The use of treated brewery effluent as a water and nutrient source in crop irrigation

A thesis submitted in fulfilment of the requirements for the degree:

Master of Science

at

Rhodes University

by

Richard Peter Taylor

December 2015

Abstract

Brewery effluent (BE) needs to be treated before it can be released into the environment, reused or used in down-stream activities. Current technologies used to address this concern at the experimental wastewater treatment plant at Ibahyi Brewery (SAB Ltd) include anaerobic digestion (AD), primary facultative ponds (PFP), high rate algal ponds (HRAP) and constructed wetlands (CW). The aim of this work was to determine if BE treated in these systems might be suitable for crop irrigation. A test crop, cabbage (*Brassica oleracea* cv. Star 3301), grew best on post-AD and post-PFP BE compared to those irrigated with post-HRAP or post-CW effluent. However, the yield was 13% lower than cabbage plants irrigated with a commercial nutrient solution and fresh water. The relatively high conductivity (3019.05 ± 48.72 $\mu s/cm^2$) of BE may be the main factor reducing the cabbage yields. Post-HRAP and post-CW BE were the least suitable for irrigated crop production due to the higher conductivity and lower nutrient content of these treated effluents. After three months, soils irrigated with post-AD and post-PFP BE had a significantly higher sodium content and sodium adsorption ratio (3919 ± 94.77 mg/kg & 8.18 ± 0.17) than soil irrigated with a commercial nutrient solution (920.58 ± 27.46 mg/kg & 2.20 ± 0.05; $p<0.05$). However, this was not accompanied by a deterioration in the soil's hydro-physical properties, nor a change in the metabolic community structure of the soil ($p>0.05$). After prolonged irrigation with treated BE, sodium is likely to build up in the soil and this can be expected to be accompanied by a deterioration in the soil physical structure. However, crops species such as millet (*Echinochloa esculenta*), lucerne (*Medicago sativa*) and saltbush (*Atriplex nummularia*) reduced the build-up of sodium in the soil. The results suggest that sodium was mainly removed from the soil through plant-assisted leaching. Of the crops grown,

lucerne showed the most promise because it improved the soil physical properties, is able to grow well in alkaline environments, is a popular fodder crop and can be harvested multiple times from a single stand. Brewery effluent is more suitable for soil production systems than hydroponic production systems because the soil was able to act as a buffer against the high pH of post-AD BE, whereas in a hydroponics systems the high pH reduced the availability of key minerals to plants. In conclusion brewery effluent contains sufficient plants nutrients to support the growth of cabbages, saltbush, lucerne and millet. However the sodium content of BE is a concern as it accumulates in the soil, and in the long-term it may lead to soil degradation. It is suggested that the brewery change the pH neutralising treatment of BE from sodium hydroxide to potassium hydroxide, or dolomitic lime (calcium and magnesium carbonate) because this would reduce the introduction of sodium into the system, and would increase the suitability of BE for crop production, given potassium and calcium are plant nutrients. The benefits of developing this nutrient and water resource could contribute to cost-reductions at the brewery, more efficient water, nutrient and energy management, create job opportunities with the potential of improving food security in the local community.

Table of contents

List of Figures

List of tables

List of abbreviations

AD	Anaerobic digestion
AFP	Air filled porosity
AS	Activated sludge
AWCD	Average well colour development
BE	Brewery effluent
BOD	Biological oxygen demand
CCI	Chlorophyll concentration index
CEC	Cation exchange capacity
CFU	Colony forming units
COD	Chemical oxygen demand
CW	Constructed wetland
DO	Dissolved oxygen
DWAF	Department of Water Affairs and Forestry
EC	Electrical conductivity
ESP	Exchangeable sodium percentage
PFP	Primary facultative pond
PC	Permanent charge
H	Shannon-Weaver index
HRAP	High rate algal pond
HSSFCW	Horizontal subsurface flow constructed wetland
NS	Nutrient solution
PVC	Polyvinylchloride
OD	Optical density
R	Richness
SAB Ltd	South African Breweries Limited
SAR	Sodium absorption ratio
TDS	Total dissolved solids
VC	Variable charge

Acknowledgements

I would first like to thank my family, Tony Pote and Tony Du Plessis for their support through this journey.

I would like to thank my supervisors Dr Clifford Jones and Prof. Mark Laing for their advice and wiliness to guide me whenever it was needed. A special thank you must also be given to staff and student of the Department of Ichthyology and Fisheries Science for their friendship, academic and administrative support. I would like to say and extra thank you to the secretaries Tina, Xoliswa and Yvain for taking care of all financial details during the past two years.

Special thanks must also to given to Prof. Joe Dames from the Microbiology Department and Prof. Brad Ripley from the Botany Department who were always willing to give me advice and let me utilise equipment in their laboratories.

Thank you to the employees of Talbot & Talbot, Olivia, Lindle, Phumeza, and Chase, who were always welcoming, helpful and supportive. Special thanks must be given to the employees at Ibhayi Brewery, especially Pravendhran, Trevor, Deon, Cor and Andries, for assisting me with information I needed and making my time spent at the brewery a pleasure.

Finally I would like to thank the Water Research Commission and South African Breweries for funding this project. Without this funding I would have not been able to carry out this research project. I hope this thesis is a fitting piece of work as we develop technologies that can be used to ensure the reuse of water.

Chapter 1: Introduction

South Africa is a water scarce country (Arnell 2004). The increase in size and number of industries over the past century has led to the production of increasing quantities of wastewater and less clean water available for human consumption (Hanjra & Qureshi 2010). Breweries are a major consumer of fresh water and producers of nutrient-rich wastewater (Braeken *et al.* 2004). The wastewater is a major liability because it poses an environmental threat, so it has to be treated prior to disposal (Braeken *et al.* 2004). Brewery effluent (BE) at Ibhayi Brewery (SAB Ltd) in Port Elizabeth is partially treated onsite after which it is sent to the municipal sewer (Jones *et al.* 2013). The current treatment process is costly and energy expensive (Power 2014, Simate *et al.* 2011); however, alternative technologies exist that could make the water and nutrients in the BE available for re-use or for use in other downstream applications. There is a need to identify potential alternative water, nutrient and energy resources and to develop the techniques needed to exploit their value (Simate *et al.* 2011, Power 2014). The irrigation of agricultural crops is a potential downstream use for water and nutrients that have been recovered from BE.

Brewery effluent is an organic effluent that contains nitrogen and phosphorous (Braeken *et al.* 2004). Nitrogen and phosphorous are essential for good plant growth (Freeman 2005). Farmers spend money buying inorganic nitrogen and phosphorus fertilisers. Brewery effluent could be used as a source of water and nutrients in crop production (Kumar *et al.* 2010, Jones *et al.* 2013). Lettuce and tomatoes have been grown hydroponically on partially treated BE (Jones *et al.* 2013, Power 2014). However, no research has been done on the possible use of BE as a source of irrigation water in agricultural crop production and the effect that this will have on crop production rates and soil characteristics. This research may

provide a way in which breweries can find an end use for their effluent, meaning they would have zero waste.

Water is one of the key challenges that will determine the future of humanity on our planet (Arnell 2004). It has even been suggested that water will soon be a bigger crisis than oil for humanity (Power 2013). It is vital that we develop technologies to ensure adequate supply for water to all users while minimising environmental degradation and water resource pollution (Simate *et al.* 2011). South Africa is faced by water, food and energy shortages (Rodda *et al.* 2011). This project has the potential to address these issues, since a "waste" product, which is currently a liability to industry, might be used to produce crops and create jobs when it is used as an irrigation source in crop production.

1.1 Problem identification

It is necessary to ensure that there will be no negative side effects that will compromise crop health or soil structure and fertility when BE is used in agriculture. Therefore, a treatment process that ensures that the use of treated BE does not compromise the integrity of the crop and soil needs to be developed.

Each treatment process in the experimental wastewater treatment plant at Ibahyi Brewery (SAB Ltd) results in BE with a different set of water quality parameters such as pH, form and concentration of nitrogen, concentration of phosphorous and electrical conductivity (EC) (Jones *et al.* 2013). These parameters have shown to directly and indirectly affect plant growth (Lucas & Davis 1961, Epstein & Bloom 2005). It is therefore essential that the most suitable pre-treatment method of BE is found so that the nutrients in the effluent are made accessible to the plants without compromising the environment in any way.

Most effluents contain relatively high concentrations of salts (total dissolved salts > 1500 mg/l) and when used for irrigation, they have the potential to add large amounts of salt to the soil (Muyen *et al.* 2011). The increase in soil salt content from irrigation with highly saline waters has resulted in major reductions in crop yields and may lead to agricultural lands becoming unusable (Qadir & Schubert 2002, Muyen *et al.* 2011). This is a global problem because the use of potable water for irrigation is not viable in most countries and they are forced to use wastewater for irrigation purposes (Muyen *et al.* 2011). Brewery effluent has a relatively high levels of dissolved salts (Table 1.1), therefore it is essential that the effect of BE on soil physical, chemical and biological fertility are determined.

The pH of irrigation waters and hydroponic solutions affect the availability of nutrients to plants (Lucas & Davis 1961, Epstein & Bloom 2005): a "pH of six ensures optimal assimilation rates of macro and micro nutrients" (Lucas & Davis 1961). Power (2014) grew tomatoes on BE and found that adjusting the pH to 6.5 doubled plant height and tomato yields.

In order to successfully assess the feasibility using BE as crop irrigation source, the following questions must be answered:

- What are the characteristics of BE and where does each characteristic originate from?
- What are the current BE treatment processes?
- What other treatment processes could be used to make BE most suitable for crop irrigation?
- Do the different forms of treated BE fall within the criteria for use as an irrigation water?
- Which characteristics of treated BE inhibit plant growth and have negative effects on the soil?
- What are the most suitable crops to grow under irrigation with BE?

1.2 Literature review

Water is one of the key challenges that will determine the future of humanity on our planet and technologies that ensure adequate supply for water to all users while minimising environmental degradation and water resource pollution need to be developed (Arnell 2004, Simate *et. al* 2011).

Agriculture is a major consumer of potable water in all countries (Muyen *et al.* 2011). Effluents that contain nutrients need to be exploited to reduce their polluting effects on our environment. If nutrient rich wastewater is used for agriculture this will free up potable water for other uses such as ecosystem health, and human and animal consumption (Muyen *et al*. 2011). Breweries are recognised as a significant global industry, with global beer production around 1.8 billion hectolitres in 2010 and a wastewater production of around 5.4 billion hectolitres (Ascher 2012). Thus breweries are an ideal target for the investigation of using nutrient rich wastewaters in agriculture. Theoretically, if all BE was used for agriculture, this would free 7.2 billion hectolitres of fresh water for other uses.

1.2.1 Components of brewery effluent

Raw BE contains macromolecules originating from wort, beer and cleaning-in-place processes (Braeken *et al.* 2004, Brito *et al.* 2007, Cilliers 2012,). These include carbohydrates such as maltose, dextrose and lactose, proteins and amino acids, hop compounds, vitamins and minerals, alcohol, yeast, yeast derived fermentation products, sodium hydroxide, nitric and phosphoric acid, sequestering agents, chelating agents, wetting agents, Kieselguhr,

silica hydrogel, small amounts of spent grain, calcium sulphate and lactic acid, and water at 85 ^{o}C (Viljoen, pers. comm., Brewing Master, Ibhayi Brewery, SAB Ltd., April 2010).

Brewery effluent has a high chemical oxygen demand (COD) from all the organic compounds (carbohydrates, sugars, starch, proteins and amino acids) it contains (Simate *et al.* 2011; Table 1.1). It usually has temperatures ranging from 25 - 38 ^{o}C. The pH values can range from 2 - 12 depending on the amount and type of chemicals used in salinization (Simate *et al.* 2011; Table 1.1). Nitrogen and phosphorous levels depend on the amount of raw material and yeast present in the effluent (Braeken *et al.* 2004).

Table 1.1 Qualities of raw brewery effluent (Simate *et al.* 2011).

Parameter	Value
pH	3 - 12
Temperature (^{o}C)	18 - 40
Chemical oxygen demand (COD) (mg/l)	2000 - 6000
Biological oxygen demand (BOD) (mg/l)	1200 - 3600
COD:BOD ratio	1.667
Volatile fatty acids (mg/l)	1000 - 2500
Phosphate (mg/l)	10 - 50
Total nitrogen (mg/l)	25 - 80
Total solids (mg/l)	5100 - 7150
Total suspended solids (mg/l)	2901 - 3000
Total dissolved solids (mg/l)	2020 - 5940

1.2.2 Treatment methods of brewery effluent

The treatment system used at iBhayi brewery consists of a combination of technologies. The full volume effluent is screened through a drum filter that removes solid wastes such as stones, plastics, glass, paper and labels from the waste stream, after which it is sent to an anaerobic digester (Cilliers 2012; Figure 1.1). After AD some is used in the experimental system and the rest is sent to the municipality for processing with sewage (Power 2014; Figure 1.1). In the experimental system AD effluent is passed through a primary facultative

pond (PFP) after which it can be sent through the HRAP or CW systems for further treatment (Figure 1.1).

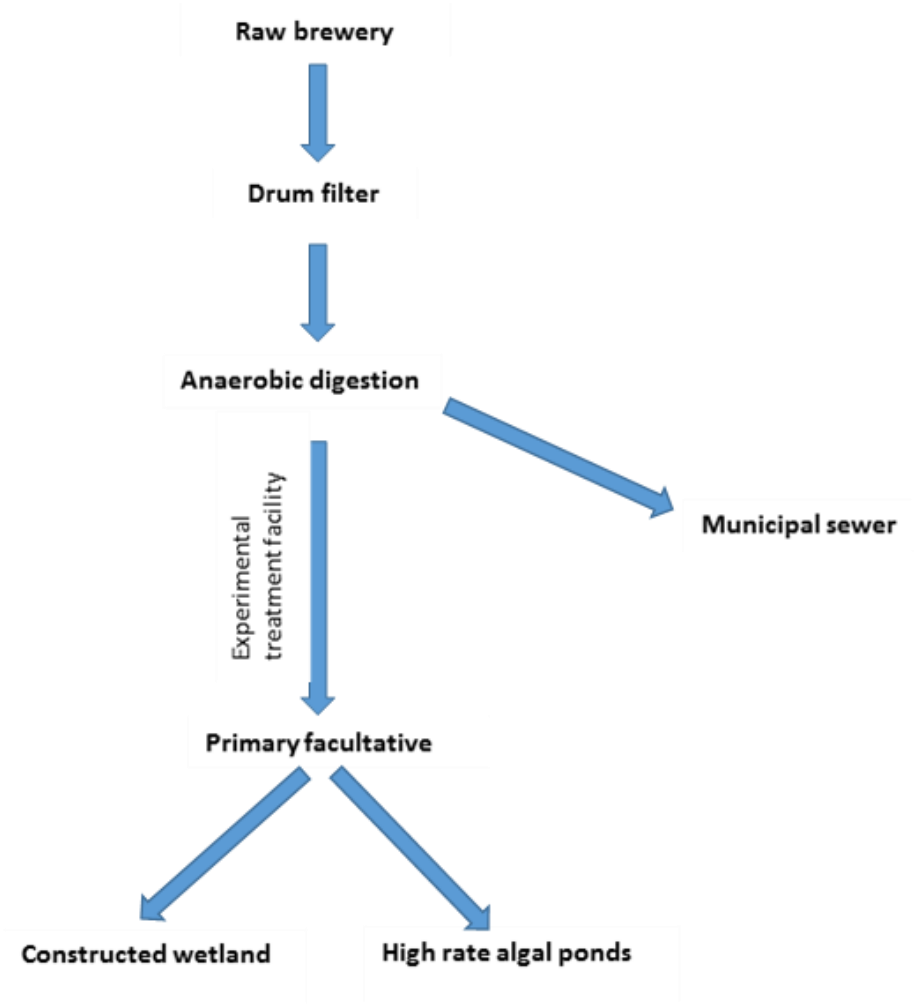

Figure 1.1 Treatment processes used at Ibhayi brewery and the experimental treatment facility.

Anaerobic digestion

Anaerobic digestion is a biological process that converts organic carbon into a gaseous mixture that is principally composed of carbon and hydrogen; methane is the most reduced carbon state, and carbon dioxide is the most oxidized carbon state (Lyberatos & Skiadas 1999). The process happens through the concerted action of a highly integrated community

of bacteria (Lyberatos & Skiadas 1999). The whole process happens in the absence of oxygen. Although the main products that form during anaerobic digestion are CO_2 and small hydrocarbons, other gases such as nitrogen, nitrogen oxides, hydrogen, ammonia, hydrogen sulphide and other volatile compounds, are also generated (Angelidaki & Sanders 2004).The initial anaerobic treatment of BE results in an effluent with high concentrations of organic acids, phosphate, ammonia, nitrate and other low molecular weight substances (Ogbonna *et al*. 2000). The phosphate, ammonia, nitrate and other low molecular weight substance could be used to fuel crop production (Juwarkar & Dutta 1990, Dakoure *et al.* 2013). Anaerobically digested BE is not a balanced irrigation water and nutrient source and may contain certain substances that will inhibit plant growth and deteriorate soil fertility and structure (Kumar *et al.* 2010, Dakoure *et al.* 2013).

The micro-organisms responsible for AD function best at a neutral pH (Lyberatos & Skiadas 1999, Liu *et al.* 2008). During AD CO_2 is produced and a fraction of this dissolves in the liquor, generating carbonic acid and carbonate alkalinity (Lyberatos & Skiadas 1999, Van Rensburg *et al.* 2003). This causes an increase in both the acidity and alkalinity of the liquor (Van Rensburg *et al.* 2003). Therefore to maintain a stable pH, the incoming BE pH (average 5.48) is neutralised with sodium hydroxide (Power 2014). This results in post-AD having a high sodium content, alkalinity and conductivity (Table 1.2, 1.4).

Table 1.2 Average water qualities of post anaerobically digested (AD), primary facultative pond (PFP), and high rate algal pond (HRAP) brewery effluent (Cillers 2012).

Parameter	AD	PFP	HRAP
Temperature (^{o}C)	22.69	21.71	21.07
pH	7.82	8.25	9.68
Chemical oxygen demand (mg/l)	153.21	135.74	95.00
Ammonia (mg/l)	42.53	39.49	1.77
Nitrite (mg/l)	0.07	0.08	1.72
Nitrate (mg/l)	1.97	1.82	13.82
Phosphate (mg/l)	12.49	9.50	5.23
Chloride (mg/l)	482.25	486.62	546.53
Electrical conductivity (µs/cm^2)	2761.85	2761.50	2867.51

Primary facultative pond

After AD brewery effuent is then passed into a primary facultative pond (PFP) where large molecules are settled out (Cilliers 2012). The purpose of the PFP is to reduce the total suspended solids from the effluent and thus lower the COD and biological oxygen demand (BOD) (Jones *et al.* 2013). Solids in the influent to the PFP and excess biomass produced in the PFP settle out and form a sludge layer at the bottom (Marais 1970, Tchobanoglous & Schroeder 1987; Figure 1.2). The bottom layer is anaerobic and results in the anaerobic breakdown of organics, which release soluble organics into the water column (Marais 1970, Tchobanoglous & Schroeder 1987). Organic matter dissolved or suspended in the water column is metabolised by heterotrophic bacteria with the uptake of oxygen (Marais 1970). The dissolved oxygen utilised by the bacteria is replaced by the photosynthetic production of oxygen by algae in the water column (Tchobanoglous & Schroeder 1987). The bacteria in turn produce CO_2 which is utilised by the algae (Marias 1970). This symbiotic relationship results in a decrease of BOD and COD in BE (Marias 1970). Effluent from the PFP has similar qualities to post-AD effluent except for the reduced COD and nutrients that are settled out in their organic form or utilised by the microorganisms in the PFP (Marias 1970, Senzia *et al.*

2002; Table 1.2). The pH of the liquor in the PFP changes diurnally due to the diurnal photosynthetic consumption of CO_2 in the day (increase pH) and the release of CO_2 at night (decrease pH) from the respiration of microorganisms (Marias 1970, Kayombo *et al.* 2002). The effluent from the PFP has a higher pH than the effluent from the AD, which causes plant nutrients to be less available to plants when than AD effluent (Table 1.2; Section 1.2.4 *pH and plant nutrients*).

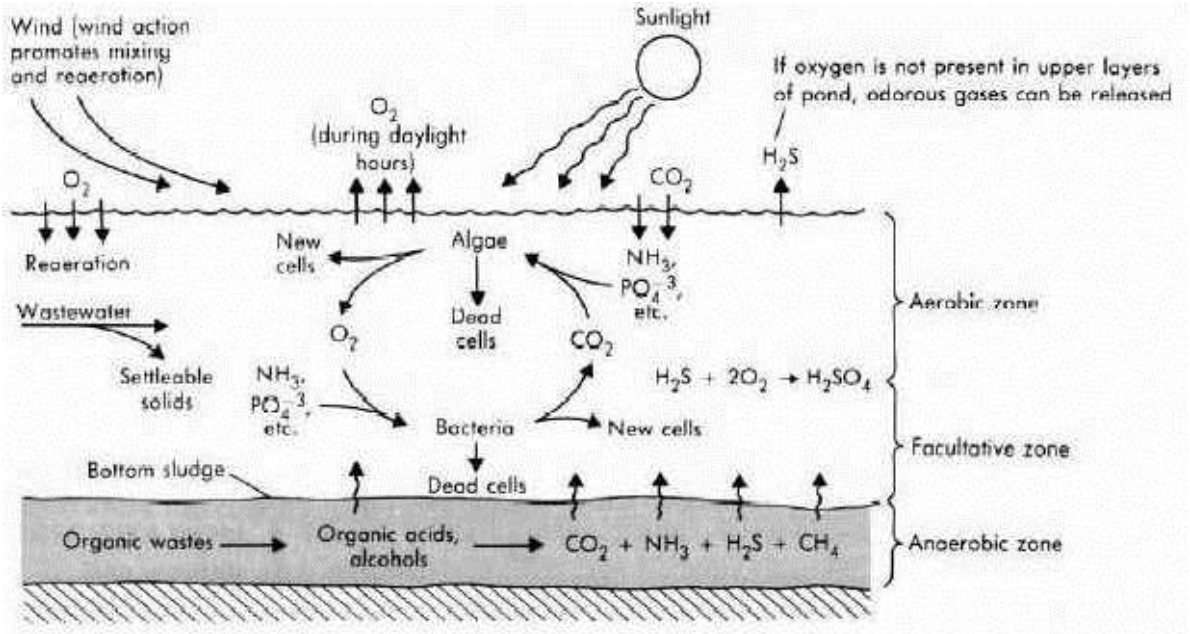

Figure 1.2 Operations of a facultative ponds (Tchobanoglous & Schroeder 1987)**.**

High rate algal pond

After the PFP BE is passed through high rate algal ponds, which efficiently remove much of the dissolved nitrogen and phosphorous (Cilliers 2012; Table 1.2). The nutrient concentration in HRAP effluent is low in ammonia (Table 1.2) and the algae may act as buffer against harmful nutrient concentration effects. However, this may be counterproductive because essential nutrients are lost as they are utilised by the algae. The

pH of effluent leaving HRAP is greater than 8.5 due to the photosynthetic consumption of CO_2 by algae (Cilliers 2012, Wells *et al.* 2003; Table 1.2). This poses a major problem as a pH above 8.5 will make certain nutrients unavailable to plants (Tyson *et al.* 2007). Power (2014) found that the high pH of HRAP effluent significantly reduced tomato plant height and yield. The evaporation of water from the HRAP system causes an increase in concentration of dissolved salts in the effluent (Cilliers 2012; Table 1.2). This can be seen by the increase in EC in HRAP effluent (Table 1.2). This poses a major problem because the high salt levels in HRAP effluent will reduce crop growth and have negative effects of soil structure and composition (Section 1.2.5 *Soil sodicity*).

Constructed wetland

Post-PFP BE can also be sent through a constructed wetland system to remove excess nutrients from the BE and make it more suitable for crop irrigation. A horizontal subsurface flow constructed wetland (HSSFCW) is used at Ibhayi brewery to treat post-PFP effluent (Jones *et al.* 2013; Figure 1.3). Horizontal flow constructed wetlands have successfully been used around the world as secondary and tertiary effluent treatment systems (Vymazal 2009). They have been shown to reduce COD, dissolved nitrogen, dissolved phosphorous and chloride among components (Cheng *et al.* 2002, Kenatu 2011, Vymazal 2009, Jones *et al.* 2013, Shepherd *et al.* 2014; Table 1.3). Horizontal flow constructed wetlands have high removal rates of organics and suspended solids but low removal rates of nutrients (Vymazal 2007; Table 1.3). The main mechanisms for nitrogen removal in HSSFCW are plant uptake, microbial uptake, adsorption and denitrification (Brix & Schierup 1989, Vymazal 2007). Phosphate removal in HSSFCW is mainly achieved by adsorption, plant uptake and microbial

uptake (Stottmeister *et al.* 2003, Vymazal 2007). Due to the low levels of removal of plant nutrients, HSSFCW could reduce the harmful effects of high nutrient concentrations while keeping nutrient concentrations high enough to support good crop growth. Constructed wetlands normally have a neutral pH and change the pH of an effluent towards a neutral pH, provided there is limited algal growth (Vyzmal 2005). This would be beneficial because the HSSFCW would reduce the pH of post-PFP effluent, thus making the minerals more readily available to plants.

Due to evaporation and evapotranspiration the concentration of dissolved salts in HSSFCW liquor will increase (Jones *et al*. 2013, Shepherd *et al.* 2014). This is a concern because an increase in dissolved salts can cause a reduction in crop growth and can have negative effects on the soil structure and composition, such as that which occurs with soil sodicity (Section 1.2.4 *pH and plant nutrients*; 1.2.5 *Soil sodicity*). Knight *et al.* (2000) and Kento (2011) found that HSSFCW planted with *Phragmities karka* reduced total dissolved solids (TDS) levels by 17 and 54% respectively. However, this is not common, and in most studies the salt removed by adsorption and plant assimilation is less than the concentrating effect caused by evaporation and evapotranspiration, resulting in an overall increase in TDS (Coleman *et al.* 2001, Stottmeister *et al.* 2003, Vymazal 2007)

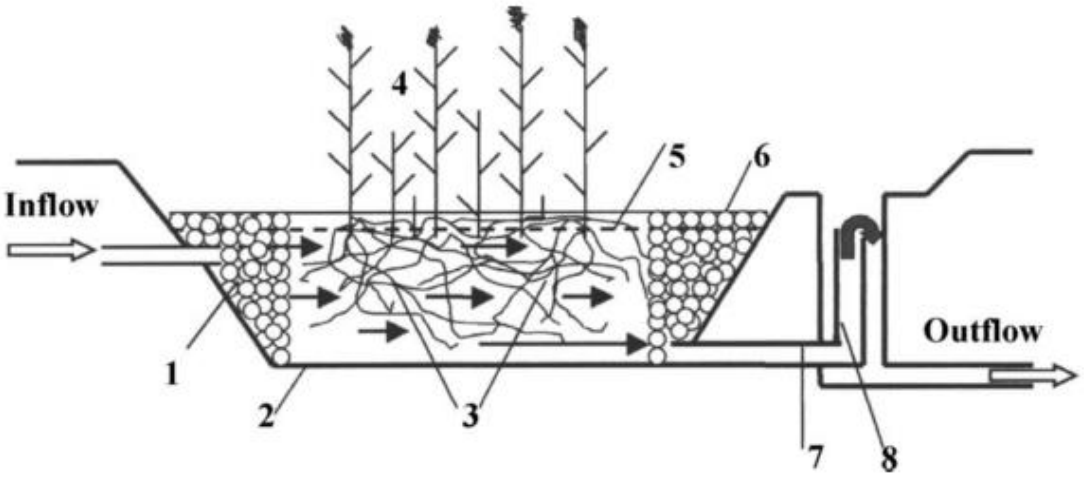

Figure 1.3 Schematic representation of horizontal sub-surface flow constructed wetland used at the research site. 1, distribution zone filled with large stones; 2, impermeable liner; 3, filtration medium (gravel, crushed rock); 4, vegetation; 5, water level in the bed; 6, collection zone filled with large stones; 7, collection drainage pipe; 8, outlet structure for maintaining of water level in the bed. The arrows indicate only a general flow pattern (Vymazal 2005).

Table 1.3 Average performance of horizontal flow constructed wetlands on industrial effluent (Vymazal 2009).

Parameter	Concentration (mg/l)	
	In	Out
BOD	652	254
COD	1856	789
TSS	239	128
TN	138	102
NH_4-N	62	48
TP	9	5

1.2.3 Irrigation standards

Irrigation of any land with water containing waste must have an electrical conductivity that does not exceed 200 $mS.m^{-1}$, a pH that is not less than six more than nine, a COD that does not exceed 400 $mg.l^{-1}$ after removal of algae, faecal coliforms must not exceed 100 000 per 100 ml and a sodium absorption ratio (SAR) must not exceed five for biodegradable industrial wastewater (DWAF 1996).

Raw BE is not suitable for irrigation because it has a COD well over 400 $mg.l^{-1}$ and contains large molecules that cannot be used by plants (Ajmal & Khan 1984, Juwarkar & Dutta 1990). Post-AD BE is suitable for irrigation because it falls within the criteria set by the Department of Water Affairs and Forestry (DWAF) in 1996 for the use of water in agriculture for irrigation purposes. The only concern is the electrical conductivity (EC) of BE because it is near the limit set by DWAF (DWAF 1999). Brewery effluent has an EC between 200 – 300 $mS.m^{-1}$. This means that post-AD BE has slight to moderate restrictions when used in irrigation (DWAF 1996, FAO 2003). The EC of BE should result in only a 10-20% decrease in crop productivity (DWAF 1996).

1.2.4 Plant production

Brewery effluent has successfully been used to grow tomatoes and lettuce in previous studies (Jones *et al.* 2013, Power 2014). Brewery effluent does contain sufficient nutrient to support plant growth. However it is not a balanced nutrient solution and may have certain characteristics that inhibit healthy crop production (Table 1.4). The high pH and salt content of BE (Table 1.4) are major concerns and will be discussed in the following section. The elements required to support good plant growth must be understood as well as deficiency and toxicity symptoms in order to assess the quality of BE as a water and nutrient source for irrigated crop production. Nutrients essential for plant growth at a given concentration can accumulate in the soil and inhibit plant growth or accumulate in the plant tissue and become toxic to organisms that consume the plant (Tyson *et al.* 2007, Lastra *et al.* 2009). It is therefore essential that the nutrients in the irrigation water, soil and plant tissue are monitored.

Table 1.4 Post primary facultative pond (PFP) effluent analysis 2010-02-19 (BemLab), TDS total dissolved solids.

Parameter	Value	Parameter	Value
pH	8.20	Magnesium (mg/l)	10.70
Sulphate (mg/l)	24	Phosphorous (mg/l)	6.95
Conductivity (µs/cm^2)	2880	Iron (mg/l)	0.49
Boron (mg/l)	0.06	Ammonium (mg/l)	24.72
Sodium (mg/l)	476.63	Chloride (mg/l)	285.20
Manganese (mg/l)	0.04	Nitrate (mg/l)	0.46
Potassium (mg/l)	12.30	Carbonate (mg/l)	165.30
Copper (mg/l)	0.01	Fluoride (mg/l)	1.20
Calcium (mg/l)	50.90	Bicarbonate (mg/l)	1066.60
Zinc (mg/l)	0.04	TDS (mg/l)	2150

Plant nutrients

Most vascular plants require 17 essential elements for good growth and reproduction (Marschner 1990, Epstein & Bloom 2005). In order to be defined as an essential element, the element must be needed for the plant to develop and reproduce normally, and it must fulfil a specific structural or metabolic role (Marschner 1990, Epstein & Bloom 2005). The 17 essential elements are carbon, hydrogen, oxygen, nitrogen, phosphorus, sulphur, potassium, calcium, magnesium, iron, manganese, zinc, copper, boron, molybdenum, chlorine and nickel (Marschner 1990, Epstein & Bloom 2005). These essential elements can be split up into two groups; macronutrients and micronutrients. Macronutrients (carbon, hydrogen, oxygen, nitrogen, phosphorus, sulphur, potassium, calcium, magnesium) are required in relatively large quantities for the production of nucleic acids, proteins, carbohydrates and phospholipids among other important molecules (Epstein & Bloom 2005). Micronutrients (iron, manganese, zinc, copper, boron, molybdenum, chlorine and nickel) are required in much smaller quantities and typically function as cofactors for specific enzymes (Epstein & Bloom 2005). Below is a table summarising the form each nutrient is available to plants, their function, the concentration they are found in plant tissue and symptoms of deficiency and toxicity.

Table 1.5 Essential elements for plant growth, their concentration in dry tissue, function, toxicity and deficiency symptoms.

Element	Concentration in dry tissue	Function	Deficiency symptoms	Toxicity symptoms
Carbon (CO_2)	45%	Substrate for photosynthesis, major component of organic molecules (Freeman 2005)	Cell death (starvation) (Freeman 2005)	N/A
Oxygen (O_2, H_2O_2)	45%	Electron acceptor in cellular respiration, major component of organic molecules (Freeman 2005)	Cell death (suffocation) (Freeman 2005)	N/A
Hydrogen (H_2O)	6%	Major component of organic compounds; electrical balance and establishment of electrochemical gradients (Freeman 2005). Water is required to maintain turgor pressure in plants (Roberto 2005)	Cell death (desiccation) (Freeman 2005)	N/A
Nitrogen (NH_4^+, NO_3)	1.5%	Component of amino acids, nucleic acids, nucleotides, proteins, hormones, coenzymes and several other metabolic entities (Epstein & Bloom 2005).	Growth is retarded and slow, leaves with signs of chlorosis or yellowing. Fruit is oftern "exceptionally well coloured". Older parts of plants show signs of stress first as nitrogen is translocated to younger, active growing sites (Epstein & Bloom 2005)	Overly vigorous growth and delayed fruit ripening (Epstein & Bloom 2005). Plants may also become more susceptible to pests (Roberto 2005).
Potassium (K^+)	1%	Control of many enzymes, cell osmotic adjustment, required for synthesis of organic molecules (Epstein & Bloom 2005, Freeman 2005)	Growth is significantly reduced. Leaves are dark green or blue green with marginal necrosis. Small spots of dead tissue develop on the leaves (Epstein & Bloom 2005)	"Excessive potassium may result in a secondary magnesium deficiency" (Roberto 2005)

Not applicable (N/A)

Table 1.5 (Continued) Essential elements for plant growth, their concentration in dry tissue, function, toxicity and deficiency symptoms.

Element	Concentration in dry tissue	Function	Deficiency symptoms	Toxicity symptoms
Copper (Cu^{+}, Cu^{2+})	0.0006%	Cofactor for enzymes involved in photosynthesis and respiration, component of lignin (Epstein & Bloom 2005, Freeman 2005)	Symptoms vary greatly depending on the species. Leaves may be chlorotic or dark green with rolled up margins. Young shoots die back and new shoots emerge from multiple buds (Epstein & Bloom 2005)	Copper toxicity often causes iron deficiency (Roberto 2005). Chlorosis may appear on leaves (Marschner 1990) Excessive copper levels affect the root zone first and can lead to malformation of the root system. If copper toxicity is suspected, both the shoots and the roots of the plant should be analysed (Marschner 1990).
Nickel (Ni^{2+})	0.000005%	A component of urease, an enzyme needed for nitrogen metabolism (Epstein & Bloom 2005)	Deficiency is rare and symptoms include small supped leaves and marginal chlorosis (Epstein & Bloom 2005)	Nickel toxicity may lead to zinc or iron deficiency and cause leaf chlorosis (Marschner 1990)
Magnesium (Mg^{2+})	0.2%	Chlorophyll component, component of plant proteins and activates many enzymes (Freeman 2005).	A plant experiencing a shortage of available magnesium it will transport magnesium form mature to young actively growing regions (Epstein & Bloom 2005). Leaves will present signs of chlorosis. Leaves will curl up or prematurely drop off the plant (Freeman 2005).	Magnesium toxicity is rare (Roberto 2005).
Phosphorus ($H_2PO_4^{-}$, HPO_4^{2-})	0.2%	Key role in all metabolites dealing with energy acquisition (ATP), component of nucleic acids, phospholipids, and several coenzymes (Epstein & Bloom 2005).	Plant growth will be stunted and leaves will discolour, appearing dark green with red, purple and brown spots along the veins (Epstein & Bloom 2005). Fruit production and root growth can also be affected (Freeman 2005).	Excessive phosphorus reduce the availability of copper and zinc resulting in copper and zinc deficiencies (Roberto 2005).
Sulphur (SO_4^{2-})	0.1%	Component of enzymes, proteins and coenzymes (Epstein & Bloom 2005).	Sulphur deficiencies are similar to nitrogen deficiencies except that the chlorosis caused by sulphur deficiency will present in the younger leaves. Plants may also appear stunted or spindly (Epstein & Bloom 2005).	Excessive sulphur slows growth and leaves are smaller (Roberto 2005).

Table 1.5 (Continued) Essential elements for plant growth, their concentration in dry tissue, function, toxicity and deficiency symptoms.

Element	Concentration in dry tissue	Function	Deficiency symptoms	Toxicity symptoms
Chlorine (Cl^-)	0.01%	Activates enzyme system of photosystem II in which water is split and oxygen released (Epstein & Bloom 2005)	Leaf tips can wilt, chlorosis or necrosis of leaves, or leaves will develop a bronze appearance characteristic of chlorine deficiency (Epstein & Bloom 2005).	Excessive chloride can cause calcium deficiencies (Freeman 2005).
Iron (Fe^{3+}, Fe^{2+})	0.01%	Required for chlorophyll synthesis, component of heme proteins, ferredoxin and iron-sulfur proteins. Iron also serves as an enzyme cofactor (Epstein & Bloom 2005, Freeman 2005).	Chlorosis between the veins of young leaves. In most species the veins become chlorotic (Epstein & Bloom 2005). Deficiency may also lead to blossom drop (Roberto 2005).	Iron toxicity is difficult to spot and rare (Roberto 2005).
Manganese (Mn^{2+})	0.005%	An enzyme activator, component of water-splitting enzyme in photosystem II and enzyme superoxide (Epstein & Bloom 2005)	The symptoms vary greatly between species. Leaves present with chlorosis between the veins and occasionally necrotic spots (Epstein & Bloom 2004). Mn deficiency can also cause failed blooms (Roberto 2005)	Excessive manganese can reduce iron availability and lead to iron deficiency (Roberto 2005)
Zinc (Zn^{2+})	0.002%	Component in enzymes or enzyme activation, function, structure or regulation. (Epstein & Bloom 2005, Freeman 2005)	Abnormal plant growth with failure of leaves to expand and internodes to elongate causes leaves at nodes to be "closely telescoped" give rise to the symptom called rosette. Leaves appear small, distorted and/or chlorotic in some species (Epstein & Bloom 2005)	Inhibition of root elongation, chlorosis in young leaves and restrict iron availability (Marschner 1990, Roberto 2005).

Table 1.5 (Continued) Essential elements for plant growth, their concentration in dry tissue, function, toxicity and deficiency symptoms.

Element	Concentration in dry tissue	Function	Deficiency symptoms	Toxicity symptoms
Calcium (Ca^{2+})	0.5%	Regulatory functions of many enzymes and cells, role in cell wall structure; essential part of membranes, controls movements of ions through membranes; plays major role in transduction of signals (Epstein & Bloom 2005).	`Symptoms appear earliest and most severely in meristematic regions and young leaves. Growing points are damaged or die. In flowers and fruit this is known as blossom end rot. The growth of roots is severely affected and damaged roots become prone to infection by bacteria and fungi (Epstein & Bloom 2005)	Calcium toxicity is extremely rare and hard to identify (Roberto 2005).
Boron (H_2BO^{3-})	0.02%	Binds to polysaccharides of the cell wall contributing to its stability it is also required for xylem differentiation. May play a role in metabolism of ribonucleic acid (Epstein & Bloom 2005)	Boron deficiency can restrict the lateral and longitudinal growth of roots causing them to appear "stubby and bushy" (Marschner 1990). Plant tissues appear hard, dry and brittle with leaves becoming distorted and stems dry and cracked. Growing tips may die and leaves may develop black necrotic spots. Flowering is also severely affected and fruit may show similar dry, corky or cracking symptoms (Epstein & Bloom 2005, Freeman 2005).	Boron toxicity may causes necrosis and chlorosis on leaf margins. (Marschner 1990) Leaf tips yellow and die off. Roberto 2005).
Molybdenum (MoO_4^{2-})	0.00001%	Molybdenum plays a significant role in nitrogen acquisition and utilisation. Component of enzymes nitrate reductase and nitrogenase (Epstein & Bloom 2005).	"Leaf margins tend curl or roll. Leaf blades become necrotic and disintegrate, leaving a much reduced strip along the midrib (Whiptail)". Chlorosis will also occur in older leaves. Thus the simultaneous appearance of "whiptail" and chlorosis which is a strong indicator of molybdenum deficiency (Epstein & Bloom 2005).	Molybdenum toxicity can cause leaves to turn yellow (Roberto 2005). However molybdenum toxicity is noted to affect animals sooner and more severely than plants (Marschner 1990).

Nitrogen

Nitrogen is an essential nutrient for plant growth and is mainly assimilated by plants in the form of ammonia or nitrate (Freeman 2005). However unfavourably high levels of nitrogen can lead to vigorous vegetative growth, delayed fruit ripening and many plants become more susceptible to pests (Roberto 2005). Most plant species grow best if they have access to both ammonia and nitrate nitrogen (Errebhi & Wilcox 1990). The form of available nitrogen has shown to affect the growth of plants, with some plants growing faster when fertilised with ammonia while others grow better when fertilised with nitrate (Borgognone *et al.* 2012, Claussen & Lenz 1999). Plants that prefer nitrogen in the form of ammonia will grow better in post-AD BE while plants that prefer nitrogen in the form of nitrate will grow better in HRAP or CW BE.

Ammonia versus nitrate

Ammonia is an important source of inorganic nitrogen for plants (Raven *et al.* 1992, Lastra *et al.* 2009). Plants have lower energy requirements for the assimilation of ammonia nitrogen than nitrate nitrogen (Barker & Mills 1980, Raven *et al.* 1992). Claussen & Lenz (1999) compared the growth and photosynthetic rate of blueberry, raspberry and strawberry fertilised with ammonia or nitrate, strawberries had a greater dry mass and photosynthetic rate when grown fertilised with nitrate. Blueberries plants had a significantly higher dry mass when fertilised with ammonia (Claussen & Lenz 1999). Lastra *et al.* (2009) found that two varieties of varieties of lettuce grew best when fed with nitrate alone while two other varieties grew best when fertilised with ammonia and nitrate. These studies concluded that form of nitrogen is dependent on species as well as soil pH, and buffer capacity of the growth medium. Vessey *et al.* (1990) found that nitrate root absorption is

preferred in acidic condition while ammonia absorption is preferred in alkaline conditions. This is probably due to the availability of H^+ ions in acidic conditions which is needed for co-transport of nitrate into the root (Stewart *et al.* 1987, Vessey *et al.* 1990). Ammonia toxicity is reduced in neutral conditions and enhanced in acidic conditions (Claussen & Lenz 1999). Therefore the most suitable treatment process used to make BE a fertiliser for crop production will be crop specific and will also depend of the characteristics of the soil.

Excessive levels of ammonia can lead to impaired uptake of metal ions, acidification of the rhizosphere, alterations in the osmotic balance, or modified phytohormone metabolic activity (Gerendás *et al.* 1997, Claussen & Lenz 1999).

Foliar nitrate has been shown to contribute significantly to human NO_3^- intake and is believed to contribute to blue baby' syndrome and cancers (Wang *et al.* 2008, Lastra *et al.* 2009). The European Union limit for foliar nitrate concentration is 3,500 to 4,500 mg N-NO3/kg fresh weight for the winter season and 2,500 mg of N-NO3/kg for the summer crops (EUROPA 2009). The foliar nitrate concentration of plants grown in this trial will be monitored to ensure it is safe for human consumption. Lettuce grown in previous trials on BE was suitable for human consumption according to European legislated limits on foliar nitrate concentration (Power 2014)

pH and plant nutrients

The pH of the soil and irrigation water affects the availability of nutrients to plants and thus the growth and yield of plants (Figure 1.4) (Lucas & Davis 1961, Tyson *et al.* 2007). The precipitation of Fe^{2+}, Mn^{2+}, phosphate, Ca^{2+} and Mn^{2+} to insoluble and unavailable salts can happen when the pH of a nutrient solution rises above 7.5 (Tyson *et al.* 2007). Power (2014) grew tomatoes on BE with and without pH adjustment. He found that adjusting the pH to

6.5 increased the height and yield of tomato plants by 100%. This is important because the pH of BE, after any treatment is above 7.5. Therefore the pH of BE will reduce the availability of nutrients to plants and should be adjusted in order to make all the nutrients available to plants. Lucas and Davies (1961) and Epstein and Bloom (2005) both suggest that the optimal pH range for most plants is between five and seven (Figure 1.4). The pH of the irrigation water will therefore be manipulated to six to ensure that all the nutrients in BE are made available to plants.

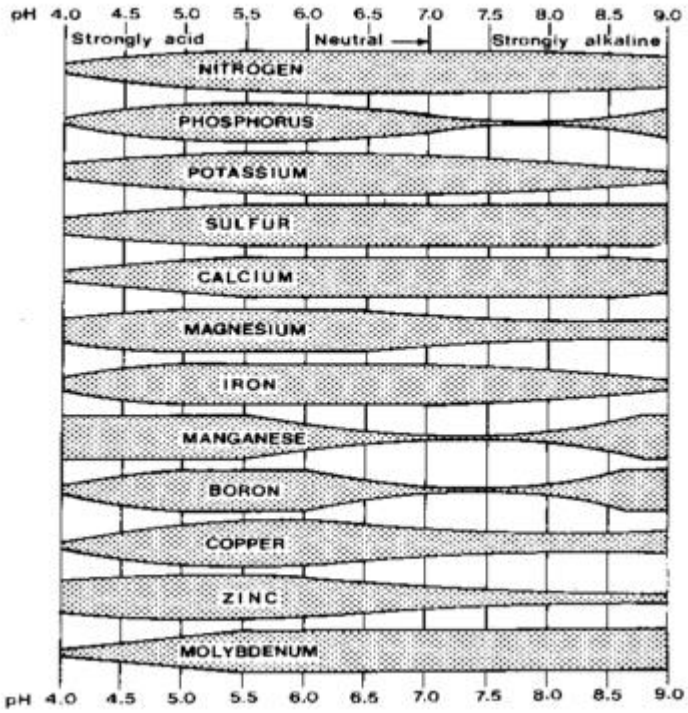

Figure 1.4 The availability of different essential elements as influenced by pH (Lucas & Davis 1961).

Salts

The high sodium content of BE poses a major problem when it is used a source of water or nutrients for crop production (Sweeney & Graetz 1991, Dakoure *et al.* 2013, Power 2014).

Salinity is a measurement of the amount of dissolved ions present in a solutions is measured as TDS in mg/l or in units of electrical conductivity. Brewery effluent has a salinity of 2700 $\mu s/cm^2$, and a TDS at this level may have negative effects of crop growth and yield. Power (2014) concluded that in order to successfully utilise BE as a source of nutrients for plants growth the salinity issue of BE needs to be addressed. The general effect of salinity is a reduced growth rate and yield of most crops (Shannon & Grieve 1999). At low to medium concentrations this is primarily due to the osmotic effects because of the reduced osmotic potential between the root plasma and soil water (Munns & Termaat 1986, Jacoby 1994). This means that plants have to spend more energy to take up water from the soil, which increases respiration and has negative effects on growth (Munns & Termaat 1986, Jacoby 1994). The severity of salinity response is species specific and is also mediated to environmental factors such as humidity, temperature, wind, light and air pollution (Shannon *et al.* 1994). High temperatures and low humidity increase the effects of salinity (Munns & Termaat 1986, Shannon & Grieve 1999). Salinity may cause ion toxicities and nutrition deficiencies, depending of the composition of the saline solution (Epstein & Bloom 2005). Salinity induced nutrient deficiency symptoms are similar to those that occur in the absence of salinity (Shannon & Grieve 1999). Chow *et al.* (1990) found that that the K^+ requirements for shoot growth are higher under high salinities than low salinities. High concentration of Na and Cl may accumulate in the leaves and cause "scorching and firing" of the leaves (Shannon & Grieve 1999). Calcium deficiencies are common when there is a high Na content in the soil water (Jacoby 1994). Not all salinity effects are bad, and spinach yields have shown to increase at a low to moderate salinity (Osawa 1963). Cabbage heads are more compact at low salinities but are less compact as salinity increases (Osawa 1961).

1.2.5 Soil

The soil is a complex system where chemical, physical and biological characteristics, climate, plant communities and agricultural practices interact to determine the fertility of the soil (Pankhurst *et al.* 1997, Abbot & Murphy 2007). This level of complexity poses constraints on our ability to predict the effect that agricultural practices will have on soil fertility (Pankhurst *et al.* 1997). Soil fertility is defined as the capacity of a soil to provide physical, chemical and biological requirements to support plant growth and reproduction (Abbot & Murphy 2007).

Soil biological fertility is the "capacity of organisms in the soil to contribute to the nutritional requirements of plants for productivity, reproduction and quality while maintaining biological processes that contribute positively to the physical and chemical state of the soil" (Abbot & Murphy 2007). Soil chemical fertility is the "capacity of soil to provide a suitable chemical and nutritional environment for plants for productivity, reproduction and quality in a way that supports beneficial soil physical and biological processes" (Abbot & Murphy 2007).

Nutrients

To ensure sustainable irrigation of nutrient rich water the application rates must be consistent with the removal rates of the nutrients by plants and soil microbes (Lazarova & Bahri 2005). If the application rates of nutrients exceed removal rates then the accumulation of nutrients, and leaching of nutrients into the groun-water can occur, resulting in environmental and human health concerns (Lazarova & Bahri 2005). Nutrient additions may affect soil microbe communities both positively and negatively, as well as

carbon and nitrogen cycling and enzyme activities (Sinsabaugh *et al.* 2004, Sinsabaugh 2010). The fact that studies have shown both detrimental and enhancing effects of nutrient additions to soils illustrates the complexity of relationships among soil microbial communities in agricultural soils (Sinsabaugh *et al.* 2004, Sinsabaugh 2010).

Nitrogen

Nitrogen is an essential element required in relatively large quantities for plant growth (Section 1.2.4 *Nitrogen*) it is also utilised by the microbes in the soil (Black 1968, Abbot & Murphy 2007). The amount of nitrogen utilised by microbes is related to the amount of carbon present in the soil (Black 1968, Abbot & Murphy 2007). This is because the carbon provides microbes with an energy supply, which is not supplied by nitrogen (Black 1968, Abbot & Murphy 2007). Black (1968) found that microbial biomass increased while mineral nitrogen decreased with the addition of sugars in fallow soil. The microbial biomass did not increase, whereas and mineral nitrogen levels increased when no sugars were present in fallow soil (Black 1968).

Iritani and Arnold (1960) investigated the effect of carbon to nitrogen ratios on the soil nitrogen removal rates of nitrogen by tomatoes. They found that that ratio of carbon to nitrogen influences the microbe mineralisation and immobilization of nitrogen. Carbon to nitrogen ratios below 22 resulted in net nitrogen mineralisation and increased soil nitrogen removal by tomato plants. A carbon to nitrogen ratio above 22 resulted a net nitrogen immobilisation and an accumulation of soil nitrogen.

Brewery effluent contains both carbon and nitrogen, therefore providing an energy source and food source to soil microbes. This could increase the microbe populations associated

with nitrogen and carbon mineralisation, and could lead to the greater mineralisation of nitrogen and carbon in the soil.

Carbon

Carbon is source of energy for soil microbes (Fontainea *et al.* 2003, Abbot & Murphy 2007). It is generally accepted that low levels of soil carbon limit the amount of energy available to microorganisms and in turn this limits enzyme production and growth of microorganisms (Fontainea *et al.* 2003) Carbon levels in the soil are strongly correlated to microbial populations and communities (Zahran 1992, Lieth & Al-Masoom 1993, Rietz & Haynes 2003). Brewery effluent contains carbon and is measured as COD. Irrigation with BE will add carbon to the soil that can be expected to increase microbial populations, especially the bacteria involved in carbon decomposition. Kannan & Oblisami (1990), Saqqar *et al.* (1997), Hati *et al.* (2007), and Senthilraja *et al. (*2013) found that the addition of treated effluent increased soil microbial populations and that this could have been due to the increase in soil carbon. However, Saqqar *et al.* (1997) found that the increase in microbial populations could have been due to the addition of microbes present in the wastewater. Juwarkar & Dutta (1990) and Kaushik *et al.* (2005) observed a 50% reduction in soil microbial populations treated with raw distillery effluent. This was attributed to the addition of high BOD because during degradation, “some organic acids are formed that may influence different microbes thereby reducing microbial populations”. The addition of carbon from effluents generally increases microbial populations but in some cases it has shown to decrease microbial populations (Saqqar *et al.* 1997, Hati *et al.* 2007, Kaushik *et al.* 2005)

Salts

Anaerobically digested BE has a high salt content (Table 1.2, 1.4) due to the addition of sodium hydroxide in the AD digestion, formation of carbonate during AD and the addition of Cl^- during CIP (Section 1.2.2 *Anaerobic digestion*). The concentration of most salts in effluent will increase in all treatment systems after AD due to evaporation and evapotranspiration. This is a major concern because salts can accumulate in the soil leading to soil salinization, sodification and alkalinisation which result in decreased soil fertility and crop production (Black 1968, Grattan & Grieve 1999, Qadir & Schubert 2002, Muyen *et al.* 2011).

Salinity

Salinity is the amount of salts in a water body or soil and is measured by TDS or EC (Qadir & Schubert 2002). Most wastewaters contain relatively high concentration of salts (TDS > 1500 mg/l) and when used for irrigation, they have the potential to add large amounts of salts to the soil (Muyen *et al.* 2011). Most of the literature on the use of wastewater as a source of irrigation have found that it increases the salinity of the soil Aljaloud *et al.* 1993, Saqqar *et al.* 1997, Bond 1998, Kaushik *et al.* 2005, Hati *et al.* 2007, Jalali *et al.* 2008, Kumar *et al.* 2010, Muyen *et al.* 2011, Dakoure *et al.* 2013). The salinity of wastewater is the major limitation for its sustainable use for irrigation purposes (Aljaloud *et al.* 1993, Muyen *et al.* 2011). This is because the build-up of salts in soils is a major cause of decreased yields in agriculture worldwide (Qadir & Schubert 2002, Muyen *et al.* 2011). Soil salinity has been shown to decrease crop yield and health due to the increase in energy required for water and nutrient uptake from saline soils and by decreasing the availability of certain plant nutrients (Section 1.2.4 *Salts*) (Black 1968, Grattan & Grieve 1999, Qadir & Schubert 2002,

Muyen *et al.* 2011). Salinity has also shown to affect the physical structure and biological activity of the soil (Garcia *et al.* 1994, Pathak & Rao 1998, Rietz & Haynes 2003).

Soil water salinity improves the aggregation and stabilization of soils by causing fine particles to bind together (Buckman & Brady 1967, Abu Sharar *et al*. 1987). This is known as flocculation and initially is beneficial in terms of soil aeration, root penetration, and root growth (Buckman & Brady 1967, Abu Sharar *et al*. 1987). Increasing soil water salinity can have positive effects of soil aggregation and stability but it also increases the osmotic stress on plants. Therefore soil salinity cannot be increased to maintain soil structure without considering potential impacts on plant health (Abu Sharar *et al*. 1987, Garcia *et al.* 1994, Pathak & Rao 1998).

Rietz & Haynes (2003) studied the changes in microbial communities at different soil salinities in sugarcane fields. They found a significant negative relationship between microbial carbon and EC, demonstrating the negative effects that salinity has on soil microbial communities. Similar results were found by Lieth & Al-Masoom (1993) and Garcia *et al.* (1994) where microbial carbon was positively related to organic carbon. They concluded that the microbial community was more stressed under increasingly saline conditions due to the increase in metabolic quotient. The increasing salinity, increased osmotic stress limits, decreased microbial growth and activity and microorganisms tend to dehydrate the soil microbes (Galinski 1995, Oren 1999). However there was still "substantial microbial activity" in the saline soils where sugarcane was not growing. Other authors have found that microbial numbers did not decrease with increasing salinity and Pankhurst *et al.* (2001) found that agriculture induced salinity caused a shift towards a "less active, less diverse, bacteria-dominated community" (Nelson *et al.* 1996). The shift in microbial

community can be detected using biology ecoplates which were used in this study to quantify the community of microbes present in the soil (Garland & Millis 1991, Gomez *et al.* 2004).

Sodicity

Soils can be classified into four groups according to their salt and sodium content (Black 1968, Qadir & Schubert 2002). Soils that have an EC above 4 $dS.m^{-1}$ are classified as saline. Soils that have an exchangeable sodium percentage (ESP) greater than 15% or a SAR > 13 are classified as sodic (Sumner *et al.* 1995, Qadir & Schubert 2002). Exchangeable sodium percentage is the percentage of exchangeable sodium ions relative to the cation exchange capacity of a soil (Sumner *et al.* 1995, Qadir & Schubert 2002). The sodium absorption ratio is a measure of the relative concentration of sodium to calcium and magnesium ions (Pescod 1992). Irrigation waters with a high sodium content can cause a severe collapse in soil structure due to the accumulation of sodium ions in the soil (Qadir & Schubert 2002). This is known as sodicity, which causes major decreases in soil fertility, accompanied by a decrease in crop yields and can result in lands becoming unsuitable for agriculture (Black 1968, Qadir & Schubert 2002).

In soils sodium ions disrupt the forces that bind clay particles together and high concentrations of sodium ions in the soil cause clay particles to expand (Agassi *et al.* 1981, Qadir & Schubert 2002). The expansion of clay particles result in soil expansion and swelling, resulting in decreased soil permeability and porosity due to the clogging of soil pores by clay particles (Agassi *et al.* 1981, Qadir & Schubert 2002). Therefore sodium induced soil dispersion results in reduced infiltration, reduced hydraulic conductivity, and increased surface crusting (Agassi *et al.* 1981, Qadir & Schubert 2002).

Salts that keep soil flocculated such as Ca^{2+} and Mg^{2+} can reduce the effects of soil dispersion by sodium because they compete for the same spaces that sodium binds to on clay particles (Agassi *et al.* 1981, Abu Sharar *et al.* 1987). Therefore irrigation water with a high salinity (provided by calcium and magnesium ions) can counter act the effects of soil dispersion by sodium ions (Agassi *et al.* 1981, Abu Sharar *et al.* 1987). The ratio of salinity to ESP/SAR of irrigation waters determines the effects of salts and sodium on soils, and whether flocculation or soil dispersion will occur (Agassi *et al.* 1981, Abu Sharar *et al.* 1987). For example, irrigation water with a high ESP/SAR and low salinity will cause soil dispersion (Agassi *et al.* 1981, Abu Sharar *et al.* 1987). However, waters with a high ESP and salinity will cause slight to moderate soil dispersion (Agassi *et al.* 1981, Abu Sharar *et al.* 1987). This is represented in Figure 1.5. However this does not mean that effluent with a high salinity and ESP/SAR can be sustainably used as an irrigation source because the high salinity and ESP/SAR will cause a long-term increase in soil salinity, which will place stress on plants and will lead to decreased crop yields (Jalali *et al.* 2008, Muyen *et al.* 2011).

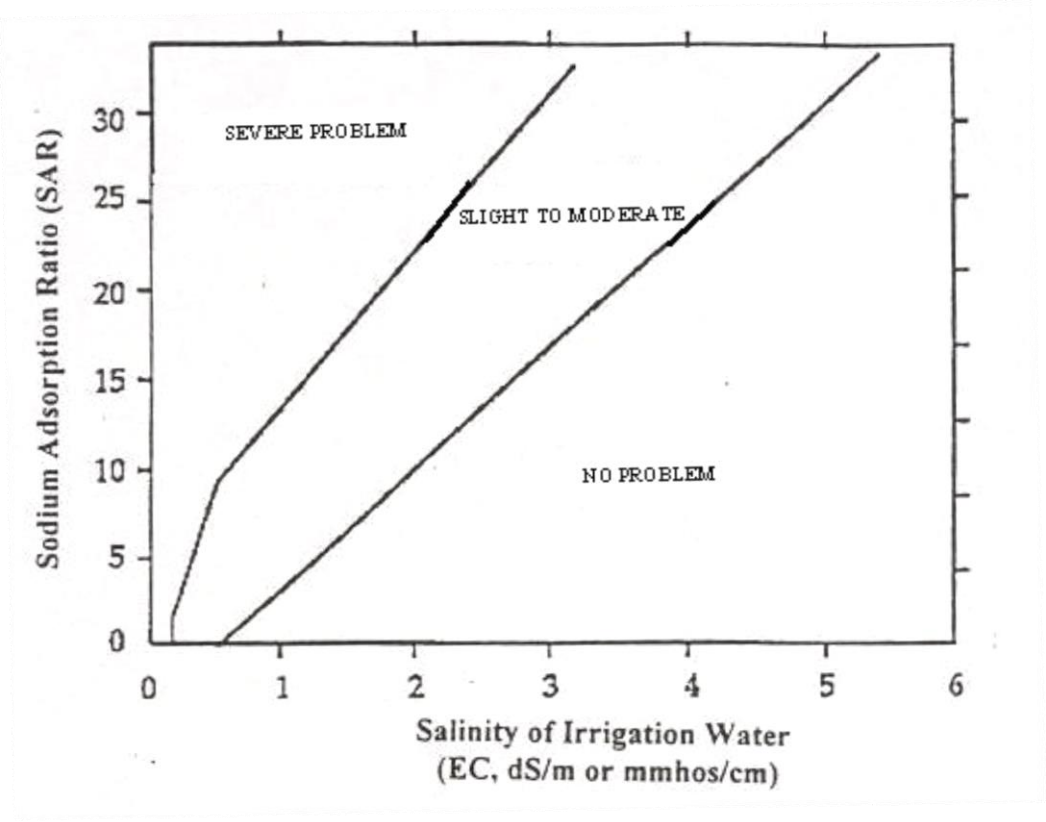

Figure 1.5 The relationship between electrical conductivity (EC), sodium absorption ratio (SAR) and infiltration rates (Pearson 2003).

Soil dispersion hardens soils and blocks infiltration, which results in reduced plant available water and increased runoff and soil erosion (Agassi *et al.* 1981, Abu Sharar *et al.* 1987, Muyen *et al.* 2011). The hardening of soils and decreased pore volume of sodium induced soil dispersion results in the reduction in soil hydraulic conductivity (Agassi *et al.* 1981, Abu Sharar *et al.* 1987, Muyen *et al.* 2011). This means that water cannot infiltrate through the surface layer easily, causing the upper layer of soil to become water logged. This results in the soil becoming anaerobic which reduces root growth, decreases organic matter decomposition and all other aerobic microbe processes (Pearson 2003).

In a number of studies soils irrigated with distillery or paper mill effluent developed soils with high levels of sodium and a 2 - 15 fold increase in soil EC (Ajmal & Khan 1984, Kaushik

et al. 2005, Kumar *et al.* 2010, Kumar & Chopra 2012, Dakoure *et al.* 2013). Dakoure *et al.* (2013) irrigated eggplants grown on ferralsol soil with BE that had been treated using stabilisation ponds. After two seasons of irrigation (2006 - 2008) they found that the effluent caused a strong degradation of hydro-structural soil properties. Soil irrigated with effluent had a decreased soil structural porosity, an increased bulk density and pH when compared to soils irrigated with tap water. This was also accompanied by a 50% reduction in eggplant yield. The soil irrigated with effluent was classifies as sodic due to the accumulation of sodium and bicarbonates in the soil. This emphasises the harmful effects of using effluent with a high alkalinity and sodium content as an irrigation source. Brewery effluent has a high sodium content (476. 63 mg/l) and can cause sodification of soil. Soil physical parameters such as infiltration rate, hydraulic conductivity, pore volume and aggregate stability were therefore recorded to quantify the effects of the salinity of brewery on soil structure.

Alkalinity and pH

The treated BE has a pH greater than eight and when applied to soils could cause the soil pH to increase. Soils are said to be alkaline when they have a pH greater than 8.4 (Dakoure *et al.* 2013). The evaporation and evapotranspiration of irrigation water containing a positive calcite residual alkalinity, such as BE, causes soil alkalinisation (Condom *et al.* 1999). This results in the accumulation of carbonates in the soil surface which increases the soil pH while calcium ions concentration decreases down to undetectable concentrations, thus sharply increasing the SAR of the soil solution (Condom *et al.* 1999). Plant nutrients become less available to plants at high pH values (Lucas & Davis 1961, Condom *et al.* 1999). Alkaline soils also cause organic matter dissolution which accumulates on the surface of the soil and

can be identified as a black layer on the soil surface known as black alkali (Condom *et al.* 1999, Al Droubi *et al.* 1980, Dakoure *et al.* 2013). Dakoure *et al.* (2013) reported alkalinisation of soil after two years of irrigation with treated BE and observed black alkali forming on the soil surface. Soil alkalization normally happens in conjunction with soil sodicity and similar effects such as clay dispersion, deterioration of soil structure and decreased microbe activity, can be expected with soil alkalization (Condom *et al.* 1999, Dakoure *et al.* 2013). However Sahrawat (1982) recorded higher nitrification rates in slightly alkaline soils than soils with a neutral pH accompanied with increased nitrate leaching and denitrification. The effect of soil alkalization on microbes is complex and species specific (Nelson *et al.* 1996). One can expect BE to cause changes in the microbial community to a community that is adapted to alkaline and high pH environments due to the high pH and alkalinity of the BE.

1.2.6 Conclusion

Based on the current water crises faced by South Africa, Africa and the globe, it is vital that technologies are developed that enable the reuse of industrial wastewater. The brewing industry is a major consumer of water and producer of nutrient rich wastewater, which poses an environmental threat when discharged. Brewery effluent contains potential plant nutrients and has the potential to be exploited as a water and nutrient resource in agriculture. However, this is offset by the high alkalinity and sodicity of the water. The effects that BE have on crop production rates, plant health and soil structure are not clear, as well as what pre-treatment methods would make the effluent most suitable for crop irrigation.

Characteristics such as pH, the form and concentration of nitrogen, the concentration of phosphorus and EC differ in BE treated in the various components of the Ibhayi Brewery experimental treatment faculty (Cilliers 2012, Jones *et al.* 2013). These parameters have been shown to directly and indirectly affect plant growth and microbial communities in the plant growth medium (Lucas & Davis 1961, Lieth & Al-Masoom 1993, Garcia *et al.* 1994). It is therefore essential that the most suitable pre-treatment method of BE is found so that the nutrients in the effluent are made accessible to the plants and to reduce their accumulation in the soil.

1.3 Aim and objectives

The aim of this study was to assess the suitability of BE as a water and nutrient source in irrigated crop production, the treatment technologies required to make BE suitable for crop production systems and the effects of BE on the health of plants and the fertility of treated soil. Experiments were also carried out which investigated the use of various crop production systems that could be used to minimise the negative effects that the sodium content of BE has on the soil. This was achieved by addressing the following objectives:

- determine the most suitable BE pre-treatment processes required to make it suitable for crop irrigation (Chapter 2);
- compare the soil chemical, physical and biological fertility when irrigated with BE subject to different pre-treatment processes (Chapter 2);
- determine the effect of pH manipulation of BE on the availability of nutrients to crops grown in soil (Chapter 2 & 3);

- identify a suitable crop species to be used in crop production systems using BE for irrigation water (Chapter 3);
- compare the build-up of sodium in the soil when various crops were irrigated with treated BE (Chapter 3);
- compare crop growth and health and the availability of nutrients to the crops when subject to BE in a hydroponic production system (Chapter 4); and
- determine the ability of different crop species to assimilate sodium from BE (Chapter 4).

Chapter 2: The effect of different effluent treatment technologies on cabbage production and physiochemical properties of soil

2.1 Introduction

Breweries are a major consumer of resource water and producers of nutrient rich wastewater. In 2010 global beer production was 1.8 billion hectolitres accompanied by a wastewater production of around 7.2 billion hectolitres (Ascher 2012). The wastewater is a major liability for brewing companies because it poses an environmental threat, so it has to be treated before it can be returned to the environment. Agriculture is a major consumer of potable water in all countries (Muyen *et al.* 2011). Effluents that contain nutrients needs to be exploited to reduce their polluting effects on the environment (Muyen *et al.* 2011, Simate *et. al* 2011). If nutrient rich wastewater could be used for agriculture, then this would free up potable water for other uses such as ecosystem health and human consumption. Theoretically, on a global scale, if BE was used for irrigation purposes in agriculture, it would free up 7.2 billion hectolitres for other uses.

Brewery effluent is an organic effluent that contains the plant nutrients nitrogen and phosphorous, and a range of organic and inorganic compounds (Senthilraja *et al.* 2013, Power 2014). Nitrogen and phosphorous are essential for good plant growth and health (Epstein & Bloom 2005). Farmers normally have to buy inorganic nitrogen and phosphorus fertilisers. Brewery effluent has the potential be used as a source of water and nutrients in irrigated crop production (Muyen *et al.* 2011, Senthilraja *et al.* 2013, Power 2014). However, BE also has properties that may inhibit the growth of plants or even diminish the fertility of soils when used to irrigate crops (Kaushik *et al.* 2005, Senthilraja *et al.* 2013, Power 2014). Various authors have found that the irrigation of soils with brewery wastewaters have led to

a deterioration of the physical profile of the soils and diminishing soil fertility (Ajmal & Khan 1984, Kaushik *et al.* 2005, Kumar *et al.* 2010, Kumar & Chopra 2012, Dakoure *et al.* 2013). The effect that BE has on crop production rates, plant health and soil structure is not clear, as well as what pre-treatment methods would make the effluent most suitable for crop irrigation.

Brewery effluent at Ibahyi Brewery (SAB Ltd, Port Elizabeth) is treated in an anaerobic digester (AD) and activated sludge system (AS) before being either piped to a municipal sewer or it is channelled back to the factory for re-use (Naiker, pers. comm., Senior Engineer, Ibhayi Brewery, SAB Ltd., July 2015). A small stream of post-AD BE is fed into an experimental treatment facility, run by Rhodes University, which uses various alternative, sustainable methods of BE treatment (Jones *et al.* 2013). This treatment facility includes bioremediation facilities such as a primary facultative pond (PFP), a high rate algal pond (HRAP) and a constructed wetland (CW). Each treatment process results in BE having different water quality parameters such as pH, form and concentration of nitrogen, and the concentration of phosphorous, sodium and other dissolved salts (Cilliers 2012, Jones *et al.* 2013). These parameters have been shown to directly and indirectly affect plant growth and soil fertility (Lucas & Davis 1961, Lieth & Al-Masoom 1993, Garcia *et al.* 1994).

Different methods of BE pre-treatment have been found to influence nutrient availability and downstream crop productivity (Power 2014, Power & Jones 2015). Dakoure *et al.* (2013) found that the sodium content of BE negatively affected the physical properties of the irrigated soil. It is therefore essential that the most suitable pre-treatment method of BE is found so that the nutrients in the effluent are made accessible to the plants while minimising any negative impacts BE may have on the soil.

2.1.1 Aims and objectives

The aim of this study was to determine the best pre-treatment method or combination of pre-treatment methods to make BE suitable for crop irrigation, and to evaluate the potential of BE as an irrigation water source. This was done by comparing the change in soil characteristics and growth of cabbages irrigated with treated BE to cabbages irrigated with a conventional irrigation solution. Cabbage plants were grown in the soil and irrigated with BE after treatment using AD, PFP, HRAP, CW and a commercial fertigation solution. All the irrigation treatments were subject to both pH adjustment and no pH adjustment. The plant height, plant weight, head weight, head diameter and health of the plants under each irrigation treatment were determined and compared between treatments. Physical, chemical and biological properties of the soil were also recorded to determine the effect that each irrigation water had on soil fertility.

The objectives of this study were to:

- compare the effect of different BE treatment processes on plant growth and soil characteristics;
- compare plant growth and soil characteristics between treatments subject to BE and conventional irrigation water;
- compare plant growth and soil characteristics between treatments subject to pH adjustment and those without pH adjustment; and
- determine if plant growth or soil characteristics are influenced by an interaction between pre-treatment process and pH adjustment.

2.2 Methods and materials

2.2.1 Experimental species

Cabbage (*Brassica oleracea* cv. Star 3301; Starke Ayres Pty Ltd, South Africa) was grown for 12 weeks to observe the short-term effects that BE has on the biological, physical and chemical properties of the soil as well as the effect it has on cabbage health and growth. *Brassica oleracea* (cv. Star 3301) was used in this trial because it is a common summer variety used by farmers and the trial was run over summer.

Two hundred cabbage seedlings were purchased from a commercial nursery (Moorland Seedlings Pty Ltd, Humansdorp). Of these 120 similar size seedlings were used for this experiment.

2.2.2 Treatments

Six irrigation solutions were applied to the cabbages which included post-AD, post-PFP, post-HRAP, post-CW, a commercial irrigation solution and municipal water (Figure 2.1, Table 2.1). The pH of each irrigation treatment was either adjusted to 6.5 with 98% sulphuric acid (Protea Chemicals Pty Ltd, South Africa) or left unadjusted. This resulted in a total of 12 irrigation treatments being tested (Table 2.1).

The plants irrigated with municipal water served as the control. The nutrient solution (NS) was comprised of a commercially available inorganic-fertilizer (Hygrotech Pty Ltd, South Africa; Registration number K5709; Act 36 of 1947), and calcium nitrate with a composition of 11.7% nitrogen and 16.6% calcium, mixed in a ratio of 1:0.8 and dissolved in municipal water to achieve an EC of 1800 µm (Table 2.2, Hygrotech Pty Ltd, South Africa). Each treatment was replicated ten times with a replicate consisting of a single pot.

Table 2.1 Irrigation treatments (T1 - T12) that were used to irrigate cabbage plants.

Irrigation solution	pH not adjusted	pH adjusted to 6.5
AD effluent	T1	T7
PFP effluent	T2	T8
HRAP effluent	T3	T9
CW effluent	T4	T10
Municipal water	T5	T11
Municipal water with inorganic fertiliser	T6	T12

Anaerobic digestion (AD), primary facultative pond (PFP), high rate algal pond (HRAP), constructed wetland (CW).

Table 2.2 The elemental composition of the inorganic fertilizer used in the nutrient solution treatments (Hygrotech Pty Ltd, South Africa).

Composition in %							Composition in mg/kg					
N	P	K	Ca	Mg	Na	S	Fe	Mn	Zn	Cu	B	Mo
6.8	4.2	20.8	-	Mg	-	6.4	1254	299	149	22	373	37

2.2.3 Experimental System

Cabbage plants were grown out doors in 23 l pots due to there being no land available at Ibhayi brewery for field trials (Figure 2.1). These pots were filled with top soil (oxidic sandy loam, 5 - 10% silt, 20 - 25% clay, 65 - 70% sand) obtained from a commercial supplier (Habata farm Pty Ltd, Sundays River Valley, South Africa). One cabbage plant was planted in each pot. Experimental treatments and their replicates were applied to pot using a complete randomisation design.

2.2.4 Irrigation regime

Cabbages were irrigated with one litre two to three times a week, depending on the moisture content of the soil. Every day a 10 cm long stick was pushed into the soil. If the stick came out

dry, then the cabbage plants were irrigated. If the stick came out muddy, then the plants were not irrigated. If cabbage plants showed signs of wilting they were also irrigated. Before each irrigation two 10 l buckets were filled with water from each of the six respective treatment water sources. The pH of the water in one of the buckets was then adjusted to 6.5. Water was taken out of the buckets using a one litre watering can and used to irrigate the cabbage plants. During irrigation care was taken not to wet the cabbage leaves.

The maximum amount of water irrigated at one time was one litre. This was done to ensure that leaching did not occur. Water was not observed draining out the bottom of the pots. In total each cabbage plant received 198.1 mm of treatment irrigation water and 91 mm of rain during the twelve week growth trial.

One month after planting, diamond back moth larvae were noticed on some of the cabbages. Cabbages plants were sprayed with Malasol (active ingredient: Mercaptothion, Efekto Agro-serve Pty Ltd) to kill the larvae. When a spraying occurred every plant was sprayed, an event that occurred five times during the trial. As a result no plants suffered severe damage from the diamond back moth larvae.

A

B

Figure 2.1 The 120 pot experimental system with the constructed wetland in the background (A), and the six different irrigation treatments, without pH adjustment (B).

2.2.5 Data collection

Irrigation water parameters

The pH, temperature and electrical conductivity (EC) of the water used in each treatment was recorded before each irrigation using an electronic probe (Hanna, HI 991300, United Kingdom). Chemical oxygen demand (COD), ammonia, nitrite, nitrate and phosphate of each irrigation solution was recorded weekly, using a spectrophotometer (Merck Spectroquant Pharo 100 spectrophotometer, product number 100706, Darmstadt, Germany) and commercially available test kits, using standard methods (Merck Pty Ltd, Darmstadt, Germany). Each sample was filtered through an eight micron filter paper prior to analysis and the following test kits were used:

- High-range ammonia cell tests (Merck Pty Ltd, product: 1.14559.0001)
- Low-range ammonium test (Merck Pty Ltd, product: 1.14752.0001)
- Nitrite test (Merck Pty Ltd, product: 1.14776.0001)
- Nitrate test (Merck Pty Ltd, product: 1.09713.0001)
- Phosphate test (Merck Pty Ltd, product: 1.14842.0001)
- Chemical oxygen demand cell test (Meck Pty Ltd, product: 1.14895.0001)

Plant parameters

The height and width of each cabbage plant was recorded (1 mm accuracy) at the start of the experiment and every four weeks until the end of the experiment. At the start of the

experiment twenty cabbage plants were randomly chosen from the population of seedlings used in the trial. These plants were used to determine the mean root mass, stem mass and leaf mass at the start of the trial. These plants were not used in the trial because of the destructive nature of the sampling method. At the end of the trial the root mass, stem mass, and leaf mass of each cabbage plant was recorded. The dry weight of each sample was also determined by oven drying samples at 80 °C for a minimum of 72 h (Borgognone *et al.* 2012) or until a constant weight was achieved.

The chlorophyll concentration index (CCI) of cabbage plant leaves was recorded using a chlorophyll content meter (CCM-200 Plus Chlorophyll Content Meter, Opti-Sciences Inc., USA). Readings were recorded at the start of the trial and every four weeks until the end of the experiment, on the uppermost fully expanded leaf of each plant.

At the beginning of the trial 12 plants were randomly chosen and used for leaf chemical analysis. These plants were not used in the experiment due to the destructive nature of the sampling. At the end of the trial three plants were randomly selected from each treatment and used for leaf chemical testing. All samples were analysed for N, P, Na, Cl, K, Al, Ca, Cu, Fe, Mn, Mg and Zn content at a commercial analytical laboratory (BemLab Pty Ltd, Strand, South Africa).

Photographs of the plants and stress symptoms of the plants were described and recorded to determine if the plants were experiencing any nutrient deficiencies or diseases. Daily temperature and rainfall data were recorded using a rainfall gauge situated next to the experiment and a thermometer (Hanna, HI 991300, United Kingdom).

Soil parameters

The physical properties of the soil that were measured included infiltration rate, moisture content, porosity, aggregate stability, bulk density, compaction and water potential. The type and texture of the soil was described at the start and end of the trial (Carter & Gregorich 2008). Infiltration rates were determined, every four weeks, by pouring one litre of irrigation treatment water into each pot in twenty seconds and recording the time it took for the water to drain into the soil. Timing was only started once all the water had been poured into the pot. Infiltration rate was then calculated using Equation 2.1:

Infiltration rate = (volume of water added/surface area of pot)/time [2.1]

Soil aggregate stability was measured at the beginning and end of the experiment (Le Bissonnais 1996). At the beginning of the trial 10 samples were taken from the soil used in the trial. At the end of the trial five composite samples were taken from each treatment. A composite sample consisted of soil obtained from two pots in each treatment. Five grams of soil sample was placed in distilled water and allowed to stand for ten minutes. The sample was then passed through a 0.05 mm sieve and aggregates >0.05 mm were collected and transferred onto a 0.50 mm sieve previously immersed in ethanol, and shaken five times with a gentle regular helical rotation movement. The >0.5 mm aggregates on the sieve were collected, dried at 40 °C, and then gently dry sieved using a column of six sieves: 2.00, 1.00, 0.50, 0.20, 0.10, and 0.05 mm. The aggregate stability was represented by the mean weight diameter (mm) of aggregates and was calculated using Equation 2.2:

$$\text{Mean weight diameter} = \sum (d \times m) / 100 \qquad [2.2]$$

where d was the mean diameter between the two sieves (mm) and m was the weight

fraction of aggregates remaining on the sieve (%).

Air filled porosity (AFP), bulk density and moisture content were measured, in each pot at the beginning and end of the trial, according to the Australian standard for potting mixes (Handreck & Black 1994). The apparatus used was a 110 mm plastic pipe with an end cap that had four 3.0 mm holes drilled into it. The pipe was bored into the soil to get an undisturbed soil sample. The soil was then run through a series of wetting cycles as follows: 30 min wetting and five min draining, 10 min wetting and five min draining, and 10 min wetting and five min draining. A gauze was placed over the top of the vessel and submerged in water to just above the surface of the soil. The holes in the bottom were then sealed and the vessel was moved into a tray, where the holes were unblocked. The vessel was left to drain for 30 min and the amount of water collected was measured. Air filled porosity was calculated using Equation 2.3. Directly after the AFP test the vessel was placed in a drying oven at 105 ^{o}C and allowed to dry for a minimum of 24 h, until a constant mass was achieved. Water holding capacity was calculated using Equation 2.4, and bulk density was then calculated using Equation 2.5.

$$\text{Air filled porosity (\%)} = (\text{volume drained}/\text{volume of soil}) \times 100 \quad [2.3]$$

$$\text{Water holding capacity (\%)} = ((\text{wet weight} - \text{dry weight})/\text{volume}) \times 100 \quad [2.4]$$

$$\text{Bulk density} = \text{dry weight}/\text{volume} \quad [2.5]$$

Soil compaction was measured at the start and end of the experiment in every pot using a pocket penetrometer (Szkurlat Pty Ltd, Poland).

A psychrometer (PST-55-15 thermocouple psychrometer/hygrometer, Psypro, Wescor Inc., Logan, UT, USA) was used to construct a soil suction test, which related gravimetric soil

water content to soil water potential. At the beginning of the trial 10 samples were taken from the soil used in the trial. At the end of the trial four composite samples were taken from each treatment. A composite sample consisted of soil taken from two pots from each treatment. Each sample was oven dried at 40 ^{o}C for 48 h, until a constant mass was achieved. Each sample was then wet with distilled water to 30% moisture content, by weight, and placed in a 30 ml air tight glass vial. Glass vials were then placed in a fridge for 48 h to allow the water to become evenly distributed throughout the soil. To determine the water potential of the soil, soil psychrometers were sealed into the vials and calibrated against standard solutions of 0.1 to 1.0 molar NaCl. Once analysed, samples were placed in an oven at 36 ^{o}C, in their vials with the lids off and allowed to dry until they reached 20% moisture content by weight. This was done by monitoring the weight of the samples every 30 min. Once samples reached 20% moisture content the lids were put back on the vials (making them air tight) and put into a fridge for 48 h to ensure the water become evenly distributed throughout the soil. After 48 h the samples were re-weighed to calculate their exact moisture content and then analysed. Once analysed the samples were then dried to 10% moisture content by weight. Once samples reached 10% moisture content the vials were sealed and placed in a fridge for 48 h. After which they were re-weighed and analysed. The psychrometer was calibrated before water potential readings were taken at each of the soil moisture contents described above.

The chemical properties of the soil that were recorded included pH, EC, cation exchange capacity (CEC), C, NH_4, P, Na, Cl, K, Ca, Cu, Mn and Mg. Electrical conductivity and pH were measured in every pot at the start and end of the experiment using a pH and conductivity meter (Hanna, HI 991300, United Kingdom) where the soil sample was mixed with distilled water at a ratio of 1:2.5 (Hati *et al.* 2007). At the beginning of the trial 10 samples were

taken from the soil used in the trial and used for soil chemical analysis. At the end of the experiment three composite soil samples from each treatment were used for soil chemical analysis. A composite sample consisted of soil taken from three pots in each treatment. These samples were sent to a commercial analytical laboratory and analysed for CEC, C, NH_4, PO_4, Na, K, Ca, Cu, Mn and Mg (BemLab Pty Ltd, Strand, South Africa). The sodium adsorption ration of the soil was also calculated using Equation 2.6, where Na, Ca, Mg and K are expressed in millequivalents per litre, (meq/l) obtained from a saturated paste soil extract (Sumner *et al.* 1995, Qadir & Schubert 2002).

$$\text{Sodium adsorption ration} = \text{Na} \div \sqrt{\frac{\text{Ca} + \text{Mg}}{2}} \qquad [2.6]$$

Community level physiological profiling was used to describe the biological health of the soil. This was done by direct inoculation of soil samples into single carbon source wells of microtitre plates (Eco Microplates BL1506, Biolog Inc, USA), followed by incubation and spectrometric detection of heterotrophic activity (Garland & Mills 1991). The microtitre plates contains 31 different carbon sources, nutrients and a redox dye (Garland & Mills 1991). Each carbon source is replicated three times and three water controls are also included (Garland & Mills 1991). At the beginning of the trial 12 samples were be randomly taken from the soil before it was placed into the pots. At the end of the trial three composite samples were taken from each treatment, where the composite samples consisted of soil taken from three pots in each treatment. Samples were analysed at the Department of Biochemistry and Microbiology, Rhodes University. One gram of soil sample was placed in 99 ml of sterile saline solution (0.2% NaCl) and allowed to settle. A further 10 x dilution was made by dispensing two millilitres into 18 ml sterile saline (10^{-3} dilution). After mixing, 150 µl was pipetted into each of the wells in the microtitre plates. The plate was

then incubated at 25 ^{o}C and readings were taken every 24 h, for five days, using a microplate reader (PowerWave HT Microplate Spectrophotometer, Biotek, USA) at a wavelength of 590 nm.

Microbial activity in each plate was expressed as average well colour development (AWCD) and was determined using Equation 2.7: (Garland & Millis 1991, Gomez *et al.* 2004)

$$\text{Average well colour development} = \sum OD_i/31 \quad [2.7]$$

where OD_i was the optical density value from each well, corrected by subtracting the blank well (inoculated, but without a carbon source) values from each plate well (Garland & Millis 1991, Gomez *et al.* 2004, Weber & Legge 2009). Richness (R) values were calculated as the number wells with a positive optical density (the number of oxidised carbon substrates, Magdalena *et al.* 2012). Shannon-Weaver index (H) values were calculated using Equation 2.8:

$$\text{Shannon Weaver index} = -\sum p_i(\ln p_i) \quad [2.8]$$

where p_i was the ratio of the activity on each substrate (OD_i) to the sum of activities on all substrates ($\sum OD_i$; Garland & Millis 1991, Magdalena *et al.* 2012). Plate reading at 119 h of incubation were used to calculate AWCD, R and H. The carbon substrates on each plate were grouped into the following five categories: (1) carbohydrates; (2) carboxylic and acetic acids; (3) amino acids; (4) polymers; and (5) amines and amides (Weber & Legge 2009). Each category was expressed as a percentage of total absorbance value of the plate corresponding to a particular treatment (Weber & Legge 2009, Chodak & Niklińska 2010).

Microbial counts were also performed by direct inoculation of soil suspension onto sterile nutrient agar plates. Five composite samples per treatment were analysed with a composite sample containing soil from two replicates. Dilutions were prepared as described above and 100 µl of the 10^{-3} dilution was pipetted onto a sterile nutrient agar plate. Then solution was spread across the surface of the nutrient agar plate using a sterile bent glass rod. Plates were then incubated at 25 ^{o}C for 24 h and the number of colonies on each plate was counted and colony forming units (CFU) were calculated using Equation 2.9.

$$CFU = \text{number of colonies} \times 10^{4} \quad [2.9]$$

2.2.6 Statistical analysis

The significant effects of treatments on measured variables were calculated using a one-way and multifactor repeated measures analysis of variance (ANOVA). When significant differences were detected treatment means were compared using a Tukey multiple range test procedure at $p<0.05$ probability level. All data were checked for equality of variance and for the normal distribution of the residuals using Levene's test and a Shapiro-Wilk plot of the residuals, respectively. If the assumptions were not met then the data were log or square-root transformed and checked for equal variance and normal distribution of residuals. If the assumptions were still not met, a non-parametric Mann-Whitney U test or a Kruskal Wallis ANOVA was used to compare the data between treatments. All analyses were performed using the Statistica (version 10) software package (StatSoft Inc, Tulsa, USA).

Statistical analysis of the data obtained from the Biolog plates was performed on the actual OD density of the wells and on transformed data where wells with an OD lower than 0.1 was

set to zero. When this was done no differences was found in the conclusions from the analysis and therefore the data presented from Biolog plates were obtained from the actual OD of the wells.

2.3 Results

2.3.1 Irrigation water chemistry

The conductivity of the BE irrigation treatments (3301.85 ± 34.46 µs/cm^2) was significantly higher than the nutrient solution (1904.91 ± 31.10 µs/cm^2) and water (595.86 ± 17.466 µs/cm^2) treatments (Kruskal Wallis, $H_{(11,264)}$=239.57, p<0.0001; Figure 2.2). The conductivity increased for all irrigation treatments when the pH was adjusted to 6.5 with sulphuric acid (Figure 2.2). Brewery effluent irrigation treatments had a higher pH than the nutrient solution (7.56 ± 0.02) and water (6.72 ± 0.05) treatments (Figure 2.3). High rate algal pond water had the highest pH (9.17 ± 0.14) while the other BE irrigation waters had a mean pH of 8.14 ± 0.03 (Figure 2.3). The COD of BE irrigation treatments was significantly higher than the NS or water irrigation treatments (Kruskal Wallis, $H_{(5,162)}$=114.27, p<0.0001; Table 2.3). The ammonia-nitrogen concentration of the nutrient solution (17.64 ± 0.69) was lower than the AD and PFP (34.74 ± 2.18) irrigation treatments but higher than the water after the HRAP and CW (2.34 ± 0.27) irrigation treatments (Kruskal Wallis, $H_{(5,162)}$=141.30, p<0.0001; Table 2.3). The nitrate and phosphate concentration was highest in the nutrient solution, AD and PFP treatment water (Kruskal Wallis, p<0.05; Table 2.3). High rate algal pond, CW and water irrigation treatments had the lowest nitrate and phosphate concentration. The chloride concentration was highest in CW and HRAP irrigation treatments, followed by AD

and PFP treatments (Kruskal Wallis, $H_{(5,162)}$=119.30, p<0.0001; Table 2.3). The nutrient solution and water irrigation treatments had the lowest chloride concentration (Table 2.3).

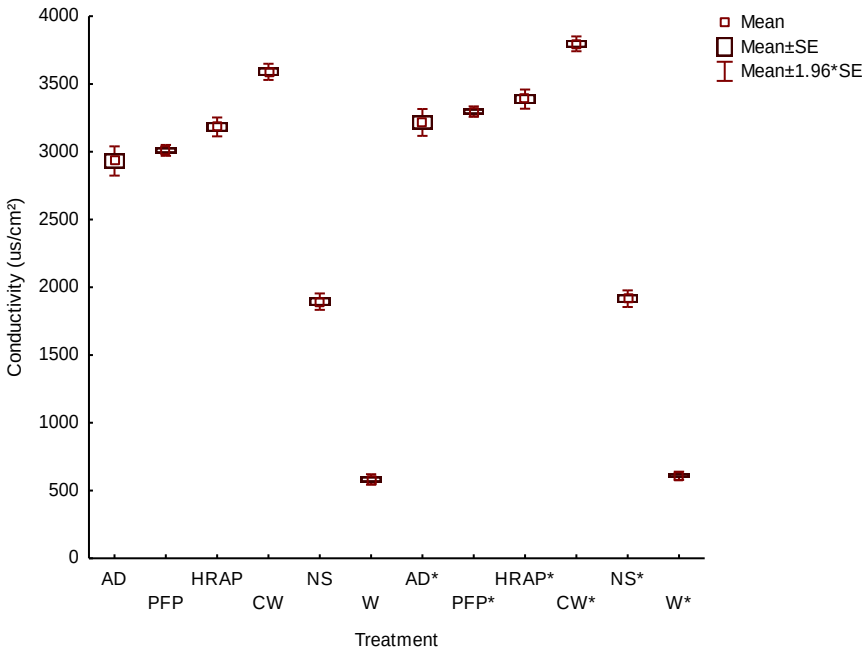

Figure 2.2 The mean conductivity of the various irrigation treatments, anaerobic digestion (AD), primary facultative pond (PFP), high rate algal pond (HRAP), constructed wetland (CW), nutrient solution (NS) and water (W) (Kruskal Wallis, $H_{(11,264)}$=239.57 p<0.0001). The irrigation water of treatments marked with * were subject pH adjustment using sulphuric acid.

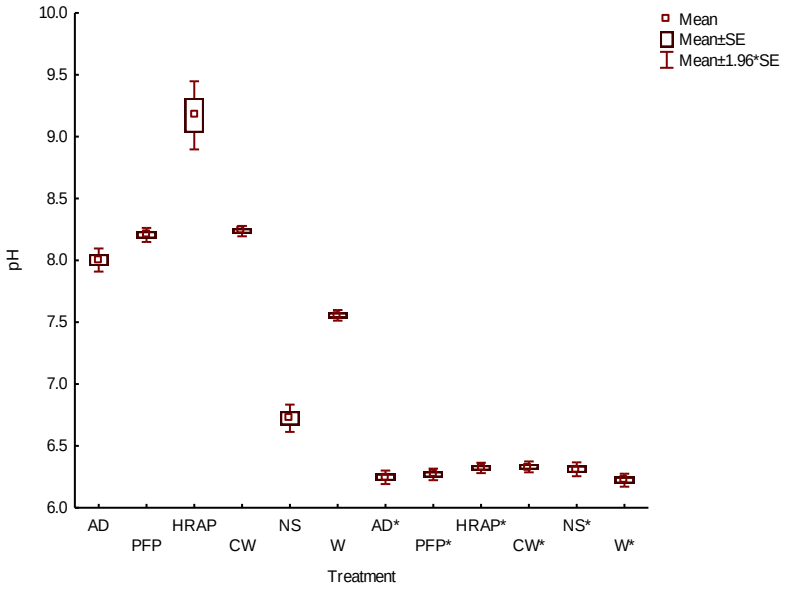

Figure 2.3 The mean pH of the various irrigation treatments, anaerobic digestion (AD), primary facultative pond (PFP), high rate algal pond (HRAP), constructed wetland (CW), nutrient solution (NS) and water (W). The irrigation water of treatments marked with * were subject pH adjustment using sulphuric acid.

Table 2.3 The mean (± standard error) water quality parameters of the various irrigation treatments. Values in the same row represented by a different superscript symbol represent significantly different means (Kruskal Wallis, P<0.05).

Property	Treatment							
	AD	PFP	HRAP	CW	NS	Water	H	P
COD (mg/l)	183.07±9.49 ^{a}	164.26±7.42 ^{a}	135.04±8.52 ^{a}	141.30±5.38 ^{a}	18.85±1.09 ^{b}	16.00±0.32 ^{b}	114.27	0.0001
NH_4-N (mg/l)	33.68±2.58 ^{a}	35.75± 1.78 ^{a}	2.52±0.19 ^{b}	2.14±0.34 ^{b}	17.04±0.69 ^{c}	0.70±0.05 ^{b}	141.30	0.0001
NO_2-N (mg/l)	0.92±0.02 ^{a}	0.87± 0.04 ^{a}	0.36±0.03 ^{b}	0.27±0.02 ^{b}	0.05±0.01 ^{c}	0.08±0.04 ^{c}	133.26	0.0001
NO_3-N (mg/l)	22.96±0.51 ^{a}	18.79±1.29 ^{a}	9.21±0.69 ^{b}	7.87±0.53 ^{b}	25.01±0.87 ^{a}	5.07±0.45 ^{b}	124.34	0.0001
PO_4-P (mg/l)	27.09±0.97 ^{a}	25.61± 1.05 ^{a}	16.12±0.95 ^{b}	17.31±0.93 ^{b}	29.88±0.12 ^{a}	6.13±0.45 ^{c}	124.51	0.0001
Chloride (mg/l)	151.92±4.82 ^{a}	153.07±4.75 ^{a}	166.85±5.13 ^{ab}	189.07±4.69 ^{b}	81.78±1.77 ^{c}	80.70±1.67 ^{c}	119.30	0.0001

Anaerobic digestion (AD), primary facultative pond (PFP), high rate algal pond (HRAP), constructed wetland (CW), nutrient solution (NS). Chemical oxygen demand (COD).

2.3.2 Plant productivity

The final mass of cabbages was not influenced by an interaction between pH regime and irrigation water source (Multifactor ANOVA, $F_{(5,108)}$=0.93, p=0.46; Figure 2.4). The pH adjustment of the irrigation waters did not affect the final mass of the cabbage plants (Multifactor ANOVA, $F_{(1,108)}$=2.83, p=0.10). Cabbage plants irrigated with NS had the greatest

mass (1223.32 ± 40.98 g) followed by cabbage plants irrigated with AD and PFP BE (478.13 ± 17.39 g; Figure 2.4). The mass of cabbaged plants irrigated with HRAP and CW effluent, and water were the smallest, with no significant difference between these three treatments (Figure 2.4). The CCI of cabbage plants was not influenced by an interaction between pH regime and the irrigation water source (Multifactor repeated measures ANOVA, $F_{(15,321)}$=0.63, p=0.85). The pH adjustment of the irrigation water sources had no effect on the CCI of cabbage plants (Multifactor repeated measures ANOVA, $F_{(5,107)}$=1.15, p=0.34). There was a significant difference in the CCI of cabbages irrigated with the various irrigation water sources, with the NS irrigated cabbages having the highest CCI, followed by AD and PFP irrigated cabbages (Multifactor repeated measures ANOVA, $F_{(15,321)}$=25.41, p<0.0001; Figure 2.5). Cabbages irrigated with water, and HRAP and CW effluent had the lowest CCI values over the course of the trial (Figure 2.5).

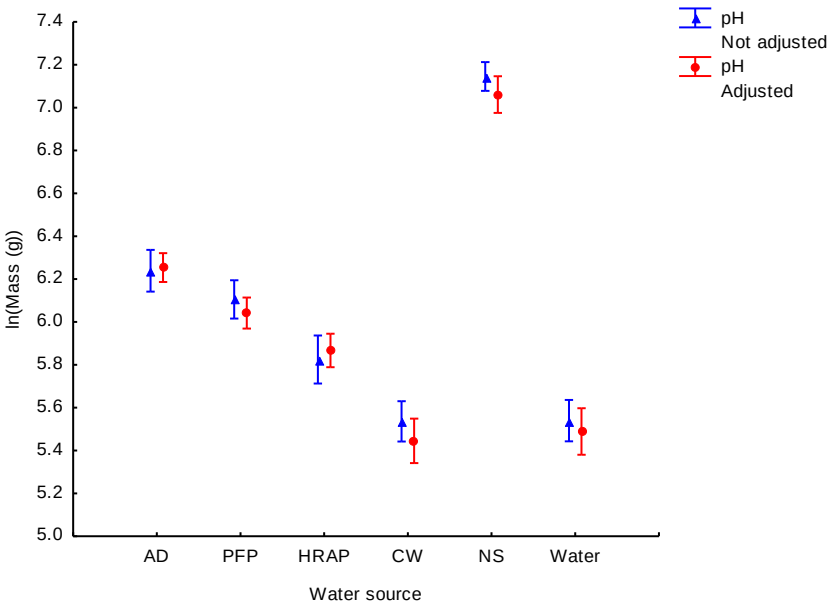

Figure 2.4 The mean (± 95% confidence interval) log transformed mass of cabbages subject to various irrigation treatments after 12 weeks, anaerobic digestion (AD), primary facultative pond (PFP), high rate algal pond (HRAP), constructed wetland (CW), nutrient solution (NS) and water (Multifactor ANOVA, $F_{(5,108)}$=0.93, p=0.46). The pH of each irrigation treatment was adjusted to 6.5 with sulphuric acid and was not adjusted.

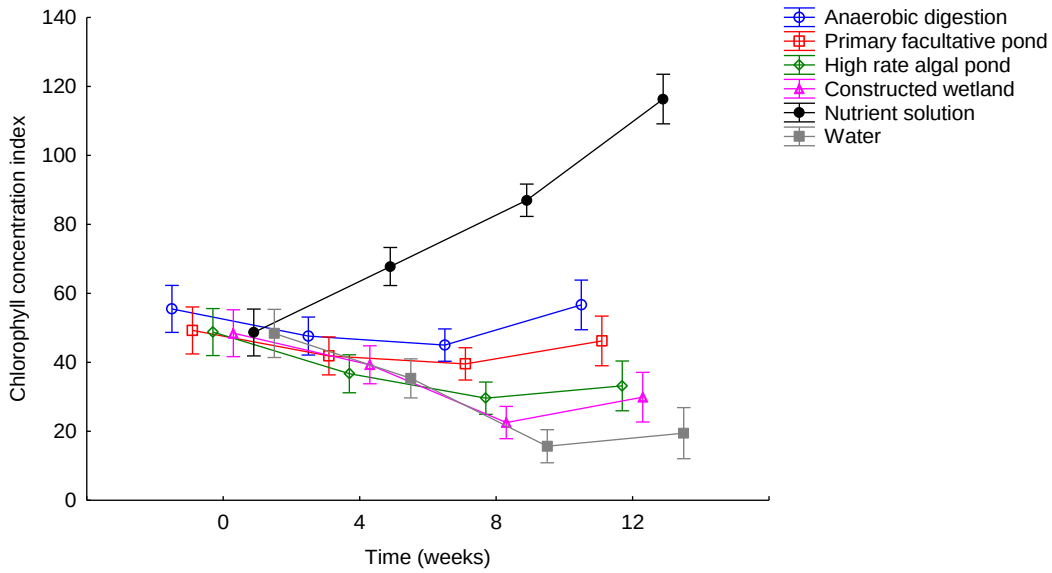

Figure 2.5 The mean (± 95% confidence interval) chlorophyll concentration index of cabbages subject to various irrigation treatments over the 12 week trial (Multifactor repeated measures ANOVA, $F_{(15,321)}$=25.41, p<0.0001).

As the ammonia, nitrate, total nitrogen and phosphate concentration in BE deceased so did the head weight of cabbages irrigated with BE (Multiple regression, P<0.05; Table 2.4). Of the measured plant nutrients, the nitrate concentration of BE had the strongest relationship to cabbage head weight (Multiple regression, y=15.33x + 161.99, R^2=0.90, $F_{(1,6)}$=55.69, p=0.0003; Figure 2.6). Cabbage weight decreased significantly with an increase in the EC of the BE (Multiple regression y=-0.33x + 1469.41, R^2=0.74, $F_{(1,6)}$=16.88, p=0.006; Table 2.4).

Table 2.4 Regression analysis results for cabbage head mass, after 12 weeks of irrigation, against water quality parameters of brewery effluent.

Property	Model	R^2	F	p
NH_4-N	y=5.53x + 285.03	0.76	19.30	0.0046
Total nitrogen	y=4.09x + 249.18	0.82	26.50	0.0021
PO_4-P	y=18.45x - 9.84	0.77	19.68	0.0044
Conductivity	y=-0.33x + 1469.41	0.74	16.88	0.0063

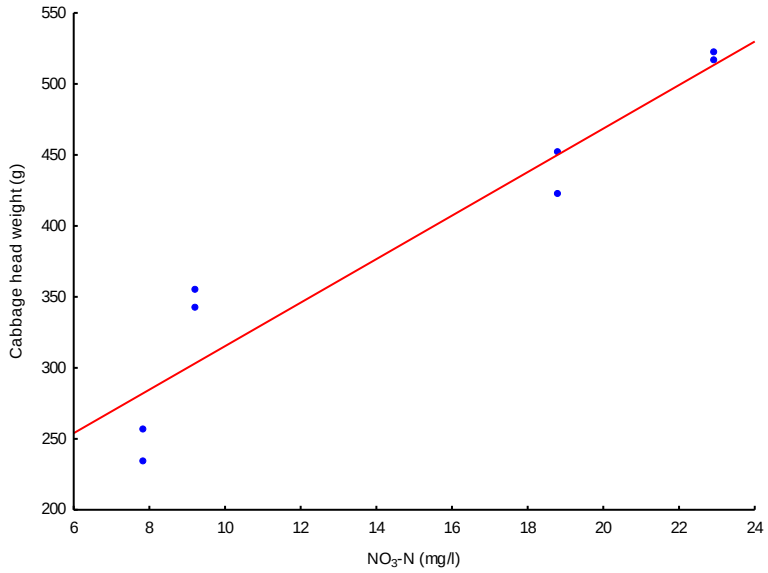

Figure 2.6 Scatterplot of the weight of cabbages against the nitrate-nitrogen concentration of brewery effluent used to irrigate the cabbages (Multiple regression, y=15.33x + 161.99, R^2=0.90, $F_{(1,6)}$=55.69, p=0.0003).

2.3.3 Chemical characteristics of plants

Cabbage plants irrigated with NS had significantly higher concentrations of N, P and K in their leaf tissue than cabbages subject to any of the BE irrigation treatments (Kruskal Wallis, p<0.05; Figure 2.7, 2.8, 2.9). The leaf concentration of N, P and K was similar for cabbages irrigated under all the BE and water irrigation treatments (Figure 2.7, 2.8, 2.9). The pH adjustment of the irrigation treatments had no effect on the N, P and K content of cabbage leaves (Figure 2.7, 2.8 & 2.9).

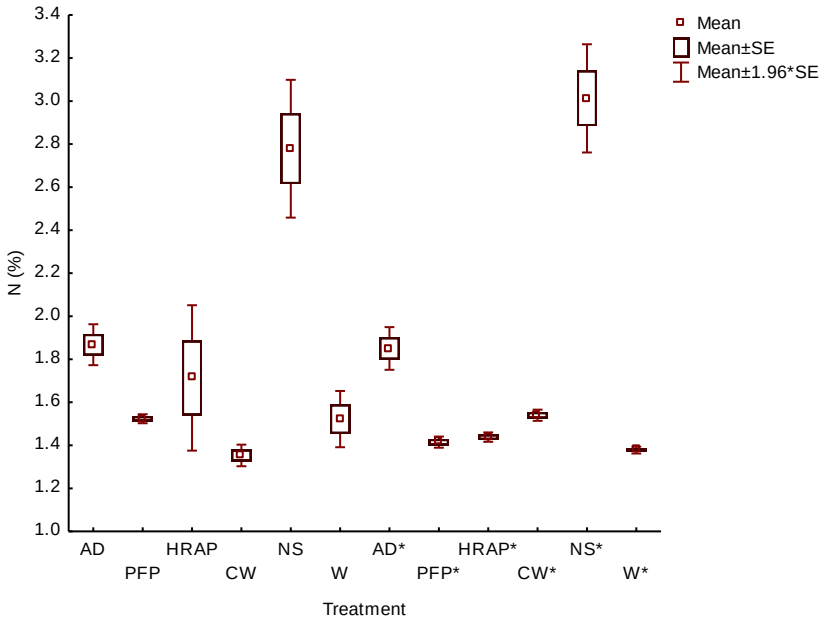

Figure 2.7 The mean nitrogen leaf content of cabbage plants irrigated under the different irrigation treatments anaerobic digestion (AD), primary facultative pond (PFP), high rate algal pond (HRAP), constructed wetland (CW), nutrient solution (NS) and water (W) (Kruskal Wallis, $H_{(11,36)}$=32.18 p=0.0007). The irrigation water of treatments marked with * were subject pH adjustment using sulphuric acid.

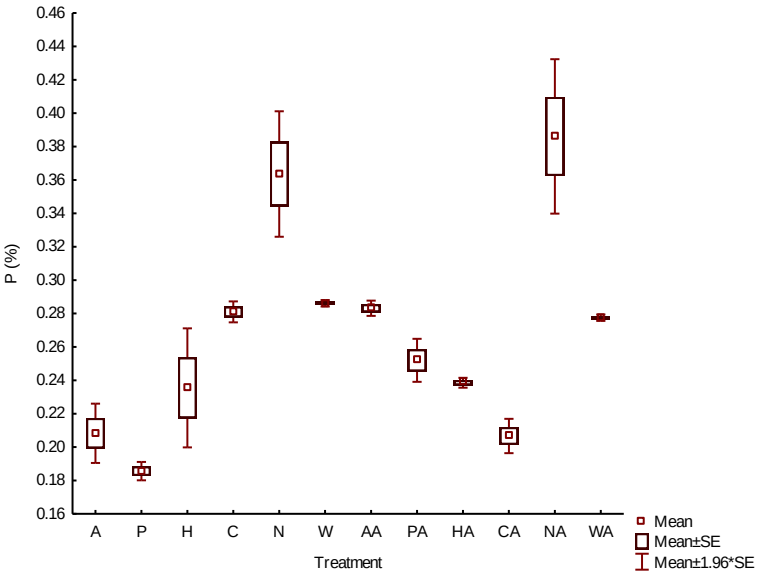

Figure 2.8 The mean phosphorous leaf content of cabbage plants irrigated under the different irrigation treatments anaerobic digestion (AD), primary facultative pond (PFP), high rate algal pond (HRAP), constructed wetland (CW), nutrient solution (NS) and water (W) (Kruskal Wallis, $H_{(11,36)}$=33.29, p=0.0005). The irrigation water of treatments marked with * were subject pH adjustment using sulphuric acid.

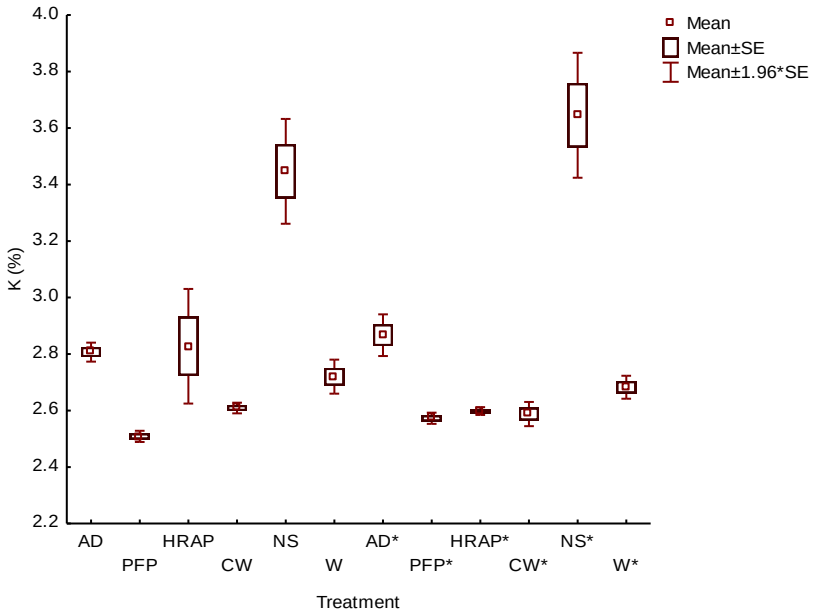

Figure 2.9 The mean potassium leaf content of cabbage plants irrigated under the different irrigation treatments anaerobic digestion (AD), primary facultative pond (PFP), high rate algal pond (HRAP), constructed wetland (CW), nutrient solution (NS) and water (W) (Kruskal Wallis, $H_{(11,36)}$=33.10, p=0.0005). The irrigation water of treatments marked with * were subject pH adjustment using sulphuric acid.

The irrigation of cabbages with BE treatment waters did not increase the sodium content of cabbage leaves when compared the sodium content of the cabbage seedlings (Table 2.5 & 2.6). The Na content of cabbage leaves was not influenced by pH, and the interaction between pH regime and water source (Multifactor ANOVA, $F_{(5,24)}$=0.85, p=0.53; Table 2.6). Cabbages plants irrigated with water had the lowest Na leaf content while cabbage plants subject to the rest of the irrigation treatments had similar Na leaf contents (Table 2.6). The pH adjustment of the irrigation solutions decreased the Na leaf concentration of cabbage plants (Multifactor ANOVA, $F_{(1,24)}$=17.48, p=0.0003).

There was no difference in the Cl, Cu, Fe, Mn and Zn leaf concentration of cabbage plants subject to all twelve experimental irrigation treatments (Multifactor ANOVA/Kruskal Wallis, p>0.05; Table 2.6). The Al leaf content of cabbages plants was generally higher for BE

irrigated plants, with the exception of plants irrigated with the acid adjusted NS treatment (Table 2.6). The Mg leaf content of cabbage plants was not influenced by pH, and the interaction between pH regime and water source (Multifactor ANOVA, $F_{(5,24)}$=1.63, p=0.19; Table 2.6). The Mg leaf content of cabbages subject to the NS irrigation treatments was significantly higher than cabbages subject to all the other irrigation treatments (Multifactor ANOVA, $F_{(1,108)}$=5.66, p=0.0014). The pH adjustment of irrigation solutions had no influence on the Mg content of cabbage leaves treatments (Multifactor ANOVA, $F_{(1,24)}$=1.68, p=0.21).

Table 2.5 The mean (± standard error) chemical composition of cabbage seedlings prior to planting.

Element											
Aluminium (mg/kg)	Calcium (%)	Chloride (%)	Copper (mg/kg)	Iron (mg/kg)	Potassium (%)	Phosphorous (%)	Magnesium (%)	Manganese (mg/kg)	Nitrogen (%)	Sodium (mg/kg)	Zinc (mg/kg)
166.67 ± 8.82	1.57 ± 0.14	1.59 ± 0.01	11.79 ± 0.23	232.37 ± 13.36	5.62 ± 0.54	0.62 ± 0.01	0.37 ± 0.01	218.97 ± 1.99	3.04 ± 0.05	6408 ± 249	31.33 ± 0.19

Table 2.6 The mean (± standard error) leaf chemical concentration for cabbage plants subject to the different irrigation treatments, after 12 weeks. Values in the same row represented by a different superscript symbol represent significantly different treatment means (Multifactor ANOVA/Kruskal Wallis, P<0.05).

	Treatment													
Element	AD	PFP	HRAP	CW	NS	Water	AD*	PFP*	HRAP*	CW*	NS*	Water*	F/H	P
Aluminium (mg/kg)	143.82 ± 2.39^{a}	248.53 ± 1.20^{b}	46.45 ± 5.13^{c}	59.16 ± 16.33^{c}	40.63 ± 4.47^{c}	63.55 ± 10.35^{c}	83.03 ± 7.12^{c}	114.21 ± 15.12^{a}	280.52 ± 26.28^{b}	113.17 ± 13.05^{a}	127.13 ± 10.24^{a}	53.06 ± 5.42^{c}	H=30.45	0.0013
Calcium (%)	0.27 ± 0.01^{a}	0.30 ± 0.01^{a}	0.29 ± 0.01^{a}	0.28 ± 0.01^{a}	0.34 ± 0.01^{b}	0.26 ± 0.01^{a}	0.23 ± 0.01^{a}	0.28 ± 0.02^{a}	0.23 ± 0.02^{a}	0.23 ± 0.01^{a}	0.35 ± 0.01^{b}	0.29 ± 0.04^{a}	H=30.06	0.0015
Chloride (%)	0.20 ± 0.04	0.19 ± 0.05	0.22 ± 0.02	0.15 ± 0.04	0.14 ± 0.03	0.21 ± 0.02	0.13 ± 0.03	0.24 ± 0.04	0.10 ± 0.01	0.12 ± 0.01	0.13 ± 0.01	0.22 ± 0.02	F=2.07	0.1041
Copper (mg/kg)	2.46 ± 0.48	3.03 ± 0.16	2.00 ± 0.71	2.96 ± 0.55	1.88 ± 0.42	3.29 ± 0.46	3.01 ± 0.16	1.88 ± 0.14	3.74 ± 0.89	2.91 ± 0.71	2.05 ± 0.46	2.03 ± 0.40	F=2.57	0.0538
Iron (mg/kg)	155.02 ± 9.10	160.35 ± 17.33	100.26 ± 31.68	91.96 ± 19.67	83.01 ± 10.21	90.21 ± 9.75	99.62 ± 11.49	143.96 ± 20.07	135.51 ± 15.93	144.67 ± 15.26	121.85 ± 20.56	89.58 ± 9.26	H=19.13	0.0588
Magnesium (%)	0.17 ± 0.01^{ab}	0.17 ± 0.01^{ab}	0.16 ± 0.01^{ab}	0.17 ± 0.01^{ab}	0.19 ± 0.01^{ab}	0.16 ± 0.01^{a}	0.15 ± 0.01^{a}	0.16 ± 0.01^{ab}	0.15 ± 0.01^{a}	0.15 ± 0.01^{a}	0.20 ± 0.01^{b}	0.17 ± 0.01^{ab}	F=1.63	0.1891
Manganese (mg/kg)	25.89 ± 0.37	27.08 ± 0.73	25.98 ± 0.07	24.91 ± 1.56	28.46 ± 0.99	24.19 ± 0.57	24.40 ± 0.71	25.04 ± 0.79	28.85 ± 2.12	25.13 ± 1.03	27.85 ± 1.50	24.72 ± 1.32	H=17.10	0.1049
Sodium (mg/kg)	6130.15 ± 253.52^{a}	5971.81 ± 149.29^{a}	6086.81 ±227.77^{a}	5829.67 ± 311.30^{ab}	5786.04 ± 115.34^{ab}	5355.56 ± 101.01^{ab}	5465.53 ±140.24^{ab}	5555.03 ±125.92^{ab}	5366.19 ±177.03^{ab}	5368.12 ± 176.10^{ab}	5748.60 ± 173.00^{ab}	5013.75 ± 139.94^{b}	F=0.85	0.5302
Zinc (mg/kg)	18.27 ± 1.55	15.92 ± 0.76	15.75 ± 0.81	17.12 ± 0.78	17.07 ± 0.11	17.25 ± 0.85	18.98 ± 0.06	16.43 ± 0.03	19.49 ± 3.15	15.71 ± 0.76	19.67 ± 0.13	16.37 ± 0.10	H=18.44	0.0719

Anaerobic digestion (AD), primary facultative pond (PFP), high rate algal pond (HRAP), constructed wetland (CW), nutrient solution (NS. Treatments marked with * were subject pH adjustment using sulphuric acid.

2.3.4 Soil physical characteristics

The water potential of soils was not influenced by an interaction between pH regime and water source (Multifactor repeated measures ANOVA, $F_{(10,72)}$=0.24, p=0.99). The pH of irrigation treatments did not influence the water potential of soils (Multifactor repeated measures ANOVA $F_{(2,72)}$=1.06 p=0.35). The water potential of soils receiving HRAP and CW irrigation treatments was consistently lower than soils subject to the other irrigation treatments (Multifactor repeated measures ANOVA $F_{(10,72)}$=45.64, p<0.0001; Figure 2.10). The difference in soil water potential of soils receiving the experimental irrigation treatments became more pronounced as the soil moisture content decreased (Figure 2.10) Soils receiving AD, PFP, NS and water irrigation treatments had similar water potentials at all soil moisture contents (Figure 2.10).

Mean weight diameter was not influenced by an interaction between pH regime and irrigation treatment (Multifactor ANOVA, $F_{(5,36)}$=0.65, p=0.66; Table 2.7). The pH adjustment of the irrigation treatments had no effect on the mean diameter of the soil particles (Multifactor ANOVA, $F_{(5,36)}$=0.26, p=0.61). Soils irrigated with AD, NS and water treatments had a higher mean diameter than soils irrigated with BE after PFP, HRAP and CW treatments (Multifactor ANOVA $F_{(5,36)}$=26.22, p<0.0001). The interaction between pH regime and irrigation treatment significantly influenced the infiltration rate of the soil (Multifactor ANOVA, $F_{(5,108)}$=4.10, p=0.002; Table 2.7). The infiltration rate of soils receiving AD, PFP and NS irrigation treatments was higher than soils subject to HRAP, CW and water irrigation treatments (Table 2.7). The pH of irrigation treatments did not influence the infiltration rate of the soil, with the exception of the PFP irrigation treatments (Table 2.7). There was no

difference in the air filled porosity, moisture content, bulk density and compaction between soils subject the irrigation treatments (Multifactor ANOVA, $p>0.05$; Table 2.7).

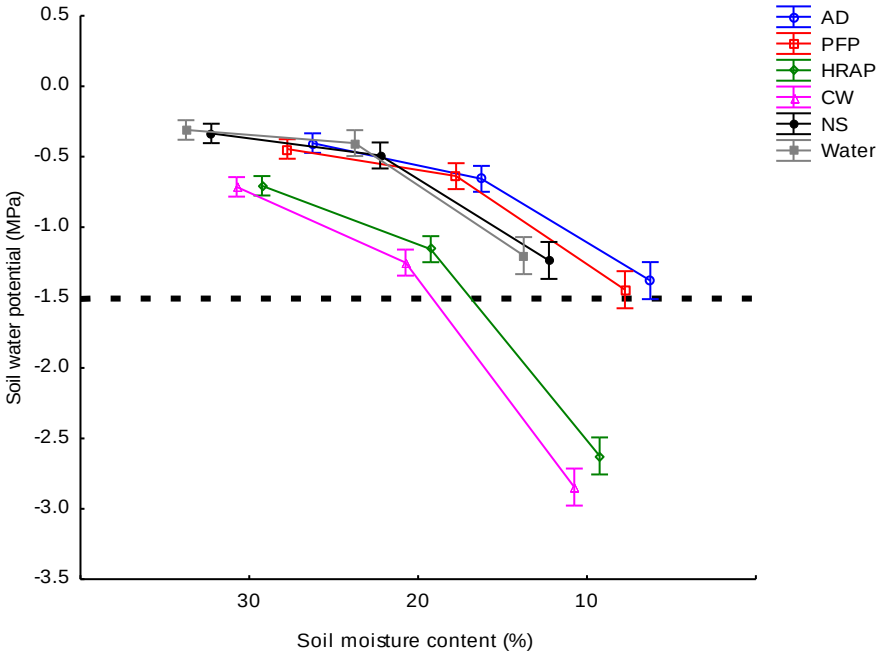

Figure 2.10 The mean (± 95% confidence interval) water potential of soil irrigated under the different irrigation treatments, anaerobic digestion (AD), primary facultative pond (PFP), high rate algal pond (HRAP), constructed wetland (CW), nutrient solution (NS) and water (Multifactor repeated measures ANOVA $F_{(10, 72)}=45.64$, $p<0.0001$). The dashed black line represents permanent wilting point, cabbages cannot access water from the soil below this line.

Table 2.7 The mean (± standard error) starting and final physical characteristics of soils subject to the different irrigation treatments. Values in the same row represented by a different superscript symbol represent significantly different treatment means (Multifactor ANOVA, P<0.05). Values from the starting column were not included in the statistical analysis.

	Treatment														
Property	Start	AD	PFP	HRAP	CW	NS	Water	AD*	PFP*	HRAP*	CW*	NS*	Water*	$F_{(5,108)}$	P
MWD (mm)	1.48 ± 0.03	1.48 ± 0.01 [a]	1.17 ± 0.04 [b]	1.21 ± 0.05 [b]	1.24 ± 0.05 [b]	1.45 ± 0.03 [a]	1.49 ± 0.05 [a]	1.45 ± 0.03 [a]	1.22 ± 0.03 [b]	1.27 ± 0.01 [b]	1.24 ± 0.03 [b]	1.50 ± 0.03 [a]	1.44 ± 0.06 [a]	1.28	0.29
Infiltration (cm/min)	2.13 ± 0.14	2.15 ± 0.26 [a]	1.95 ± 0.23 [a]	1.02 ± 0.15 [b]	0.94 ± 0.19 [b]	2.37 ± 0.26 [a]	0.47 ± 0.05 [b]	1.38 ± 0.25 [a]	1.60 ± 0.11 [a]	0.79 ± 0.10 [b]	0.84 ± 0.24 [b]	1.80 ± 0.16 [a]	0.68 0.07 [b]	4.10	0.0019
Air filled porosity (%)	13.45 ± 0.19	8.53 ± 0.24	8.31 ± 0.27	7.60 ± 0.17	8.34 ± 0.22	7.77 ± 0.48	7.70 ± 0.29	7.71 ± 0.34	7.87 ± 0.43	7.80 ± 0.39	8.13 ± 0.55	7.97 ± 0.26	7.35 ± 0.56	0.56	0.7283
Moisture content (%)	27.31 ± 0.93	27.78 ± 0.85	29.21 ± 1.20	30.82 ± 0.81	30.95 ± 1.04	28.18 ± 1.26	32.27 ± 1.18	30.96 ± 0.53	31.37 ± 1.03	29.69 ± 0.71	61.54 ± 1.03	29.52 ± 1.17	29.86 ± 1.03	2.12	0.0679
Bulk density (g/cm^3)	0.99 ± 0.01	1.06 ± 0.03	1.12 ± 0.04	1.10 ± 0.03	1.10 ± 0.04	1.10 ± 0.03	1.08 ± 0.03	1.07 ± 0.02	1.13 ± 0.03	1.05 ± 0.03	1.08 ± 0.03	1.11 ± 0.03	1.10 ± 0.04	0.31	0.9044
Compaction (kg/cm^2)	4.83 ± 0.86	14.18 ± 1.12	13.08 ± 0.91	13.27 ± 1.01	7.71 ± 0.89	17.94 ± 1.66	15.62 ± 1.01	14.24 ± 0.49	16.53 ± 1.35	11.68 ± 1.08	11.71 ± 1.12	19.49 ± 1.24	17.22 ± 0.85	0.82	0.5410

Treatments marked with * were subject to pH adjustment using sulphuric acid. Anaerobic digestion (AD), primary facultative pond (PFP), high rate algal pond (HRAP), constructed wetland (CW), nutrient solution (NS), mean weight diameter (MWD).

2.3.5 Soil chemical characteristics

Brewery effluent irrigation treatments increased the pH, conductivity and sodium content of the soil while NS and water treatments did not (Table 2.8, Figure 2.11, 2.12 & 2.13). Soils irrigated with BE irrigation treatments had a higher pH (9.49 ± 0.07) than soils irrigated with NS or water (8.49 ± 0.06) treatments (Figure 2.11). The pH adjustment of HRAP and CW irrigation treatments decreased the soils pH when compared to unadjusted HRAP and CW irrigation treatments (Figure 2.11). The conductivity of the soil was not influenced by an interaction between pH regime and irrigation treatment (Multifactor ANOVA, $F_{(5,108)}$=2.05, p=0.08; Figure 2.12). Irrigation treatments significantly affected the conductivity of the soil where soils subject to HRAP and CW irrigation treatments had the highest conductivity, followed by soils irrigated with AD and PFP BE (Multifactor ANOVA, F=131.92, p<0.0001). Soils irrigated with NS and water had the lowest conductivity with a combined mean of 1025.86 ± 50.11 µs/cm^2 (Figure 2.12). Soils subject to BE irrigation treatments had significantly higher concentrations of sodium (3919 ± 94.77 mg/kg) than soils irrigated with NS or water (920.58 ± 27.46 mg/kg, Kruskal Wallis, $H_{(11,36)}$=32.62, p=0.0006; Figure 2.13). After 12 weeks of irrigation, soils subject to BE irrigation treatments had a significantly higher SAR (8.18 ± 0.17) than soils irrigated with NS or water (2.20 ± 0.05, Kruskal Wallis, $H_{(11,36)}$=33.25, p=0.0005; Figure 2.14).

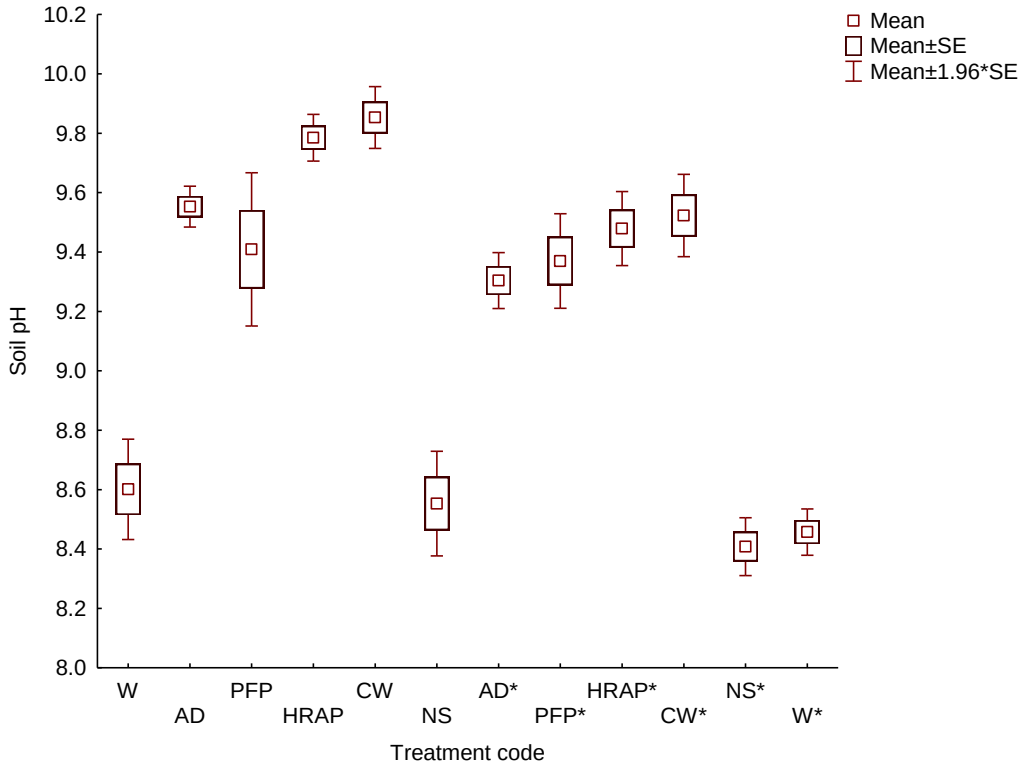

Figure 2.11 The mean pH of soil irrigated under the different irrigation treatments after 12 weeks, anaerobic digestion (AD), primary facultative pond (PFP), high rate algal pond (HRAP), constructed wetland (CW), nutrient solution (NS) and water (W). The irrigation water of treatments marked with * were subject pH adjustment using sulphuric acid.

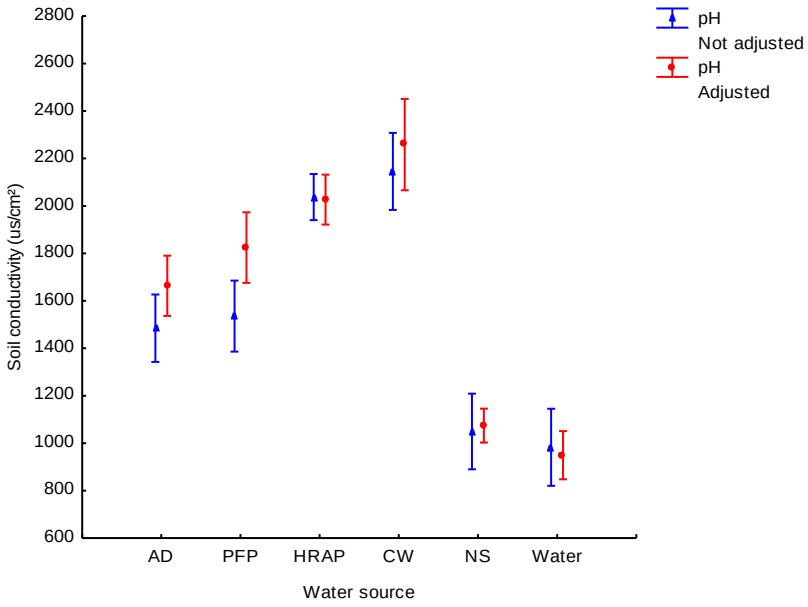

Figure 2.12 The mean (± 95% confidence interval) conductivity of soil irrigated under the different irrigation treatments (Multifactor ANOVA, $F_{(5,108)}$=2.05, p=0.08). Anaerobic digestion (AD), primary facultative pond (PFP), high rate algal pond (HRAP), constructed wetland (CW), nutrient solution (NS). The pH of each irrigation treatment was adjusted to 6.5 with sulphuric acid and was not adjusted.

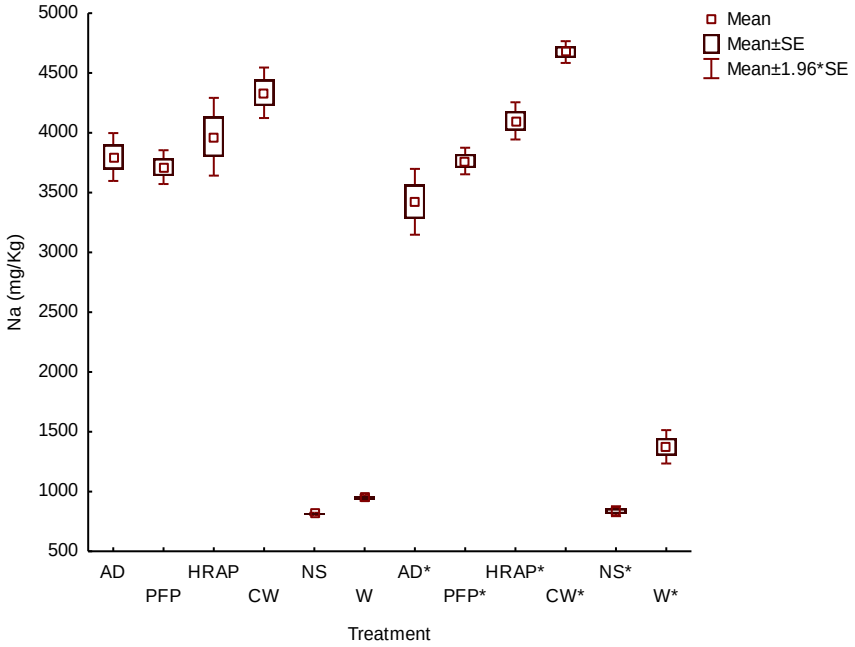

Figure 2.13 The mean sodium content of soil irrigated under the different irrigation treatments after 12 weeks (Kruskal Wallis, $H_{(11,36)}$=32.62, p=0.0006). Anaerobic digestion 1(AD), primary facultative pond (PFP), high rate algal pond (HRAP), constructed wetland (CW), nutrient solution (NS) and water (W). The irrigation water of treatments marked with * were subject pH adjustment using sulphuric acid.

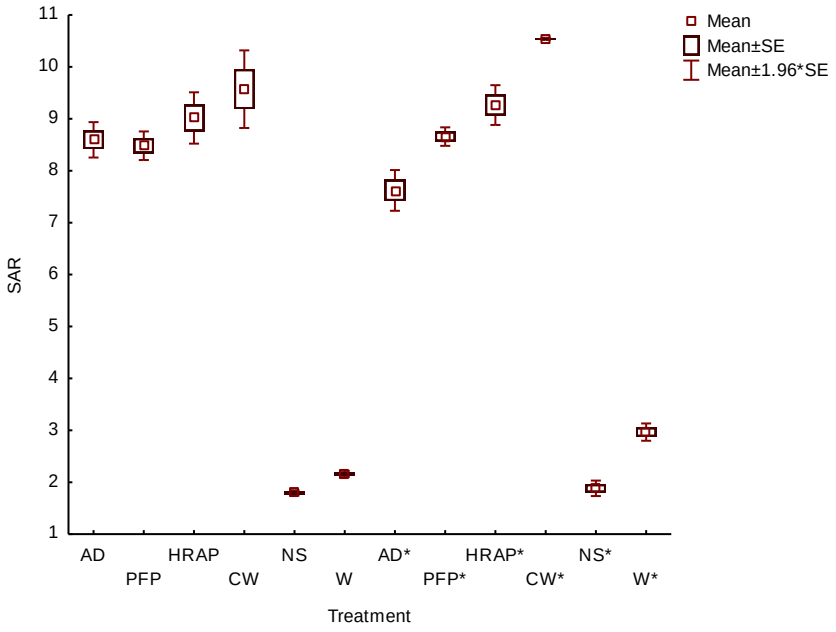

Figure 2.14 The mean sodium adsorption ratio (SAR) of soil irrigated under the different irrigation treatments after 12 weeks (Kruskal Wallis, $H_{(11,36)}$=33.25, p=0.0005). Anaerobic digestion (AD), primary facultative pond (PFP), high rate algal pond (HRAP), constructed wetland (CW), nutrient solution (NS) and water (W). The irrigation water of treatments marked with * were subject pH adjustment using sulphuric acid.

The CEC of the soil did not change during the trial and no difference was observed between soils subject to the different irrigation treatments (Kruskal Wallis, $H_{(11,36)}$=11.74, p=0.09; Table 2.8 & 2.9). Soils irrigated with CW effluent and water unadjusted for pH had significantly higher Cl concentrations than soils irrigated with the other irrigation treatments (Kruskal Wallis, $H_{(11,36)}$=31.44, p=0.0009; Table 2.9). There was no difference in the concentration of C, Ca, Cu, Mg, Mn, and Zn between soils subject to the different irrigation treatments (Multifactor ANOVA/Kruskal Wallis, p>0.05; Table 2.9). After 12 weeks of irrigation the NH_4 concentration of the soil was influenced by an interaction between pH regime and water source (Multifactor ANOVA $F_{(5,24)}$=4.10, p=0.008; Table 2.9) Soils irrigated with AD, PFP and NS treatments had higher ammonia concentrations than soils irrigated with water from the HRAP, CW or water treatments. The pH adjustment of AD and PFP irrigation solutions increased the ammonia concentration of soils whereas pH adjustment of HRAP, CW, NS and water treatments did not influence the ammonia concentration of the soil (Table 2.9).

Table 2.8 The mean (± standard error) chemical characteristics of the soil at the start of the experiment.

Element														
pH	Conductivity (µS/cm)	CEC (cmol(+)/kg)	Ammonia (mg(N)/kg)	Carbon (%)	Calcium (cmol(+)/kg)	Chloride (mg/kg)	Copper (mg/kg)	Magnesium (cmol(+)/kg)	Manganese (mg/kg)	Sodium (mg/kg)	Phosphorous (mg/kg)	Potassium (mg/kg)	Zinc (mg/kg)	SAR
8.52 ± 0.79	840.17 ± 73.64	13.60 ± 0.79	0.08 ± 0.01	0.79 ± 0.02	56.35 ± 2.52	574.26 ± 83.18	1.71 ± 0.01	13.63 ± 0.05	29.47 ± 0.64	946.80 ± 5.98	13.42 ± 0.25	444.20 ± 2.73	0.81 ± 0.02	2.21 ± 0.05

Sodium adsorption ration (SAR).

Table 2.9 The mean (± standard error) chemical characteristics of soils subject to the different irrigation treatments. Values in the same row represented by a different superscript symbol represent significantly different treatment means (Multifactor ANOVA/Kruskal Wallis, P<0.05).

Element	AD	PFP	HRAP	CW	NS	Water	AD*	PFP*	HRAP*	CW*	NS*	Water*	F/H	P
CEC (cmol(+)/kg)	15.55 ± 2.76	15.36 ± 1.23	16.36 ± 2.31	15.46 ± 2 .50	14.14 ± 1.08	14.39 ± 0.47	13.13 ± 0.94	17.14 ± 0.89	16.89 ± 1.92	13.45 ± 1.12	15.36 ± 0.79	14.16 ± 1.02	H=17.47	0.0948
Ammonia (mg(N)/kg)	9.38 ± 1.33 [a]	7.67 ± 0.52 [ab]	5.24 ± 0.75 [b]	4.94 ± 0.52 [b]	12.71 ± 1.34 [a]	5.10 ± 0.28 [b]	14.38 ± 0.50 [a]	12.27 ± 0.60 [a]	5.37 ± 0.44 [b]	6.61 ± 0.11 [b]	14.05 ± 0.28 [a]	4.33 ± 0.54 [b]	F=4.10	0.0079
Carbon (%)	0.72 ± 0.01	0.71 ± 0.02	0.68 ± 0.01	0.67 ± 0.02	0.71 ± 0.02	0.69 ± 0.02	0.71 ± 0.02	0.70 ± 0.02	0.76 ± 0.01	0.69 ± 0.02	0.70 ± 0.03	0.74 ± 0.03	F=1.36	0.2758
Calcium (cmol(+)/kg)	61.40 ± 1.05	60.82 ± 0.44	61.50 ± 2.13	65.65 ± 2.89	63.72 ± 1.34	60.05 ± 1.13	63.90 ± 2.37	59.07 ± 0.70	61.47 ± 0.37	61.75 ± 1.59	61.18 ± 3.29	67.58 ± 3.71	H=15.33	0.1679
Chloride (mg/kg)	315.23 ± 11.60 [a]	205.71 ± 57.36 [b]	180.15 ± 10.61 [b]	447.94 ± 9.96 [c]	176.55 ± 10.38 [b]	362.60 ± 27.19 [c]	139.98 ± 15.54 [b]	304.27 ± 9.46 [a]	261.70 ± 14.35 [a]	324.90 ± 10.54 [a]	192.32 ± 21.64 [b]	325.00 ± 9.13 [a]	H=31.44	0.0009
Copper (mg/kg)	1.59 ± 0.02	1.56 ± 0.02	1.54 ± 0.02	1.52 ± 0.02	1.60 ± 0.01	1.56 ± 0.02	1.57 ± 0.03	1.53 ± 0.03	1.56 ± 0.02	1.55 ± 0.02	1.52 ± 0.02	1.53 ± 0.00	F=1.43	0.2497
Magnesium (cmol(+)/kg)	12.59 ± 0.04	12.47 ± 0.08	12.61 ± 0.17	12.48 ± 0.08	13.11 ± 0.18	12.82 ± 0.11	12.64 ± 0.16	12.59 ± 0.05	12.78 ± 0.13	12.75 ± 0.14	12.93 ± 0.06	13.78 ± 0.19	F=0.53	0.7462
Manganese (mg/kg)	37.18 ± 1.02	35.17 ± 0.40	35.52 ± 0.40	37.77 ± 0.58	39.79 ± 0.41	37.19 ± 0.16	40.05 ± 0.35	39.67 ± 0.63	41.63 ± 0.40	39.40 ± 0.92	41.75 ± 0.53	35.86 ± 0.18	H=16.61	0.1200
Phosphorous (mg/kg)	24.00 ± 3.51 [a]	28.00 ± 2.87 [a]	25.33 ± 3.33 [a]	40.67 ± 3.88 [a]	79.33 ± 5.92 [b]	25.67 ± 2.33 [a]	34.33 ± 3.33 [a]	32.67 ± 4.33 [a]	35.33 ± 4.33 [a]	38.67 ± 3.33 [a]	83.33 ± 8.25 [b]	16.67 ± 2.33 [a]	H=34.02	0.0004
Potassium (mg/kg)	411.67 ± 1.76 [a]	410.33 ± 0.88 [a]	435.00 ± 2.52 [a]	455.00 ± 1.53 [a]	725.33 ± 1.76 [b]	437.33 ± 3.38 [a]	411.67 ± 2.73 [a]	418.67 ± 1.76 [a]	441.33 ± 5.70 [a]	442.33 ± 2.91 [a]	811.00 ± 12.66 [b]	427.33 ± 3.38 [a]	H=32.91	0.0005
Zinc (mg/kg)	2.41 ± 0.18	2.16 ± 0.26	2.00 ± 0.22	1.98 ± 0.05	2.25 ± 0.12	2.58 ± 0.10	2.58 ± 0.21	2.19 ± 0.18	2.20 ± 0.10	2.15 ± 0.16	2.41 ± 0.11	2.42 ± 0.18	H=14.65	0.1993

Treatments marked with * were subject to pH adjustment using sulphuric acid. Anaerobic digestion (AD), primary facultative pond (PFP), high rate algal pond (HRAP), constructed wetland (CW), nutrient solution (NS).

2.3.6 Soil biological characteristics

No significant difference was observed in carbon source utilisation of soils subject to the experimental irrigation treatments (Kruskal Wallis, $p>0.05$). On average soils contained 36.02% carbohydrate, 19.31% carboxylic and acetic acid, 22.11% amino acid, 18.45% polymer and 4.09% amine utilising bacteria (Figure 2.15). The interaction between pH regime and irrigation treatment had no influence on all the recorded soil biological indices (Multifactor ANOVA, $P>0.05$). No difference was observed for all soil biological indices recorded between pH adjusted and pH unadjusted irrigation treatments (Multifactor ANOVA, $p>0.05$). Therefore the data presented in Table 2.10 represents combined pH adjusted and pH unadjusted irrigation treatments. The AWCD was significantly higher for soils irrigated with AD, PFP and HRAP irrigation treatments than soil irrigated with CW and NS irrigation treatments (ANOVA $F_{(5,24)}=11.21$, $p<0.0001$; Table 2.10). Soils irrigated with water had significantly lower AWCD, colony forming units, Shannon Weaver index and richness compared to soils subjected to the other irrigation treatments (ANOVA, $P<0.05$; Table 2.10). Soils subject to AD, PFP, HRAP, CW and NS irrigation treatments had similar colony forming units, Shannon Weaver index and richness (Table 2.10).

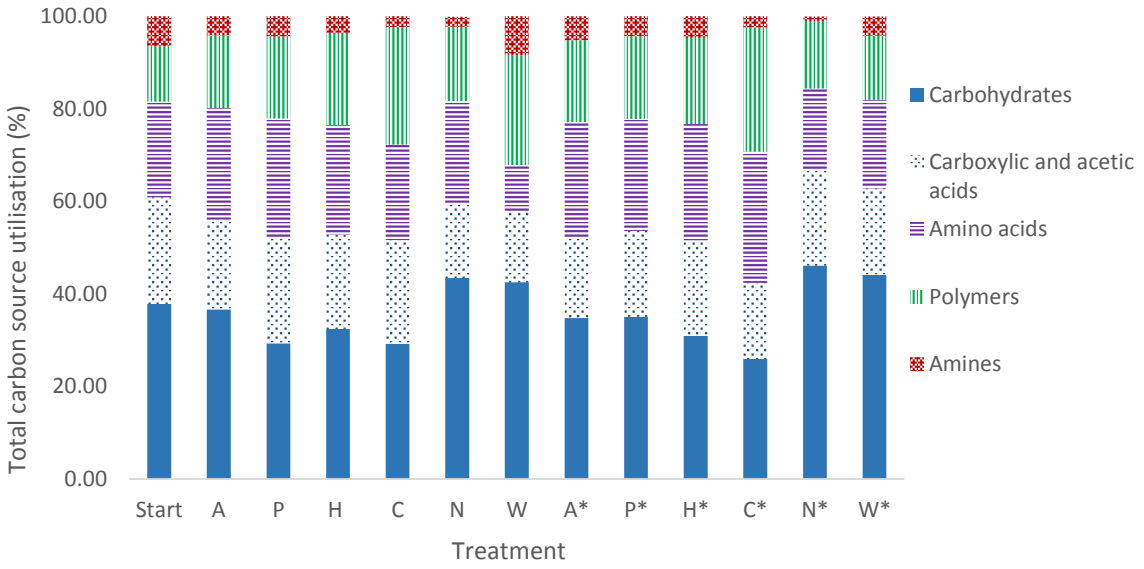

Figure 2.15 The mean percent of carbon source utilisation of soil irrigated under the different irrigation treatments after 12 weeks, treatments marked with * were subject pH adjustment using sulphuric acid. Anaerobic digestion (A), primary facultative pond (P), high rate algal pond (H), constructed wetland (C), nutrient solution (N) and water (W).

Table 2.10 The mean (± standard error) biological characteristics of soils subject to the different irrigation treatments. Values in the same row represented by a different superscript symbol represent significantly different treatment means (ANOVA, P<0.05). Values in the start column were not included in the statistical analysis.

	Treatment								
	Start	AD	PFP	HRAP	CW	NS	Water	F	P
Average well colour development	1.74 ± 0.22	1.61 ± 0.08 ^{a}	1.81 ± 0.19 ^{a}	1.75 ± 0.15 ^{a}	1.21 ± 0.21 ^{b}	1.08 ± 0.22 ^{b}	0.38 ± 0.09 ^{c}	11.21	0.0001
Shannon weaver index	3.97 ± 0.34	3.85 ± 0.24 ^{a}	3.65 ± 0.11 ^{a}	3.82 ± 0.25 ^{a}	2.94 ± 0.36 ^{a}	3.75 ± 0.08 ^{a}	2.37 ± 0.50 ^{b}	5.72	0.0013
Richness	57.33 ± 3.17	56.33 ± 2.08 ^{a}	53.50 ± 2.81 ^{a}	55.50 ± 2.28 ^{a}	27.67 ± 7.00 ^{b}	50.67 ± 3.56 ^{a}	18.33 ± 5.42 ^{b}	16.87	0.0001
Colony forming units/ gram soil	$7.94 \times 10^{5} \pm 2.15 \times 10^{4}$	$7.78 \times 10^{5} \pm 2.73 \times 10^{4}$ ^{a}	$8.17 \times 10^{5} \pm 2.94 \times 10^{4}$ ^{a}	$5.83 \times 10^{5} \pm 2.77 \times 10^{4}$ ^{a}	$5.22 \times 10^{5} \pm 2.64 \times 10^{4}$ ^{a}	$4.98 \times 10^{5} \pm 5.11 \times 10^{4}$ ^{a}	$2.95 \times 10^{5} \pm 2.31 \times 10^{4}$ ^{b}	16.35	0.0001

Anaerobic digestion (AD), primary facultative pond (PFP), high rate algal pond (HRAP), constructed wetland (CW), nutrient solution (NS).

2.3.7 Visual indicators

Cabbage plants irrigated with NS, AD and PFP treatments had green and healthy leaves (Figure 2.16). Cabbage plants subject to HRAP, CW and water irrigation treatments showed signs of nutrient deficiency with their outer leaves becoming purple and orange in colour (Figure 2.17). After 12 weeks of irrigation soils subject to AD, PFP, HRAP, CW and water irrigation treatments had surface cracking (Figure 2.18, 2.19). Only soils subject to the NS irrigation treatments had little to no surface cracking (Figure 2.19). Four and three out of the ten replicates irrigated with HRAP and CW effluent, respectively, had a visual build-up of sodium on the surface of the soil, as depicted in Figure 2.19. Soils subjected to AD and PFP irrigation treatments were slightly darker than soils subject to the other irrigation treatments, after twelve weeks of irrigation (Figure 2.18, 2.19).

Figure 2.16 Cabbage plants ten weeks after planting. From left to right the cabbages were irrigated with nutrient solution, anaerobically digested effluent and primary facultative pond effluent.

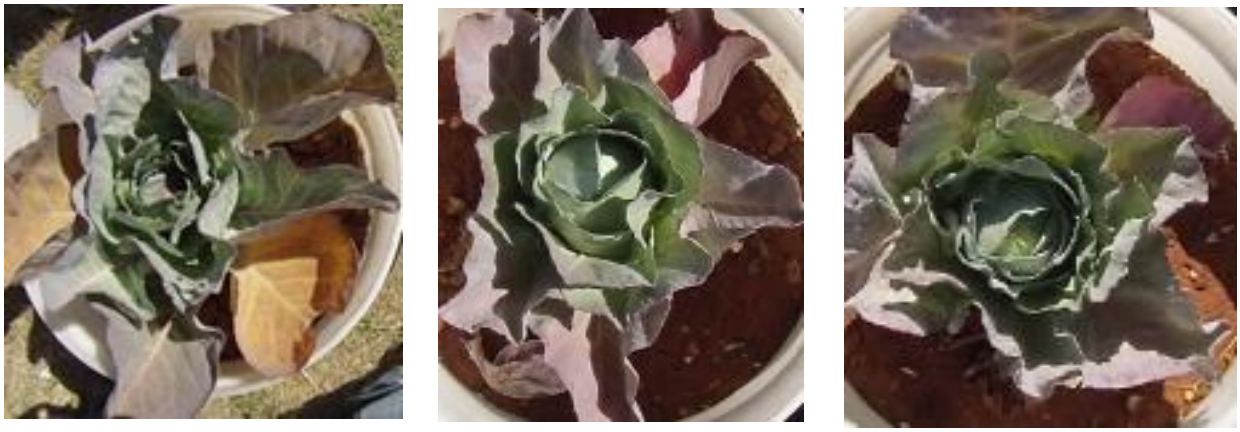

Figure 2.17 Cabbage plants ten weeks after planting. From left to right the cabbages were irrigated with high rate algal pond effluent, constructed wetland effluent and water.

Figure 2.18 Images of the soils twelve weeks into the experiment. From left to right soils were subject to irrigation treatments: anaerobic digested effluent, primary facultative effluent and high rate algal pond effluent.

Figure 2.19 Images of the soils twelve weeks into the experiment. From left to right soils were subject to irrigation treatments: constructed wetland, nutrient solution and water.

2.4 Discussion

2.4.1 Plant growth and health

Each of the experimental irrigation treatments contained different concentrations of plant nutrients (Table 2.3), which should affect the growth and health of plants they are used to irrigate. Cabbages irrigated with NS, AD and PFP irrigation treatments were significantly bigger than plants irrigated with HRAP, CW and water irrigation treatments (Figure 2.4, 2.5). In order to sustain vigorous and healthy growth plants require sufficient quantities of macro and micro nutrients (Marschner 1990, Epstein & Bloom 2005). The high rate algal pond and CW treatment processes utilise plants and algae which decrease the amount of nutrients in the effluent as it is utilised to support plant growth. This is probably the main reason why plants irrigated with HRAP and CW irrigation treatment water were significantly smaller than plants irrigated with NS, AD and PFP irrigation treatments. To further support this conclusion, plants subject to HRAP and CW irrigation treatments showed signs of nutrient deficiency. Their outer leaves were yellow-orange and/or dark red purple in colour, which is known as chlorosis and necrosis, and is a sign of nitrogen, phosphorous and potassium deficiency (Figure 2.17; Epstein & Bloom 2005). Effluent treatment processes that remove plant nutrients are counterproductive when using effluents as an irrigation source for plants because they remove valuable nutrients that are needed to support plant growth.

Brewery effluent is not an ideal plant nutrient solution and has certain characteristics that could inhibit plant growth. Anaerobically digested and post-PFP BE contained slightly higher concentrations of nitrogen and phosphorous than the NS (Table 2.3). However cabbages that were irrigated with BE subject to AD and PFP were smaller than cabbages irrigated with NS (Figure 2.4, 2.5), but showed no signs of nutrient deficiency (Figure 2.16). Therefore

certain characteristics of BE either inhibit the uptake of nutrients by cabbages or put stress on the plants resulting in less energy being spent on growth. It has previously been identified that the high conductivity, sodicity and pH in BE may decrease the growth and health of plants (Ajmal & Khan 1984, Juwarkar & Dutta 1990, Sweeney & Graetz 1991, Sukanya & Meli 2004, Senthilraja *et al.* 2013, Power 2014).

The pH of nutrient solution plays a major role in the availability of macro and micro nutrients to plants, with the optimal range for most plants being between five and seven (Chapter 1, Figure 1.4; Lucas & Davies 1961, Epstein & Bloom 2005, Power & Jones 2015). The unadjusted BE irrigation treatments had pH values around 8.14 with HRAP irrigation treatment having a mean pH of 9.17 (Table 2.3). Surprisingly no difference was observed in the growth, CCI and chemical composition of cabbages subject to BE irrigation treatments with or without pH adjustment. The pH range for good quality irrigation water is between 6.5 and 7.5 (Epstein & Bloom 2005). High pH values above 8.5 can cause the precipitation of Fe^{2+}, Mn^{2+}, PO_4, Ca^{2+} and Mn^{2+} to insoluble and unavailable salts (Tyson *et al.* 2007, Bauder & Brock 2001). However there was no difference in the growth, CCI or chemical composition of cabbages treated with pH unadjusted HRAP effluent (pH 9.17) and pH adjusted HRAP effluent (pH 6.5). Soils have the ability of resist pH change, which is known as their buffering capacity (Buckman & Brady 1967). The buffering capacity could have counteracted the pH adjustment of BE.

The salinity of irrigation water is one of the concerns when using effluents as irrigation waters since salinity causes reduced growth and yield of most crops (Shannon & Grieve 1999, Muyen *et al.* 2011). The mean EC of BE was 3301.85 $\mu s/cm^2$ (Figure 2.2), which should reduce cabbage crops yields by 10 – 20% (DWAF 1996). The mean mass of cabbages

irrigated with AD and PFP effluents was 13% lower than cabbages irrigated with NS irrigation treatments. The high EC probably of AD and PFP irrigation treatments probably caused the reduced yield of cabbages when compared to NS irrigated cabbages because AD and PFP irrigation treatments contained higher concentrations of N and P than the NS irrigation treatment. Medium salinity levels in irrigation water (2000 - 3000 $\mu s.cm^{-1}$) causes a decrease in yield in most crops (Shannon & Grieve 1999, DWAF 1996). This is primarily due to the osmotic effects by decreasing the osmotic potential between the root plasma and soil water (Munns & Termaat 1986, Jacoby 1994). This means that plants have to spend more energy to take up water from the soil, which increases respiration and has negative effects on growth (Munns & Termaat 1986, Jacoby 1994). The severity of the crop response salinity is species specific and is also mediated to environmental factors such as humidity, temperature, wind, light and air pollution (Shannon *et al.* 1994). The major contributors to salinity are discussed in Chapter 5 and recommendations are given to reduce the salinity. It is important to select salt tolerant crops when using effluents that contain moderate and higher salinities as they will be less affected.

2.4.2 Soil fertility

Over the course of the experiment the soil level in each pot dropped. The bulk density and compaction of soil in each pot increased from 1.01 g/cm^3 and 4.83 kg/cm2 to 1.08 g/cm^3 and 14.39 kg/cm^2 respectively. While the air filled porosity and infiltration rate of the soil in each pot decreased from 13.45% and 2.13 cm/min to 7.92% and 1.33 cm/min respectively. This was because the soil in the pots had not a settled soil and was not in a stable state and therefore it compacted over time especially, when irrigated because water weakens the bonds holding the soil aggregates together, causing them to compact (Van & Hill 1995).

However, the increase in soil compaction and decrease in soil porosity was similar for all treatments.

Soil water potential quantifies the tendency of water to move from one area to another area and is mainly affected by the concentration of salts in the soil (Bauder & Brock 2001, Tuller *et al.* 2003). It gives provides a measure of how easily soil water will move into the root of a plant. Soils subject to irrigation with HRAP and CW more had significantly reduced water potentials at all soil moisture contents than soils subject to the other irrigation treatments. As the salinity of the irrigation water and/or soil increases, the water potential will decrease (Barbour *et al.* 1998; Bauder & Brock 2001). This means that plants have to spend more energy to get water from the soil which will in turn compromise the growth of the plant (Barbour *et al.* 1998, Bauder & Brock 2001). The high salinity of BE probably increased the energy that plants invested in obtaining water, and this is a possible cause for the decreased growth of plants in these treatments. Most plants (including cabbages) cannot access water in the soil when the water potential decreases below -1.5 MPa; this is the dashed black line in Figure 2.10 (Lambers *et al.* 2008, Chapin *et al.* 2011). This means that in soils irrigated with HRAP and CW treated BE, plants could not access water in the soil when the gravimetric soil moisture content dropped below 15% (Figure 2.10). With the other irrigation treatments, plants could not access soil water when the gravimetric soil moisture content dropped below 10%. The negative affect of saline irrigation waters on the availability of water to plants demonstrated that the treatment process that results in BE having the lowest salinity would be the most suitable for crop irrigation.

The salinity of the various irrigation treatments had an effect on the salinity and SAR of the soils which, in turn could affect the physical characteristics of the soil. At the beginning of

the trial the soils had an SAR of 2.21 ± 0.05. After 12 weeks of irrigation, the soil SAR subject to BE irrigation treatments rose to 8.18 ± 0.17 while the SAR of soils subject NS and water irrigation remained the same throughout the trial. In most studies conducted on the use of wastewaters as an irrigation water source the SAR of the receiving soil has increased (Ajmal & Khan 1984, Kaushik *et al.* 2005, Kumar *et al.* 2010, Kumar & Chopra 2012, Dakoure *et al.* 2013). Dakoure *et al.* (2013) irrigated eggplants grown on ferralsol soil with BE that had been treated using stabilisation ponds. After two seasons of irrigation (2006 - 2008) they found that the effluent caused an increase in the SAR and ESP of the soil accompanied by a strong degradation of hydro structural soil properties. Soil irrigated with effluent had a decreased soil structural porosity, an increased bulk density and pH when compared to soils irrigated with tap water (Dakoure *et al.* 2013). During this study the increase in SAR of the soil did not seem to negatively affect the physical structure of the soil with the exception of the stability of soil aggregates which was slightly lower than soils irrigated the NS irrigation treatment. However this trial was only run for 12 weeks and after prolonged irrigation of BE on increase in the SAR of the soil accompanied by a decrease in the soils physical structure would be expected. This emphasises the point that the BE treatment which results in the lowest sodium, chloride and salinity concentration will be most suitable for irrigation.

There were signs of surface cracking in most of the soils in the experiment (Figure 2.18, 2.19). The only treatment which did not show signs of surface cracking were soils irrigated with NS. Soils irrigated with BE or municipal water all had surface cracking. Irrigation water containing a high concentration of sodium (BE irrigation treatments) can cause extreme soil particle flocculation which in turn can cause surface cracking (Miller & Donahue 1995, Buckman & Brady 1967). Hanson *et al.* (1999) found that irrigation water with a conductivity less than 500 $\mu S/cm^2$ cause soil aggregate dispersion. The low EC of the water irrigation

treatments dispersed soil aggregates causing the soil surface to clot and crack (Hanson *et al.* 1999). Whereas the moderate EC (1000 - 2000 µS/cm^2) of the NS irrigation water caused soil particle flocculation as the cations in the water help bind the micro aggregates together (Hanson *et al.* 1999).

A similar trend was observed with the infiltration rates of the soil, with soils subject to HRAP, CW and water treatments developing reduced infiltration rates. These irrigation treatments either had the highest sodium content (HRAP & CW) or the lowest conductivity (water). A high sodium content in irrigation causes extreme flocculation, resulting in the formation of a soil crust and decreased infiltration rates while the low conductivity (< 500 µS/cm^2) of the water irrigation treatments caused soil particle dispersion resulting in a decreased soil structure and infiltration rates (Miller & Donahue 1995, Hanson *et al.* 1999, Bauder & Brock 2001). A decrease in infiltration rates is normally associated with a decrease in porosity of the soil (Agassi *et al.* 1981, Abu Sharar *et al.* 1987, Hanson *et al.* 1999, Muyen *et al.* 2011). However this was not observed in this trial because the porosity of soils subject to all irrigation treatments were similar. This could be due to the method used to calculate porosity because the wetting cycles used to determine porosity could have caused the soil to compact and any differences in porosity would have been undetectable. In future studies, methods that do not require wetting the soil to determine air filled porosity of the soil are recommended.

To sustainably use effluents as an irrigation source the build-up of elements and molecules in the soil needs to a kept to a minimum. There was no increase or difference in the concentration of carbon, calcium, copper, magnesium, manganese, and zinc between soils receiving the experimental irrigation treatments or when comparing beginning and end

concentrations. The application of distillery effluent and wastewater to soils did not increase the levels of elements in the soils to toxic levels and could be used in irrigated agriculture (Kaushik *et al.* 2005, Hati *et al.* 2007, Kiziloglu *et al.* 2007). This supports the idea that post-AD or post-PFP BE can successfully be used as an irrigation source.

Brewery effluent does have a relatively high conductivity, sodium and chloride content which could potentially build up in the soil. Soils subjected to BE irrigation treatments had in an increase in the sodium, chloride and conductivity. Most studies that investigated the use of industrial effluents as an irrigation source found that they increased the sodium, chloride and conductivity of the soil after six months of irrigation (Ajmal & Khan 1984, Kaushik *et al.* 2005, Kumar *et al.* 2010, Kumar & Chopra 2012, Dakoure *et al.* 2013). With continued irrigation the build-up of sodium and chloride will have negative effects on the physical structure of the soil (described above) and will result in osmotic stress on the plants thus compromising their growth and yield. Therefore it is important to use effluents with the lowest possible conductivity and to irrigate salt tolerant crops or crops that are able to remove sodium and chloride from the soil and water.

It is important to understand whether the application of BE onto soils will affect the community of microbes in the soil and thus the functions they provide (Black 1968, Abbot & Murphy 2007). No significant difference was observed in the carbon source utilisation of soils subject to the various irrigation treatments (Figure 2.15). Soils were dominated by carbohydrate (36. 03%) utilising bacteria followed by amino acid (22.11%), carboxylic and acetic acid (19.31%), and polymer (18.45%) utilising bacteria. The literature shows both detrimental and enhancing effects of effluent irrigation on soil microbial population and communities, illustrating the complexity of relationships among soil microbial communities

in agricultural soils (Sinsabaugh *et al.* 2004, Sinsabaugh 2010). From this study it can be concluded that the application of BE to agricultural soils does not affect the overall functioning and processes performed by the soil microbe community, in the short term. It may have changed the species composition of soil microbes but the overall metabolic community structure of microbes present was not affected. Future studies should investigate the changes in species composition of soil microbes using metagenomics and conduct the study over a longer timescale.

Soils subject to BE and NS irrigation treatments had significantly higher colony forming units per gram of soil than soils irrigated with tap water. To add to this the AWCD of the Biolog plates inoculated with soil subjected BE irrigation treatments was significantly higher those inoculated with soil irrigated with water. The same results were observed when looking at the diversity and richness of the Biolog plates. In previous studies the application of treated effluents onto soil had no effect or increased the microbial population in the soil (Kannan & Oblisami 1990, Saqqar *et al.* 1997, Hati *et al.* 2007, Senthilraja *et al.* 2013). These authors concluded that the increase in microbial populations could have been due to the increase in soil carbon. However, Saqqar *et al.* (1997) stated that the increase in microbial populations could be due to the addition of microbes present in the wastewater. Juwarkar & Dutta (1990) and Kaushik *et al.* (2005) observed a 50% reduction in soil microbial populations treated with raw distillery effluent. The reduction in soil microbes in tap water irrigated treatments could have been due to two factors: firstly, tap water is chlorinated and has been treated to kill microbes, therefore its application to soil should decrease soil microbe populations if residual hypochlorite ions were still at an effective level, and the pH range was between 7.0 and 7.6, which the hypochlorite ion requires in order to be active as an oxidising agent. Vidali (2002) found that the application of tap water onto soils decreased

the diversity and population of soil microbes. Secondly, the tap water contained very little to no carbon, which means that no energy source was supplied to the microbes from the water, which would have resulted in a decrease in microbe numbers. The underlying cause however is not clear. Soils subject to NS irrigation treatments had lower soil microbe numbers that soils irrigated with BE treatments, however the difference was not significant. In this case, the NS solution provided a high level of nitrogen and phosphate. If organic carbon was present in the soil already, then carbon to nitrogen ratio and cognitive phosphate ratio could be met, which would result in a high level of microbial activity taking place in the soil.

In conclusion, the application of BE had no effect on the soil microbial populations in terms of numbers and metabolic diversity. However, the prolonged use of BE will result in a build-up of salt in the soil, which may have negative effects on soil microbial populations, as observed by (Condom *et al.* 1999, Dakoure *et al.* 2013), as well as shift in diversity to more salt tolerant species (Nelson *et al.* 1996, Pankhurst *et al.* 2001).

2.4.3 Conclusion

Brewery effluent can be used as an irrigation water source for cabbage production and contains sufficient nutrients to support crop growth. The pH adjustment of BE had no effect on plant growth or the biological activity, chemical and physical fertility of the soil. Post-AD or post-PFP BE is the most suitable for crop irrigation because it contains the highest concentration of plant nutrients and the lowest conductivity. However BE is an inferior irrigation water source when compared to a commercial irrigation water source with added inorganic fertiliser. Post-HRAP and CW BE are the least suitable for crop irrigation due to them having the lowest concentration of nutrients and the highest concentration of salts.

The sodium and chloride concentrations, and overall salinity (conductivity) are the biggest issues when using BE because the combination results in an increase in the SAR and conductivity of the soil, which puts osmotic stress on the plants, resulting in reduced growth. The application of post-AD and PFP BE did not significantly decrease the biological and physical factors of the soil. However after prolonged use it may negatively affect the soil's physical structure and reduce the soil's biological activity due to the sodium and chloride present in the effluent. Future studies should investigate the long term effects of irrigating soils with post-AD or PFP BE.

Chapter 3: The responses of selected crop species to brewery effluent irrigation

3.1 Introduction

Brewery effluent (BE) can be used as an irrigation water source in the irrigation of cabbages (Chapter 2). Post anaerobically digested (AD) or post primary facultative pond (PFP) BE is most suitable because it has the lowest conductivity and the highest concentration of plant nutrients (Chapter 2, Jones *et al.* 2013, Power 2014). In the irrigated cabbage production experiment the sodium concentration and sodium adsorption ratio (SAR) of the soil increased significantly (Chapter 2). The long term use of BE will lead to a build-up of sodium which can result in the deterioration of the soil's physical qualities, reduce crop yield and even render the soil permanently unusable for agriculture. Dakoure *et al.* (2013) found that only after two years of irrigation with treated BE there was a noticeable deterioration in the soils physical qualities due to the build-up of sodium in the soil. The accumulation of sodium in the soil from irrigation with treated wastewaters is the major limitation to using wastewater in agriculture and practices need to be developed in reduce the accumulation of sodium in soils (Qadir *et al.* 2003, Muyen *et al.* 2011).

Studies have shown that saline irrigation waters can be sustainably used for crop production systems and soil management (Grattan & Rhoades 1990, Oster 1994, Sharma & Rao 1998, Moreno *et al.* 2001). The key to the successful use of saline irrigation waters depends on the following: cultivation of salt and sodium tolerant crops, adequate leaching of sodium while avoiding deterioration of the soils physical profile, and the integrated use of saline and non-saline water (Qadir & Oster 2004). It is therefore important that to identify useful crop species that can reduce the build-up of sodium in the soil when irrigated with treated BE.

Certain crop species are more salt tolerant than others and some have been shown to aid in the removal of sodium from the soil (Qadir *et al.* 2001, Qadir *et al.* 2005). Halophytes are plants that have adapted to grow and reproduce in saline and sodic soils (Flowers & Colmer 2008). Many species of halophytes have been used in crop production systems using saline or sodic irrigation waters and soils (Miyamoto *et al.* 1996, Brown *et al.* 1999, Glenn *et al.* 1999, Qadir *et al.* 2005). Saltbush (*Atriplex nummularia*) has been used numerously in saline water irrigation systems; it can also be used as an animal fodder due to its high protein content (Brown *et al.* 1999, Glenn *et al.* 1999). Therefore saltbush was tested as a potential crop that can be used in crop production systems irrigated with BE.

In general the most suitable crops to be used in saline water irrigation have a greater biomass production, together with the ability to tolerate soil and irrigation water salinity and tolerance of periodic inundation (Qadir *et al.* 2001). Some of the plant species that have been shown to aid in the reclamation on salt affected soils are: Japanese millet (*Echinochloa esculenta*), amaranth (*Amaranthus cruentus*), lucerne (*Medicago sativa*), kallar grass (*Leptochloa fusca*) and saltbush (*Atriplex nummularia*) (Gritsenko & Gritsenko 1999, Oster *et al.* 1999, Barret-Lennard 2002).

Lucerne and millet are moderately tolerant to salinity and have the potential to be used as crops in agricultural systems where irrigation with slightly saline water is an option (Ilyas *et al.* 1993, Gritsenko & Gritsenko 1999, Qadir *et al.* 2003, Qadir *et al.* 2004). Based on the results from Chapter 2 post-PFP BE is the most suitable source of treated BE for irrigated crop production and hence this effluent was used to irrigate the crops in this trial. Millet and lucerne are both popular forage crops for livestock farming and could potentially be

irrigated with treated BE, to produce a valuable crop while minimising the build-up of sodium in the soil.

3.1.1 Aims and objectives

The aim of this experiment was to identify a crop species that could be sustainably irrigated with BE without this irrigation resulting in an increase in the soil sodium levels. This was tested by irrigating saltbush, millet and lucerne with treated BE and recording the effects that each cropping and irrigation regime had on the soils chemical and physical fertility, as well as recording the biomass produced by each crop.

The objectives of this experiment were to:

1) compare the biomass production and chemical characteristics of different crops irrigated with BE with and without pH adjustment; and
2) compare the effect of different crops irrigated with BE with and without pH adjustment have on the soil's physical and chemical characteristics.

3.2 Methods and materials

3.2.1 Experimental species

Three plants species were grown for three months to determine which plant reduced the build-up of salt in the soil when irrigated with BE. Thirty saltbush (*Atriplex nummularia*) seedlings were purchased from a commercial nursery (Mountain herb estate, Pty Ltd, Pretoria). Japanese millet (*Echinochloa esculenta*) and lucerne (*Medicago sativa*) seeds were obtained from a commercial seed supplier (Agricol Pty Ltd, Port Elizabeth) and were planted in polystyrene planter trays, filled with a mixture of 40% soil and 60% compost. These seeds

were germinated and allowed to grow for three weeks. Similar sized plants for each plant species were used in the experiment.

3.2.2 Treatments

The four crop treatments used in this experiment were as follows: lucerne, millet, saltbush and No Crop. The No Crop treatment served as the control. Each crop treatment was irrigated with post-PFP effluent, where the pH was adjusted with 98% sulphuric acid (Protea Chemicals Pty Ltd, South Africa) to 6.5 or left unadjusted (Table 3.1). This resulted in eight experimental treatments being tested (Table 3.1).

Table 3.1 The eight treatments (T1-T8) used in this experiment.

Crop	pH not adjusted	pH adjusted to 6.5
Saltbush	T1	T2
Millet	T3	T4
Lucerne	T5	T6
No Crop	T7	T8

3.2.3 Experimental system

Plants were grown out doors in 23 l pots (Figure 3.1). These pots were filled with the same top soil (oxidic sandy loam, 5 - 10% silt, 20 - 25% clay and 65 - 70% sand) used in the experiment in Chapter 2. One saltbush plant was planted in each pot and 10 millet or lucerne plants were planted in each pot (Figure 3.1). Experimental treatments and their replicates were applied to pot using a complete randomisation design.

Figure 3.1 Saltbush, lucerne and millet plants eight weeks after planting (left) and the 40 pot experimental system 12 weeks after planting (right).

3.2.4 Irrigation regime

Plants were irrigated with one litre of post-PFP BE two to three times a week, depending on the moisture content of the soil, according to the method described in Chapter 2 (Section 2.2.4). The maximum amount of water irrigated at one time was one litre. This was done to ensure that leaching did not occur; water was not observed draining out the bottom of the pots after irrigation. However, after heavy rainfall events water was noticed leaching out of the bottom of the pots. In total each pot received 212 mm of irrigation water and 78 mm of rain during the twelve week growth trial.

3.2.5 Data collection

Irrigation water parameters

The water quality parameters measured during the experiment included ammonia, nitrite, nitrate, phosphate, chloride, chemical oxygen demand (COD), pH and electrical conductivity

(EC). These parameters were measured before each irrigation using the same equipment and techniques described in Chapter 2 (Section 2.2.5).

Plant productivity

At the beginning of the trial the mass of each plant planted in the pots was recorded (0.1 g). At the end trial the mass of the each plant was also recorded (0.1 g). The height and width of plants was recorded (1 mm accuracy) at the start of the trial and every four weeks until the end of the trial. Chlorophyll concentration index (CCI) readings were recorded at the start of the trial and every four weeks until the end of the experiment, on the uppermost fully expanded leaf of each plant (CCM-200 Plus Chlorophyll Content Meter, Opti-Sciences Inc., USA). At the end of the trial three pots from each treatment were randomly selected and leaves from the plants in the pots were sent to a commercial analytical laboratory (BemLab Pty Ltd, Strand, South Africa). Therefore three leaf samples per treatment were analysed. These samples were tested for N, P, Na, Cl, K, Al, Ca, Cu, Fe, Mn, Mg and Zn content.

Photographs of the plants and stress symptoms of the plants were described and recorded to determine if the plants were experiencing any nutrient deficiencies or diseases. Daily temperature and rainfall data were recorded using a rainfall gauge situated next to the experiment and a thermometer (Hanna, HI 991300, United Kingdom).

Soil monitoring

The physical soil properties recorded included bulk density, moisture content, air filled porosity, infiltration rate and mean weight diameter (MWD). Each property was measured

at the start and end of the trial in every pot. The same methods and equations used to calculate the above properties in Chapter 2 (Section 2.2.5) were used in this experiment.

The chemical soil properties recorded included pH, EC, cation exchange capacity (CEC), C, NH_4, P, Na, Cl, K, Ca, Cu, Mn, Mg and SAR. The pH and EC of the soil was recorded in each pot at the start and end of the trial according to the methods described in Chapter 2 (Section 2.2.5). At the beginning of the trial 10 samples were randomly taken from the soil before it was placed into the pots. At the end of the trial three samples were analysed per treatment, where a sample was soil from one pot. These samples were analysed for CEC, C, NH_4, P, Na, Cl, K, Ca, Cu, Mn and Mg (BemLab Pty Ltd, Strand, South Africa). Sodium adsorption ration was calculated using the same formula described in Chapter 2 (Section 2.2.5).

3.2.6 Statistical analysis

The same statistical analysis used in Chapter 2 (Section 2.2.6) was used to analyse the data in this experiment.

3.3 Results

3.3.1 Irrigation water quality

Post-PFP BE had a mean ammonia, nitrite and nitrate concentration of 34.56 ± 2.4, 0.99 ± 0.07 and 21.33 ± 0.79 mgN/l respectively (Table 3.2). The mean phosphate concentration of post-PFP effluent was 28.76 ± 0.56 mgP/l (Table 3.2). The addition of sulphuric acid to adjust the pH of post-PFP effluent, had no influence on the water quality parameters except for conductivity (ANOVA, $P>0.05$; Table 3.2). The conductivity of post-PFP effluent, on average, increased from 3019.05 ± 48.72 to 3167.68 ± 64.34 $\mu s/cm^2$ when the pH was adjusted (Table

3.2). Post-PFP BE had a mean unadjusted pH of 8.34 ± 0.06 and adjusted pH of 6.43 ± 0.03 (Table 3.2).

Table 3.2 The mean (± standard error) water quality parameters of post primary facultative pond brewery effluent used to irrigate the experimental crops.

	Parameter							
pH	pH	Conductivity ($\mu s/cm^{2)}$	NH4-N (mg/l)	NO2-N (mg/l)	NO3-N (mg/l)	PO4-P (mg/l)	Cl (mg/l)	COD (mg/l)
Not Adjusted	8.34 ± 0.06	3019.05 ± 48.72	34.37 ± 2.80	0.95 ± 0.02	21.29 ± 0.84	28.80 ± 0.54	173.14 ± 5.17	226.00 ± 5.01
Adjusted	6.43 ± 0.03	3167.68 ± 64.34	34.75 ± 2.00	1.02 ± 0.11	21.37 ± 0.74	28.72 ± 0.57	174.69 ± 4.19	224.52 ± 5.15

3.3.2 Plant Productivity

There was no significant interaction between pH regime and crop type on the mean mass gained per pot (Multifactor ANOVA, $F_{(2,24)}$=0.53, p=0.59; Figure 3.2). There was a significant difference between the mean mass gained of the different crops, where it was highest for millet, followed by saltbush and lucerne (Multifactor ANOVA, $F_{(2,24)}$=1911.4, p<0.0001). The pH adjustment of post-PFP effluent had no effect on the mean mass gained of the experimental crops (Multifactor ANOVA, $F_{(2,24)}$ =1.20, p=0.28). The CCI of the experimental crops generally increased over the period of the trial, with CCI not being influenced by an interaction between plant type and pH regime (Multifactor repeated measures ANOVA, $F_{(6,72)}$=0.21, p=0.97; Figure 3.3). There was, however, a significant difference in CCI between crops, where lucerne and saltbush had similar CCI values over the course of the experiment and were higher than the CCI of millet plants (Multifactor repeated measures ANOVA,

$F_{(2,24)}$=40.15, p<0.0001). The pH adjustment of post-PFP BE had no influence on the CCI of all experimental crops (Multifactor repeated measures ANOVA, $F_{(1,24)}$=0.48, p=0.50).

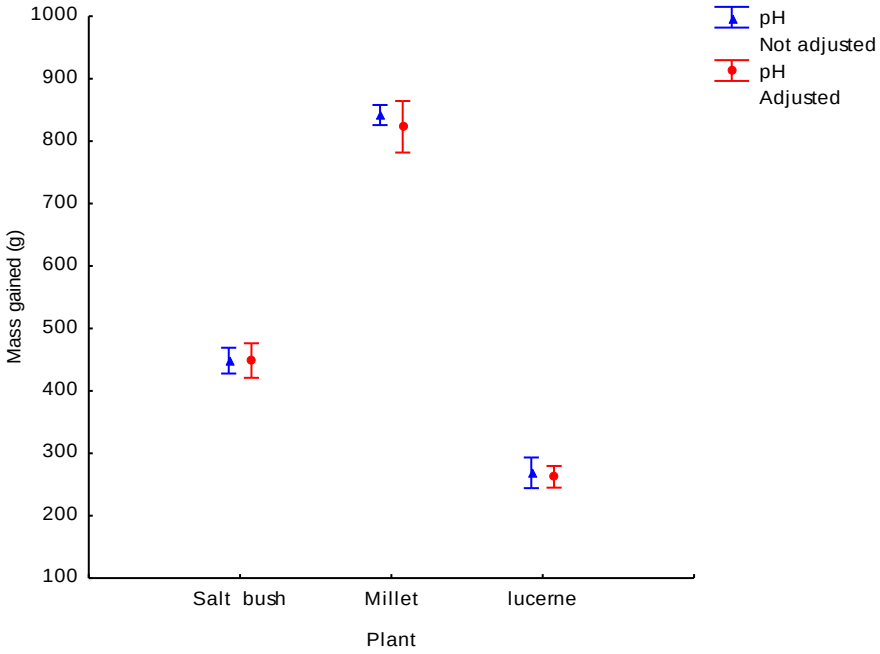

Figure 3.2 The mean (± 95% confidence interval) weight gained per pot of plants irrigated with post primary facultative pond brewery effluent with pH adjustment using sulphuric acid or without pH adjustment. (Multifactor ANOVA, $F_{(2,24)}$=0.53, p=0.59).

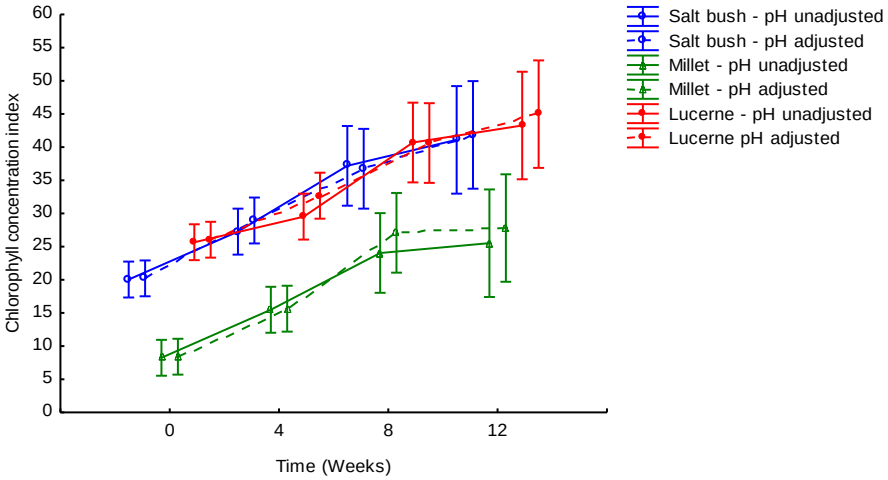

Figure 3.3 The mean (± 95% confidence interval) chlorophyll concentration index of plants irrigated with post primary facultative effluent with pH adjustment using sulphuric acid and without pH adjustment (Repeated measures ANOVA, $F_{(6,72)}$=0.28, p=0.99).

3.3.3 Plant chemical composition

Saltbush leaves had the highest Mg content (0.60 ± 0.01%) followed by millet (0.39 ± 0.01%) and lucerne (0.25 ± 0.01%; Kruskal Wallis, $H_{(5,18)}$=16.11, p=0.007; Figure 3.4). The pH adjustment of post-PFP effluent increased the leaf Mg content of saltbush and millet but not lucerne (Figure 3.4). The leaf Mn content was highest for saltbush plants (78.60 ± 0.74 mg/kg) followed by lucerne (43.58 ± 2.13 mg/kg) and millet (24.15 ± 0.40 mg/kg; Kruskal Wallis, $H_{(5,18)}$=16.25, p=0.006; Figure 3.5). The leaves of saltbush and millet plants irrigated with pH adjusted post-PFP effluent had higher Mn contents whereas lucerne plants did not (Figure 3.5). The was a significant interaction between pH regime and crop type on the Na content of the leaves of experimental crops (Multifactor ANOVA, $F_{(2,12)}$=105.77, p<0.0001; Figure 3.6). Saltbush leaf tissue had much higher Na concentrations (38024.78 ± 199.28 mg/kg) than millet or lucerne leaves (Figure 3.6). The Na leaf content of lucerne plants (4088.24 ± 106.85 mg/kg) was higher than millet plants (898.68 ± 45.26mg/kg; Figure 3.6). The pH adjustment of post-PFP effluent did not have an effect on the Na content of lucerne and millet leaves but decreased the Na content in saltbush leaves (Figure 3.6).

The Al and Fe leaf content of was influenced by an interaction between pH regime and crop type (Multifactor ANOVA, $P<0.05$). The pH adjustment of BE increased the Al and Fe leaf content of saltbush plants but not millet or lucerne plants (Table 3.3). Lucerne plants had a higher Al and Fe leaf content than saltbush and millet plants (Table 3.3). The Cu leaf content of experimental crops was influenced by an interaction between pH regime and crop type (Multifactor ANOVA, $F_{(2,12)}=23.30$, $p=0.0001$; Table 3.3). The pH adjustment of post-PFP effluent increased the Cu concentration of lucerne leaves whereas it did not influence the Cu content of saltbush or millet leaves (Table 3.3). Lucerne plants had the highest Ca, N and P leaf content (Kruskal Wallis, $p<0.007$; Table 3.3). The Ca and Cu leaf content of millet and saltbush plants were similar (Table 3.3). Saltbush plants had the lowest P leaf content (0.16 ± 0.0 1%) while millet plants had the lowest N (1.07 ± 0.02%) leaf content (Table 3.3). Saltbush leaves had the highest Cl content (1.72 ± 0.01 %), followed by lucerne (0.58 ± 0.02%) and then millet (0.36 ± 0.02%; Kruskal Wallis, $H_{(5,18)}=16.49$, $p=0.006$; Table 3.3). The K and Zn leaf content was highest in saltbush plants followed by millet and then lucerne plants (Table 3.3). The pH adjustment of PFP effluent did not seem to affect the Al, Ca, Cl, Fe and N leaf concentration of the experimental plants (Table 3.3). However the pH adjustment of PFP effluent did increase the Zn leaf content of all experimental plants (Table 3.3).

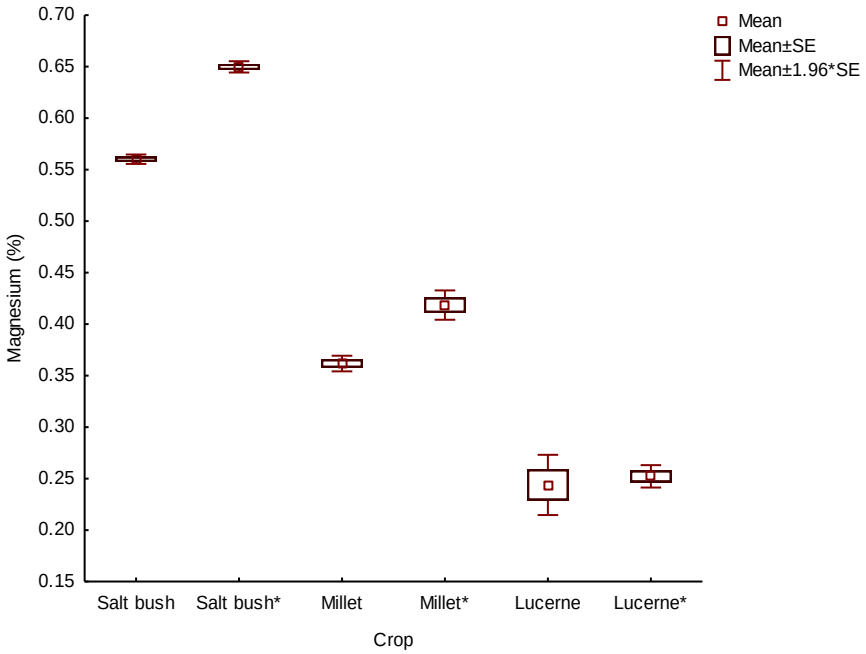

Figure 3.4 The mean leaf magnesium content of plants irrigated with post primary facultative effluent with and without pH adjustment (Kruskal Wallis, $H_{(5,18)}$=16.11, p=0.007). The irrigation water of crops marked with * were subject pH adjustment using sulphuric acid.

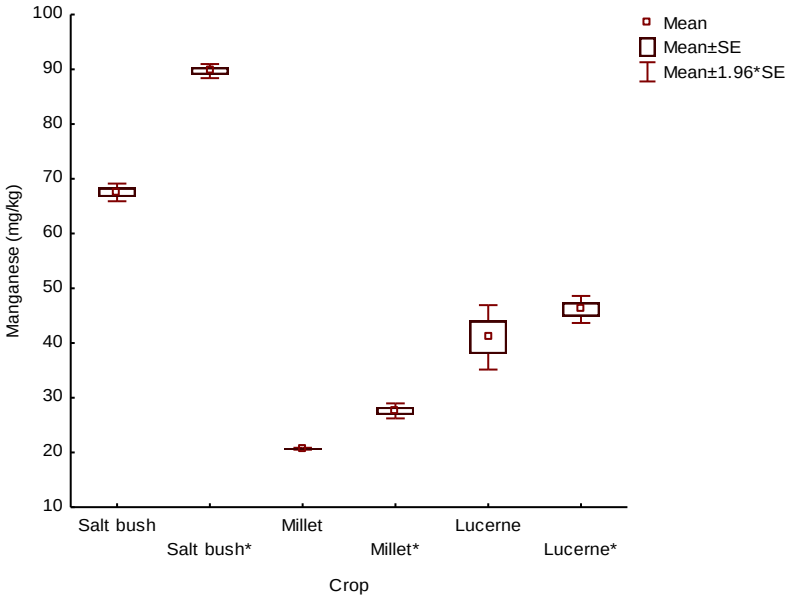

Figure 3.5 The mean leaf manganese content of plants irrigated with post primary facultative effluent with and without pH adjustment (Kruskal Wallis, $H_{(5,18)}$=16.25, p=0.006). The irrigation water of crops marked with * were subject pH adjustment using sulphuric acid.

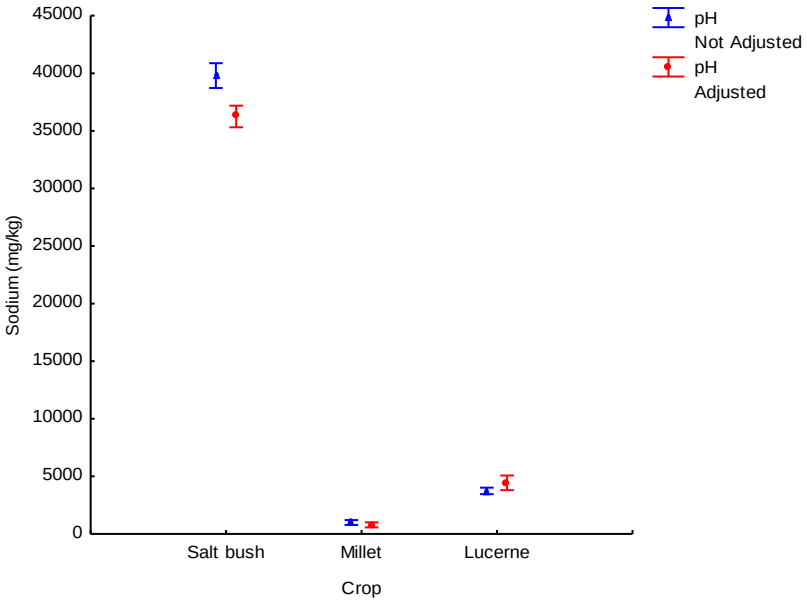

Figure 3.6 The mean (± 95% confidence interval) leaf sodium content of crops irrigated with post primary facultative effluent with pH adjustment using sulphuric acid or without pH adjustment (Multifactor ANOVA, $F_{(2,12)}$ =105.77, p<0.0001).

Table 3.3 The mean (± standard error) leaf chemical concentration of different plants irrigated with post primary facultative pond effluent at the end of the experiment. Values in the same row represented by a different superscript symbol represent significantly different treatment means (Multifactor ANOVA/Kruskal Wallis, P<0.05).

	Treatment							
Element	Saltbush	Saltbush *	Millet	Millet *	Lucerne	Lucerne *	F/H	P
Aluminium	220.23	114.74	73.59	88.04	634.13	714.77	F=20.14	0.0001
(mg/kg)	± 20.45 ^{a}	± 9.84 ^{b}	± 1.30 ^{b}	± 2.80 ^{b}	± 49.05 ^{c}	± 69.34 ^{c}		
Calcium	0.62	0.64	0.30	0.46	1.25	1.31	H=15.69	0.0078
(%)	± 0.02 ^{a}	± 0.01 ^{a}	± 0.01 ^{b}	± 0.02 ^{ab}	± 0.12 ^{c}	± 0.02 ^{c}		
Chloride	1.72	1.73	0.40	0.32	0.56	0.60	H=16.49	0.0056
(%)	± 0.01 ^{a}	± 0.01 ^{a}	± 0.00 ^{b}	± 0.03 ^{b}	± 0.02 ^{c}	± 0.01 ^{c}		
Copper	2.07	1.82	7.58	6.34	11.07	29.28	F=23.30	0.0001
(mg/kg)	± 0.23 ^{a}	± 0.26 ^{a}	± 0.25 ^{b}	± 0.14 ^{bc}	± 0.49 ^{bc}	± 3.75 ^{d}		
Iron	269.34	142.10	124.14	145.47	579.16	707.83	F=22.30	0.0001
(mg/kg)	± 22.37 ^{a}	± 10.87 ^{b}	± 3.41 ^{b}	± 4.30 ^{b}	± 39.96 ^{c}	± 76.25 ^{c}		
Nitrogen	1.81	1.96	1.03	1.11	3.12	2.89	H=16.58	0.0054
(%)	± 0.01 ^{a}	± 0.01 ^{a}	± 0.02 ^{b}	± 0.01 ^{b}	± 0.11 ^{c}	± 0.03 ^{c}		
Phosphorous	0.15	0.17	0.24	0.18	0.31	0.27	H=16.11	0.0065
(%)	± 0.01 ^{a}	± 0.00 ^{a}	± 0.01 ^{a}	± 0.01 ^{a}	± 0.01 ^{b}	± 0.00 ^{b}		
Potassium	3.53	3.63	2.52	2.89	2.04	2.08	H=16.58	0.0054
(%)	± 0.01 ^{a}	± 0.01 ^{a}	± 0.03 ^{b}	± 0.08 ^{b}	± 0.01 ^{c}	± 0.01 ^{c}		
Zinc	52.17	61.56	40.62	46.99	38.71	43.82	H=15.97	0.0069
(mg/kg)	± 0.82 ^{a}	± 0.11 ^{b}	± 0.85 ^{c}	± 2.17 ^{ac}	± 1.96 ^{c}	± 1.46 ^{c}		

The irrigation water of treatments marked with * were subject pH adjustment using sulphuric acid.

3.3.4 Soil physical properties

The MWD of soils was not influenced by an interaction between pH regime and crop type (Multifactor ANOVA, $F_{(3,32)}$=1.38, p=0.27; Figure 3.7). Soils planted with millet and lucerne had a higher MWD than soils had No Crops or saltbush plants (Multifactor ANOVA, $F_{(3,32)}$=24.73, p<0.0001). Infiltration rate was not influenced by an interaction between pH regime and crop type (Multifactor ANOVA, $F_{(3,32)}$=0.10, p=0.96; Table 3.4). The infiltration rate of the soil subject to No Crops was lower than the infiltration rate of soils subject to the experimental crops with lucerne planted soils had significantly higher infiltration rates than millet or saltbush planted soils (Multifactor ANOVA, $F_{(3,32)}$=18.54, p<0.0001). At the end of the trial there was no difference in the AFP, WHC and bulk density of the soils under the various crop treatments (Multifactor ANOVA/Kruskal Wallis, p>0.05; Table 3.4). All the physical parameters of the soil were not influenced by an interaction between pH regime and crop type (Multifactor ANOVA, p>0.05; Table 3.4), and the pH adjustment of PFP effluent had no effect on any of the physical soil parameters (Figure 3.7, Table 3.4).

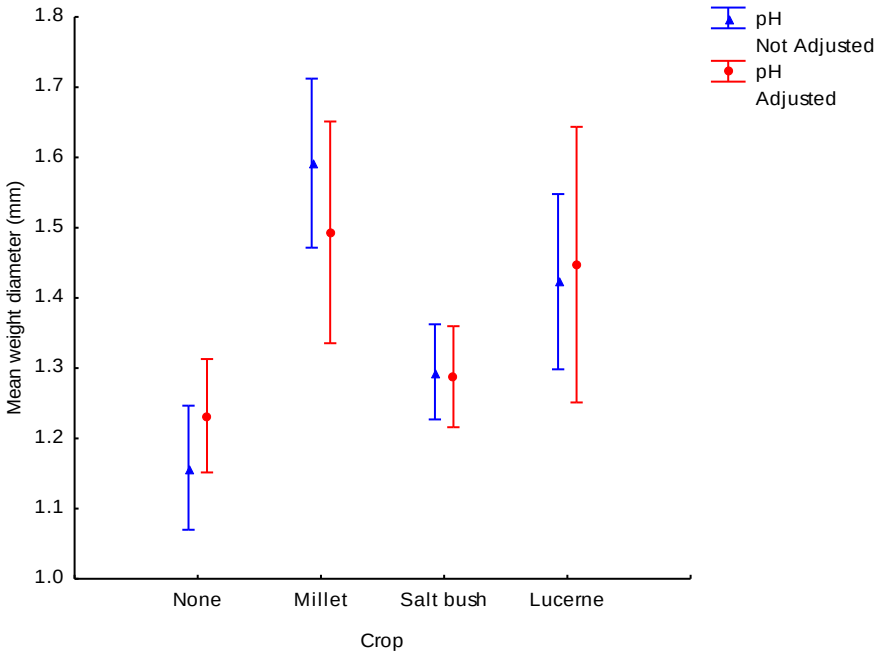

Figure 3.7 The average (± 95% confidence interval) mean weight diameter of soil from the different crop treatments, irrigated with post primary facultative pond effluent with pH adjustment using sulphuric acid or without pH adjustment (Multifactor ANOVA, $F_{(3,32)}$=1.38, p=0.27).

Table 3.4 The mean (± standard error) physical properties of soil from the different crop treatments, irrigated with post primary facultative pond effluent with and without pH adjustment at the start end of the experiment. Values in the same row represented by a different superscript symbol represent significantly different treatment means (Multifactor ANOVA/Kruskal Wallis, $p<0.05$).

		Treatment									
Property	Start	Saltbush	Saltbush*	Millet	Millet*	Lucerne	Lucerne*	None	None*	F/H	P
Air filled porosity (%)	12.47 ± 0.16	6.05 ± 0.71	6.89 ± 0.94	6.65 ± 0.38	9.03 ± 1.37	7.34 ± 0.89	5.86 ± 1.14	5.78 ±1 .10	5.87 ± 0.78	F=1.41	0.2584
Bulk density (g/cm^3)	1.01 ± 0.03	1.05 ± 0.04	1.04 ± 0.08	0.97 ± 0.12	0.96 ± 0.02	1.09 ± 0.05	1.06 ± 0.05	1.09 ± 0.06	1.05 ± 0.03	H=12.00	0.1004
WHC (%)	31.37 ± 0.98	32.26 ± 1.23	33.26 ± 1.86	32.22 ± 4.22	33.21 ± 0.60	29.58 ± 0.77	28.28 ± 0 .55	27.32 ± 3.31	32.47 ± 1.16	H=12.45	0.0866
Infiltration rate (cm/min)	2.94 ± 0.13	3.49 ± 0.94^{a}	2.91 ± 0.51^{a}	4.81 ± 0.98^{a}	4.19 ± 1.12^{a}	8.24 ± 0.49^{b}	6.85 ± 1.40^{ab}	1.81 ± 0.34^{c}	1.12 ± 0.11^{c}	F=0.30	0.8262

The irrigation water of treatments marked with * were subject pH adjustment using sulphuric acid. Values in the start column were not included in the statistical analysis. Water holding capacity (WHC).

3.3.5 Soil chemical composition

The Cl concentration of soil with no plants was the highest, with a combined (pH unadjusted no crop and pH adjusted no crop treatments) mean of 290.28 ± 10.97 mg/kg (Kruskal Wallis, $H_{(7,24)}$=26.68, p=0.004; Figure 3.8). Soils planted with the experimental crops had similar Cl levels with lucerne planted soils having the lowest (Figure 3.8). The interaction between pH regime and crop type influenced the Na concentration of the soil (Multifactor ANOVA, $F_{(3,16)}$=13.71, p=0.0001; Figure 3.9). The Na content of the soil was higher in pH unadjusted irrigation treatments for all treatments except for lucerne (Figure 3.9). Soils with No Crop treatment had the highest Na content (3447.00 ± 66.10 mg/kg) while all crop treatments had similar Na concentrations (2570.44 ± 44.69 mg/kg; Figure 3.9). The same trend was observed for SAR where No Crop soils had the highest SAR and the pH adjustment of effluent decreased the soil SAR of all treatments with the exception of lucerne (Figure 3.10). The ammonia concentration in the soil was influenced by an interaction between pH regime and crop type (Multifactor ANOVA, $F_{(3,16)}$=25.95, p<0.0001; Figure 3.11). The pH adjustment of post-PFP BE increased soil ammonia concentrations, with the increase being greater in No Crop treatments when compared to crop treatments (Figure 3.11).

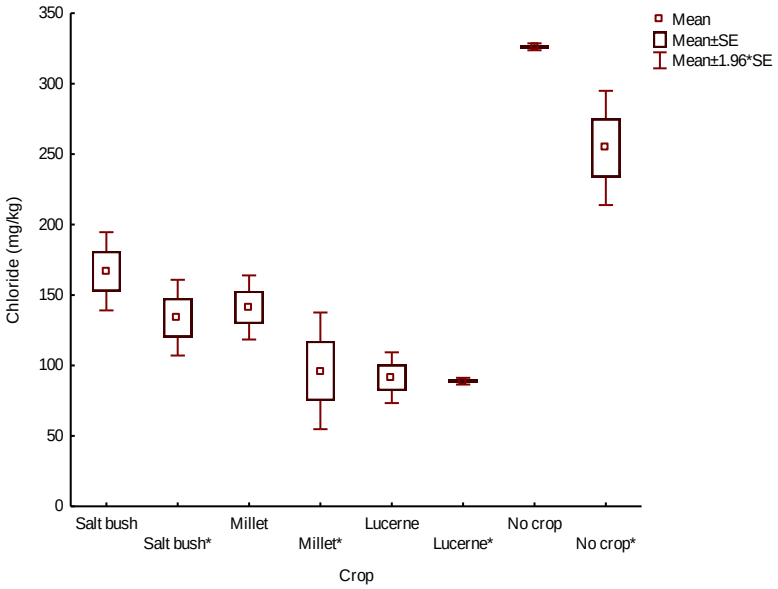

Figure 3.8 The mean chloride concentration of soil from the different crop treatments, irrigated with post primary facultative pond effluent with and without pH adjustment (Kruskal Wallis, $H_{(7,24)}$=26.68, p=0.004). The irrigation water of treatments marked with * were subject pH adjustment using sulphuric acid.

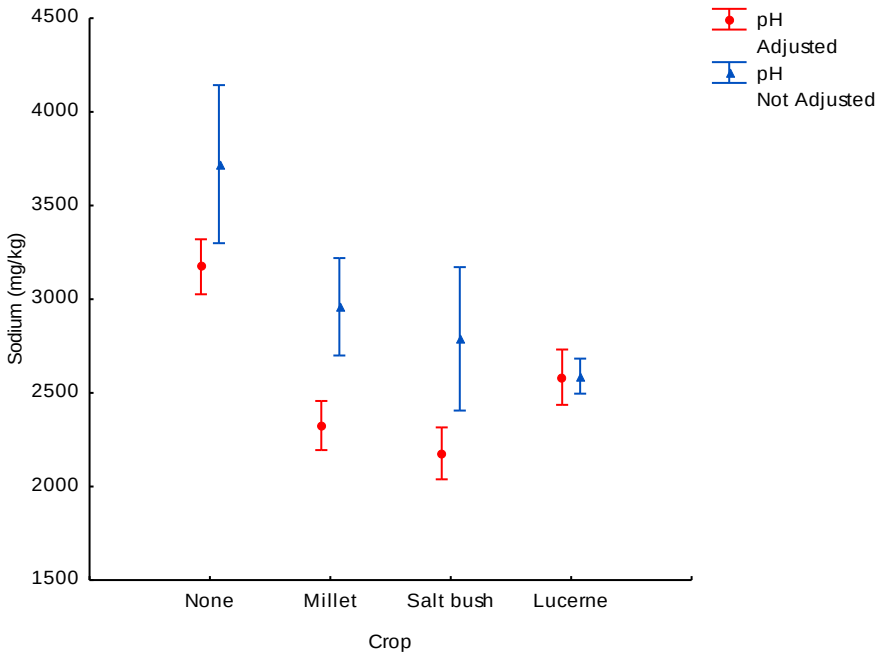

Figure 3.9 The mean (± 95% confidence interval) sodium content of soil from the different crop treatments, irrigated with post primary facultative pond effluent with pH adjustment using sulphuric acid or without pH adjustment (Multifactor ANOVA, $F_{(3,16)}$=13.71, p=0.0001).

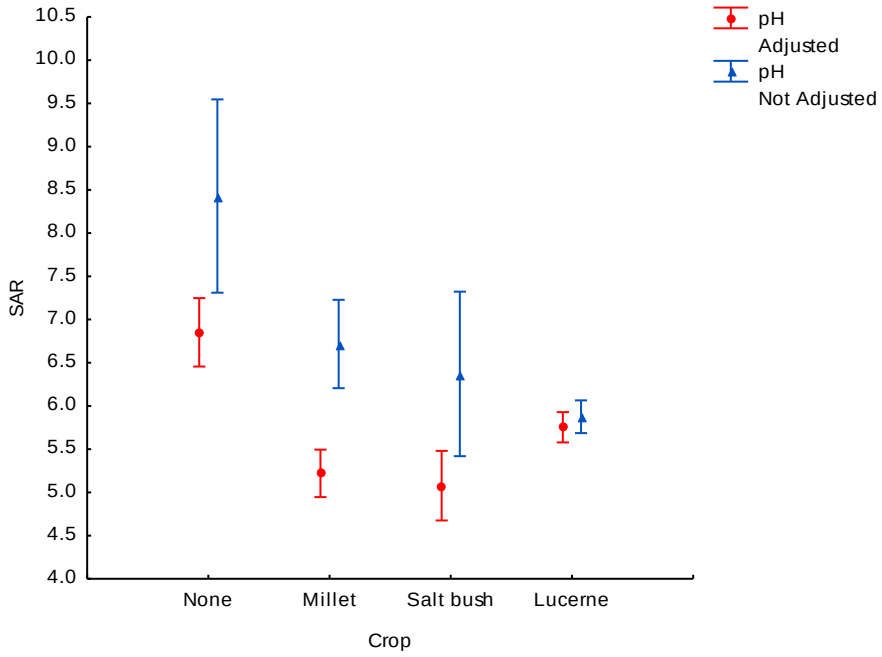

Figure 3.10 The mean (± 95% confidence interval) sodium adsorption ratio of soil from the different crop treatments, irrigated with post primary facultative pond effluent with pH adjustment using sulphuric acid or without pH adjustment (Multifactor ANOVA, $F_{(3,16)}$ =11.80, p=0.0003).

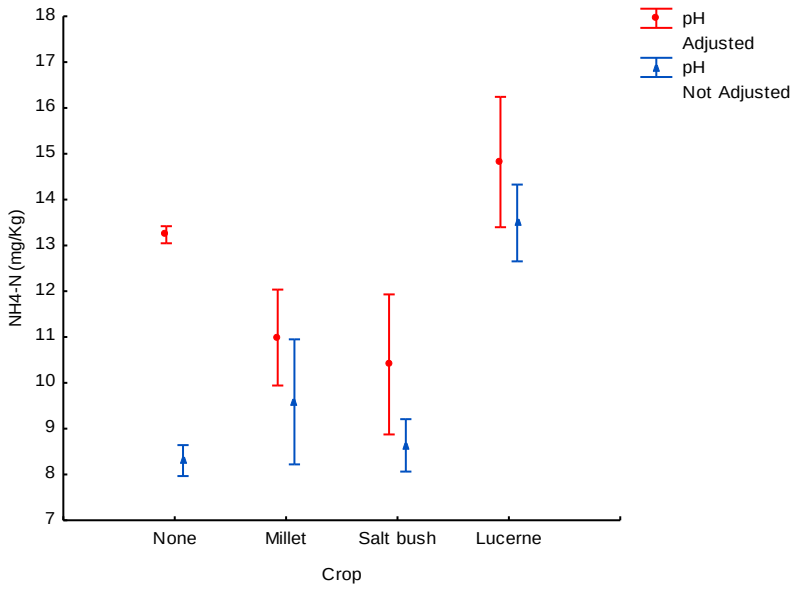

Figure 3.11 The mean (± 95% confidence interval) ammonia content of soil from the different crop treatments, irrigated with post primary facultative pond effluent with pH adjustment using sulphuric acid or without pH adjustment (Multifactor ANOVA, $F_{(3,16)}$ =25.95, p=0.0001).

After 12 weeks of irrigation the pH, conductivity and sodium concentration of the soil increased in all treatments (Table 3.5, 3.6, Figure 3.9). At the end of the trial the soil pH was similar between all treatments with the exception of the pH adjusted No Crop treatment, which had a lower soil pH (Table 3.6). Soil conductivity was not influenced by an interaction between pH regime and crop type (Multifactor ANOVA, $F_{(3,32)}$=2.88, p=0.051; Table 3.6). The soil conductivity was similar between all crop treatments (1343.60 ± 70.69 µs/cm^2) but was significantly higher in the No Crop treatments (2139.90 ± 91.74 µs/cm^2; Multifactor ANOVA, $F_{(3,32)}$=57.82, p<0.0001). The soil CEC was not influenced by and interaction between pH regime and crop type and was similar between all treatments (Multifactor ANOVA, $F_{(3,16)}$=2.29, p=0.12; Table 3.6). The soil Cu content was influenced by an interaction between pH regime and crop type (Multifactor ANOVA, $F_{(3,16)}$=5.33, p=0.01; Table 3.6). The pH adjustment of post-PFP effluent resulted in lower Cu concentrations in the soil for No Crop and millet treatments whereas pH adjustment did not effects the Cu content in the soil for lucerne and saltbush treatments (Table 3.6). Soils planted with lucerne had significantly higher K levels than all other treatments, which had similar K levels (Table 3.6). Soils from the No Crop treatments had higher Zn concentrations than all crop treatments (Table 3.6). The Ca, C, Mg, Mn and P concentration of the soil were similar between all treatments, at the end of the trial (Table 3.6).

Table 3.5: The mean (± standard error) chemical characteristics of the soil at the start of the experiment.

Element														
pH	Conductivity (µS/cm)	CEC (cmol(+)/kg)	Ammonia (mg(N)/kg)	Carbon (%)	Calcium (cmol(+)/kg)	Chloride (mg/kg)	Copper (mg/kg)	Magnesium (cmol(+)/kg)	Manganese (mg/kg)	Sodium (mg/kg)	Phosphorous (mg/kg)	Potassium (mg/kg)	Zinc (mg/kg)	SAR
8.49 ± 0.85	879.63 ± 92.41	13.51 ± 0.63	0.08 ± 0.01	0.85 ± 0.04	51.47 ± 2.93	521.24 ± 71.49	1.64 ± 0.02	15.12 ± 0.15	32.73 ± 0.89	938.94 ± 6.71	15.05 ± 0.37	463.12 ± 2.80	0.86 ± 0.03	2.24 ± 0.07

Table 3.6 The mean (± standard error) chemical characteristics of soils from the different crop treatments, irrigated with post primary facultative pond effluent with and without pH adjustment. Values in the same row represented by a different superscript symbol represent significantly different treatment means. (Multifactor ANOVA/Kruskal Wallis $p<0.05$).

	Treatment									
Element	Saltbush	Saltbush*	Millet	Millet*	Lucerne	Lucerne*	None	None*	F/H	P
pH	9.35 ± 0.05	9.20 ± 0.09	9.21 ± 0.10	9.01 ± 0.11	9.20 ± 0.09	9.09 ± 0.09	9.25 ± 0.05	8.56 ± 0.06		
Conductivity ($\mu s/cm^2$)	1312.60 ± 74.49[a]	1674.00 ± 85.19[a]	1295.80 ± 70.79[a]	1137.60 ± 54.06[a]	1400.00 ± 71.04[a]	1241.00 ± 8.56[a]	2253.00 ± 51.71[b]	2427.00 ± 183.36[b]	F=2.88	0.0514
CEC (cmol(+)/kg)	14.13 ± 0.24	12.17 ± 0.37	12.92 ± 0.98	12.62 ± 0.72	14.55 ± 0.28	13.74 ± 0.63	12.75 ± 0.91	14.01 ± 0.39	F=2.29	0.1169
NH4-N (mg/kg)	8.64 ± 0.13 [ac]	10.40 ± 0.36 [b]	9.59 ± 0.32 [c]	10.99 ± 0.24 [b]	13.49 ± 0.19 [c]	14.82 ± 0.33 [c]	8.30 ± 0.08 [a]	13.23 ± 0.04 [c]	F=25.95	0.0001
Calcium (cmol(+)/kg)	61.27 ± 0.59	62.37 ± 3.25	62.53 ± 0.67	64.60 ± 1.03	61.55 ± 0.40	64.32 ± 1.14	62.45 ± 1.06	64.03 ± 3.31	H=5.02	0.6578
Carbon (%)	0.76 ± 0.01	0.79 ± 0.02	0.75 ± 0.01	0.73 ± 0.03	0.82 ± 0.01	0.77 ± 0.01	0.73 ± 0.02	0.74 ± 0.03	H=12.66	0.0809
Copper (mg/kg)	1.80 ± 0.04 [a]	1.64 ± 0.02 [ab]	1.57 ± 0.01 [ab]	1.43 ± 0.02 [b]	1.75 ± 0.03 [a]	1.71 ± 0.02 [ab]	2.27 ± 0.11 [a]	1.86 ± 0.05 [c]	F=5.33	0.0097
Magnesium (cmol(+)/kg)	11.38 ± 0.20	11.32 ± 0.67	11.36 ± 0.33	11.26 ± 0.62	12.09 ± 0.14	12.11 ± 0.15	11.48 ± 0.06	11.93 ± 0.80	H=5.13	0.6437
Manganese (mg/kg)	40.92 ± 1.34	39.99 ± 1.41	39.49 ± 0.45	41.16 ± 0.99	43.36 ± 0.79	42.92 ± 0.62	38.34 ± 0.45	43.25 ± 1.52	F=2.58	0.0897
Phosphorous (mg/kg)	39.67 ± 1.20	35.67 ± 1.76	35.67 ± 1.20	37.00 ± 1.15	41.33 ± 0.88	43.33 ± 2.73	33.67 ± 1.67	36.00 ± 2.52	F=1.18	0.3495
Potassium (mg/kg)	435.33 ± 9.77 [a]	435.33 ± 9.42 [a]	434.33 ± 3.38 [a]	428.66 ± 6.06 [a]	517.67 ± 5.36 [b]	506.67 ± 6.36 [b]	425.00 ± 8.14 [a]	446.00 ± 2.52 [a]	F=1.69	0.2097
Zinc (mg/kg)	1.97 ± 0.03 [a]	1.98 ± 0.06 [a]	2.20 ± 0.14 [a]	2.03 ± 0.05 [a]	2.06 ± 0.04 [a]	2.29 ± 0.14 [a]	1.69 ± 0.04 [b]	1.46 ± 0.05 [b]	F=3.33	0.0461

The irrigation water of treatments marked with * were subject pH adjustment using sulphuric acid.

3.4 Discussion

3.4.1 Plant productivity

Different crops have different growth rates and have different conditions needed for good growth and health (Lucas & Davis 1961, Tyson *et al.* 2007). Millet planted in pots had the highest yield followed by saltbush and lucerne. The mean biomass production ranges were 6 to 12 ton/hectare for millet (Newman *et al.* 2010), 1 to 2 ton/hectare for saltbush (Honeysett *et al.* 2004) and 1 to 3 ton/hectare for lucerne during the first yield (Undersander *et al.* 2011). Therefore millet should generate a higher yield than saltbush or lucerne. Once all pots were harvested the lucerne in the lucerne planted pots grew back quickly and after six weeks it was ready to harvest again. This harvest would have been bigger than the first harvest. Lucerne can be grown for 5 - 7, years with yields reaching a maximum after the third harvest (Undersander *et al.* 2011).

The pH effects the availability of macro and micro-nutrients to plants and thus their growth, with 6 - 7 being the optimum range for most plants (Lucas & Davis 1961, Epstein & Bloom 2005). The pH adjustment of irrigation waters had no influence on the growth, yield or CCI of the experimental crops. Soils have the ability to withstand pH change and this known as soil buffering capacity (Buckman & Brady 1967). The higher the buffering capacity of the soil the more resistant it is to pH change (Buckman & Brady 1967). The buffering capacity of a soil is affected by the CEC, organic matter content, clay content and type of clay, and the levels of basic cations, Ca^{2+} and Mg^{2+} (Buckman & Brady 1967, de Villiers & Jackson 1967, McLean & Owen 1969). Soils with a higher organic matter content and clay content have a higher CEC, meaning they can hold more ions which buffer the soil against pH change. The buffering capacity of the soil could have neutralised the effect of the pH adjustment of BE

thus resulting in no significant difference in the growth yield, and CCI of crops irrigated with pH adjusted and pH unadjusted BE.

The bioavailability of plant nutrients is affected by pH which in turn can affect the chemical content of plant tissue (Lucas & Davis 1961, Epstein & Bloom 2005). The pH adjustment of post-PFP BE increased the magnesium and manganese leaf content of saltbush and millet plants but not lucerne plants. The Copper and zinc leaf content of all crops increased with the pH adjustment of BE. The mean pH of post-PFP BE was 8.34 ± 0.06. At this pH certain plant nutrients such as phosphorous, magnesium, manganese become less available to the plants Figure 3.12 (Lucas & Davis 1961, Epstein & Bloom 2005). Agboola & Corey (1973) found that organic matter and pH affected the elemental composition of maize tissue. The pH adjustment of BE increased the leaf chemical concentration of certain elements due to them becoming more available to plants. This effect was observed more in saltbush and millet plants with the chemical composition on lucerne plants being least affected by the alkaline pH of BE. This may be because millet and saltbush prefer a more acidic pH, whereas lucerne grows best in more alkaline soils (Honeysett *et al.* 2004, Newman *et al.* 2010, Undersander *et al.* 2011). According to Lucas & Davis (1961) the availability of P, Mg, Mn, Cu, Zn and B become restricted at pH levels above 7.5. The leaf analysis of the crop plants showed reduced levels of Mg, Mn, Cu and Zn in crops irrigated with pH unadjusted BE. However, the increased levels of certain plant nutrients in the experimental crops did not correlate with an increased yield of growth of the experimental crops.

Saltbush, millet and lucerne leaf tissue have different chemical compositions. Lucerne plants had the highest calcium, copper, nitrogen and phosphorus leaf content chemical composition. Different plant species each have different sodium and chloride leaf contents.

Saltbush plants had the highest sodium and chloride (38024.78 ± 199.28 mg/kg; 1.73 ± 0.01%) content followed by lucerne (4088.24 ± 106.85 mg/kg, 0.58 ± 0.02%) and then millet (889.68 ± 50.07mg/kg, 0.36 ± 0.02%) respectively. The sodium leaf content of saltbush generally increases as the sodium content of the soil-water interphase increases with reported ranges from 20 – 50 g/kg (Glenn *et al.* 1999, Silveira *et al.* 2009, Diaz *et al.* 2013). Therefore the sodium content in the soil should be related to the sodium content and growth of the crops. However this was not the case in this experiment because the sodium content of soils was similar between all crop treatments. This suggests that there are probably other plant-soil interactions that aid in the removal from sodium the soil such as plant assisted leaching (Chaudhri *et al*. 1964, Gritsenko & Gritsenko 1999, Owens 2001).

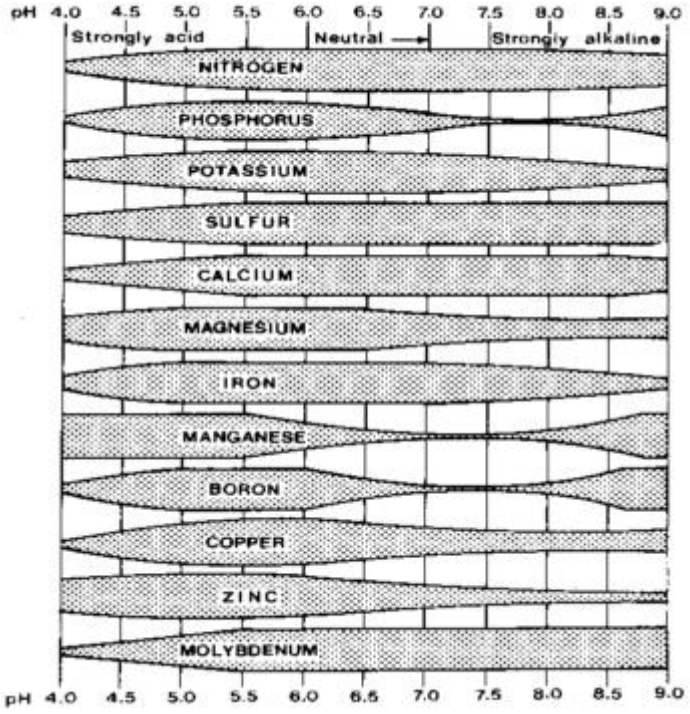

Figure 3.12 The availability of different essential elements as influenced by pH (Lucas & Davis 1961).

3.4.2 Soil fertility

Over the course of the trial the height of soil in each pot dropped. The mean AFP for all pots in the experiment decreased from 12.47 ± 0.16 to 5.95 ± 0.91%. The mean bulk density increased slightly during the trial while the water holding capacity remained similar throughout the experiment. This was because the soil in the pots was not a settled soil and in a stable state and naturally would compact over time especially when irrigated because water weakens the bonds holding the soil aggregates together causing them to compact (Van & Hill 1995). However the increase in soil bulk density and decrease in soil porosity was similar between all treatments.

The addition of sodium from irrigation waters has the ability to affect the soils physical characteristics. Soils from the No Crop treatments had a lower stability than soils from the crop treatments. Dakoure *et al.* (2013) found that the application of BE decreased the hydro structural soil properties after two years of irrigation. Sodium ions disrupt the forces that bind clay particles together and high concentrations of sodium ions in the soil cause clay particles to expand (Agassi *et al.* 1981, Qadir & Schubert 2002). The expansion of clay particles result in soil expansion and swelling, resulting in decreased soil permeability and porosity due to the clogging of soil pores by clay particles (Agassi *et al.* 1981, Qadir & Schubert 2002). The sodium content of soils with crop treatments was lower than the sodium content of soils with the No Crop treatment. This lower sodium concentration of soils from the crop treatments probably resulted in the soil having a greater stability. Crops aid in the reduction in the build-up of sodium in the soil which in turn will decrease the degradation of the soils physical profile when irrigated with slightly saline effluent (Chaudhri *et al*. 1964, Gritsenko & Gritsenko, 1999, Owens 2001, Qadir *et al*. 2003).

The infiltration rate of soils can be influenced by the crops grown in it and the sodium content of the soil. The infiltration rate of soils from the No Crop treatment decreased throughout the trial and was significantly lower than the infiltration rate of soils from the crop treatments. The addition of sodium cations to soils causes soil dispersion, reforming of the soil into a tightly packed, unstructured and cement-like crust (Agassi *et al.* 1981, Qadir & Schubert 2002). This is then linked with decreased infiltration rates, reduced hydraulic conductivity and surface crusting (Agassi *et al.* 1981, Qadir & Schubert 2002). Since the No Crop soils had the highest sodium content, it should have the lowest infiltration rate. All crop treatments increased the infiltration rate of the soil while soils planted with lucerne having the highest infiltration rate. Plant roots form macrospores in the soil and contribute to the formation of soil aggregates which aid in fluid transport and hence increase the infiltration rate of a soil (Angers & Caron 1998). Meek *et al.* (1990) found that lucerne caused a fourfold increase in the soils infiltration rates when compared to bare soils. The growth of crops on soils increases the soils hydro-physical properties compared to unplanted soils. Lucerne is a good crop to use in wastewater irrigation agricultural systems because it enhances the physical profile of the soil more than millet and saltbush.

Cation exchange capacity is a measure of a soil's ability to hold exchangeable cations (Robertson *et al*. 1999). There were no differences in the CEC values of the soils as a result of any of the irrigation treatments. Cation exchange capacity is mainly influenced by pH, organic matter and clay particles (Sollins *et al*. 1988, Robertson *et al*. 1999). The rise in pH increases the CEC of oxidic soils (Sollins *et al*. 1988). The irrigation of BE, with or without pH adjustment had no effect on the CEC of oxidic sandy loam. However after prolonged use the irrigation of BE may cause a change the CEC and pH of the soil, especially in variably charged soils (Sollins *et al*. 1988, Sumner & Naidu 1998). The irrigation of wastewaters onto soils has

been shown to both increase and decrease the CEC of soils (Kiziloglu *et al.* 2007, Kiziloglu *et al.* 2008, Kumar *et al.* 2010).

Different crop may result in different elements building up in the soil when BE is used as an irrigation water source. The soil sodium and chloride concentrations were significantly higher in the treatment with No Crop when compared to those with crops whereas there was no difference in the soil sodium or chloride concentrations with the crop treatments. Crops such as saltbush, lucerne and millet can be used to decrease the sodium content in soils and to reduce its build up (Chaudhri *et al*. 1964, Gritsenko & Gritsenko 1999, Owens 2001, Qadir *et al*. 2003). This happens through two processes. Firstly sodium and chloride are assimilated into the plant tissue thus reducing the salt content of the soil (Chaudhri *et al*. 1964, Gritsenko & Gritsenko 1999, Owens 2001). Secondly, the phenomenon occurs whereby plant roots "increase the dissolution rate of calcite, resulting in enhanced levels of Ca^{2+} in soil solution to replace Na^{+} from the cation exchange complex" (Qadir *et al.* 2005). Qadir *et al.* (2003) found that soil sodium removal by lucerne plant tissue accounted for less than five percent of the total sodium removed from the soil. The majority of the sodium was removed from the soil through leaching (Qadir e*t al.* 2003). The leaching of sodium through the soil was higher in lucerne planted soil compared to unplanted soil (Qadir e*t al.* 2003). Even though the leaf sodium content and yield of the cops in this experiment was different, the sodium content of the soil remained the same. This supports the idea that sodium is mainly removed from the soil through plant assisted leaching.

Qadir *et al.* (2003 & 2005) has identified the following plant-soil process which increases the dissolution rate of calcite which in turn increases the levels of Ca^{2+} in soil solution that can replace Na^{+} from the cation exchange complex. The increase in soil atmosphere $C0_2$ from

the respiration of roots and associated microorganism's increases the dissolution of $C0_2$ in water to form carbonic acid. Carbonic acid then dissociates into a hydrogen ion (H^+) and bicarbonate. The reaction of the hydrogen ion with soil calcium carbonate produces Ca^{2+} and HCO_3^-. The Ca^{2+} can now exchange with sodium at the soils cation exchange sites resulting in sodium ions being leached out in the soils percolating water which in turn reduces the soils sodium content and SAR (Qadir *et al.* 2003, Qadir *et al.* 2005). The pH adjustment of BE would have increased the concentration of hydrogen ions in the irrigation water and soil. The pH adjustment of BE resulted in a decrease in the sodium content of the soil of all crop treatments except for lucerne. This might have been be due to the addition of hydrogen ions during pH adjustment which increased the dissolution rate of calcite in the soil and thus increased the levels of Ca^{2+} in soil solution that can replace Na^+ from the cation exchange complex. The leaching of sodium in soil is dependant of the pH of the irrigation water with acidic irrigation waters increasing sodium leaching through the soil due to the addition of hydrogen ions which can react with calcium carbonate. To support this information future experiments should collect the leachate at the bottom of the pots to determine its composition.

The pH affects the availability of plant nutrients and the pH adjustment of irrigation waters will make certain nutrients more available to plants, which may affect the build-up/reduction of certain elements in the soil (Epstein & Bloom 2005). The soil copper concentration was lower in all pH adjusted pots. The leaf copper concentration was higher in all experimental crops. The leaf copper concentration is influenced by pH and organic matter of the soil (Agboola & Corey 1973, Parker & Pedler 1997, Epstein & Bloom 2005). The pH adjustment of irrigation waters changes the availability of certain plant nutrients which can affect their build up in the soil. However, the leaf Mn and Mg content was high in pH

adjusted treatments, but the soil Mn and Mg content was similar between all treatments. This could mean that there are other factors such as leaching that are affecting the concentration of nutrients in the soil.

Different soils have different nutrient cycling processes and the influence of pH on the nutrient mobility can also be different (Sollins *et al.* 1988). Soils can be divided into two classes based on the major factors affecting nutrient mobility: permanent charge (pc) and variable charge (vc) surfaces, which hold plant nutrients in the soil (Sollins *et al.* 1988). Moist soils are comprised of both surfaces but are dominated by one or the other. The soil used in this experiment was an oxidic sandy loam which are dominated by vc surfaces (Sollins *et al.* 1988). There was no difference in the soil phosphate, phosphorous, magnesium, manganese and calcium content of all the treatments. The mobility of P and K in vc dominated soils is hardly regulated by pH and leaching of P and K is minimal in most soils except for sandy soils (Sollins *et al.* 1988, Pieri 1989). Magnesium, manganese and calcium are leached quite easily out of soils (Sollins *et al.* 1988, Lehmann & Schroth 2003). The leaching process is pH dependant in vc dominated soils with more leaching occurring as the pH decreases (Sollins *et al.* 1988, Lehmann & Schroth 2003). The pH adjustment of BE did not seem to affect the mobility and leaching of P, K, Mn, Mg and Ca in the vc dominated oxcidic sandy loam. This could be because vc soils normally have a high buffering capacity and the pH of the effluent had little effect on the pH of the soil (Sollins *et al.* 1988). The dynamics between soil charge type, pH and nutrient mobility are complex and future studies should collect the leachate from the pots to determine exactly what is being leached out the soil.

Nitrosomas and *Nitrobacter* are nitrifying bacterial species that convert ammonia nitrogen to nitrate nitrogen (Antoniou *et al.* 1989). Their growth and performance is pH dependant, with an optimum range is 7.5 – 8.2 (Antoniou *et al.* 1989). Nitrifying bacteria growth rates are reduced at pH levels below 6.5 (Hall 1974, Painter and Loveless 1983, Antoniou *et al.* 1989). In all crop treatments the soil ammonium levels were higher in pH adjusted irrigation treatments. The pH adjustment of BE may have decreased the growth and activity of soil nitrifying bacteria which lead to the increase in ammonia in the soil when compared to unadjusted irrigation treatments. Acidic irrigation waters can be expected to increase the nitrogen leaching of soils (Lehmann & Schroth 2003). The pH of irrigation waters can affect the activity of soil bacteria which will influence the buildup/removal of certain plant nutrients such as ammonia (Sollins *et al.* 1988, Lehmann & Schroth 2003).

3.4.3 Conclusion

Post primary facultative BE supported crop growth and all crops irrigated with post-PFP effluent showed no signs of nutrient deficiency. The pH adjustment had no influence on crop growth and health, which was probably due to the buffering capacity of the soil. However some pH related responses where noticed in the chemical properties of the soil and plant tissue. This illustrates the importance of understanding pH effects, and how they affects the cation exchange capacity of soils and the mobility of certain nutrients and elements. Future research should focus on the ideal soil type to be used in agricultural production systems where saline effluents are used as a source of irrigation. Variable charged soils with a high CEC should be most suitable for irrigation with alkaline effluents such as BE. The crop treatments reduced the build-up of sodium in the soil but not but stop it. The primary way in which sodium was removed from the soil was probably leaching and this was found to be pH dependant, with increased sodium leaching occurring at lower pH

values. Future pot studies should collect the leachate coming out of the pots to confirm this. In this way, a mass balance could be done to determine exactly how much sodium was removed by the plant tissue and by leaching through the soil. Of the crops grown, lucerne showed the most promise because it improved the soils physical properties. It is also a popular fodder crop, can grow well in alkaline environments and can be harvested multiple times from one stand. Future studies should quantify the quantities of sodium that are taken up by the lucerne plants and the effect that irrigation frequency and duration have on sodium lost by leaching, in the presence of lucerne plants.

Chapter 4: Hydroponic production

4.1 Introduction

Brewery effluent (BE) can successfully be used as an irrigation source in irrigated crop production; however, there are still uncertainties regarding how suitable it is for this purpose (Chapters 1 & 2). The issues that need to be addresses are: how much sodium can be removed by crop biomass production, the effect of alkalinity and pH of BE on nutrient availability, plant growth and health and does it lack certain plant nutrients?

When BE is used to irrigate plants in the soil the soil may buffer the plants against the some of the unsuitable properties of BE (Pope & Vasey 1976, Van Breemen *et al.* 1983). Soils may buffer the high pH and alkalinity of BE (Buckman & Brady 1967). The results from a cabbage production experiment suggested that the soil was able to buffer the effect of the high pH BE.

Cation exchange capacity (CEC) is a measure of a soil's ability to hold onto exchangeable cations, and specifically to plant nutrient cations, which are attached to negatively charged clay and organic matter particles in the soil (Robertson *et al*. 1999, Epstein & Bloom 2005). The soil solution is the most important source of nutrients for terrestrial plants (Epstein & Bloom 2005). Cabbage plants grown on post-primary facultative pond (PFP) effluent showed no signs of nutrient deficiency. However, the soil solution could be supplying plants with nutrients that are lacking in BE. To understand whether BE lacks any key plant nutrients, plants need to be grown in soilless culture systems where the supply of nutrients from the soil is eliminated. Hydroponic plant production is one way to grow plants in the absence of

soil, and could be used to better understand the suitability of BE as a nutrient source for their growth.

Another question raised in Chapter 3 is how much sodium plants are able to assimilate into their tissue. In hydroponic systems it is possible to monitor the sodium concentration in the hydroponic solution and thus determine how much sodium is removed by plants. The nutrient removal rates in hydroponic solutions can also be measure to determine what proportion of the minerals in BE is utilised by plants.

The same crops as grown in previous experiments (Chapters 2 & 3) were grown in hydroponic systems to get a better understanding of how the properties of post-PFP BE directly affect plant growth and health. It also allows for the comparison of hydroponic and soil production systems to determine which is more suitable for the use of BE to grow irrigated crops.

4.1.1 Aims and objectives

The aim of this study was to determine what nutrients plants are able to remove from BE, the influence of pH on their removal rate, and the effect of alkalinity and pH on crop growth and plant health in a hydroponic production system. This was done by conducting two experiments: 1) a nutrient solution experiment; and 2) a crop performance experiment.

Nutrient solution experiment

Cabbages were grown on three different nutrient solutions, in a recirculating hydroponic system, where the pH of each nutrient solution was or was not adjusted. The objectives were to determine:

1) the effect of nutrient solution type and pH on the health, growth and leaf chemical composition of cabbage plants grown in a hydroponic production system; and
2) the effect of pH on the removal of nutrients and elements from nutrient solutions by cabbages plants grown in a hydroponic production system.

Crop performance experiment

Cabbage, saltbush and millet plants were grown in a recirculating hydroponic system fed with pH adjusted or pH unadjusted post-PFP BE. The objectives of this experiment were:

1) to compare the growth, health and chemical composition of cabbage, saltbush and millet plants grown in a hydroponic system fed with BE with and without pH adjustment; and
2) to compare the effect of pH on the nutrient, sodium and chloride removal rates of cabbage, saltbush and millet from post-PFP BE grown in a hydroponic production system.

4.2 Methods and materials

The two experiments were carried out simultaneously in identical recirculating hydroponic systems. The first was a nutrient solution experiment where cabbages were grown using different irrigation sources. This experiment corresponds with a prior experiment on the production cabbage (Chapter 2). The second experiment was a crop performance experiment where different crops were grown on post-PFP BE, which corresponded to a previously conducted crop suitability experiment (Chapter 3).

4.2.1 Nutrient solution experiment

Experimental species

Cabbage (*Brassica oleracea* cv. Star 3301; Starke Ayres Pty Ltd, South Africa) was grown for 12 weeks in identical recirculating hydroponic systems to observe their nutrient removal capabilities from BE, concurrently measuring their health and growth. Two hundred cabbage seedlings were purchased from a commercial seedling supplier (Moorlands Seedlings Pty Ltd, Humansdorp). Of these, 90 similar size seedlings were used for this experiment.

Treatments

Three irrigation water sources were used which included post-PFP BE, tap water (Water) and a conventional irrigation source consisting of an inorganic fertiliser and tap water (NS). The pH of each irrigation water source was either adjusted to 6.5 with 98% sulphuric acid (Protea Chemicals Pty Ltd, South Africa) or left unadjusted. This resulted in six irrigation treatments (Table 4.1). The conventional irrigation source was comprised of commercially available inorganic-fertilizer (Hygroponic®, Hygrotech Pty Ltd, South Africa; Registration number K5709; Act 36 of 1947), and calcium nitrate with a composition of 11.7% nitrogen and 16.6% calcium, mixed in a ratio of 1:0.8 and dissolved in municipal water to achieve an EC of 1800 µm (Chapter 2; Table 2.2).

Table 4.1 Irrigation treatments (T1 – T6) that were used to irrigate cabbage plants.

Water source	pH not adjusted	pH adjusted to 6.5
Primary facultative pond	T1	T2
Municipal water	T3	T4
Municipal water + inorganic fertiliser	T5	T6

Experimental system

The experiment was carried out in 18 identical recirculating hydroponic growing systems each containing five pots (Figures 4.1, 4.2, 4.3). Each treatment was replicated three times with a replicate consisting of an entire hydroponic system.

The system was a variation of the Dutch bucket hydroponic system (Roberto 2005). Each channel was made of one 1500 mm long 160 mm diameter polyvinylchloride (PVC) pipe with a series of 110 mm holes drilled in the top to accommodate the pots (Power 2014; Figure 4.1). Each pot was a 120 mm common plastic garden pot which was extensively perforated with a 5.0 mm drill bit (Power 2014). The pots were filled with rinsed and dried 10 mm diameter crushed gravel which served as physical support for the plants but provided no nutritional benefit to the plants (Power 2014).

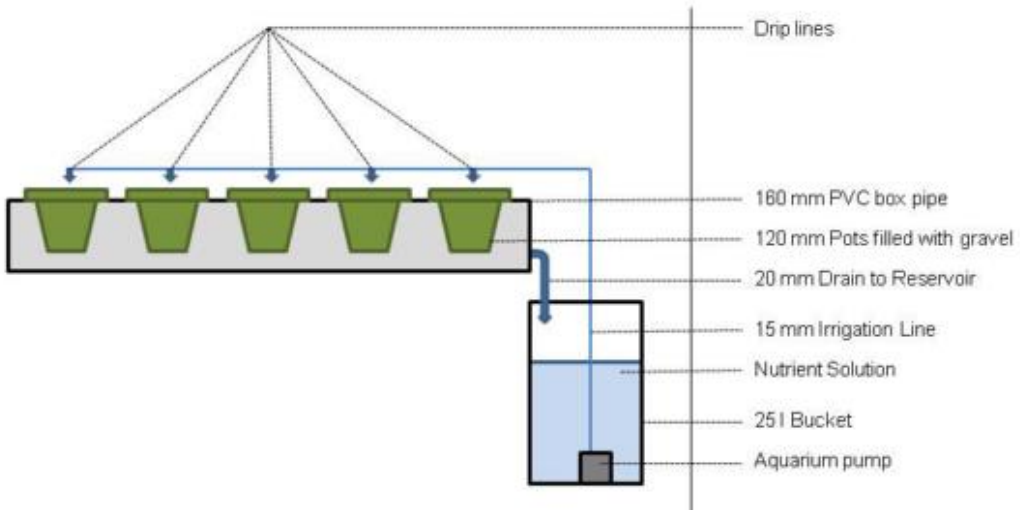

Figure 4.1 Cross-section of an individual hydroponic system with pots in which plants were grown, nutrient solution sump (drain reservoir), pump and irrigation line (not drawn to scale; Power 2014).

Figure 4.2 Views of the completed growth channels including reservoir, pump, irrigation connections, gravel-filled pots, and drain (Power 2014).

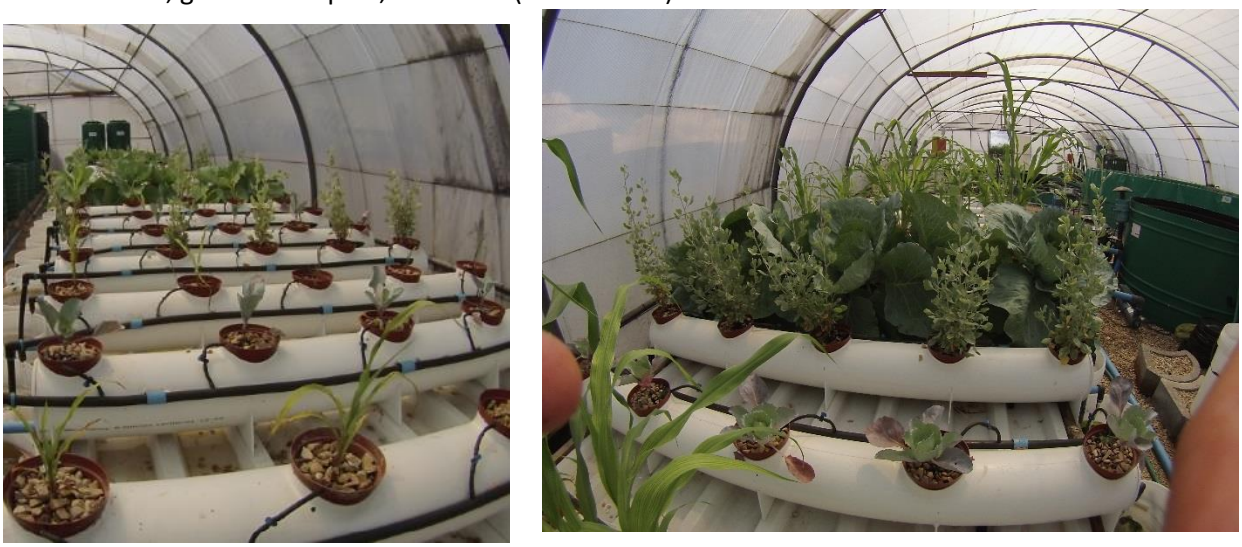

Figure 4.3 The hydroponic experimental system six (left) and ten (right) weeks after planting.

The nutrient solution for each channel was contained in a 25 L plastic bucket stored on the ground at the foot of each channel (Power 2014). An 18 watt submersible aquarium pump (Resun, Model: SP-2500, China) fed the nutrient solutions up through a 15 mm irrigation line along the length of the channel. A 5.0 mm spaghetti tube, fitted with a micro-valve to ensure even discharge along the pipe, connected the 15 mm main line to each of the gravel filled pots (Power 2014).

A 20 mm drain hole was cut into the end of each channel (Power 2014). A 20 mm PVC pipe was fitted into this hole and extended both into and out of the 160 mm channel (Power 2014). A 20 mm plastic elbow joint was fitted on either end of the 20 mm PVC pipe (Power 2014). The elbow inside the channel was orientated upwards to raise the drainage level of the channels to roughly 50 mm and to create a submerged root zone for the plants (Figure 4.2; Power 2014). The outer elbow was orientated downwards, towards the nutrient solution reservoir (Power 2014). The outer elbow was connected to the reservoir by a 20 mm plastic hose (Power 2014). This created a closed, recirculating system (Power 2014).

At the start of the trial one plant was planted in each pot filled with gravel. The nutrient solution in each hydroponic system was replaced every seven days or when the water level in the reservoir was less than 25% full, whichever came first.

Data collection

Water quality parameters where measured in newly added nutrient solutions and old nutrient solutions, just before replacement with a new solution. The water quality parameters measured during the experiment included ammonia, nitrite, nitrate, phosphate, chloride, chemical oxygen demand (COD), dissolved oxygen (DO) pH and electrical conductivity (EC). These parameters were measured using the same equipment and techniques described in Chapter 2 (Section 2.2.5). Dissolved oxygen was measured using a hand held probe (Hanna, HI 991300, United Kingdom).

Total nitrogen (total N) was calculated by using Equation 4.1:

$$\text{Total nitrogen} = [\text{N-NH}_4^+] + [\text{N-NO}_2^+] + [\text{N-NO}_3^+] \quad [4.1]$$

At the beginning of the trial the mass of each plant planted in each pot was recorded (0.1 g accuracy). At the end trial the mass of the each plant was also recorded (0.1 g accuracy). The height and width of plants was recorded at the start of the trial and every week until the end of the trial (1 mm accuracy). Chlorophyll concentration (CCI) readings were recorded at the start of the trial and every four weeks until the end of the experiment, on the uppermost fully expanded leaf of each plant (CCM-200 Plus Chlorophyll Content Meter, Opti-Sciences Inc., USA). At the end of the trial plant leaves from each replicate were sent to a commercial analytical laboratory (BemLab Pty Ltd, Strand, South Africa). These samples were analysed for N, P, Na, Cl, K, Al, Ca, Cu, Fe, Mn, Mg and Zn from each treatment, with a sample consisting of plant leaves from one hydroponic system.

Photographs of the plants and stress symptoms of the plants were described and recorded to determine if the plants were experiencing any nutrient deficiencies or diseases. Daily temperature data were recorded using a thermometer (Hanna, HI 991300, United Kingdom).

Statistical analysis

The same statistical analysis that was used in Chapter 2 (Section 2.2.6) was used to analyse the data in this experiment.

4.2.2 Crop performance experiment

Experimental species

Cabbage (*Brassica oleracea* cv. Star 3301), saltbush (*Atriplex nummularia*) and Japanese millet (*Echinochloa esculenta*) were grown in recirculating hydroponic systems to observe

their capacity to remove sodium from BE. Sixty saltbush seedlings were purchased from a commercial nursery (Mountain herb estate, Pty Ltd, Pretoria). Japanese millet seeds were obtained from a commercial seed supplier (Agricol Pty Ltd, Port Elizabeth) and were planted in polystyrene planter trays, filled with a mixture of 40% soil and 60% compost. These seeds were germinated and allowed to grow for three weeks, prior to the start of the experiment. Similar size plants for each plant species were used.

Treatments

The four crop treatments used in this experiment were cabbage, millet, saltbush and No Crop. The No Crop treatment served as the control. Each crop treatment was irrigated with post-PFP effluent, were the pH was either adjusted to 6.5 with 98% sulphuric acid (Protea Chemicals Pty Ltd, South Africa) or left unadjusted. This resulted in eight experimental treatments being tested (Table 4.2). Treatments T1 and T2 (Table 4.2) are shared with the nutrient solution experiment presented in this chapter (Table 4.1).

Table 4.2 The eight (T1 and T2 and T7 – T12) treatments used in this experiment.

Plant	pH not adjusted	pH adjusted to 6.5
Cabbage	T1	T2
Saltbush	T7	T8
Millet	T9	T10
No Crop	T11	T12

Experimental system

Plants were grown in 24 recirculating hydroponic systems which were identical to the ones used in the nutrient solution experiment (*Experimental system*, Section 4.2.1; Figures 4.1, 4.2, 4.3) Six of the hydroponic systems were (i.e. the replicates for T1 and T2) were shared with this experiment and the nutrient solution experiment (Section 4.2.1). There were thus a total of 36 systems used for both experiments combined (Figures 4.1, 4.2, 4.3).

Experimental treatments and their replicates were applied to pot using a complete randomisation design.

Data collection

The same data collected in the nutrient solution experiment (Section 4.2.1) were collected in this experiment.

Statistical analysis

The same statistical analyses that were used as in Chapter 2 (Section 2.2.6) were used to analyse the data in this experiment.

4.3 Results

4.3.1 Nutrient solution

Water quality

At the start post-PFP BE had the highest pH (8.36 ± 0.03) followed by tap water (7.69 ± 0.04) and then nutrient solution (7.34 ± 0.06; Table 4.3). The pH in all systems increased over time (Table 4.3 & 4.4). The mean pH of the effluent systems, prior to replacement, (8.71 ± 0.06) was higher than the NS (8.07 ± 0.04) or Water treatments (8.07 ± 0.05; Table 4.4). Just before replacement, the pH of effluent unadjusted treatments (9.06 ± 0.06) was higher than effluent adjusted treatments (8.36 ± 0.05; Table 4.4). Effluent treatments had the highest starting conductivity (3068.53 ± 42.69 µs/cm^2) followed by the NS (1838.61 ± 40.77 µs/cm^2) and the Water treatments (579.22 ± 9.00 µs/cm^2; Table 4.3). The conductivity of BE treatments increased from 2913.80 ± 46.78 to 3223.26 ± 38.59 µs/cm^2 when the pH was adjusted (Table 4.3). The conductivity of BE and Water treatments did not change while the

conductivity of NS treatments decreased (Table 4.3, 4.4). The pH adjustment of irrigation solutions had no effect on the conductivity of old irrigation solutions (Table 4.4).

Dissolved oxygen was not influenced by an interaction between pH regime and water source for fresh or old nutrient solutions (Multifactor ANOVA, $p>0.05$; Table 4.3, 4.4). The initial DO levels where significantly higher in NS and Water treatments than any of the BE treatments (Multifactor ANOVA, $F_{(2,192)}=99.72$, $p<0.0001$). The DO of old irrigation solutions was similar in all hydroponic systems (Multifactor ANOVA, $F_{(2,192)}=0.72$, $p=0.49$). Chemical oxygen demand decreased in BE treatments from a mean of 214.83 ± 6.37 to 106.00 ± 3.17 mg/l (Table 4.3, 4.4). In NS and Water treatments the COD increased from a mean of 15.30 ± 0.38 to 22.20 ± 0.72 mg/l (Table 4.3, 4.4).

The BE systems had the highest starting total N (56.21 ± 2.60 mg/l) followed by NS (40.65 ± 1.74 mg/l) and water (10.08 ± 0.39 mg/l; Table 4.3). Total N decreased in all experimental systems, with the BE systems having the highest end total N, followed by NS and then Water (Kruskal Wallis, $H_{(5,198)}=130.06$, $p<0.0001$; Table 4.4) . The pH adjusted BE systems had a lower mean end total N (14.89 ± 0.55 mg/l) than the unadjusted BE systems (23.14 ± 0.49 mg/l); this was not observed in NS of Water systems (Table 4.4). The same trend was observed for nitrate, because nitrate made the bulk of total N (Table 4.4). Effluent systems had the highest starting ammonia concentration (36.73 ± 2.28 mg/l) followed by NS (17.36 ± 0.85 mg/l) and then water (1.67 ± 0.11 mg/l; Table 4.3). The ammonia concentration of old irrigation solutions was similar between all experimental systems (Kruskal Wallis, $H=9.54$, $p=0.09$; Table 4.4). The nitrite concentration in new irrigation solutions was higher in BE systems (0.83 ± 0.05 mg/l) while NS and Water systems (0.04 ± 0.01 mg/l) had similar starting nitrite concentrations (Table 4.3). All experimental systems had similar old irrigation

solution nitrite concentrations (Kruskal Wallis, H=8.94, p=0.11; Table 4.4). Effluent and NS systems had the highest starting phosphate concentration (28.01 ± 0.60 mg/l), while water systems had the lowest (12.24 ± 0.52 mg/l; Table 4.3). At replacement the BE systems had the highest phosphate concentration followed by NS and then Water systems (Table 4.4). The pH adjusted BE systems had lower end phosphate concentrations (18.61 ± 0.60 mg/l) than the pH unadjusted BE systems (26.49 ± 0.72 mg/l; Table 4.4).

Effluent hydroponic systems had a higher starting sodium concentration than Water or NS systems (Table 4.3). The sodium concentration increased in all systems with BE systems having the highest end sodium concentration followed by NS and water systems (Kruskal Wallis, H=133.60, P<0.0001; Table 4.4). The pH adjustment of hydroponic systems had no influence on the sodium concentration of the old nutrient solutions (Table 4.4). The mean starting chloride concentration of BE systems (191.34 ± 5.05 mg/l) was higher than NS and Water systems (104.23 ± 1.53 mg/l). The chloride concentration increased in all systems with BE systems having the highest end chloride concentration of 225.55 ± 6.47 mg/l (Table 4.3, 4.4). Nutrient solution and Water systems had a combined mean end chloride concentration of 128.21 ± 2.21 mg/l (Table 4.4).

Table 4.3 The mean (± standard error) water quality parameters of fresh irrigation solutions. Values in the same row represented by a different superscript symbol represent means that are significantly different (Multifactor ANOVA/Kruskal Wallis, P<0.05).

Parameter	PFP	PFP*	NS	NS*	Water	Water*	F/H	P
pH	8.36	6.54	7.34	6.72	7.69	6.57		
	± 0.03	± 0.10	± 0.06	± 0.07	± 0.04	± 0.07		
Conductivity	2864.44	3207.83	1888.22	1924.25	567.06	594.08	H=194.53	0.0001
(µs/cm²)	± 38.94 [a]	± 38.71 [a]	± 15.14 [b]	± 15.90 [b]	± 8.83 [c]	± 9.22 [c]		
DO	79.38	78.63	91.15	88.97	89.98	89.21	F=1.37	0.2559
(mg/l)	± 1.14 [a]	± 1.00 [a]	± 0.69 [b]	± 0.84 [b]	± 0.83 [b]	± 0.73 [b]		
COD	213.21	216.44	17.23	18.84	17.41	15.51	H=161.66	0.0001
(mg/l)	± 6.68 [a]	± 6.06 [a]	± 0.41 [b]	± 0.55 [b]	± 0.39 [c]	± 0.16 [c]		
Total N	57.59	54.83	40.67	40.63	10.15	10.00	H=178.64	0.0001
(mg/l)	± 2.46 [a]	± 2.74 [a]	± 1.74 [b]	± 1.74 [b]	± 0.41 [c]	± 0.36 [c]		
NH_4-N	36.55	36.91	17.22	17.50	1.66	1.67	H=190.36	0.0001
(mg/l)	± 2.30 [a]	± 2.25 [a]	± 0.85 [b]	± 0.84 [b]	± 0.11 [c]	± 0.10 [c]		
NO_2-N	0.85	0.80	0.04	0.04	0.03	0.03	H=148.03	0.0001
(mg/l)	± 0.04 [a]	± 0.05 [a]	± 0.01 [b]	± 0.01 [b]	± 0.01 [b]	± 0.01 [b]		
NO_3-N	20.19	21.13	23.37	23.13	8.31	8.45	H=182.88	0.0001
(mg/l)	± 0.50 [a]	± 0.69 [a]	± 0.92 [b]	± 0.92 [b]	± 0.32 [c]	± 0.35 [c]		
PO_4-P	27.86	27.28	28.12	28.77	12.36	12.12	H=165.99	0.5683
(mg/l)	± 0.51 [a]	± 0.70 [a]	± 0.63 [a]	± 0.55 [a]	± 0.56 [b]	± 0.47 [b]		
Cl	188.62	194.08	104.72	108.36	103.20	100.62	H=156.24	0.0001
(mg/l)	± 4.98 [a]	± 5.12 [a]	± 1.96 [b]	± 1.22 [b]	± 1.61 [b]	± 1.31 [b]		
Na	613.54	27.05	151.59	154.26	144.64	143.79	H=147.63	0.0001
(mg/l)	± 9.33 [a]	± 9.06 [a]	± 2.46 [b]	± 3.21 [b]	± 2.82 [b]	± 2.60 [b]		

Treatments marked with * were subject to pH adjustment using sulphuric acid. Primary facultative pond (PFP), nutrient solution (NS), chemical oxygen demand (COD), dissolved oxygen (DO).

Table 4.4 The mean (± standard error) water quality parameters of old irrigation solutions. Values in the same row represented by a different superscript symbol represent means that are significantly different (Multifactor ANOVA/Kruskal Wallis, P<0.05).

Parameter	PFP	PFP*	NS	NS*	Water	Water*	F value	P value
pH	9.06	8.36	8.09	8.04	8.14	7.99		
	± 0.06	± 0.05	± 0.04	± 0.04	± 0.04	± 0.05		
Conductivity	3184.36	3202.61	916.36	979.36	523.00	540.67	H=189.24	0.0001
(µs/cm²)	± 55.49 [a]	± 41.40 [a]	± 30.02 [b]	± 19.34 [b]	± 8.67 [c]	± 7.22 [c]		
DO	86.11	85.83	89.29	85.59	89.13	86.41	F=0.60	0.5487
(mg/l)	± 2.07	± 1.53	± 1.44	± 1.44	± 1.50	± 1.56		
COD	105.85	106.15	22.64	24.03	21.27	20.84	H=148.83	0.0001
(mg/l)	± 3.60 [a]	± 2.73 [a]	± 0.69 [b]	± 0.83 [b]	± 0.70 [b]	± 0.69 [b]		
Total N	23.14	14.89	11.20	11.46	6.25	6.66	H=138.88	0.0001
(mg/l)	± 0.49 [a]	± 0.58 [b]	± 0.95 [b]	± 0.93 [b]	± 0.32 [c]	± 0.51 [c]		
NH_4-N	0.63	0.56	0.66	0.61	0.32	0.25	H=9.54	0.0893
(mg/l)	± 0.22	± 0.21	± 0.24	± 0.24	± 0.07	± 0.04		
NO_2-N	0.10	0.10	0.07	0.09	0.08	0.08	H=8.94	0.1114
(mg/l)	± 0.01	± 0.01	± 0.01	± 0.01	± 0.01	± 0.02		
NO_3-N	22.40	14.23	10.76	10.46	5.99	5.84	H=141.93	0.0001
(mg/l)	± 0.63 [a]	± 0.58 [b]	± 0.75 [c]	± 0.77 [c]	± 0.29 [d]	± 0.29 [d]		
PO_4-P	26.49	18.61	16.57	17.29	9.76	9.09	H=158.42	0.0001
(mg/l)	± 0.72 [a]	± 0.60 [b]	± 0.72 [b]	± 0.61 [b]	± 0.48 [d]	± 0.45 [d]		
Cl	227.00	224.09	132.55	132.09	122.76	125.45	H=140.34	0.0001
(mg/l)	± 5.62 [a]	± 7.32 [a]	± 2.80 [b]	± 2.37 [b]	± 1.71 [b]	± 1.96 [b]		
Na	723.79	727.45	179.03	178.61	170.27	172.33	H=133.60	0.0001
(mg/l)	± 4.91 [a]	± 5.41 [a]	± 3.52 [b]	± 3.79 [b]	± 3.37 [b]	± 2.93 [b]		

Treatments marked with * were subject to pH adjustment using sulphuric acid. Primary facultative pond (PFP), nutrient solution (NS), chemical oxygen demand (COD), dissolved oxygen (DO).

Plant productivity

The final weight of cabbages were influenced by an interaction between the pH regimes and the water sources (Multifactor ANOVA, $F_{(2,12)}$=85.50, p<0.0001; Figure 4.4). Cabbages from the pH adjusted BE systems were significantly bigger than cabbages from the pH unadjusted BE systems, whereas pH adjustment had no influence on cabbage size in the Water and NS treatments (Figure 4.4) After twelve weeks, cabbages from the NS systems were bigger than cabbages from all other treatments (Figure 4.4, 4.5). Similarly the height and CCI of cabbages was influenced by an interaction between the pH regime and the water source (Multifactor repeated measures ANOVA, P<0.05, Figure 4.5, 4.6). Cabbages grown in the NS system cabbages had the highest height throughout the trial, followed by pH adjusted BE grown cabbages (Figure 4.5). Cabbages grown on Water and pH unadjusted BE systems were the smallest plants (Figure 4.4, 4.5). The CCI of cabbages grown in Water and pH unadjusted BE hydroponic systems decreased over time (Figure 4.6). The CCI of cabbages grown in NS and pH adjusted BE systems increased over time, with NS grown cabbages having the highest CCI throughout the trial (Figure 4.6).

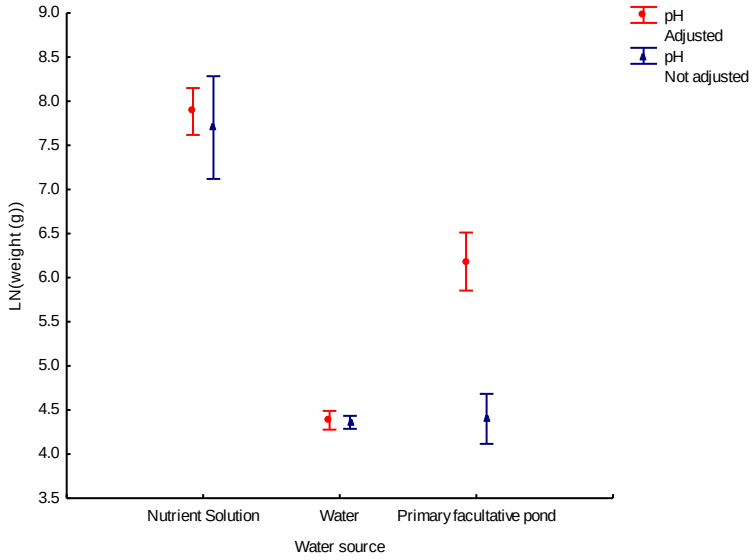

Figure 4.4 The mean (± 95% confidence interval) log transformed individual weight of cabbages, after 12 weeks, grown on the various irrigation solutions (Multifactor ANOVA, $F_{(2,12)}$=85.50, p<0.0001).

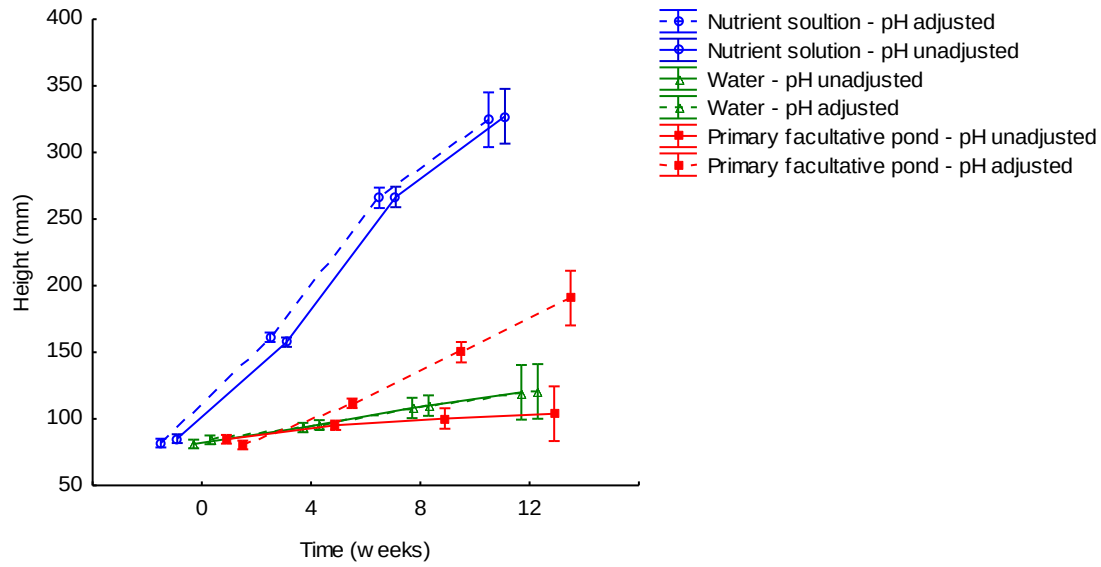

Figure 4.5 The mean (± 95% confidence interval) height of cabbage plants subject the various irrigation solutions, over the 12 week experiment (Multifactor repeated measures ANOVA, $F_{(15,36)}$=87.15, p<0.0001).

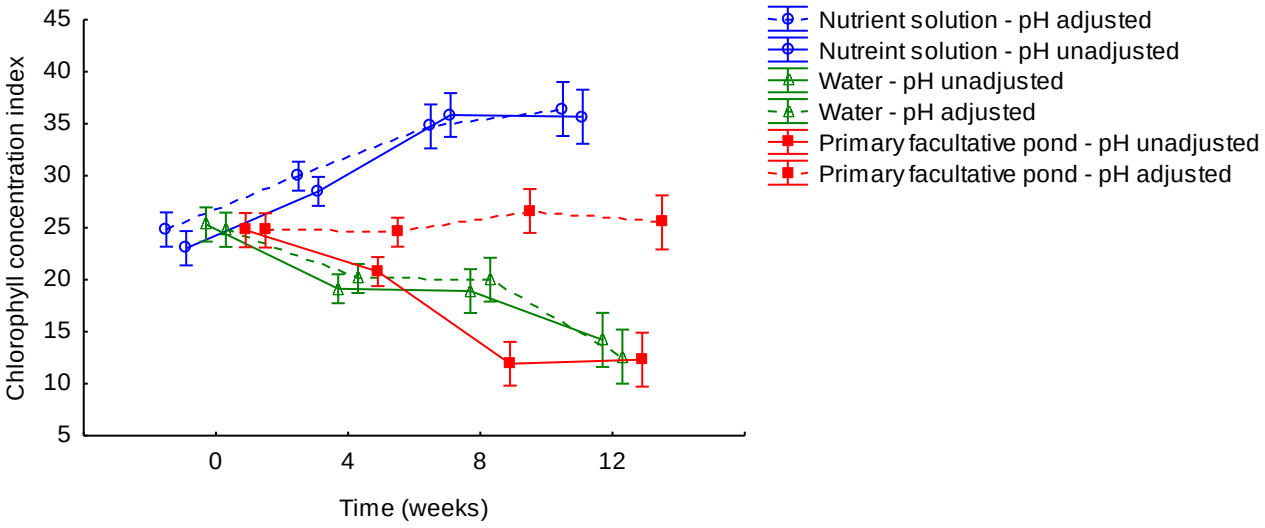

Figure 4.6 The mean (± 95% confidence interval) chlorophyll concentration index of cabbage plants subject the various irrigation solutions, over the 12 week experiment (Multifactor repeated measures ANOVA $F_{(15,36)}$=34.76, p<0.0001).

Plant chemical composition

The Na leaf content was highest in BE grown cabbages (21936.16 ± 408.39 mg/kg) followed by NS (9858.54 ± 416.71 mg/kg) and water grown cabbages (3057.63± 620.51 mg/kg; Kruskal Wallis, $H_{(5,18)}$=15.74, p=0.008; Figure 4.7). Cabbages grown in pH adjusted BE systems had a lower Na content (14766.26 ± 541.97 mg/kg) than cabbages grown in BE unadjusted systems (29106.03 ± 274.81 mg/kg; Figure 4.7). The leaf concentration of all the measured macro and micro nutrients was higher in cabbages grown in pH adjusted systems compared to pH unadjusted BE systems, with the exception of Zn (Table 4.5). The N content of cabbage leaves was similar between BE and NS grown cabbages (2.92 ± 0.05%) with water grown cabbages having the lowest leaf N content (0.79 ± 0.02%; Table 4.5). Nutrient solution grown cabbages had the highest P and K leaf concentration followed by BE and then water grown cabbages (Table 4.5). The leaves of BE grown cabbages had lower Mg and

Mn concentrations than NS and water grown cabbages (Table 4.5). Cabbages grown in pH unadjusted BE systems had lower leaf Fe and Cu concentrations than cabbages from all other experimental systems (Table 4.5). Effluent and NS grown cabbages had higher leaf Cl and Al concentrations than water grown cabbages (Table 4.5). Water grown cabbages had the highest Ca content followed by NS grown cabbages and then BE grown cabbages (Table 4.5).

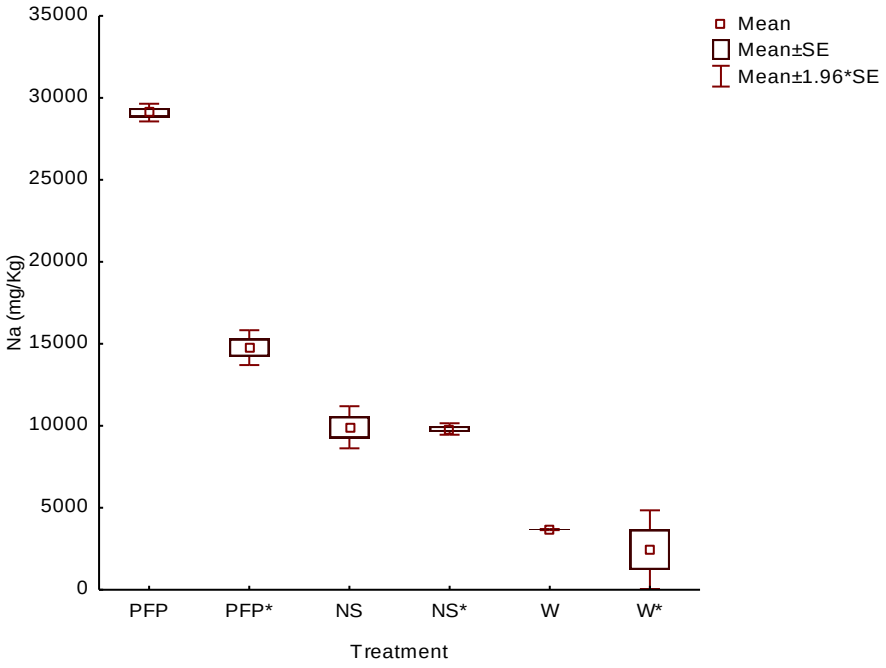

Figure 4.7 The leaf sodium content of cabbage plants grown on the different irrigation solutions (Kruskal Wallis, $H_{(5,18)}$=15.74, p=0.008). Nutrient solution (NS), water (W), primary facultative pond (PFP). The irrigation water of treatments marked with * were subject pH adjustment using sulphuric acid.

Table 4.5 The mean (± standard error) chemical leaf content of cabbage plants subject to the experimental irrigation solutions. Values in the same row represented by a different superscript symbol represent means that are significantly different (Multifactor ANOVA/Kruskal Wallis, P<0.05).

Element	PFP	PFP*	NS	NS*	Water	Water*	F/H	P
Aluminium (mg/kg)	50.74 ± 0.57 [a]	41.07 ± 5.43 [a]	34.35 ± 1.57 [ab]	33.67 ± 1.86 [ab]	15.86 ± 2.10 [b]	17.00 ± 1.15 [b]	H=13.30	0.0207
Calcium (%)	0.34 ± 0.01 [a]	0.74 ± 0.02 [b]	1.42 ± 0.11 [c]	1.60 ± 0.03 [c]	2.85 ± 0.06 [d]	2.87 0.07 [d]	H=15.92	0.0071
Chloride (%)	0.77 ± 0.01 [a]	0.73 ± 0.03 [a]	0.74 ± 0.04 [a]	0.75 ± 0.04 [a]	0.54 ± 0.03 [b]	0.55 ± 0.04 [b]	F=0.42	0.6660
Copper (mg/kg)	0.23 ± 0.13 [a]	2.89 ± 0.59 [b]	3.04 ± 0.09 [b]	3.03 ± 0.09 [b]	2.69 ± 0.16 [b]	2.60 ± 0.26 [b]	H=10.37	0.0655
Iron (mg/kg)	25.60 ± 2.08 [a]	66.03 ± 9.89 [b]	70.29 ± 5.47 [b]	67.00 ± 4.00 [b]	40.45 ± 0.06 [ab]	40.67 ± 1.20 [ab]	H=14.44	0.0130
Nitrogen (%)	2.77 ± 0.01 [a]	3.28 ± 0.02 [b]	2.82 ± 0.11 [a]	2.80 ± 0.07 [a]	0.82 ± 0.01 [c]	0.75 ± 0.03 [c]	H=14.82	0.0112
Magnesium (%)	0.17 ± 0.01 [a]	0.28 ± 0.01 [b]	0.40 ± 0.02 [c]	0.47 ± 0.05 [c]	0.87 ± 0.01 [d]	0.86 ± 0.02 [d]	H=15.83	0.0073
Manganese (mg/kg)	8.86 ± 0.70 [a]	16.94 ± 1.33 [b]	27.97 ± 2.17 [c]	28.67 ± 2.03 [c]	30.14 ± 0.79 [c]	31.33 ± 1.20 [c]	F=4.00	0.0466
Phosphorous (%)	0.17 ± 0.01 [a]	0.43 ± 0.01 [b]	0.46 ± 0.01 [c]	0.47 ± 0.01 [c]	0.21 ± 0.01 [d]	0.22 ± 0.01 [d]	F=255.64	0.0001
Potassium (%)	1.04 ± 0.01 [a]	2.57 ± 0.04 [b]	3.24 ± 0.10 [c]	3.33 ± 0.05 [c]	1.54 ± 0.01 [d]	1.51 ± 0.02 [d]	F=442.67	0.0001
Zinc (mg/kg)	36.98 ± 3.69 [a]	42.76 ± 2.01 [a]	37.55 ± 0.76 [a]	36.33 ± 0.88 [a]	35.37 ± 038 [b]	34.77 ± 0.44 [b]	F=2.02	0.1756

Treatments marked with * were subject to pH adjustment using sulphuric acid. Primary facultative pond (PFP), nutrient solution (NS).

Visual indicators

Immediately after planting all cabbage seedlings planted in BE systems showed signs of wilting (Figure 4.8). This lasted for three to five days after which no wilting was observed in any cabbage plants. Cabbages grown in the NS hydroponic systems were the biggest, followed by pH adjusted BE grown cabbages and then by pH unadjusted BE grown cabbages (Figure 4.8).

A B

Figure 4.8 Cabbage seedlings in an effluent system (left) and water system (right) one day after planting (A), and cabbages grown in hydroponic systems filled with from left to right water, pH unadjusted PFP effluent, pH adjusted PFP effluent and NS (B).

4.3.2 Crop productivity

Water quality

The mean starting pH of pH unadjusted systems was 8.41 ± 0.27 which increased to 9.04 ± 0.05 in old irrigation solutions (Table 4.6). The pH adjusted hydroponic systems had a mean starting pH of 6.51 ± 0.59 which increased to 8.27 ± 0.05 in old irrigation solutions (Table 4.6, 4.7). The pH adjustment of hydroponic systems increased the conductivity from 3243.13 ± 27.76 to 3329.64 ± 31.37 $\mu s/cm^2$ (Table 4.6). There was no difference in the conductivity of old irrigation solutions between all hydroponic systems with the exception of pH adjusted No Crop hydroponic systems which had a significantly higher conductivity than all other systems (Kruskal Wallis $H_{(7,264)}$=33.52, p<0.0001; Table 4.7). The COD and DO concentration in old irrigation solutions was not influenced by an interaction between pH regime and crop type (Multifactor ANOVA, p>0.05; Table 4.7). Dissolved oxygen was similar between all treatments (Multifactor ANOVA, $F_{(3,280)}$=0.94, p=0.33). All hydroponic systems lowered the COD of the irrigation water from a combined mean of 211.95 ± 2.84 to 109.55 ± 5.11 mg/l, with no difference between systems (Multifactor ANOVA, $F_{(3,280)}$0.42, p=0.66). The sodium content in all hydroponic systems increased during each cycle and was not influenced by an interaction between pH regime and crop type (Multifactor ANOVA, $F_{(3,280)}$=0.88, p=0.45; Table 4.7). The sodium content in old hydroponic irrigation water was significantly lower in saltbush treatments and similar between all other treatments (Multifactor ANOVA, $F_{(3,280)}$=6.87, p=0.002). The pH adjustment of hydroponic systems had no influence on the sodium concentration (Multifactor ANOVA, $F_{(1,280)}$=1.38, p=0.24). The chloride concentration increased in all hydroponic systems with no difference between treatments (Kruskal Wallis H=$_{(7,264)}$1.32, p=0.99; Table 4.7).

The ammonia and nitrite concentrations in the hydroponic systems decreased from 36.73 ± 0.92 & 0.82 ± 0.02 to 0.10 ± 0.01 & 0.61 ± 0.09 mg/l respectively with no difference being observed between treatments (Kruskal Wallis $p>0.05$; Table 4.7). The pH adjusted millet and cabbage treatments as well as both saltbush treatments had the lowest total N concentration in old irrigation solutions (Table 4.7). The No Crop and pH unadjusted millet and cabbage treatments had the highest total N concentration in old irrigation solutions (Table 4.7). The same trend was observed with nitrate because nitrate made up the bulk of total N (Table 4.7). The phosphate concentration hardly dropped in No Crop and pH unadjusted millet and cabbage planted systems (Table 4.6, 4.7). The pH adjusted millet and cabbage and both saltbush treatments had the lowest phosphate concentration in old irrigation solutions (Table 4.7).

Table 4.6 The mean (± standard error) water quality parameters of start irrigation solutions.

	Parameter										
pH	pH	Conductivity ($\mu s/cm^{2)}$	DO (mg/l)	NH4-N (mg/l)	NO2-N (mg/l)	NO3-N (mg/l)	PO4-P (mg/l)	Cl (mg/l)	COD (mg/l)	Total N (mg/l)	Na (mg/l)
Not Adjusted	8.41 ± 0.27	2902. 90 ± 41.39	78.84 ± 1.16	36.90 ± 1.29	0.78 ± 0.03	21.33 ± 0.51	27.15 ± 0.75	193.69 ± 5.03	212.38 ± 6.54	58.37 ± 2.97	612.83 ± 8.40
Adjusted	6.51 ± 0.59	3238.82 ± 37.69	80.82 ± 0.93	36.56 ± 1.31	0.82 ± 0.03	20.42 ± 0.52	27.87 ± 0.51	188.85 ± 5.06	210.87 ± 7.27	57.83 ± 2.36	610.38 ± 8.43

Chemical oxygen demand (COD), dissolved oxygen (DO).

Table 4.7 The mean (± standard error) water quality parameters of old irrigation solutions. Values in the same row represented by a different superscript symbol represent means that are significantly different. (Multifactor ANOVA/Kruskal Wallis, P<0.05).

Parameter	Cabbage	Cabbage	Saltbush	Saltbush*	Millet	Millet*	None	None*	F/H	P
pH	9.06 ± 0.06	8.36 ± 0.05	8.96 ± 0.05	8.23 ± 0.06	9.13 ± 0.05	8.18 ± 0.04	9.03 ± 0.05	8.36 ± 0.05		
Conductivity ($\mu s/cm^2$)	3184.36 ± 55.49 [a]	3202.61 ± 41.40 [a]	3233.21 ± 41.49 [a]	3199.70 ± 54.97 [a]	3240.21 ± 41.58 [a]	3249.24 ± 44.14 [a]	3255.97 ± 41.55 [a]	3540.03 ± 44.31 [b]	H=33.52	0.0001
DO (%)	86.12 ± 1.52	85.85 ± 2.06	86.96 ± 1.78	85.12 ± 1.49	86.16 ± 1.71	87.13 ± 1.53	85.22 ± 1.43	86.21 ± 1.38	F=0.16	0.9261
COD (mg/l)	105.85 ± 3.60	106.15 ± 2.73	109.85 ± 2.32	106.12 ± 2.62	110.52 ± 2.74	109.24 ± 3.16	111.30 ± 2.97	112.69 ± 2.95	F=0.29	0.8314
Total N (mg/l)	23.14 ± 0.49 [a]	14.89 ± 0.58 [b]	17.34 ± 0.51 [b]	14.41 ± 0.52 [b]	30.53 ± 6.88 [a]	12.17 ± 0.87 [b]	24.85 ± 0.25 [a]	23.72 ± 0.23 [a]	H=195.18	0.0001
NH_4-N (mg/l)	0.63 ± 0.21	0.56 ± 0.21	0.65 ± 0.21	0.53 ± 0.19	0.56 ± 0.21	0.60 ± 0.20	0.65 ± 0.27	0.66 ± 0.23	H=4.23	0.7524
NO_2-N (mg/l)	0.11 ± 0.01	0.11 ± 0.01	0.11 ± 0.01	0.09 ± 0.01	0.09 ± 0.01	0.11 ± 0.01	0.10 ± 0.01	0.13 ± 0.02	H=4.46	0.7258
NO_3-N (mg/l)	22.40 ± 0.63 [a]	14.23 ± 7.36 [b]	16.58 ± 0.46 [b]	13.79 ± 0.46 [b]	29.87 ± 6.90 [a]	11.46 ± 0.76 [b]	24.10 ± 0.32 [b]	23.94 ± 0.32 [b]	H=189.90	0.0001
PO_4-P (mg/l)	26.50 ± 0.72 [a]	18.61 ± 0.60 [b]	20.78 ± 0.58 [ab]	18.10 ± 0.50 [b]	27.18 ± 0.73 [a]	16.20 ± 0.63 [b]	27.33 ± 0.68 [a]	26.93 ± 0.75 [a]	H=151.18	0.0001
Cl (mg/l)	227.00 ± 5.62	224.09 ± 7.32	226.55 ± 6.66	227.18 ± 6.51	227.45 ± 5.72	228.36 ± 7.02	223.52 ± 6.35	218.79 ± 7.43	H=1.32	0.9880
Na (mg/l)	725.50 ± 4.73	730.06 ± 5.28	711.38 ± 6.06	705.69 ± 6.29	725.16 ± 6.30	735.64 ± 6.13	723.56 ± 4.50	734.06 ± 4.06	F=0.88	0.4500

Treatments marked with * were subject to pH adjustment using sulphuric acid. Chemical oxygen demand (COD), dissolved oxygen (DO).

Plant productivity

The pH adjustment of post-PFP brewery significantly affected the growth of all plants with plants gaining significantly more weight when irrigated with pH adjusted BE (Kruskal Wallis, $H_{(5,18)}$=16.16, p=0.006; Figure 4.9). Millet planted in pH unadjusted systems hardly grew and gained on average 5.73 ± 3.34 g whereas millet planted in pH adjusted system gained on average 553.12 ± 77.86 g, over the 12 week trial, a tenfold increase (Figure 4.9).

The height of millet plants grown in pH adjusted systems increased steadily throughout the trial while millet plants grown in the pH unadjusted systems stayed the same height throughout the experiment (Figure 4.10). The pH adjustment of BE did not influence the height of saltbush or cabbage plants over the 12 week trial (Figure 4.10). The CCI of saltbush and millet plants grown in pH adjusted systems increased throughout the trial while the CCI of saltbush and millet plants grown in pH unadjusted systems deceased or stayed the same throughout the trial (Figure 4.11). The CCI of cabbage plants grown in pH adjusted hydroponic system remained constant whereas the CCI of cabbage plants grown in pH unadjusted systems decreased over the 12 week experiment (Figure 4.11).

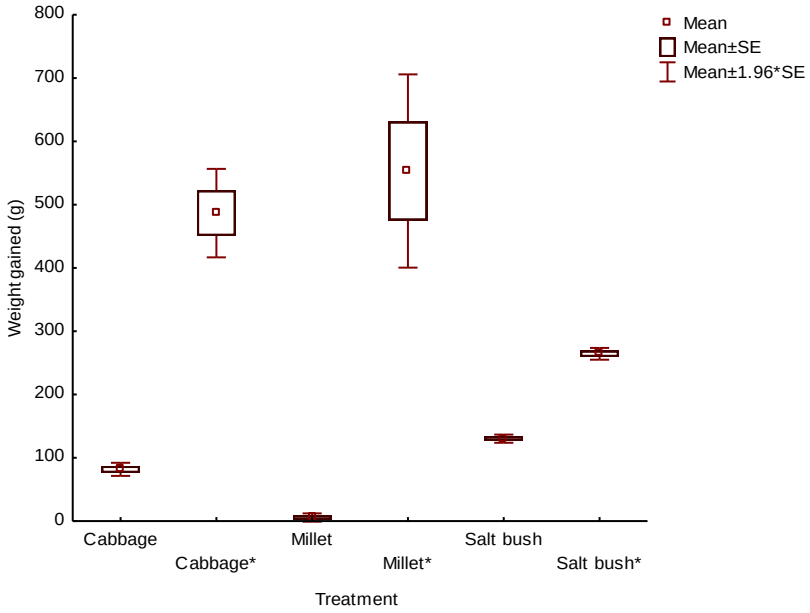

Figure 4.9 The mean individual weight gain of cabbage, millet and saltbush plants grown in hydroponic systems fed with post primary facultative pond effluent (Kruskal Wallis, $H_{(5,18)}$=16.16, p=0.006). Treatments marked with * were subject to pH change to 6.5 with sulphuric acid.

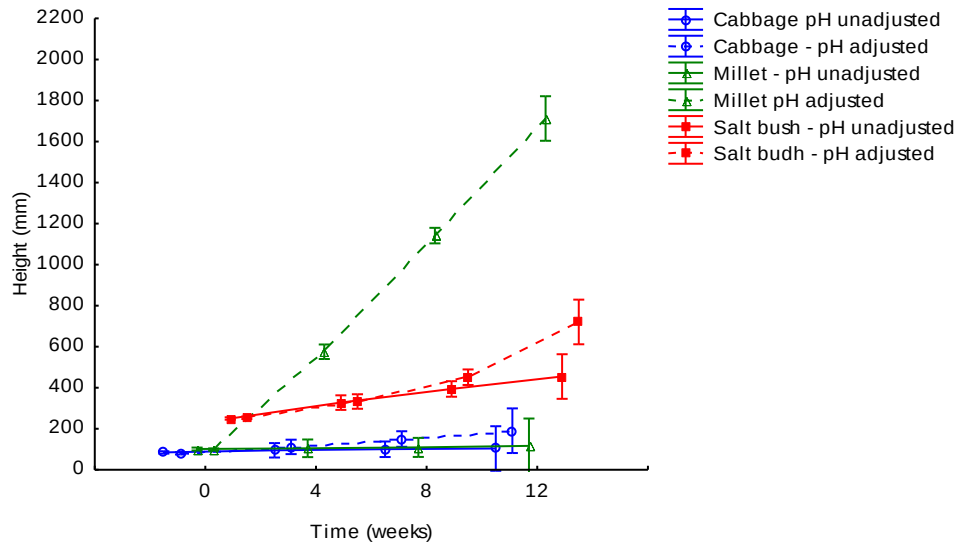

Figure 4.10 The mean (± 95% confidence interval) height of cabbage, millet and saltbush plants grown irrigated with post primary facultative pond effluent, over the 12 week experiment (Multifactor repeated measures ANOVA, $F_{(15,36)}$=85.53, p<0.0001).

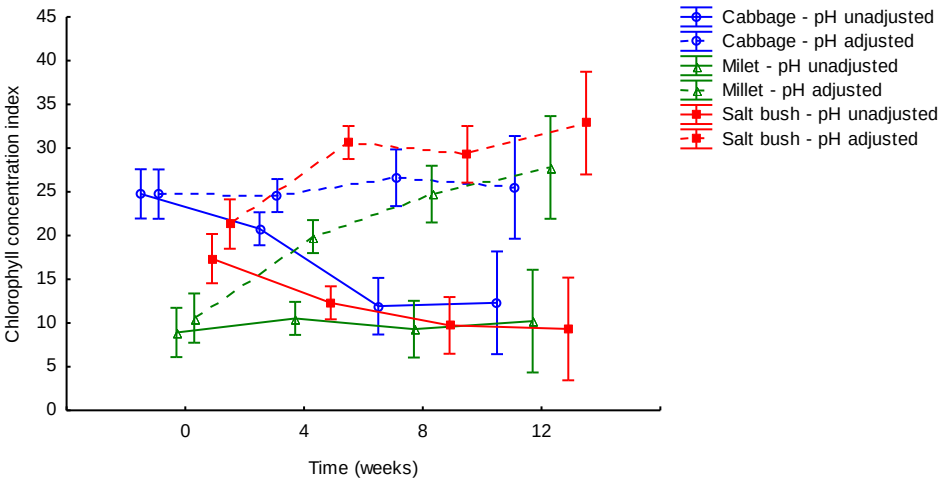

Figure 4.11 The mean (± 95% confidence interval) chlorophyll concentration index of cabbage, millet and saltbush plants irrigated with post primary facultative pond effluent, over the 12 week experiment (Repeated measures ANOVA, $F_{(15,36)}$=12.40, p<0.0001).

Plant chemical composition

Saltbush plants had a significantly higher leaf Na concentration (50995.25 ± 719.44 mg/kg) than millet (3001.07 ± 679.95 mg/kg) or cabbage plants (21685.82 ± 317.14 mg/kg; Kruskal Wallis, $H_{(5,17)}$=15.59, p=0.008; Figure 4.12). The pH adjustment of BE resulted in a decrease in the Na content of cabbage, millet and saltbush plants (Figure 4.12).

The pH adjustment of BE increased the N, P and Cu leaf content of cabbage, saltbush and millet plants (Table 4.8). The K, Mn, Fe and Cu content of cabbages leaves increased with pH adjustment whereas pH adjustment had no influence on the K, Mn, Fe and Cu content of saltbush and millet leaves (Table 4.8). The pH adjustment of post-PFP BE had no influence on the Mg, Zn, Cl, Al, and Mg content of cabbage, saltbush and millet leaves.

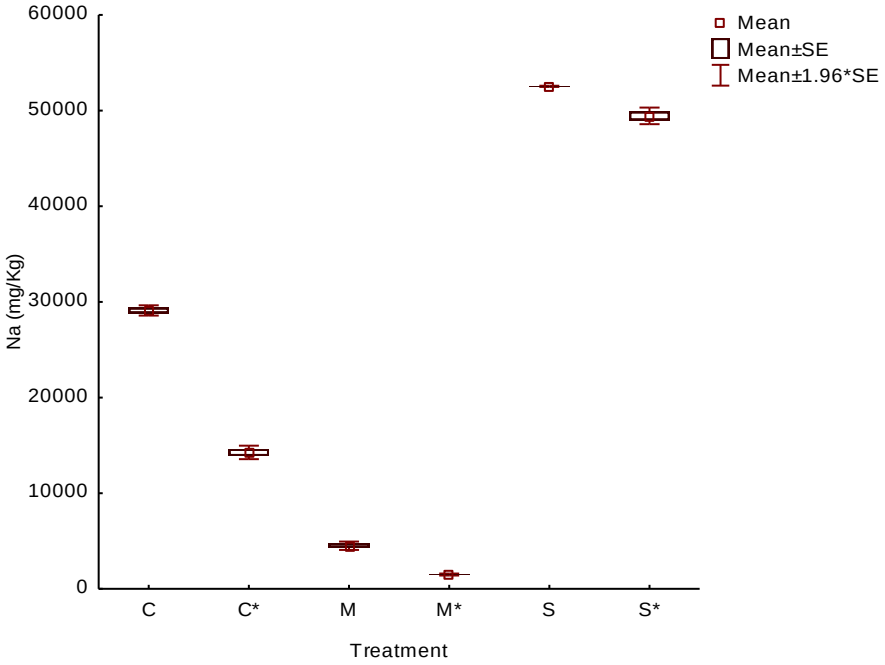

Figure 4.12 The mean (± 95% confidence interval) leaf sodium content of cabbage, millet and saltbush plants irrigated with post primary facultative pond effluent with and without pH adjustment (Kruskal Wallis, $H_{(5,17)}$=15.59, p=0.008).

Table 4.8 The mean (± standard error) chemical leaf content of plants irrigated with post primary facultative pond effluent. Values in the same row represented by a different superscript symbol represent means that are significantly different (Multifactor ANOVA/Kruskal Wallis, P<0.05).

.

Element	Cabbage	Cabbage*	Saltbush	Saltbush*	Millet	Millet*	F/H	P
Aluminium (mg/kg)	50.73 ± 0.57	45.76 ± 14.18	72.17 ± 9.38	78.66 ± 9.46	58.33 ± 10.74	64.53 ± 4.13	F=0.27	0.7707
Calcium (%)	0.34 ± 0.01 [a]	0.73 ± 0.03 [b]	0.84 ± 0.01 [b]	0.74 ± 0.01 [b]	0.63 ± 0.07 [b]	0.60 ± 0.01 [b]	H=13.03	0.0231
Chloride (%)	0.77 ± 0.01 [a]	0.76 ± 0.01 [a]	1.71 ± 0.03 [b]	1.69 ± 0.01 [b]	0.75 ± 0.02 [a]	0.70 ± 0.03 [a]	F=1.60	0.2462
Copper (mg/kg)	0.23 ± 0.13 [a]	3.25 ± 0.82 [b]	0.14 ± 0.04 [a]	0.87 ± 0.08 [c]	0.79 ± 0.30 [c]	11.52 ± 0.64 [d]	H=13.82	0.0168
Iron (mg/kg)	25.60 ± 2.08 [a]	74.68 ± 21.23 [b]	42.57 ± 5.57 [a]	30.72 ± 2.10 [a]	63.67 ± 18.48 [ab]	79.08 ± 8.40 [b]	H=14.05	0.0153
Nitrogen (%)	2.77 ± 0.01 [a]	3.27 ± 0.03 [b]	2.23 ± 0.15 [ab]	3.30 ± 0.01 [b]	1.74 ± 0.01 [c]	2.38 ± 0.09 [a]	H=14.95	0.0106
Magnesium (%)	0.17 ± 0.01 [a]	0.27 ± 0.01 [a]	0.74 ± 0.03 [b]	0.71 ± 0.05 [b]	0.47 ± 0.06 [c]	0.60 ± 0.03 [bc]	F=2.39	0.1371
Manganese (mg/kg)	8.76 ± 0.70 [a]	16.88 ± 2.30 [b]	14.71 ± 0.77 [b]	12.96 ± 1.02 [ab]	41.59 ± 2.34 [c]	41.47 ± 0.10 [c]	H=14.23	0.0142
Phosphorous (%)	0.17 ± 0.01 [a]	0.42 ± 0.01 [b]	0.15 ± 0.01 [a]	0.28 ± 0.01 [b]	0.14 ± 0.01 [a]	0.24 ± 0.01 [b]	F=40.06	0.0001
Potassium (%)	1.04 ± 0.01 [a]	2.54 ± 0.02 [b]	1.63 ± 0.01 [a]	1.85 ± 0.01 [a]	1.80 ± 0.08 [a]	1.67 ± 0.03 [a]	H=13.08	0.0227
Zinc (mg/kg)	36.98 ± 3.69 [a]	42.21 ± 3.34 [a]	83.60 ± 1.44 [b]	76.00 ± 5.69 [b]	78.24 ± 1.89 [b]	79.11 ± 0.87 [b]	H=12.55	0.0280

Treatments marked with * were subject to pH adjustment using sulphuric acid.

Visual indicators

Saltbush plants grown on PFP unadjusted BE had lighter and yellower leaves than saltbush plants grown on pH adjusted PFP BE (Figure 4.13). Millet plants grown on PFP adjusted BE were much bigger than millet plants grown on pH unadjusted BE (Figure 4.13)

A B

Figure 4.13 Saltbush (A) and millet (B) plants grown on pH unadjusted (plant on left) brewery effluent and pH adjusted (plant on right) brewery effluent.

4.4 Discussion

4.4.1 Nutrient solution experiment

Water chemistry

The post-PFP BE has a high alkalinity. It took about three millilitres of 98% sulphuric acid to decrease the pH of 20 l BE from 8.41 ± 0.27 to 6.5. The pH in all pH adjusted hydroponic systems system increased to a pH between 8.0 and 9.0 after seven days (Table 4.4). The pH adjustment of BE was only done once, at the beginning of each replacement because

constant pH adjustment would increase the already high conductivity of the BE systems thus putting more osmotic stress on the plants. The high alkalinity of BE is generated from the addition of sodium hydroxide to BE before it goes through anaerobic digestion and the generation of carbonate, bicarbonate and ammonium alkalinity during anaerobic digestion (Van Rensburg *et al.* 2003, Power 2014). The pH of anaerobic supernatants has been shown to increase with aeration of the liquor (Musvoto *et al.* 2000, Power 2014). The high alkalinity of BE is an issue when using BE in hydroponic crop production systems because it difficult to maintain a stable pH between 6.5 - 7.0 in hydroponic systems (Power 2014).

The hydroponic systems had an influence on the COD of the irrigation solutions. The COD in BE systems generally decreased from 214.83 ± 6.37 to 106.00 ± 3.17 mg/l (Table 4.4). In water and NS systems the COD increased slightly from 17.25 ± 0.38 to 22.20 ± 0.73 (Table 4.3, 4.4). Chemical oxygen demand is a measure of the amount of oxygen required to oxidise all organic matter and is thus affected by the quantity of organic compounds in the irrigation solutions (Penn *et al.* 2009). The main mechanisms for COD removal in constructed wetlands include sedimentation, filtration, adsorption, plant uptake and microbial metabolism (Lee *et al.* 2004, El-Khateeb *et al.* 2009, Ong *et al.* 2009, Fan *et al.* 2013). The plants and associated microorganisms were able to reduce the relatively high amount of organics in the BE hydroponic systems, while they increasing the amount of organics in low organic load nutrient solutions.

The total N in the fresh irrigation solutions were composed of generally equal amounts of ammonia and nitrate (Table 4.3). In old nutrient solutions, total N was compromised mainly of nitrate. The mean dissolved oxygen concentration hydroponic systems was 85.83 ± 2.06% (Table 4.4). Nitrifying bacteria are aerobic bacteria that oxidise ammonia into nitrate

(Antoniou *et al.* 1989, Tyson *et al.* 2007). The aerobic conditions in the hydroponic systems were suitable for nitrifying bacteria, thus the almost complete reduction of ammonia to nitrate in hydroponic systems.

Plant productivity

Brewery effluent is not a complete nutrient solution and may contain certain properties that inhibit crop growth. Cabbages grown in the NS hydroponic systems grew faster and had a higher end weight than BE grown cabbages. Power (2014) and Jones *et al.* (2013) all found that irrigation with post-PFP BE reduced tomato and lettuce growth when compared to plants irrigated with a hydroponic nutrient solution. They concluded that the pH and high conductivity were the major factors contributing to this. The post-PFP BE contained higher concentrations of ammonia nitrogen and similar P concentrations than to the NS used in this experiment. Ammonia toxicity increases because the proportion of its cationic form increases as the pH increases (Britto & Kronzucker 2002, Bar-Yosef *et al.* 2009, Borgognone *et al.* 2012). Ammonia toxicity is linked with specific nutrient uptake restrictions in crops, specifically other cations such as Ca, P and Mg (Britto & Kronzucker 2002, Bar-Yosef *et al.* 2009, Borgognone *et al.* 2012). Brewery effluent grown cabbages had lower Ca, P and Mg leaf concentrations than NS or water grown cabbages. This suggests that ammonia toxicity may have played a role in putting stress on the BE irrigated plants thus reducing their growth. However, the ammonia in BE is unstable. The aeration of post-AD BE encourages the biological nitrification of ammonium to nitrate (Tyson *et al.* 2007). This results in a decrease in ammonia toxicity and in ammonia generated alkalinity (Gallert *et al.* 1998, Britto & Kronzucker 2002). Therefore it is probably not ammonia generated alkalinity or toxicity that is caused the reduction in growth of BE grown cabbages. However the pH of hydroponic

systems still rose thus indicating that other factors such as the carbon acid/base system were contributing to the high alkalinity observed in the hydroponic systems.

It is probably not the lack of nutrients in BE that is resulted in the decreased plant growth but other properties such as high conductivity and pH (Jones *et al.* 2013, Power 2014). The conductivity and salinity of most effluents limit their use in irrigated crop production (Pescod 1992, Muyen *et al.* 2011). For the first five days after planting all cabbage seedlings planted in BE hydroponic systems showed signs of wilting while cabbages from the NS and Water systems did not. Wilting is a sign of osmotic stress in plants and, this reflects the conductivity and / or salinity of the BE used to irrigate the plants (Epstein & Bloom 2005, Castillo 2011). Five days after planting, the cabbages stopped wilting and regained their regular shape. Plants have the ability to adjust to moderate levels of salinity via various physiological adaptions, such as ion compartmentalization, osmotic adjustment, succulence, selectivity and uptake of ions, enzyme responses, and the balance of nutrient uptake (Munns 2002, Castillo 2011, Camilla *et al.* 2012). Initially, the cabbage seedlings were not used to the conductivity levels of BE and took five days to adjust to the high conductivity of BE systems.

The salinity of irrigation waters between 2000 - 3000 µs/cm^2 causes a decrease yield in most crops (Shannon & Grieve 1999, DWAF 1996). The threshold salinity of cabbages is 1800 µs/cm^2 and the conductivity of BE (3068.23 ± 42.69 µs/cm^2) should result in a 10 -20% decreased in yield (Shannon & Grieve 1999, DWAF 1996). This is primarily due to the osmotic effects is causes by decreasing the osmotic potential between the root plasma and soil water (Munns & Termaat 1986, Jacoby 1994). This means that plants have to spend more energy to take up water from the soil, which increases respiration and has negative

effects on growth (Munns & Termaat 1986, Jacoby 1994). The impact salinity is species specific and is also mediated by environmental factors such as humidity, temperature, wind, light and air pollution (Shannon *et al*. 1994). The conductivity of post-PFP BE put stress on crops, which could be expected to be accompanied by a decrease in growth and yield.

The bioavailability of most plant nutrients are affected by pH with the availability of most nutrients decreasing heavily at pH values above 7.5 (Lucas & Davis 1961, Tyson *et al.* 2007). The pH adjustment of BE significantly increased the growth, yield and CCI of cabbages. The yield and CCI of cabbages grown in pH unadjusted BE systems and water systems (nutrient limited) were similar. Brewery effluent with no pH adjustment is not a suitable irrigation source for hydroponic production of cabbages. The high pH of BE in pH unadjusted systems decreased the availability of nutrients to cabbages, resulting in cabbages growing at the same rate as nutrient limited cabbages.

The effect of pH on the availability of micronutrients to cabbage plants became more pronounced when looking at the leaf chemical composition. The leaf concentration of all the measured macro and micro nutrients was higher in cabbages grown in pH adjusted systems compared to pH unadjusted BE systems, with the exception of zinc (Table 4.5). Most micronutrients become less available to plants when the pH rises above 7.5 (Lucas & Davis 1961, Epstein & Bloom 2005, Tyson *et al.* 2007). Plants often display nutritional disorders when grown under saline environments and these disorders can become even more pronounced when the pH of the environment in unfavourable (Grattan & Grieve 1999).

There was a marked difference in the Na content of cabbage leaves subject to the various irrigation treatments. Effluent grown cabbages had the highest sodium leaf content (21936.16 ± 408.39 mg/kg) followed by NS (9858.54 ± 416.71 mg/kg) and water grown

cabbages (3057.63± 620.51 mg/kg; Figure 4.7). The sodium content of plant tissue has been found to increase as the sodium content of irrigation waters increases (Glenn *et al.* 1999, Silveira *et al.* 2009, Diaz *et al.* 2013). Sodium puts osmotic stress on plants and can reduce the availability of certain nutrient to plants such as Ca, K and Mg (Grattan & Grieve 1999). High concentrations of sodium in saline waters increases the ratio of Na to nutrient cations such as Ca, K and Mg, resulting in increased Na uptake and decreased nutrient cation uptake (Maas & Grieve 1987, Alam *et al.* 1989, Ehret *et al.* 1990). Plants have to spend more energy on obtaining water and nutrients thus reducing the amount of energy spent on growth therefore reduced yield (Alam *et al.* 1989, Ehret *et al.* 1990, Grattan & Grieve 1999). The interaction between salinity and nutrient deficiencies is extremely complex and is influenced by crop type, soil type and the environment in which the crops are grown (Grattan & Grieve 1999).

4.4.2 Crop productivity

Water chemistry

The pH of the hydroponic systems was not stable. The pH increased from about 8.4 to more than pH 9.0 in the unadjusted BE treatments over the course of a single replacement cycle. The pH adjusted treatments all started at 6.5, but this increased to above pH 8.0 before the end of the replacement cycle. The high alkalinity of BE was able to resist the change in pH. During AD sodium hydroxide is added to the BE to neutralise the pH in the anaerobic digester and during AD carbonate and bicarbonate alkalinity is generated through the production of carbon dioxide (Van Rensburg *et al.* 2003). When BE is aerated or exposed to the atmosphere (such as in hydroponic systems) the volatile carbon dioxide expressed as carbonic acid is gassed off whereas the carbonate alkalinity is more stable and remains in

the water (Musvoto *et al.* 2000, Van Rensburg *et al.* 2003). This results in a decrease in acidity of BE and an increase in pH (Musvoto *et al.* 2000, Van Rensburg *et al.* 2003). The high alkalinity of BE is a major issue because it is difficult to keep BE at a constant pH, without continuously adding acid, in order to optimise the availability of nutrients to plants when it is used as a hydroponic nutrient source. The continual addition of acid would increase the conductivity of BE and would put more osmotic stress on the plants in hydroponic systems.

One of the objective of this experiment was to determine how much sodium could be removed from BE by various crops. The sodium concentration increased in all hydroponic systems as the time that the plants were exposed to the solution increased. This increased in sodium concentration with time was probably due to a process called evapotranspiration, where salt concentration increases due to the removal of water (Lymbery *et al.* 2006, Muyen *et al.* 2011, Rozema 2014). The irrigation solutions from the hydroponic systems planted with saltbush had the lowest concentration of sodium, whereas the systems planted with millet and cabbage had similar sodium concentration to the hydroponic systems with No Crops. Halophytes such as saltbush have been shown to assimilate sodium from soil water complexes (Miyamoto *et al.* 1996, Brown *et al.* 1999, Glenn *et al.* 1999). However, this rate of sodium assimilation was slower than the concentrating effect caused by evapotranspiration, which accounts for the increase in sodium in all the treatments (Rozema 2014).

Plant productivity

The level of sodium found in BE has been identified as a major restriction to the widespread use of BE as a water and nutrient source in irrigated crop production. Salinity poses two major threats to plants: osmotic stress due to the relatively high solute concentrations

irrigation waters; and ion specific stresses resulting from the k^+/Na^+ ratios and Na^+ concentrations that are harmful to plants (Blumwald *et al.* 2000). The sodium leaf content was lower in millet, saltbush and cabbage plants grown in pH adjusted BE systems when compared to pH unadjusted BE systems. The influx of Na^+ into root cells is passive, while the efflux of Na^+ out of the root cells in active (Blumwald *et al.* 2000, Blumwald 2000, Apse & Blumwald 2007). An increase in the extracellular Na^+ concentration will cause an increased electrochemical gradient that will favour the movement of Na^+ into the root cell (Blumwald *et al.* 2000). Sodium enters the cell through K^+ carriers in the membrane because Na^+ and K^+ ions have similar hydraulic radii (Blumwald *et al.* 2000). The efflux of Na^+ is an active process because they have to be transported against their electrochemical gradient (Cramer *et al.* 1985, Blumwald *et al.* 2000). Sodium extrusion is mediated by the plasma membranes H^+-ATPase which pump out H^+ generating an electrochemical gradient against the natural H^+ gradient (Blumwald *et al.* 2000). This allows the Na^+/H^+ antiporters to couple the movement of H^+ into the cell with the extrusion of Na^+ (Hassidim *et al.* 1990, Blumwald *et al.* 2000). The increase in extracellular H^+ has been found to aid in the efflux of sodium through the plasma membrane and to enhance the salt tolerance of cells (Nass *et al.* 1997, Blumwald *et al.* 2000). Therefore the pH adjustment of BE may have enhanced the efflux of Na^+ in the root cells, thus decreasing the level of Na^+ in the plant tissue. Future research should investigate the influence of irrigation water pH on the Na^+ influx and efflux of plants and the salt tolerance of plants under acidic and alkaline conditions.

The growth of plants in hydroponic systems should be accompanied by a decrease in the nutrient concentration of the irrigation solutions. Phosphorous and total N concentrations decreased over time in all hydroponic systems. At the end of each cycle cabbage, saltbush and millet pH adjusted treatments had a lower total N and P concentrations than the No

Crop, cabbage and millet pH unadjusted hydroponic systems. Nitrogen and P are macronutrients needed by plants to support growth (Lucas & Davis 1961, Epstein & Bloom 2005). There were also algae and other microorganisms that contributed to the nutrient decrease in all hydroponic systems. Millet grown on pH unadjusted BE hardly grew and did not remove more nutrients than the hydroponic systems with No Crops. This emphasises the importance of pH when using BE as a hydroponic irrigation source.

The pH of a hydroponic solution can influence the growth and health of plants. The CCI families of cabbage, saltbush and millet plants were all higher when grown in the pH adjusted hydroponic systems. Millet plants grown in pH unadjusted BE hydroponic systems hardly grew. Chlorophyll concentration index is a direct measurement of the photosynthetic potential of a leaf and can be indirectly related to the nitrogen nutrient status of a leaf (Filella *et al.* 1995, Moran *et al.* 2000). Chlorophyll concentration index is also closely related to plant stress and age (Peñuelas & Filella 1998, Merzlyak *et al.* 1999). The nitrogen content of cabbage, millet and saltbush plants was higher in pH adjusted BE systems which can be linked to the higher CCI of their leaves as most of leaf nitrogen is incorporated into chlorophyll (Filella *et al.* 1995, Moran *et al.* 2000). The pH adjustment increased the photosynthetic potential of millet and saltbush plants which intern means they were able to grown faster.

4.4.3 Conclusion

The pH adjustment of post-PFP BE had a major influence on the growth, health and chemical composition of plants grown in hydroponic systems. The macro and micronutrient concentrations of cabbage leaves increased when the pH of post-PFP BE was adjusted to 6.5 at the start of each irrigation cycle. Post-PFP BE that is not pH adjusted is not a suitable

water and nutrient source for the hydroponic production of cabbage and millet plants. However, pH adjustment of BE however renders it much more suitable for hydroponic crop production. The high alkalinity of BE is a major issue, firstly for deceasing the availability of nutrients in BE, and, secondly for making it hard to maintain a pH range of between 6.5 and 7.0 to optimise the availability of nutrients to the plants. Continual pH adjustment would increase the conductivity of the BE, putting more osmotic stress onto the irrigated plants. The generation of alkalinity needs to be fully understood and technologies or practices need to be investigated that can reduce the alkalinity of BE. The pH plays a major role in the availability of nutrients to plants as well as influx/efflux of cations and anions through the plasma membrane. This needs to be further investigated because there is evidence that pH can influence the sodium efflux rate and sodium tolerance of plants.

Chapter 5: Discussion

Brewery effluent (BE) has the potential to be used in irrigated crop production. This was demonstrated in Chapter 2 and 3, where cabbages, saltbush, millet and lucerne were successfully grown using treated BE as a nutrient source and for irrigation water. The different BE treatment processes at the experimental treatment facility affected the concentration of nutrients and elements in BE which impacted on the growth of plants, as well as the fertility of the soil. Post high rate algal pond (HRAP) and constructed wetland (CW) effluent was not suitable for irrigated crop production because cabbages irrigated with these treatments had the same yield as cabbages irrigated with a nutrient limited irrigation source. This was because HRAP and CW treatment processes utilise processes that remove nutrients that could be used to support crop growth. Post anaerobic digestion (AD) and post primary facultative pond (PFP) BE contained similar or higher concentrations of ammonia, nitrate and phosphate to the commercial nutrient solution (NS). However the yield of cabbages irrigated with post-AD or post-PFP effluent was lower by 13% when compared to NS irrigated cabbages. Brewery effluent had certain properties (salinity) that reduced the growth of crops and it may be lacking in certain micronutrients.

5.1 Salts

The relatively high concentration of dissolved salts in BE is a concern when using BE in irrigated crop production systems. The electrical conductivity (EC), sodium absorption ratio (SAR) and sodium concentration all increased in all the soils irrigated with BE. This resulted in a decreased osmotic potential between the soil water and root plasma, meaning that plants spent more energy on obtaining water and less on growth (Munns & Termaat 1986, Jacoby 1994). Therefore the BE treatment process that resulted in the lowest EC should be

the most suitable for crop irrigation. Post-AD and post-PFP BE were measured with the lowest EC values, and the lowest concentrations of sodium, which identified them as being the most suitable for irrigated crop production systems. Evapotranspiration during the HRAP and CW treatments processes increase the sodium concentration of BE, thus decreasing its suitability for irrigated crop production.

Sodium enters root cells passively through K^+ transporters in the root plasma membrane via an electro-chemical gradient (Blumwald *et al.* 2000, Apse & Blumwald 2007). However the efflux of sodium out of the cell is an active process (Blumwald *et al.* 2000, Apse & Blumwald 2007). This means that plants irrigated with BE had to spend more energy on osmoregulation and sodium efflux, which in turn compromised plant growth. Sodium extrusion is mediated by the plasma membranes H^+- ATPase which pump out H^+ generating an electrochemical gradient H^+ gradient which allows the Na^+/H^+ antiporters to couple the movement of H^+ into the cell with the extrusion of Na^+ (Hassidim *et al.* 1990, Blumwald *et al.* 2000). An increase in extracellular H^+ has been shown to increase the efflux of sodium through the plasma membrane (Nass *et al.* 1997, Blumwald *et al.* 2000). The pH adjustment of BE decreased the sodium leaf content of cabbages, saltbush and lucerne. This could have been due to two reasons; firstly the addition of H^+ would decrease the ratio of Na/positively charged ions thus, decreasing the sodium electrochemical gradient between the root plasma and extracellular water; and secondly, the addition of H^+ may enhance sodium efflux. Future research should investigate this to determine how pH affects the sodium tolerance and sodium efflux of plants.

Sodium has also been shown to effect the fertility of the soil (Agassi *et al.* 1981, Qadir & Schubert 2002). The addition of sodium ions to the soil increases the Na/plant nutrient

cation ratios which has been shown to decrease the uptake of certain plant nutrient cations such as K^+ (Maas & Grieve 1987, Alam *et al.* 1989, Ehret *et al.* 1990). Plants grown in a high saline environment often display nutrient cation ion deficiencies, of elements such as Ca, K and Mg, (Maas & Grieve 1987, Alam *et al.* 1989, Ehret *et al.* 1990). Post-AD and post-PFP irrigated plants did not show signs of nutrient deficiencies but after prolonged irrigation the sodium may build up in the soil to levels which cause sodium induced nutrient deficiencies.

Sodium also affects the dispersion and flocculation of soil aggregates (Agassi *et al.* 1981, Qadir & Schubert 2002). Irrigation with waters with a SAR above 9.0 cause soil dispersion, which is accompanied by a deterioration in the hydro-physical properties of the soil (Buckman & Brady 1967, Khouri *et al.* 1994, Miller & Donahue 1995). The physical properties of soils irrigated with post-AD and post-PFP BE did not show signs of deterioration. However, the sodium concentration of the soils increased, and after prolonged use of BE irrigation, the build-up of sodium in the soil will be accompanied by a reduction in the stability, rainfall infiltration rate, and porosity of the soil, as observed by numerous authors (Agassi *et al.* 1981, Abu Sharar *et al.* 1987, Muyen *et al.* 2011, Kumar & Chopra 2012, Dakoure *et al.* 2013).

The accumulation of sodium in the soil from irrigation with treated wastewaters is the major limitation to using wastewater in agriculture and practices need to be developed in reduce the accumulation of sodium in soils (Qadir *et al.* 2003, Muyen *et al.* 2011). The key to the successful use of saline irrigation waters depends on the following: cultivation of salt and sodium tolerant crops; adequate leaching of sodium while avoiding deterioration of the soils physical profile; and the integrated use of saline and non-saline water (Qadir & Oster 2004). Certain crop species have been shown to aid in the removal of sodium from the soil (Qadir

et al. 2001, Qadir *et al.* 2005).It is therefore important that crop species are identified that can reduce the build-up of sodium in the soil.

None of the crops grown in the crop suitability experiment (Chapter 3) were able to stop the build-up of sodium in the soil. They were able to reduce the build-up when compared to soils irrigated with No Crops. The sodium content in the soil was not related to the sodium content and growth of the crops. This suggests that there are probably other plant-soil interactions that aided in the removal of sodium from the soil, such as plant assisted leaching (Chaudhri *et al.* 1964, Gritsenko & Gritsenko 1999, Owens 2001). Sodium is removed from the soil through two processes; firstly sodium is assimilated into the plant tissue thus reducing the salt content of the soil (Chaudhri *et al.* 1964, Gritsenko & Gritsenko 1999, Owens 2001). Secondly, "the ability of plant roots to increase the dissolution rate of calcite, resulting in enhanced levels of Ca^{2+} in soil solution to replace Na^{+} from the cation exchange complex" (Qadir *et al.* 2005). Qadir *et al.* (2003) found that soil sodium removal by lucerne plant tissue accounted for less than five percent of the total sodium removed from the soil. The majority of the sodium was removed from the soil through leaching (Qadir e*t al.* 2003). The leaching of sodium through the soil was higher in lucerne planted soil compared to unplanted soil (Qadir e*t al.* 2003). Respiration from roots and associated microorganisms released CO_2 into the soil water which forms carbonic acid. The generation of H^{+} from carbonic acid dissociation can then react with soil calcium carbonate to produce Ca^{2+} and HCO_3^{-}. The Ca^{2+} can now exchange with sodium at the soils cation exchange sites resulting in sodium ions being leached out in the soils percolating water which in turn reduces the soils sodium content and SAR (Qadir *et al.* 2003, Qadir *et al.* 2005). The addition of H^{+} in pH adjusted irrigation treatments increases the dissolution rate of calcite in the soil and thus increased the levels of Ca^{2+} in soil solution that can replace Na^{+} from the cation

exchange complex. Acidic irrigation waters should increase sodium leaching through the soil due to the addition of H^+ which can react with calcium carbonate. Future research should be done to determine the effect of acidic irrigation waters on the leaching rate of sodium from soils as this may aid in the reclamation of salt affected soils.

Saline water can successfully be used for crop irrigation provided proper management strategies are put in place to reduce the negative impacts it has on the soil (Murtaza *et al.* 2006). These include the cyclic use of non-saline water and saline water in crop production systems, cultivation of crops that aid in the assimilation and leaching of soil water sodium and the addition of gypsum to the soil or irrigation water (Qadir *et al.* 2001, Murtaza *et al.* 2006). Future research needs to investigate the use of BE in soil crop production systems where these management practices are optimised to ensure sodium does not build in in the soil to levels where it becomes detrimental to the soil's fertility and impacts upon crop yields.

5.2 Soil vs hydroponic culture systems

By growing the same crops in soil and in hydroponic systems, it is possible to compare the performance of crops grown in each system, with respect to the utilisation of treated BE. In hydroponic systems plants are exposed directly to the negative qualities of BE, whereas the soil may buffer the plants against some these effects.

The cation exchange complex of soils acts as a nutrient bank from which to supply plants with the majority of minerals they need (Epstein & Bloom 2005). If BE lacks certain micronutrients then soil can supply these nutrients to the plants. However, the gravel in hydroponic beds do not provide any nutrient buffering for the crops grown in the hydroponic systems. Plants irrigated with post-PFP effluent in hydroponic systems and in

the soil showed no signs of nutrient deficiency. This implies that post-PFP effluent is not lacking in macro and micro nutrients. However, plants in both production systems did grow slower than plants irrigated with a commercial nutrient solution. The direct cause for the reduced growth cannot be clearly identified and it was probably a combination of salt stress, effluent alkalinity and possibly the lack of some micronutrients in BE.

The effect of pH manipulation on the growth and chemical composition was different when comparing plants grown in the soil and in hydroponic systems. The pH manipulation of BE had no influence on the growth, CCI and N, P and K composition of plants grown in the soil. However, in hydroponic systems the pH adjustment of BE increased the mass, CCI and N content of the same plants. Millet and cabbage plants grown in hydroponic systems fed with pH unadjusted post-PFP BE hardly grew over the 12 week trial, while plants grown in pH adjusted hydroponic systems grew significantly larger (Chapter 4).

Soils have the ability to withstand pH change and this known as its buffering capacity (Buckman & Brady 1967, de Villiers & Jackson 1967, McLean & Owen 1969). Therefore the pH of BE did not influence that growth and CCI of plants grown in the soil as the soil was able to resist the pH of BE. In hydroponic systems the plants are directly exposed to the pH effects of BE. The availability of most nutrients decreases substantially when the pH rises above 7.5 (Lucas & Davis 1961, Tyson *et al.* 2007). The high pH of BE in pH unadjusted hydroponic systems decreased the availability of nutrients to cabbages, resulting in cabbages growing at the same rate as nutrient limited cabbages. When using BE in hydroponic crop production systems it is vital to adjust the pH to 6.5 – 7.0 to ensure the maximum availability of nutrients to plants and, hence, good crop growth.

A problem observed in the hydroponics trials was the high alkalinity of BE. After pH adjustment to 6.5 the pH of hydroponic system still rose to 8.0 after 5 - 7 days. Further pH adjustment of BE is not feasible because this would increase the conductivity of the hydroponic systems, thus increasing the osmotic stress on the plants. The high alkalinity of BE arises because of the addition of sodium hydroxide to raw BE before it goes through anaerobic digestion, and the generation of carbonate, bicarbonate and ammonium alkalinity during anaerobic digestion (Van Rensburg *et al.* 2003, Power 2014). The pH of anaerobic supernatants has been shown to increase with aeration of the liquor (Musvoto *et al.* 2000, Power 2014). The high alkalinity of BE is an issue when using BE in hydroponic crop production systems as large amounts of acid need to be used to correct the pH (Power 2014). The generation of the high alkalinity in BE comes from the AD process (Van Rensburg *et al.* 2003, Power 2014). The anaerobic digestion process also adds to the sodium content of BE. The high sodium and alkalinity of BE is the main limitation when using BE in hydroponic and soil production systems. The origin of these properties needs to be identified and solutions to address these problems are discussed in the following section.

5.3 Origin of sodium and alkalinity in brewery effluent

There are two contributors to the sodium content of BE: sodium hydroxide is used in the bottle washing process; and it is added to the incoming effluent before it goes through anaerobic digestion. Sodium hydroxide is used because of its low cost, good cleaning capabilities and efficiency in neutralising acids (Potgieter, *pers. comm.,* Process Specialist, Ibhayi Brewery, SAB Ltd., August 2015). An alternative to sodium hydroxide would be other highly soluble alkaline compounds such as potassium hydroxide. However potassium

hydroxide is more expensive and less readily available than sodium hydroxide (Potgieter, *pers. comm.,* Process Specialist, Ibhayi Brewery, SAB Ltd., August 2015). It is therefore unlikely that the brewery industry will change from using sodium hydroxide in their cleaning processes. The use of potassium hydroxide instead sodium hydroxide would decrease the sodium content of and would increase the potassium concentration of the effluent, which should increase the suitability of the effluent for irrigated crop production.

Carbon dioxide is generated during the AD of organic waste in the effluent (Batstone *et al.* 2002). Some of the CO_2 generated during AD dissolves in the liquor and generates carbonic acid and carbonate alkalinity (Van Rensburg *et al.* 2003). This causes an increase both the alkalinity and acidity of the liquor (Van Rensburg *et al.* 2003). In order to maintain a stable pH that is suitable for the methanogens in the anaerobic digester sodium hydroxide is added to the incoming effluent (Power 2014). When BE is exposed to the atmosphere the volatile carbon dioxide dissolved as carbonic acid is gassed off whereas the carbonate alkalinity is more stable and remains in the water (Musvoto *et al.* 2000, Van Rensburg *et al.* 2003). This results in a decrease in acidity of BE and an increase in pH (Musvoto *et al.* 2000, Van Rensburg *et al.* 2003). This is why post-AD BE has a high pH and alkalinity.

An opportunity exists to decrease the reliance on the addition of sodium hydroxide during AD. If post-AD effluent is exposed to atmospheric conditions it will lose its carbonic acidity while maintaining its alkalinity (Musvoto *et al.* 2000, Power 2014). A fraction of this degassed post-AD effluent can then be recycled back with incoming effluent going into the digester which will stabilise the pH of the digester (Power 2014). This will increase the influent alkalinity and potentially reduce the amount of sodium hydroxide added during

anaerobic digestion. This in turn will decrease the sodium concentration of the BE, making it more suitable for downstream crop production.

5.4 Conclusion

Brewery effluent contains sufficient plants nutrients to support the growth of cabbages, saltbush, lucerne and millet. However BE contain certain components that inhibit the growth of plants. In soil culture systems the primary concern is the high salinity/conductivity of BE. In hydroponic systems it is the combination of the high alkalinity and salinity/conductivity of the effluent. The buffering capacity of soils was able to resist the pH of BE where as in hydroponic systems the high pH of BE decreased the availability of nutrients to plants, which resulted in plants growing at the same rate as plants grown in nutrient limited systems The high alkalinity of BE is an issue, firstly for deceasing the availability of nutrients in BE, and secondly making it hard to maintain a pH range of between 6.5 and 7.0 to optimise the availability of nutrients of the plants.

A major concern when using BE in soil crop production systems is the accumulation of sodium in the soil. After prolonged used the accumulation of sodium in the soil will be accompanied by the deterioration of the soils hydro-physical properties and decrease in crop yield. Sodium removal from the soil water can be achieved by plant uptake and leaching. The results from this study suggests that the main mechanisms for sodium removal was through plant assisted leaching. Further sodium leaching was increased as the acidity of the irrigation water increased (pH decreased). Of the crops grown, lucerne showed the most promise as it improved the soils physical properties, it is also a popular fodder crop, can grow well in alkaline environments and can be harvested multiple times from one stand.

The concept of a single-use, treatment and discharge system will not be able to address the water resources needs of society in the future. With the current water status of South Africa, it is vital that we develop technologies and change industry practices, to enable the reuse of wastewater, for example, by changing the treatment of BE from sodium hydroxide to potassium hydroxide or calcium carbonate (or hydroxide). Brewery effluent has three major resources that could to be exploited: the biogas potential from the carbon content in the effluent; the plant nutrients in the effluent, as a source of fertilisers; and the water component of the effluent for irrigation purposes or industrial use. The benefits of developing this energy, nutrient and water resource could contribute to cost-reductions at the brewery, more efficient water, nutrient and energy management at the brewery, and offer opportunities for job creation and benefit local food security.

References

Abbot LK, Murphy DV. (2007) *Soil biological fertility: A key to sustainable land use in agriculture.* Springer, Dordrecht, Netherlands.

Abu Sharar TM, Bingham FT, Rhoades JD. (1987) Stability of soil aggregates as affected by electrolyte concentration and composition. *Soil Science Society of America Journal* **51:** 309-314.

Agassi M, Shainberg I, Morin J. (1981) Effect of electrolyte concentration and soil sodicity on infiltration rate and crust formation. *Soil Science Society of America Journal* **48:** 848-51.

Agboola AA, Corey RB. (1973) The relationship between soil pH, organic matter, available phosphorus, exchangeable potassium, calcium, magnesium, and nine elements in the maize tissue. *Soil Science* **115:** 331-400.

Ajmal M, Khan AU. (1984) Effects of brewery effluent on agricultural soil and crop plants. *Environmental Pollution* **33:** 341-351.

Alam SM, Naqvi SSM, Amzi AR. (1989) Effect of salt stress on growth of tomato. *Pakistan Journal of Scientific and Industrial Research* **32:** 110-113.

Al Droubi A, Fritz B, Gac JY, Tardy Y. (1980) Generalized residual alkalinity concept; application to prediction of the chemical evolution of natural waters by evaporation. *American Journal of Science* **280:** 560-572.

Aljaloud AA, Hussain G, Alsaati AJ, Karimullah S. (1993) Effects of wastewaters on plant growth and soil properties. *Arid Soil Research and Rehabilitation* **7:** 173-179.

Angelidaki I, Sanders W. (2004) Assessment of the anaerobic biodegradability of macro pollutants. *Environmental Science and Bio/Technology* **3:** 117-129.

Angers DA, Caron J. (1998) Plant-induced changes in soil structure: Processes and feedbacks. *Biogeochemistry* **42:** 55-72.

Antoniou P, Hamilton J, Koopman B, Jain R, Holloway B, Lyberatos G, Svoronos SA. (1989) Effect of temperature and pH on the effective maximum specific growth rate of nitrifying bacteria. *Water Research* **24:** 97-101.

Apse MP, Blumwald E. (2007) Na^+ transport in plants. *Federation of European Biochemical Societies* **581:** 2247-2254.

Arnell NW. (2004) Climate change and global water resources: SRES emissions and socio-economic scenarios. *Global Environmental Change* **14:** 31-52.

Ascher B. (2012) Global beer: The road to monopoly. Report for the American Antitrust Institute http://antitrustinstitute.org/~antitrust/sites/default/files/Global%20Beer%20Road%20to%20Monopoly_0.pdf (Accessed: 2014/06/19).

Barbour MG, Burk JH, Pitts WD, Gillian FS, Schwartz MN. (1998) *Terrestrial plant ecology.* Cummings, California, USA.

Barker AV, Mills HA. (1980) Ammonium and nitrate nutrition of horticultural crops. *Horticultural Reviews* **2:** 395-423.

Bar-Yosef B, Mattson NS, Lieth HJ. (2009) Effects of NH4:NO3:urea ratio on cut roses yield, leaf nutrients content and proton efflux by roots in closed hydroponic system. *Scientia Horticulturae* **122:** 610-619.

Batstone DJ, Keller J, Angelikadi I, Kalyuzhnyi SV, Pavlostathis SG, Rozzi A, Sanders WTM, Siegrist H, Vavilin VA. (2002) The IWA Anaerobic Digestion Model No 1 (ADM1). *Water Science and Technology* **45:** 65-73.

Bauder JW, Brock TA. (2001) Irrigation water quality, soil amendment, and crop effects on sodium leaching. *Arid Land Research and Management* **15:** 101-113.

Black CA. (1968) *Soil plant relationships.* John Wiley and sons Inc, New York, USA.

Blumwald E. (2000) Sodium transport and salt tolerance in plants. *Current Opinion in Cell Biology* **12:** 431-434.

Blumwald E, Aharon GS, Apse MP. (2000) Sodium transport in plant cells. *Biochimica et Biophysica acta* **1465:** 140-151.

Bond WJ. (1998) Effluent irrigation an environmental challenge for soil science. *Australian Journal of Soil Research* **36:** 543-555.

Borgognone D, Colla G, Rouphael Y, Cardarelli M, Rea E, Schwarz D. (2012) Effect of nitrogen form and nutrient solution pH on growth and mineral composition of self- grafted and grafted tomatoes. *Scientia Horticulturae* **149:** 61-69.

Braeken L, Van der Bruggen B, Vandecasteele C. (2004) Regeneration of brewery waste water using nanofiltration. *Water Research* **38:** 3075-3082.

Brito AG, Peixoto J, Oliveira JM, Costa C, Nogueira R, Rodrigues A. (2007) Brewery and winery wastewater treatment: some focal points of design and operation. *Utilization of By-products and Treatment of Waste in the Food Industry* **3:** 109-131.

Britto DT, Kronzucker HJ. (2002) $NH4^+$ toxicity in higher plants: a critical review. *Journal of Plant Physiology* **159:** 567-584.

Brix H, Schierup HH. (1989) The use of macrophytes in water pollution control. *Ambio* **18:** 100-107.

Brown JJ, Glenn EP, Fitzsimmons KM, Smith SE. (1999) Halophytes for the treatment of saline aquaculture effluent. *Aquaculture* **175:** 255-268.

Buckman HL, Brady NC. (1967) *The nature and properties of soils 6th edition.* Macmillian company, New York, USA.

Camilla P, Mancuso S, Shabala S. (2012) Physiology of acclimation to salinity stress in pea (*Pisum sativum*). *Environmental and Experimental Botany* **84:** 44-51.

Carter MR, Gregorich EG. (2008) *Soil sampling and methods of analysis second edition*. Taylor and Francis, New York.

Castillo N. (2011) A hydroponic approach to evaluate responses to salinity stress in cotton. Doctor of philosophy thesis, Texas Technology University, Lubbock, USA.

Chapin FS, Matsou PA, Vitousek P. (2011) *Principles of Terrestrial Ecosystem Ecology.* Springer, New York City, USA.

Chaudhri II, Shah BH, Naqvi N, Mallick IA. (1964) Investigations on the role of *Suaeda fruticosa* in the reclamation of saline and alkaline soils in West Pakistan. *Plant and Soil* **21:** 1-7.

Cheng S, Grosse W, Karrenbrock F, Thoennessen M. (2002) Efficiency of constructed wetlands in decontamination of water polluted by heavy metals. *Ecological Engineering* **18:** 317-325.

Chow WS, Ball MC, Anderson JM. (1990) Growth and photosynthetic responses of spinach to salinity: implications of K^+ nutrition for salt tolerance. *Australian Journal of Plant Physiology* **17:** 563-578.

Cilliers A. (2012) The treatment of brewery effluent using an integrated high rate algal ponding system. Masters thesis, Rhodes University, Grahamstown, South Africa.

Claussen W, Lenz F. (1999) Effect of ammonium or nitrate nutrition on net photosynthesis, growth, and activity of the enzymes nitrate reductase and glutamine synthetase in blueberry, raspberry and strawberry. *Plant and Soil* **208:** 95-102.

Chodak M, Niklińska M. (2010). Effect of texture and tree species on microbial properties of mine soils. *Applied Soil Ecology* ***46*****:** 268-275.

Coleman J, Hench K, Garbutt K, Sexstone A, Bissonnette G, Skousen J. (2001) Treatment of domestic wastewater by three species in constructed wetlands. *Water, Air and Soil Pollution* **128:** 283-295.

Condom N, Kuper M, Marlet S, Vallies V, Kijne J. (1999) Salinization, alkalization and sodification in Punjab (Pakistan): Characterization of the geochemical and physical processes of degradation. *Land Degradation Development* **10:** 123-140.

Cramer GR, Lauchli A, Polito VA. (1985) Displacement of Ca^{2+} by Na^+ from the plasmalemma of root cells: a primary response to salt stress? *Plant Physiology* **79:** 207-211.

Dakoure MY, Mermoud A, Yacouba H, Boivin P. (2013) Impacts of irrigation with industrial treated wastewater on soil properties. *Geoderma* **200:** 31-39.

Department of Water affairs and Forestry's (DWAF). (1996) *Water Quality Guidelines. Volume 4: Agricultural use: Irrigation*.

Department of Water Affairs and Forestry (DWAF). (1999) Engaging in a controlled activity, identified as such in section 37(1): irrigation of any land with waste or water containing waste generated through any industrial activity or by a waterwork. Government Gazette 20526.

de Villiers JM, Jackson ML. (1967) Cation exchange capacity variations with pH in soil clays. *Soil Science Society America Proceedings* **31:** 614-619.

Diaz FJ, Benes SE, Grattan SR. (2013) Field performance of halophytic species under irrigation with saline drainage water in the San Joaquin Valley of California. *Agricultural Water Management* **118:** 59-69.

Ehret DL, RE Redmann, BL Harvey, Cipywnyk A. (1990) Salinity-induced calcium deficiencies in wheat and barley. *Plant Soil* **128:** 143-151.

El-Khateeb MA, Al-Herrawy AZ, Kamel MM, El-Gohary FA. (2009) Use of wetlands as post-treatment of anaerobically treated effluent. *Desalination* **245:** 50-59.

Epstein E, Bloom A. (2005) *Mineral Nutrition of Plants: Principles and Perspectives.* Sinauer Associates, Sunderland, USA.

Errebhi M, Wilcox GE. (1990) Plant species response to ammonium-nitrate concentration ratios. *Journal of Plant Nutrition* **13:** 1017-1029.

European Union scientific for food (EUROPA). (2009) Opinion on nitrate and nitrite. Disponível em http://ec.europa.eu/ (Accessed: 2014/07/10).

Fan J, Zhang B, Zhang J, Ngo HH, Guo W, Liu F, Guo Y, Wu H. (2013) Intermittent aeration strategy to enhance organics and nitrogen removal in subsurface flow constructed wetlands. *Bioresource Technology* **141:** 117-122.

Filella I, Serrano I, Serra J, Penuelas J. (1995) Evaluating wheat nitrogen status with canopy reflectance indices and discriminant analysis. *Crop Science* **35:** 1400-1405.

Flowers TJ, Colmer TD. (2008) Salinity tolerance in halophytes. *New Phytologist* **179:** 945-963.

Fontainea B, Mariottib A, Abbadie L. (2003) The priming effect of organic matter: a question of microbial competition? *Soil Biology and Biochemistry* **35:** 837-843.

Freeman S. (2005) *Biological Science 2nd Ed*. Pearson Prentice Hall, New Jersey, USA.

Hanjra MA, Qureshi ME. 2010. Global water crisis and future food security in an era of climate change. *Food Policy* **35:** 365-377.

Galinski EA. (1995). Osmo-adaptation in bacteria. *Advances in Microbial Physiology* **37:** 273-328.

Gallert C, Bauer S, Winter J. (1998) Effect of ammonia of the anaerobic digestion of protein by a mesophilic and thermophilic biowaste population. *Applied Microbiology and Biotechnology* **50:** 495-501.

Garcia C, Hernandez T, Costa F. (1994) Microbial activity in soils under Mediterranean environmental conditions. *Soil Biology and Biochemistry* **26:** 1185–1191.

Garland JL, Mills AL. (1991) Classification and characteristics of heterotrophic microbial communities on the basis of patterns of community-level sole-carbon-source-utilization. *Applied and Environmental Microbiology* **57:** 2351-2359.

Gerendás J, Zhu Z, Bendixen R, Sattelmacher B. (1997) Physiological and biochemical processes related to ammonium toxicity in higher plants. *Journal of Plant Nutrition and Soil Science* **160:** 239-251.

Glenn EP, Brown JJ, Blumwald E. (1999) Salt tolerance and crop potential of halophytes. *Plant Sciences* **18:** 227-255.

Gomez E, Garland JL, Conti M. (2004) Reproducibility in the response of soil bacterial community level physiological profiles from a land use intensification gradient. *Applied Soil Ecology* **26:** 21-30.

Grattan SR, Grieve CM. (1999) Salinity-mineral nutrient relations in horticultural crops. *Scientia Horticulurae* **78:** 127-157.

Grattan SR, Rhoades JD. (1990) Irrigation with saline ground water and drainage water. Agricultural salinity assessment and management, manuals and reports on engineering practices No. 71. American Society of Civil Engineers, New York.

Gritsenko GV, Gritsenko AV. (1999) Quality of irrigation water and out- look for phytomelioration of soils. *Eurasian Soil Science* **32:** 236-242.

Hall IR. (1974) Some studies on nitrification in the activated-sludge process. *Water Pollution Control* **73:** 538-547.

Handreck KA, Black ND. (1994) *Growing media for ornamental plants and turf*. New South Wales Press, Sydney.

Hanson B, Grattan SR, Fulton A. (1999) *Agricultural Salinity and Drainage*. University of California Irrigation Program. University of California, Davis.

Hassidim M, Braun Y, Lerner HR, Reinhold L. (1990) Na^+/H^+ and K^+/H^+ antiport in root membrane vesicles isolated from the halophyte *Atriplex* and the glycophyte Cotton. *Plant Physiology* **94:** 1795-1801.

Hati KM, Biswas AK, Bandyopadhyay KK, Misra AK. (2007) Soil properties and crop yields on a vertisol in India with application of distillery effluent. *Soil and Tillage Research* **92:** 60-68.

Honeysett BM, Milthorpe PL, Wynne MJ. (2004) Getting the best from old man saltbush, Agricultural research and advisory station, Condobolin, Australia. http://www.dpi.nsw.gov.au/__data/assets/pdf_file/0016/162223/Getting-the-best-from-old-man-saltbush.pdf, (Accessed: 2015/10/28).

Ilyas M, Miller RW, Qureshi RH. (1993) Hydrualic conductivity of saline-sodic soil after gypsum application and cropping. *Soil Science Society of America Journal* **57:** 1580-1585.

Iritani WM, Arnold CY. (1960) Nitrogen release of vegetable crop residues during incubation as related to their chemical composition. *Soil Science* **89:** 74-82.

Jacoby B. (1994) *Mechanisms involved in salt tolerance by plant. Handbook of Plant and Crop Stress.* Marcel Dekker, New York, pp. 97-123.

Jalali M, Merikhpour H, Kaledhonkar MJ, Van Der Zee SE. (2008) Effects of wastewater irrigation on soil sodicity and nutrient leaching in calcareous soils. *Agricultural Water Management* **95:** 143-153.

Jones CLW, Britz PJ, Cilliers A, Laubscher R, Power S, Scheepers R. (2013) *Environmentally sustainable beneficiation of brewery effluent: algal ponding, constructed wetland, hydroponic vegetables and aquaculture (Final draft).* Report to the Water Research Commission (K5/2008), South Africa.

Juwarkar A, Dutta SA. (1990) Impact of distillery effluent application to land and soil microflora. *Environmental Monitoring and Assessment* **15:** 201-210.

Kannan K, Oblisami G. (1990) Effect of pulp and paper mill effluent irrigation on carbon dioxide evolution in soils. *Journal of Agronomy and Crop Science* **164:** 116-119.

Kayombo S, Mbwette TSA, Mayo AW, Katima THY, Jorgensen SE. (2002) Diurnal cycles of variation of physical–chemical parameters in waste stabilization ponds. *Ecological Engineering* **18:** 287-291.

Kaushik A, Nisha R, Jagjeeta K, Kaushik CP. (2005) Impact of long and short term irrigation of a sodic soil with distillery effluent in combination with bioamendments. *Bioresource Technology* **96:** 1860-1866.

Kenatu A. (2011) Evaluation of the performance of constructed wetland system for the treatment of brewery wastewater. Masters thesis, Addis Ababa University, Addis Ababa, Ethopia.

Khouri N, Kalbermatten JM, Bartone CR. (1994) *The reuse of wastewater in agriculture: A guide for planners.* World Bank water and sanitation programme, World Bank, Washington, USA.

Kiziloglu FM, Turan M, Sahin U, Angin I, Anapali O, Okuroglu M. (2007) Effects of wastewater irrigation on soil and cabbage-plant (*Brassica olerecea* var. capitate cv. yalova-1) chemical properties. *Journal of Plant Nutrition and Soil Science* **170:** 166-172.

Kiziloglu FM, Turan M, Sahin U, Kuslu Y, Dursun A. (2008) Effects of untreated and treated wastewater irrigation on some chemical properties of cauliflower (*Brassica olerecea* L. var. botrytis) and red cabbage (*Brassica olerecea* L. var. rubra) grown on calcareous soil in Turkey. *Agricultural Water Management* **95:** 716-724.

Knight RL, Payne VW, Borer RE, Clarke RA, Pries JH. (2000) Constructed wetlands for livestock wastewater management. *Ecological Engineering* **15:** 41-55.

Kumar V, Chopra AK, Pathak C, Pathak S. (2010) Agro-potentiality of paper mill effluent on the characteristics of *Trigonella foenum-graecum* (Fenugreek). *New York Science Journal* **3:** 68-77.

Kumar V, Chopra AK. (2012) Effects of paper mill effluent irrigation on agronomical characteristics of *Vigna radiata* in two different seasons. *Soil Science and Plant Analysis* **43:** 2142-2166.

Lambers H, Chapin FS, Pons TL. (2008) *Plant Physiological Ecology.* Springer, New York City, USA.

Lastra O, Tapia ML, Razeto B, Rojas M. (2009) Response of hydroponic lettuce cultivars to different treatments of nitrogen: Growth and foliar nitrate content. IDESIA (Chile) **27:** 83-89.

Lazarova V, Bahri A. (2005) *Water Reuse for Irrigation, Agriculture, Landscape and Turf Grass*. CRC Press, New York, USA.

Le Bissonnais Y. (1996) Aggregate stability and assessment of soil crustability and erodibility: I. Theory and methodology. *European Journal of Soil Science* **47:** 425-437.

Lee CY, Lee CC, Lee FY, Tseng SK, Liao CJ. (2004) Performance of subsurface flow constructed wetland taking pretreated swine effluent under heavy loads. *Bioresource Technology* **92:** 173-179.

Lehmann J, Schroth G. (2003) *Trees, crops and soil fertility.* Centre for Agriculture and Bioscience International, Oxfordshire, United Kingdom.

Lieth H, Al-Masoom AA. (1993) *Towards Rational Use of High Salinity Tolerant Plants, Deliberations about High Salinity Tolerant Plants and Ecosystems* Kluwer Academic Publishers, Dordrecht, Netherlands.

Liu C, Yuan X, Zeng G, Li W, Li J. (2008) Prediction of methane yield at optimum pH for anaerobic digestion of organic fraction of municipal solid waste. *Bioresource Technology* **99:** 882-888.

Lucas RE, Davis JF. (1961) Relationships between pH values of organic soils and availabilities of 12 plant nutrients. *Soil Science* **92:** 177-182.

Lyberatos G, Skiadas IV. (1999) Modelling of anaerobic digestion – a review. *Global Nest: The International Journal* **1:** 63-76.

Lymbery AJ, Doupe RG, Bennet T, Starcevich MR. (2006) Efficacy of a subsurface-flow wetland using estuarine sedge *Juncus krassii* to treat effluent from inland saline aquaculture. *Aquaculture Engineering* **34:** 1-7.

Maas EV, Grieve CM. (1987) Sodium –induced calcium deficiency in salt-stressed corn. *Plant Cell Environment* **10:** 559-564.

Magdalena F, Karolina O, Jerzy L. (2012) Community level physiological profile (CLPP), characterization and microbial activity of soil amended with dairy sewage sludge. *Sensors* **12:** 3253-3268.

Marais GVR. (1970) Dynamic behaviour of oxidation ponds. Proceedings 2nd International Symposium on Waste Treatment Lagoons, Kansas City.

Marschner H (1990) *Mineral Nutrition of Higher Plants*. Academic Press, London, United Kingdom.

Mclean EO, Owen J. (1969) Effects of pH on the contribution of organic matter and clay to soil cation exchange capacity. *Soil Science Society America Proceedings* **6:** 150-156.

Meek BD, Detar WR, Rolph D, Rechel ER, Carter LM. (1990) Infiltration rate as affected by an alfalfa and no-till cotton cropping system. *Soil Science Society of America Journal* **54:** 505-508.

Merzlyak MN, Gitelson AA, Chivkunova OB, Rakitin VY. (1999) Non-destructive optical detection of leaf senescence and fruit ripening. *Plant Physiology* **106:** 135-141.

Miller RW, Donahue RL. (1995) *Soils in Our Environment 7th Edition*. Prentice Hall, New Jersey, USA.

Miyamoto S, Glennt EP, Olsent MW. (1996) Growth, water use and salt uptake of four halophytes irrigated with highly saline water. *Journal of Arid Environments* **32:** 141-159.

Moran JA, Mitchell AK, Goodmanson G, Stockburger KA. (2000) Differentiation among effects of nitrogen fertilization treatments on conifer seedlings by foliar reflectance: a comparison of methods. *Tree Physiology* **20:** 1113-1120.

Moreno F, Cabrera F, Fernandez-Boy E, Giron IF, Fernandez JE, Bellido B. (2001) Irrigation with saline water in the reclaimed marsh soils of south-west Spain: impact on soil properties and cotton and sugar beet crops. *Agricultural Water Management* **48:** 133-150.

Munns R. (2002) Comparative physiology of salt and water stress. *Plant Cell Environment* **25:** 239-250.

Munns R, Termaat A. (1986) Whole-plant responses to salinity. *Plant Physiology* **13:** 143-160.

Murtaza G, Ghafoor A, Qadir M. (2006) Irrigation and soil management strategies for using saline-sodic water in a cotton-wheat rotation. *Agricultural Water Management* **81:** 98-114.

Musvoto EV, Wentzel MC, Ekama GA. (2000) Integrated chemical-physical process modelling–II Simulating aeration treatment of anaerobic digester supernatants. *Water Research* **34:** 1868-1880.

Muyen Z, Moorea GA, Wrigley RJ. (2011) Soil salinity and sodicity effects of wastewater irrigation in South East Australia. *Agricultural Water Management* **99:** 33-41.

Nass R, Cunningham KW, Roa R. (1997) Intracellular sequestration of sodium by a novel Na^+/H^+ exchanger in yeast is enhanced by mutations in the plasma membrane H^+-ATPase. *Journal of Biological Chemistry* **272:** 26145-26152.

Nelson PN, Ladd JN, Oades JM. (1996) Decomposition of ^{14}C- labelled plant material in a salt-affected soil. *Soil Biology and Biochemistry* **28:** 433-441.

Newman Y, Jennings E, Vendramini J, Blount A. (2010) Pearl millet (*Pennisetum glaucum*): overview and management, University of Florida http://edis.ifas.ufl.edu/ag347, (Accessed: 2015/10/28).

Ogbonna JC, Yoshizawa H, Tanaka H. (2000) Treatment of high strength organic wastewater by a mixed culture of photosynthetic microorganisms. *Journal of Applied Phycology* **12:** 277-284.

Ong SA, Uchiyama K, Inadama D, Yamagiwa K. (2009) Simultaneous removal of colour, organic compounds and nutrients in azo dye-containing wastewater using up-flow constructed wetland. *Journal of Hazardous Materials* **165:** 696-703.

Oren A. (1999) Bioenergetic aspects of halophilism. *Microbiology and Molecular Biology* **65:** 334-348.

Osawa T. (1961) Studies of salt tolerance of vegetable crops in sand cultures, II leafy vegetables. *Japanese Society for Horticulture* **30:** 48-56.

Osawa T. (1963) Studies of salt tolerance of vegetable crops with special reference to osmotic effects and specific ion effects. *Japanese Society for Horticulture* **32:** 211-223.

Oster JD. (1994) Irrigation with poor quality water. *Agricultural Water Management* **25:** 271-297.

Owens S. (2001). Salt of the earth. *European Molecular Biology Organization* **2:** 877-879.

Painter HA, Loveless JE. (1983) Effect of temperature and pH value on the growth-rate constants of nitrifying bacteria in the activated-sludge process. *Water Research* **17:** 237-248.

Pankhurst CE, Doube BM, Gupta SR. (1997) *Biological Indicators of Soil Health.* CAB International, Wallingford, United Kingdom.

Pankhurst CE, Yu S, Hawke BG, Harch BD. (2001) Capacity of fatty acid profiles and substrate utilization patterns to describe differences in soil microbial communities associated with increased salinity or alkalinity at three locations of South Australia. *Biology and Fertility of Soils* **33:** 204-217.

Parker DR, Pedler JF. (1997) Re-evaluating the free-ion activity model of trace metal availability to higher plants. *Plant Soil* **196:** 223-228.

Pathak H, Rao DLN. (1998) Carbon and nitrogen mineralization from added organic matter in saline and alkali soils. *Soil Biology and Biochemistry* **30:** 695-702.

Pearson KE. (2003) *The Basics of salinity and sodicity effects on soil physical properties*. Montana State University, http://waterquality.montana.edu/docs/methane/basics_highlight.shtml, (Accessed: 2014/06/19).

Penn MR, Pauer JJ, Mihelcic JR. (2009) *Environmental and ecological chemistry volume 2.* Encyclopedia of Life Support Systems, Oxford, United Kingdom.

Peñuelas J, Filella I. (1998) Visible and near-infrared reflectance techniques for diagnosing plant physiological status. *Trends in Plant Science* **3:** 151-156.

Pieri CJMG. (1989) *Fertility of soils. A future for farming in the West African savannah* Springer-Verlag, Berlin, Germany.

Pope PE, Vasey RB. (1976) Effect of soil buffer capacity on soil reaction (pH) modification and subsequent effects on growth and nutrient uptake of *Platanus occidentalis* L. seedlings. *Arkansas Academy of Science Proceedings* **30:** 67-70.

Power SD. (2014) The water and nutrient potential of brewery effluent for hydroponic tomato production. Masters thesis, Rhodes University, Grahamstown, South Africa.

Power SD, Jones CLW. (2015 in review) Anaerobically digested brewery effluent as a medium for hydroponic crop production – The influence of algal ponds and pH. *Cleaner production*: In review.

Pescod MB. (1992) *Wastewater Treatment and Use in Agriculture - FAO Irrigation and Drainage paper 47.* FAO, Rome, Italy.

Qadir M, Ghafoor A, Murtaza G. (2001) Use of saline-sodic waters through phytoremediation of calcareous saline-sodic soils. *Agricultural Water Management* **50:** 197-210.

Qadir M, Noble AD, Oster JD, Schubert S, Ghafoor A. (2005) Driving forces for sodium removal during phytoremediation of calcareous sodic and saline –sodic soils: a review. *Soil Use and Management* **21:** 173-180.

Qadir M, Oster JD. (2004) Crop and irrigation management strategies for saline-sodic soils and waters aimed at environmentally sustainable agriculture. *Science of the Total Environment* **323:** 1-19.

Qadir M, Schubert S. (2002) Degradation processes and nutrient constraints in sodic soils. *Land Degradation Development* **13:** 275-294.

Qadir M, Steffens D, Yan F, Schubert S. (2003) Sodium removal from a calcareous saline-sodic soil through leaching and plant uptake during phytoremediation. *Land Degradation and Development* **14:** 301-307.

Raven JA, Wollenweber RB, Handley LL. (1992) A comparison of ammonium and nitrate as nitrogen sources for photolithotrophs. *New Phytologist* **121:** 19-32.

Rietz DN, Haynes RJ. (2003) Effects of irrigation-induced salinity and sodicity on soil microbial activity. *Soil Biology and Biochemistry* **35:** 845-854.

Roberto K (2005) *How-To Hydroponics 4th Ed*. Future garden Press. New York, USA.

Robertson CP, Colemen DC, Bledsoe CS, Sollins P. (1999) *Standard soil methods for mong-term ecological research.* Oxford University press, United Kingdom.

Rodda N, Carden C, Armitage N, du Plessis HM. (2011) Development of guidance for sustainable irrigation use of greywater in gardens and small-scale agriculture in South Africa. *Water South Africa* **37:** 727-738.

Rozema ER. (2014) Can plants be used to remove Na^+ and Cl^- from nutrient solution in greenhouse production? Masters Thesis, The University of Guelph, Guelph, Canada.

Saqqar MR, Al-Nakshabandi GA, Shatanawi MM, Fayyad M, Al-Horani M. (1997) Some environmental problems associated with the use of treated wastewater for irrigation in Jordan. *Agricultural Water Management* **34:** 81-94.

Sahrawat KL. (1982) Nitrification in some tropical soils. *Plant and Soil* **65:** 281-286.

Senthilraja A, Jothimani P, Rajannan G. (2013) Effect of brewery wastewater on growth and physiological changes in maize, sunflower and sesame crops. *International Journal for Life Sciences and Educational Research* **1:** 36-42.

Senzia MA, Mayo AW, Mbwette TSA, Katima JHY, Jorgensen SE. (2002) Modelling nitrogen transformation and removal in primary facultative ponds. *Ecological Modelling* **154:** 207-215.

Shannon MC, Grieve CM. (1999) Tolerance of vegetable crops to salinity. *Scientia Horticulturae* **78:** 5-38.

Shannon MC, Grieve CM, Francois LE. (1994) *Plant environment interactions.* Elsevier, New York, USA.

Sharma DP, Rao KVGK. (1998) Strategy for long term use of saline drainage water for irrigation in semi-arid regions. *Soil Tillage Research* **48:** 287-295.

Shepherd HL, Grismer ME, Tchobanoglous G. (2014) Treatment of high-strength winery wastewater using a subsurface-flow constructed wetland. *Water Environmental Research* **73:** 394-403.

Silveira JAG, Araujo SAM, Lima JPMS, Viegas RA. (2009) Root and leaves display constrasting osmotic adjustment mechanisms in response to NaCl-salinity in *Atriplex nummularia. Environmental and Experimental Botany* **66:** 1-8.

Simate GS, Cluett J, Lyuke SE, Musapatika ET, Ndlovu S, Walubita LF. (2011) The treatment of brewery wastewater for reuse: State of the art. *Desalination* **273:** 235-247.

Sinsabaugh RL, Zak DR, Gallo M, Lauber C, Amonette R. (2004) Nitrogen deposition and dissolved organic carbon production in northern temperate forests. *Soil Biology and Biochemistry* **36:** 1509-1515.

Sinsabaugh RL. (2010) Phenol oxidase, peroxidase and organic matter dynamics of soil. *Soil Biology and Biochemistry* **42:** 391-404.

Sollins P, Robertson GP, Uehara G. (1988) Nutrient mobility in variable and permanent charge soils. *Biogeochemistry* **6:** 181-199.

Stewart GR, Sumar N, Patel M. (1987) *Inorganic nitrogen metabolism.* Springer, New York, USA.

Stottmeister U, Wiebner A, Kuschk P, Kappelmeyer U, Kastner M, Bederski O, Muller RA, Moormann H. (2003) Effects of plants and microorganisms in constructed wetlands for wastewater treatment. *Biotechnology Advances* **22:** 93-117.

Sukanya TS, Meli SS. (2004) Feasibility of distillery effluent as nitrogen source and its effect on nutrient uptake and soil nutrient status in wheat production. *Karnataka Journal of Agricultural Sciences* **17:** 410-416.

Sumner ME, Naidu R. (1998) *Sodic soils: distribution, properties, management and environmental consequences.* Oxford University press, United Kingdom.

Sumner ME, Naidu R, Rengasamy P. (1995) *Australian Sodic Soils: Distribution, Properties and Management.* CSIRO, Melbourne.

Sweeney DW, Graetz DA. (1991) Application of distillery waste anaerobic digester effluent to St. Augustine grass. *Agriculture, Ecosystems and Environment* **33:** 341-351.

Tchobanoglous G, Schroeder E. (1987) *Water Quality: Characteristics, Modeling, Modification.* Addison-Wesley, Massachusetts, USA.

Tuller M, Or D, Wrauth JM. (2003) Soil water potential [Online] Available: http://www.researchgate.net/publication/37451005_Soil_Water_Potential (Accessed 2015/12/10).

Tyson RV, Simmone EH, Davis M, Lamb EM, White JM, Treadwell DD. (2007) Effect of nutrient solution, nitrate-nitrogen concentration, and pH on nitrification rate in perlite medium. *Journal of Plant Nutrition* **30:** 901-913.

Undersander D, Cosgrove D, Cullem E, Grau C, Rice ME, Renz M, Sheaffer C, Shewmaker G, Sulc M. (2011) Alfalfal management guide, American society of Agronomy. https://www.crops.org/files/publications/alfalfa-management-guide.pdf, (Accessed: 2015/10/28).

Van Breemen N, Mulder J, Driscoll CT. (1983) Acidification and alkalinisation of soils. *Plant and Soil* **75:** 283-308.

Van HM, Hill RL. (1995) *Soil compaction and soil structure degradation. Crop residue management to reduce erosion and improve soil quality*, United States Department of Agriculture, Conservation report No 41.

Van Rensburg P, Musvoto EV, Wentzel MC, Ekama A. (2003) Modelling multiple mineral precipitation in anaerobic digester liquor. *Water Research* **37:** 3087-3097.

Vessey JK, Henry LT, Chaillou S, Raper CD. (1990) Root-zone acidity affects relative uptake of nitrate and ammonium from mixed nitrogen sources. *Journal of Plant Nutrition* **13:** 95-116.

Vymazal J. (2005) Horizontal sub-surface flow and hybrid constructed wetlands systems for wastewater treatment. *Ecological Engineering* **25:** 478-490.

Vymazal J. (2007) Removal of nutrients in various types of constructed wetlands. *Science of the Total Environment* **380:** 48-65.

Vymazal J. (2009) The use of constructed wetlands with horizontal sub-surface flow for various types of wastewater. *Ecological Engineering* **35:** 1-17.

Wang H, Wu L, Zhu Y, Tao Q. (2008) Growth, nitrate accumulation, and macronutrient concentration of pakchoi as affected by external nitrate-N: amino acid-N ratio. *Journal of Plant Nutrition* **31:** 1789-1799.

Weber K, Legge R. (2009) One-dimensional metric for tracking bacterial community divergence using sole carbon source utilization patterns. *Journal of Microbiology Methods* **2009:** 55-61.

Wells CD, Dekker LG, Clark S, Hart OO, Neba A, Rose PD. (2003) Integrated algal ponding systems (IAPS) in the treatment of domestic wastewater: A nine year performance evaluation. Environmental Biotechnology Research Unit, Rhodes University, Grahamstown, South Africa.

Zahran HH. (1992) Diversity, adaptation and activity of the bacterial flora in saline environments. *Biology and Fertility of Soils* **25:** 211-223.