW), control medium (CM) and organically (O).

Nutritional quality (wet weight)	**Silver beet**			**Tomatoes**		
	WW	**CM**	**O**	**WW**	**CM**	**O**
Ascorbic acid (mg/100g)	20 ± 5	17 ± 1	21 ± 2	13 ± 3	21 ± 3	19 ± 4
Total carotenoids (mg/g)	687 ± 0	730 ± 28	700 ± 30	215 ± 24	281 ± 48	91 ± 11
Total soluble solids (% Brix)	7.4 ± 0.3	7.1 ± 2	< 4.5	5 ± 0.2	5 ± 0.5	6 ± 0.3

6.4 DISCUSSION

Tomatoes are a popular fruit used all over the world as they are available year round and have lots of nutritional value, even when consumed in small quantities (Abushita *et al.,* 2000). They are one of the highest contributors of vitamin C (Gahler *et al.,* 2003; Wilcox *et al.,* 2003). According to a study conducted by Abushita *et al* (2000), the mean ascorbic acid content in their tomatoes was 17mg/100g while, another study showed that greenhouse tomatoes have a vitamin C content of between 12-17mg/100g (Gahler *et al.* (2003). In this study, ascorbic acid concentration the WW tomato (13mg/100g) was the lowest, however there was no significant difference between all the samples.

According to the USDA (2004), the ascorbic acid estimation in silver beet is 2.8mg/leaf and in tomatoes it is 15.6mg/tomato. Overall in this study, the tomatoes had a lower concentration than the silver beet. This may be due to the amount of nitrogen available to them through the growth medium. It has been reported that an increase in nitrogen concentration decreases the ascorbic acid concentration in tomatoes, but it increases the concentration in spinaches (Wunderlich *et al.,* 2007).

Lycopene (red carotenoid pigment) production in tomatoes is dependent on the potassium concentration (Zdravković *et al.,* 2007). This study found that fertilisers with increased phosphorus did not significantly increase the level of lycopene. There was a significant difference ($p<0.05$) in total carotenoids between O tomatoes and both WW and CM tomatoes (Table 6.1). According to the USDA (2004), carotenoids estimation found in silver beet was 563μg/leaf and in tomatoes it was 552μg/tomato.

According to Nichols (2006), harvested tomato fruits have a Brix level of about 5%. However, TSS in wild tomatoes can reach 15%, which is three times more than that in domestic varieties. According to the results in table 6.1 both fruits grown in the channels had the same Brix level of 5%. The tomatoes that had been purchased had a higher Brix level, however this was not statistically significant. Daiss *et al.* (2008) found that eight weeks after sowing silver beet in two treatments (EM-Bokashi+EM and Greengold®) contained about 4.5 Brix and 4.3 Brix respectively. In this experiment the Brix levels in WW (7.4 Brix) and CM (7.1 Brix) were both higher (Table 6.1) and the organically purchased silver beet had low readings on the meter.

Nutritional quality of foods are important because without it, it can increase susceptibility to infection, reduction in academic performance, especially in children (United Nations., 2007). This study has shown that secondary treated domestic effluent can provide food crops with required nutrients for good nutritional value, which can positively influence the Millennium Development Goals.

6.5 CONCLUSION

According to this study, the fruits and vegetables grown in treated wastewater showed similar nutritional quality in terms of ascorbic acid, TSS and carotenoids as the commercial medium grown and organically grown produce. This highlights the potential for utilising secondary treated effluent for producing high-quality produce through hydroponics systems.

CHAPTER 7

RISK OF PATHOGEN CONTAMINATION OF CROPS IN WASTEWATER HYDROPONICS

7.1 INTRODUCTION

The risk of pathogen contamination of edible food crops has limited the use of treated wastewater for crop production. The risk to humans through contact with wastewater reuse involves transmission of pathogens including infectious enteroviruses (Mignotte *et al.,* 1999), especially in edible food crops. When growing crops in effluent, it is necessary to consider the risks involved, especially if the crops are for sale and consumption (Ottoson *et al.,* 2005). Amahmid *et al.* (1999) found *Giardia* cysts and *Ascaris* eggs on crops irrigated with raw wastewater, however, not on crops irrigated with treated wastewater.

Different types of irrigation systems can be used in reducing the risk of transferring contaminants to plants and field workers. Irrigation systems such as sprinkler and open irrigation, where humans are in contact with the effluent are not widely accepted due to the associated health risks. There may be risks involved with using effluent for soil irrigation as it may contaminate edible food crops due to direct contact with the plants (Rosas *et al.,* 1984). Soil and groundwater contamination with pathogens and parasites is also possible through soil irrigation.

The nutrient film technique (NFT) and water culture (WC) are forms of hydroponics that may reduce these risks compared to other irrigation systems. If the hydroponics technique is used for growing leafy and fruit crops the edible parts of the plant are not in contact with the wastewater because of a physical barrier between the plant parts and the medium. The other advantage of this system is that it is a type of intensive agriculture where farmers/communities are able to grow substantial amount of crops in limited space.

This study looked at the possibility of bacterial contamination of plants if wastewater was used to grow edible crops in hydroponics systems using the NFT and WC system. This is to determine the risk of pathogen contamination of crops (silver beet and tomatoes) grown in secondary treated effluent for human consumption. It also examined the bacterial pathogen die off rate in the solution at different nutrient solution retention times. Though parasites are the main concern in WW irrigation in most developing countries, their level in secondary treated effluent from developed countries like Australia can be negligible therefore only bacterial pathogens were tested (WHO, 2006a,b).

7.2 MATERIALS AND METHODS

The NFT experimental set-up was the same as that described in section 3.2 (Figure 7.1) and had the same nutrient solution retention time as described in Chapter 4. This experiment was run at the same time as the one described in Chapter 4. Tomatoes were also grown (in triplicate) in water culture (WC) hydroponic set-up (Figure 7.2). In the WC system, the plants were grown in tubs containing the nutrient medium. It was a closed-system without recirculation of the nutrient medium. The retention time in WC nutrient solution was 14 days. The tomato seedlings were planted in 10cm x 10cm nursery pots, filled with expanded clay balls and then suspended into the tubs to allow the roots to grow into the nutrient solution.

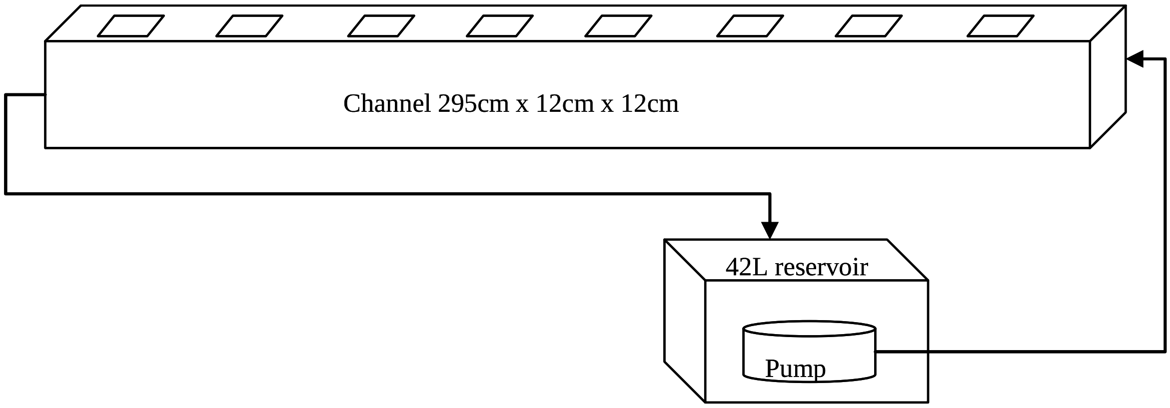


Figure 7.1: Nutrient film technique (NFT) experiment design

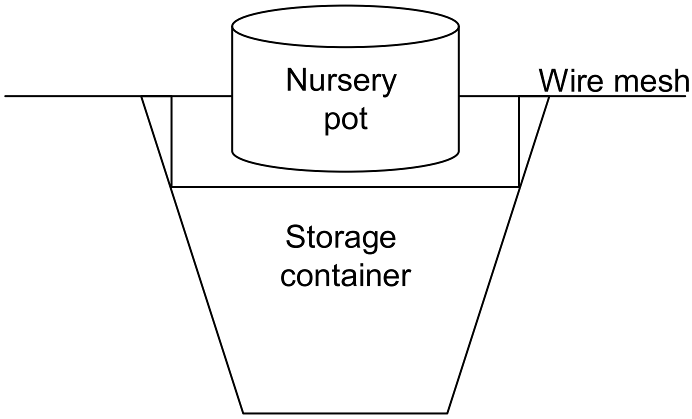


Figure 7.2: Water culture (WC) experiment design

The commercial nutrient solutions in both hydroponic systems were inoculated with *S.typhimurium* and *E.coli*. Pure cultures of *Salmonella typhimurium* (ATCC14028) and *Escherichia coli* (WACC4) used for this experiment were grown in buffered peptone water and lauryl tryptose broth respectively. Serial dilutions were prepared according to Standards Australia (1991). The pathogens from the pure culture were pipetted (1mL) into Mcartney bottles containing 9mL sterile water, this was then spiked to the treatment (42L storage containers). Sampling of wastewater (WW), control medium (CM) and spiked control medium (CMS) was conducted every 7 days and after every 14 days when the medium was changed and pathogens were reinoculated. The experiment was conducted for 28 days and was spiked two times. All treatments were conducted in triplicate.

For analysis, the water samples were collected in sterile 250mL schott bottles and tested following the methods by Standards Australia for both *E.coli* (Standards Australia., 1995a) and *S.typhimurium* (Standards Australia., 1995b).

When the plants were ready for harvest, the edible parts of the plants were separated and were tested. Organically grown silver beet and tomatoes were purchased from a supermarket for comparison of the quality. The edible parts of the plants were washed with sterile water and the wash water was analysed for *E.coli* (Standards Australia., 1995a) and *S.typhimurium* (Standards Australia., 1995b). Then the edible parts of the plants were analysed for *E.coli* (Standards Australia., 1992) and *S.typhimurium* (Standards Australia., 2004).

The statistical analysis was conducted using the same methods as described in Chapter 3.

7.3 RESULTS

Nutrient Solution

Overall, NFT had a better pathogen reduction rate than the WC within the first seven days (Table 7.1). There were no detectable pathogens after 14 days in both systems (Table 7.2).

Table 7.1: Reduction of bacterial pathogens in the solution of nutrient film technique (NFT) systems

	Plant	**Pathogen**	**Reduction in pathogens (total number) in NFT**								
			0 days			**7 days**			**14 days**		
			WW	**CM**	**CMS**	**WW**	**CM**	**CMS**	**WW**	**CM**	**CMS**
1st spike	**Silver beet**	***E.coli***	59±2	0	1800±780	<1	0	<1	<1	0	<1
		S.typhimurium	13±1	0	2800±2068	<1	0	<1	<1	0	<1
	Tomato	***E.coli***	59±2	0	1350±37	<1	0	<1	<1	0	<1
		S.typhimurium	13±1	0	1100±443	<1	0	<1	<1	0	<1
2nd spike	**Silver beet**	***E.coli***	85±3	0	575±307	<1	0	<1	<1	0	<1
		S.typhimurium	7±2	0	800±529	<1	0	<1	<1	0	<1
	Tomato	***E.coli***	399±50	0	634±393	<1	0	<1	<1	0	<1
		S.typhimurium	13±3	0	360±159	<1	0	<1	<1	0	<1

Table 7.2: Reduction of bacterial pathogens in the solutions water culture (WC) systems

	Pathogen	**Reduction in pathogens (total number) in WC**								
		0 days			**7 days**			**14 days**		
		WW	**CM**	**CMS**	**WW**	**CM**	**CMS**	**WW**	**CM**	**CMS**
1st spike	***E.coli***	399±50	0	1330±300	180±16	0	360±150	<1	0	<1
	S.typhimurium	13±5	0	2460±370	2±0.8	0	530±33	<1	0	<1
2nd spike	***E.coli***	7±3	0	16733±8870	<1	0	2167±167	<1	0	67±15
	S.typhimurium	15±3	0	21667±5608	<1	0	550±300	<1	0	67±20

Harvest

Pathogen concentration in wash water

The wash water from WW, CM, CMS (tomatoes) and organically grown silver beet had no pathogens detected (Table 7.3). The wash water from CMS silver beet had *S.typhimurium* present at a very low concentration of 2cfu in 100mL.

Table 7.3: Number of bacterial pathogen contamination in silver beet and tomato wash water (cfu/100mL) in nutrient film technique (NFT), water culture (WC) and organically grown (O).

Plant	**Pathogen**	**NFT**			**WC**			**Supermarket**
		WW	**CM**	**CMS**	**WW**	**CM**	**CMS**	**O**
Silver beet	*E.coli*	n.d.	n.d.	n.d.	n/a	n/a	n/a	n.d.
	S.typhimurium	n.d.	n.d.	2	n/a	n/a	n/a	n.d.
Tomatoes	*E.coli*	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	S.typhimurium	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.

n/a – not applicable

n.d. – not detected

Pathogen concentration on the edible parts of the plants

There were no detectable concentrations of *E.coli* or *S.typhimurium* in any of the edible parts of the plants grown in WW, CM, CMS and O silver beet and tomatoes.

7.4 DISCUSSION

The die-off rate was quite significant in most cases (Tables 7.1 and 7.2). Pathogens had significantly reduced in most samples by the 7th day, which is less time (between 14 – 21 days) than was noted by Oyama *et al.* (2008). The die off rate was higher in the NFT than WC and this may be due to the aeration in the NFT system. The microbial effluent quality after 14 days was within the World Health Organisation guidelines (WHO, 1989). The revised guidelines state that drip irrigation with treated wastewater should contain less than 1000cfu/100mL *E.coli* for low growing crops and less than 100000cfu/100mL *E.coli* for higher growing crops (WHO, 2006b). The ARMC (Australia) *et al.* (2000) guidelines for raw edible food crops in contact with treated effluent should have thermotolerant coliform count of less than 10cfu/100ml and crops not in direct contact with treated effluent should have a thermotolerant coliform count of less than 1000cfu/100ml. The effluent quality of the medium was well below these guidelines. The validity of using faecal coliforms as an indicator of pathogens has been questioned, however, Harwood *et al.* (2005) found that it was relatively cautious to use in order to protect human health.

A study conducted by Teltsch and Katzenelson (1978) showed a strong possibility that enteric bacteria and viruses can be spread in the air via spray irrigation. Studies have shown the possibility of contaminated water used for spray irrigation may play a big role in contaminating vegetables (Rosas *et al.,* 1984; Islam *et al.,* 2004) which, could be due to contaminated water being in contact with the vegetables. A study conducted by Pollice *et al.* (2004) found that there were low bacterial contamination in tomato and fennel crops irrigated with tertiary treated wastewater and concluded that the higher the treatment the safer the produce will be from bacterial contamination.

It was observed that crops like lettuce and parsley irrigated with raw wastewater were more contaminated compared to crops like tomatoes and pimento (Melloul *et al.,* 2001). The most likely reason given was that leafy vegetables that develop at the soil surface have more foliage, which offers more area for contamination from water spray (Rosas *et al.,* 1984; Melloul *et al.,* 2001). Samples of lettuce and radish grown in soil fertilised with manure and fertiliser are observed to be contaminated with faecal coliforms which, could have been due to contact of the vegetables and soil (Machado *et al.,* 2006).

Using alternative methods for wastewater irrigation can reduce the exposure of edible parts to pathogens (NRMMC *et al.,* 2006). This study revealed that growing vegetables using the nutrient film technique in hydroponics system may reduce the exposure risk. However, a study showed that hydroponic tomatoes grown in an inoculated nutrient solution was found to take up *S.typhimurium* to the stems and leaves of young plants (before fruit maturation) (Guo *et al.,* 2002). In this study (Table 7.3), vegetables grown in WW and CMS were not contaminated with the pathogens. One reason may be because there is no contact between the edible parts of the plant and the contaminated medium.

Tomato fruits have the ability to promote salmonella growth, depending on the handling methods (Zhuang *et al.,* 1995). Abdul-Raouf *et al.* (1993) found that it was possible to contaminate salad vegetables with *E.coli* O157:H7 during production, harvest, processing, marketing and preparation, as a result care should be taken when handling the vegetables. Another possible source of contamination of food crops is through the water sprinkled on vegetables in order for them to look fresh for marketing purposes (Hamilton *et al.,* 2006).

As bacterial survival depends on climatic conditions, the results may vary from region to region (Vaz da Costa-Vargas *et al.,* 1991). In this study, pathogen contamination of vegetables using secondary treated domestic wastewater is significantly low and as a result can be recommended for safe consumption. The secondary treated domestic wastewater, after passing through the hydroponics system, for a period of 7 – 14 days showed thorough elimination of *E.coli* and *S. typhimurium,* which made the final effluent safe for open irrigation.

7.5 CONCLUSIONS

This study has shown that edible parts of silver beet and tomatoes are safe from bacterial pathogens when wastewater or a solution containing pathogens is used as the nutrient medium. The effluent after passing through the hydroponics system was completely deprived of *E. coli* and *Salmonella* sp. between 7 to 14 days. The effluent after going through these systems (nutrient film technique and water culture) can be used safely for further irrigation. However, it has to be noted that the safety depends on the system hygiene.

CHAPTER 8

GENERAL DISCUSSION

8.1 INTRODUCTION

It is important that the reuse of wastewater for agriculture/horticulture be regulated through on site dependent factors. The WHO (2006b) give out general guidelines for the safe use of wastewater. In countries where guidelines are not available, these are the best to follow. In countries where there is capital available to follow more stringent guidelines, then USEPA (2004) guidelines can be adopted. Most countries have their own guidelines based on the economic and social conditions without compromising environmental and health safety.

However, highly cautious procedure is required when using treated wastewater for food production mainly due to the unknown risks to humans and their health. This research studied the overall aspects in order to assess suitability of using secondary treated domestic wastewater for food production using the hydroponics technique. The main aspects that were studied through this research were the adequacy of plant nutrients in secondary treated wastewater for optimum production of horticulture crops, the nutritional quality of the produce and the microbial safety of the system.

8.2 AVAILABILITY OF NUTRIENTS FOR PLANT GROWTH

In Chapter 3, tests were conducted to use up the nutrient and water completely in a secondary treated domestic effluent through a hydroponic system by growing silver beet, tomatoes and carnations. Therefore the nutrient water was recycled through the system for about five weeks until the nutrient water was depleted. It was observed that there were inadequate nutrients available when the nutrient solution retention time was retained until all the solution was exhausted (Chapter 3 and Chapter 5). The poor performance of silver beet and tomatoes was attributed to low nutrient availability in the wastewater. However, carnations produced similar crop as in the commercial medium revealing its potential as an ideal crop for reusing secondary treated effluent.

In order to rectify this there were two available options, the first was to add fertiliser to the wastewater to increase nutrient availability. The second was to reduce nutrient retention time was reduced to 14 days facilitating more nutrient availability. With the revised reduced retention time of the nutrient solution (Chapter 4), the silver beet produced 51% more and tomatoes produced 49% more crops than the previous trial. In the case of silver beet, the revised wastewater nutrient solution retention time produced more silver beet than the commercial treatment. The silver beet also had more nitrogen and phosphorus in the edible parts of the plant (Chapter 5). However, the numbers of tomatoes grown in secondary treated domestic wastewater were still lower than the number of tomatoes grown in commercial medium. The major difference was that the overall height of the tomato plant in the wastewater treatment was better in the study discussed in Chapter 4 than in Chapter 3.

The chemical quality of the effluent after both trials showed that it could be either disposed off safely or used for irrigation as it met the disposal guidelines (WHO, 1989; EPP, 1995; ANZECC and ARMCANZ, 2000; ARMC (Australia) *et al.*, 2000; USEPA, 2004)**.**

8.3 PATHOGEN CONTAMINATION OF PRODUCE

The *E.coli* and *S.typhimurium* sps selected as the indicators of microbial contamination in the effluent after passing through two hydroponics systems (nutrient film technique – NFT, water culture – WC) were reduced to a safe quantity. However, the reduction was higher in NFT than WC (Chapter 5). The results obtained from the WC were similar to those found by Oyama *et al.* (2005) where the reduction in faecal coliform was achieved to between 73% to 97% in the spiked medium (CMS) and between 69% to 99% in the wastewater treatment in the first seven days and took 14 days for complete elimination. The *S. typhimurium* was not found in any of the treatments after 14 days and was also not present in the edible parts of the plant.

Only the bacteria spiked treatment (CMS) had *S.typhimurium* in the wash water of tomatoes at a very low level of 2cfu/100mL which may have occurred due to the handling procedures. It is important to reduce the risk of contamination by ensuring proper hygiene techniques (Blumenthal *et al.,* 2000). Leafy vegetable are prone to contamination especially with irrigation water as the surface area is large (Rosas *et al.,* 1984; Melloul *et al.,* 2001), however the silver beet wash water did not have any *E. coli* present.

There were no pathogens detected in the tomatoes or silver beet in any of the produce. There is less risk of accumulation of contaminants in a hydroponics system as compared to soil, where the contamination varies depending on the element (Weber *et al.,* 2006; Rosabal *et al.,* 2007).

The findings of this study are quite positive for the use of secondary treated domestic wastewater for food production through the hydroponics system. However, in order to use treated effluent for food production, as an added safety measure, people handling the foods should be aware of avoiding direct contact with the effluent and strict hygienic procedures need to be followed when using wastewater.

8.4 NUTRITIONAL QUALITY OF PLANTS

The nutritional quality (ascorbic acid, total carotenoids and total soluble solids) of silver beet and tomatoes in the wastewater-grown produce were of similar quality to organic produce available at a supermarket (Table 6.1). The nutritional quality of silver beet and tomatoes grown in the secondary treated domestic wastewater were found to be comparable to the USDA (2004), which provides more evidence for the benefits of using secondary treated domestic wastewater for food crop production. Lack of adequate vitamins and minerals can increase the risk of death in childhood diseases (Food and Agriculture Organisation., 2005). This study has demonstrated that secondary treated domestic wastewater can provide food cops with adequate nutritional quality.

8.5 CONCLUSION

The findings of this study are quite positive for the use of treated domestic wastewater in conjunction with hydroponics for food production as the risk of contamination was reduced, while producing comparable quality of produce for tomatoes and silver beet. The nutritional quality of the produce was also similar to an organic produce. The effluent after passing through this hydroponics system met the guidelines for open irrigation/ disposal or further use. However, there are still unknown factors, like the effects of pharmaceuticals on plant production, uptake of viruses and Helminths, which can be more prevalent in developing countries or rural communities. It also demonstrates the ability of using the hydroponics technique and combining it with further wastewater treatment while producing commercially important crops, which assists in meeting the Millennium Development Goals. In developed countries such as Australia, water shortages have sent the requirement for wastewater reuse. This method demonstrates the ability to grow commercially important crops with minimal risk to both human and environmental health in urban or rural isolated communities.

CHAPTER 9

CONCLUSIONS AND RECOMMENDATIONS FOR FURTHER RESEARCH

This research found that secondary treated domestic effluent when used as a hydroponics medium

- Contains the required nutrients for production of healthy carnations with more flowers produced in the effluent than the commercial hydroponics solution.
- Contains the required nutrients for healthy edible food growth when the retention time of the wastewater in the hydroponics channels is adjusted accordingly (14 days).
- Reduces the risk of microbial pathogen contamination to the edible parts of the plant.
- Produces products with good nutritional quality comparable to soil grown produce.
- The effluent after use can be used further for open irrigation as it meets the guidelines with regards to the nitrogen and microbial pathogen levels.

Recommendations for further research are to

- Understand the effect (if any) of pharmaceuticals on the edible parts of the plants.
- Analyse the effect of viruses on workers and on the edible parts of the plant.
- Issues in relation to scaling up to commercial levels.

CHAPTER 10

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