The Effects of Irrigation with Laundry Greywater on Berea Red Soil Characteristics and Growth and Yield of Swiss Chard (*Beta vulgaris* L.) and Sweet Pepper (*Capsicum annuum* L.)

By

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24 November 2013

As the candidate's supervisor I agree/do not agree to the submission of this dissertation.

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"The loveliest theories are being overthrown by these damned experiments; it's no fun being a chemist anymore." Justus von Liebig (German Chemist, 1803-1873)

"In an age when man has forgotten his origins and is blind even to his most essential needs for survival, water along with other resources has become the victim of his indifference." Rachel Carson (American Conservationist, 1907-1964)

"Water is life's matter and matrix, mother and medium. There is no life without water." Albert Szent-Gyorgyi quotes (Hungarian Biochemist, 1937 Nobel Prize for Medicine, 1893-1986).

"We never know the worth of water 'till the well is dry." Thomas Fuller, Gnomologia, 1732

ABSTRACT

For many regions of the world, water security is becoming a rapidly growing concern driven primarily by a rapidly growing human population, an increasingly uncertain future global climate and compounded further by the strong dependency that exists between water security and food security. Reuse of laundry greywater for irrigation is a potential means of recovering water and nutrients that would otherwise be lost since it represents a considerable proportion of domestic waste-water discharge. This approach may be of substantial benefit for communities wishing to irrigate plants more sustainably and economically. However, laundry greywater chemistry is often characterised by high solute loads, which as irrigation water may be detrimental to plant growth, development and survival, and also adversely affect soil physicochemical properties. In this study, the physiological effects of laundry greywater irrigation regimes on the growth and development of Swiss Chard (*Beta vulgaris*) and Sweet Pepper (*Capsicum annuum*) were evaluated in relation to environmental conditions, soil pH, soil fertility, specific hydraulic conductivity of soils (K_s), plant nutrient uptake and fruit development in *C. annuum*.

Potted *B. vulgaris* and *C. annuum* plants were irrigated daily for 96 day and 135 days respectively at two experimental sites characterised by different environmental conditions. At the Unilever site, plants were exposed to full sunlight and strong sea breezes, whilst at the University site plants were partially shaded and sheltered from wind. Laundry greywater generated from two powder formulations (PG, CG) and a liquid laundry detergent (LG) were used as irrigation media, while a balanced nutrient solution (NS) and tap water (TW) treatment served as positive and negative controls respectively. *B. vulgaris* and *C. annuum* irrigated with NS attained the highest growth rates. For both crops, irrigation with greywater generated from the phosphate-based laundry powder (PG) produced similar growth and yield as TW irrigated treatments. Crops irrigated with laundry greywater generated from the carbonate-based laundry powder (CG) reflected the lowest mean biomass among all experimental treatments. Comparisons of tissue accumulations among treatments, together with nutrient uptake levels recorded, are suggestive of macronutrient deficiencies in greywater- and TW-irrigated treatments. Total mean fresh edible biomass values of NS irrigated *B. vulgaris* was significantly higher than all other treatments whilst for *C. annuum* proportional biomass allocation to fruit was highest for CG- irrigated individuals but reflected the lowest absolute biomass among treatments. Irrigation treatment was also found to have significant influence on fruit shape quality indices.

The combination of high electrical conductivities, Na concentrations and Sodium Adsorption Ratios among greywaters and greywater-irrigated soils suggest the long-term application of laundry greywater to soil may cause adverse physiochemical change in soils. Recorded K_s values among treatment soils indicated that the application of the LG formulation to soils induced severe soil hydrophobicity. Soil pH was found to mostly increase among treatments but varied considerably in accordance with methodology and plant species. Plant growth and soil attribute trends established for both sites were similar, suggesting environmental factors had a negligible influence overall. A repeated growth cycle undertaken at the Unilever site re-using treated soils found all trends consistent with those established for the first growth cycle, although anatomical biomass among *B. vulgaris* and *C. annuum* treatments were found to have declined significantly in most cases. The similar growth and biomass productivity of TW-irrigated *C. annuum* and *B. vulgaris* relative to greywater- irrigated treatments supports the indicated use of certain laundry greywater formulations as alternative irrigation mediums for crop production but long-term benefits may be offset by losses in soil quality.

PREFACE AND DECLARATIONS

All field work described in this dissertation was conducted on a split, dual-site basis, with experimental sites located at the University of KwaZulu-Natal (UKZN) Howard College campus, Glenwood, Durban and at the Unilever South Africa Head Office, Durban North. Part of the experimental work described in this dissertation was carried out at Unilever SA from July 2008 until November 2010 and the remainder at UKZN (Howard College and Westville Campuses) from April 2010 until November 2010.

These studies represent original work by the author and have not otherwise been submitted in any form for any degree or diploma to any other tertiary institution. Where use has been made of the work of others, it is duly acknowledged in text.

DECLARATION 1 - PLAGIARISM

I, GRAHAM JOHN TAYLOR declare that:

(i) The research reported in this dissertation, except where otherwise indicated, is my original work.

(ii) This dissertation has not been submitted for any degree or examination at any other university.

(iii) This dissertation does not contain other persons' data, pictures, graphs or other information, unless specifically acknowledged as being sourced from other researchers.

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a) Their words have been re-written but the general information attributed to them has been referenced;
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DECLARATION 2 – PUBLICATIONS

DETAILS OF CONTRIBUTION TO PUBLICATIONS that form part and/or include research presented in this thesis:

Publication 1

Taylor, G.J., Rodda, N., Pammenter, N., 2010. The Effects of Powder and Liquid Laundry Detergents on Swiss Chard and Soil Irrigated with Laundry Greywater. 1st Pan African Chemistry Network Green Chemistry Conference, UNECA, Addis Ababa, 15-17 November 2010.

Publication 2

Singh, U., Taylor, G.J., Mhlongo, W., Duys, L., Loxley, C., Townsend, R., Pammenter, N., Rodda, N., 2010. Effect of Powder and Liquid Laundry Detergents on Swiss Chard Plants and Soil Irrigated with Laundry Greywater. WISA Biennial Conference, Durban, 19-21 April 2010.

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LIST OF SYMBOLS AND ACRONYMS USED

a	Well radius, in cm
α^*	Macroscopic capillary length parameter representing ratio of gravity to capillary forces experienced during drainage or infiltration, dependent on soil texture-structure
C_1	Numerically generated shape factor dependent on H/a ratio of permeameter measurements
COD	Chemical Oxygen Demand
DAP	Days After Planting
DM	Dry Mass
DO	Dissolved Oxygen
EC	Electrical conductivity
EDTA	Ethylenediaminetetraacetic acid
ESP	Exchangeable Sodium Percentage
e*(T)	Saturation Vapour Pressure at temperature T, in KPa
FM	Fresh Mass
GP	Guelph Permeameter
H_1	Well height, in cm
ICP	Inductively Coupled Plasma
K_{fs}	Field-saturated hydraulic conductivity, in cm s^{-1}
K_s	Soil specific hydraulic conductivity, in mm h^{-1}
LDMC	Leaf Dry Matter Content
LT	Leaf Thickness
MIR	Mid-Infra Red
NTU	Nephelometric Turbidity Units
PVC	Polyvinyl Chloride
Q_1	Mathematical gradient describing the steady-state discharge rate of liquid from permeameter into soil, in cm^3 s^{-1}
°R	Degrees Rankine
RH	Relative Humidity
RH_i	Relative Humidity of air at a specified time interval, as %
SAR	Sodium Adsorption Ratio
SLA	Specific Leaf Area
SLW	Specific Leaf Weight
TAED	Tetra Acetyl Ethylene Diamine

CHAPTER I: INTRODUCTION AND LITERATURE REVIEW

1.1. INTRODUCTION

There is increasing interest in wastewater reuse in many regions from both the developed and developing world (Eriksson *et al*., 2002). Economic and environmental considerations by consumers have also been cited as drivers of domestic wastewater reuse since reuse reduces the burden on wastewater treatment works (Asano and Levine, 1996; Eriksson *et al*., 2002). Wastewater reuse efforts had initially focused on large-scale projects such as using wastewater for the recharge of groundwater or for the watering of golf courses (Asano *et al*., 1985). However, it has also been recognised that potential reuse of wastewater need not be restricted to projects supplied by large-scale wastewater treatment (Casanova *et* al., 2001). One area where water reuse can be successfully implemented is at the domestic household level, particularly since wastewater streams can be relatively easily separated at source (Singh *et al*., 2010). In this way, the desired wastewater stream can be collected and reused for domestic activities whilst also reducing water costs for consumers. Moreover there are indications that general attitudes towards wastewater reuse in this way are becoming more positive (Al-Jayyousi, 2003; Miller, 2006) and hence the notion of wastewater reuse is a viable option for an increasingly receptive consumer base. Among domestic wastewater streams, greywater in particular has received much attention for reuse applications.

Greywater may be defined as that domestic wastewater produced from everyday activities arising from the use of bathtubs, hand basins, showers, laundry machines and kitchen sinks without any wastewater inputs from toilets (blackwater) (Eriksson *et al*., 2002). The interest in greywater reuse has arisen from the possibility that greywater may serve as a source of both water and nutrients for plants (Patterson, 2000; Friedler, 2004; Mara, 2004) and as such may be suitable for garden or agricultural irrigation (Gerba *et al*, 1995; Neal, 1996; Otterpohl *et al.*, 1999; Eriksson *et al.*, 2006; Rodda *et al.*, 2011a; Rodda *et al.*, 2011b). Therefore greywater reuse may offer the means to significantly reduce domestic water consumption in a two-way solution to water sustainability. Although the quality of greywater is typically poorer than that of tap water (Eriksson *et al*., 2002; Ottoson and Stenström, 2003; Palmquist and Hanaeus, 2005), it is also generally less polluted than other municipal wastewater streams due to the relatively low content of human excreta (urine and faeces) and the absence of toilet tissue (Pinto *et al*., 2010). The low organic and pathogenic loadings typical of some

greywater streams also enhance its storage and reuse potential. However, it is generally known that the application of low quality, untreated wastewater such as greywater to soils can result in numerous changes to both soils and plants. Thus the effects of greywater as a water and potential nutrient source must be balanced against the potential negative effects it may exert on plants and soils.

The notion of greywater reuse for irrigation to promote the conservation of water and the use of associated nutrients is not a new one (Rodda *et al.*, 2011a). Greywater has already been used successfully in many parts of the world to overcome shortfalls in local water supplies. In particular, greywater reuse has been practiced extensively in arid regions with high evaporation rates and low rainfall. In arable areas where rainfall is usually sufficient to support most food crops, the impetus for reuse has been less but is growing as changing climate patterns together with net growing human populations mean that growing freshwater demands are unable to be met on a consistent basis. Despite the general movement towards greywater reuse for a variety of applications, a significant risk component exists. For small-scale irrigation projects, possible risk associated with greywater reuse may fall into 3 categories (Rodda *et al*., 2011a): adverse effects on human health; adverse effects on plant growth and yield and adverse effect on the general environment, with particular reference to the ability of soil to support plant growth. Human health concerns associated with greywater have resulted in some inertial resistance to greywater reuse particularly within economically developed societies (Domènech and Saurí, 2010). Ironically, human health concerns are likely to be greatest for low-income communities where water may be scarce and vegetables irrigated with greywater are eaten raw or unwashed (Jackson *et al*., 2006). However, in a study that assessed bacterial risk to human health in ground and leafy vegetables, Jackson *et al*. (2006) found no significant risk compared with vegetables treated with either hydroponics fertilizer or tap water. By contrast, the collective risks associated with greywater application to plants and soils are relatively unknown. Greywater is often released into the environment for reuse applications without any pre-treatment, a practice which may not be safe (Gross *et al.*, 2003). Al-Hamaiedeh and Bino (2010) suggest that when greywater is utilized without pre-treatment, it has the potential to cause a number of environmental problems. The greatest concern associated with greywater for reuse applications appears to centre on the elevated levels of solutes particularly sodium (Unkovich *et al*., 2004), phosphates and COD (Friedler, 2004) typical of greywater which may be environmentally damaging, especially when environmental discharge occurs on a long-term basis. In

particular, soil physical and chemical properties may be altered (Misra and Sivongxay, 2009), which may ultimately lead to soil degradation (Ottoson and Stenström, 2003). For the application of greywater to soil where ionic sodium concentrations are characteristically high, infiltration, hydraulic conductivity and aggregate stability of soils may all be affected (Abu-Sharar and Salameh, 1995; Suarez *et al.*, 2006; Eltaif and Gharaibeh, 2007). The significance of induced changes to soil in the context of reuse potential for agriculture is that they may in turn have adverse implications for plant growth and development (Minhas *et al.*, 1994). However, whilst the impact of highly saline irrigation waters such as greywater on soil physicochemical properties has been well documented and fairly well understood (Minhas *et al*., 1994), studies that have integrated its effects on both plants and soils in which they are grown, appear to be relatively limited. The high solute loads associated with greywater, especially those pertaining to long-term application of sodium, are considered detrimental to plant growth and survival (DWAF, 1996). The effect of salinity ultimately depends on several factors, including the salt type and quantity present in irrigation waters; the soil type to which it is applied; the particular plant species to be grown and growth stage thereof; and the quantity of irrigation media leached below the root zone (Ayers and Westcott, 1976; Rhoades, 1977; Western Fertilizer Handbook, 1995; Hanson *et al*., 1999; Bauder, 2001; USDA, Natural Resources Conservation Service, 2002). However, the high levels of total P and phosphates present in some greywater household fractions (Jefferson *et al.*, 2004) need not necessarily be viewed as environmentally detrimental since P is an essential plant requirement and therefore environmentally beneficial below excess quantities (Christova-Boal, 1996). Since greywater generally has relatively low amounts of faecal contamination (Ottoson and Stenström, 2003) and a significantly higher rate of degradability of pollutants relative to blackwater (Karlgren *et al*., 1967), the need for high level treatment of domestic greywater has been questioned (Jackson and Ord, 2000; Jefferson *et al*., 2001), A study of greywater as a potential irrigation medium for agriculture practice must therefore encompass both its direct effects on plants in relation to the indirect effects that its usage has on soils (Suarez *et al.*, 2006).

Among greywater sources, laundry greywater for reuse applications in particular has only recently received attention (Misra and Sivongxay, 2009; Pinto *et al*., 2010) largely because of the number of advantages it may present over other greywater streams. Laundry greywater is a major fraction of the wastewater stream in a household, may have a low organic fraction compared to other domestic greywater streams, may be a high source of P, and laundry

detergent used in its generation represents one of the greatest consumables among household detergents. However, like other greywater streams, it is also generally characterised by high solute loadings and a high pH which may be negative for reuse applications.

Given the growing interest and usage of greywater in subsistence agriculture, a greater research-led understanding of the value of laundry greywater as a potential source of irrigation water for the successful cultivation of crops is therefore essential. Such research would need to adopt an integrated approach in an investigation of the effects of greywater reuse on both soils and plants alike due to the close link shared between these environmental components. The nature and extent of environmental problems may be influenced significantly by specific environmental factors such as soil type, climate, and the greywater sources and detergent products used. The reuse of greywater for agriculture as a means to augment existing water supplies holds promise but needs to address various environmental concerns in relation to greywater quality and its usage.

1.2. Aims and Objectives

From the above contexts, this study aimed to holistically evaluate the physiological effects of laundry greywater irrigation treatment on vegetable crops and the associated effects on soil characteristics. For this, the growth performances of two crop species will be examined, namely Swiss Chard (*Beta vulgaris*) and Sweet Pepper (*Capsicum annuum*). In fulfilment of these aims, the study objectives are as follows:

(i) To determine the physicochemical composition and temporal changes of greywater derived from three laundry detergent product formulations.

(ii) To evaluate changes in plant growth parameters and post-harvest biomass investments in the anatomical components among treatments as general indicators of plant growth performances.

(iii) To determine uptake of select macro- and micro- elements by various anatomical components of the study species among irrigation treatments.

(iv) To quantify the influence of irrigation treatment on flowering behaviour and overall fruit yield and general fruit quality at harvest among *C. annuum* treatments using fruit shape factors and pulp thickness measures.

(v) To determine effects of irrigation with greywater on soil physicochemical characteristics and hydraulic conductivity.

(vi) To perform the experiment at two experimental sites contrasting in abiotic conditions to determine the influence of environment on overall trends in plant growth and soil characteristics under different irrigation treatments. To determine the effect of longer-term applications of greywater to soils and assess any cycle-on-cycle changes in crop growth trends among treatments, a repeat of the experiment undertaken at one of the experiment sites.

(vii) To determine from the above, the suitability and sustainability of greywater irrigation for crop production in view of the potential impacts on soils and on edible biomass yields from successive growth cycles.

It is broadly hypothesised that: laundry greywater can be used as a source of unamended irrigation water without detrimental effects on plants and that greywater can provide low level of nutrients in the absence of added nutrient fertilizers for use in food gardens in the context of low-income South African households. Thus greywater is not expected to contribute adversely to soil properties and plant physiology in comparison to the situation where tap water alone is used for crop irrigation.

1.3. LITERATURE REVIEW

1.3.1. Greywater Production and Reuse

Domestic greywater production and freshwater consumption are closely linked. Globally, the use of freshwater at the household-level is inequitable and consequently the amount of greywater produced is highly variable across regions (Alcock, 2002). This is due to the consumption of water in households without water-borne sewerage being considerably less than that typically associated with Western-style households (Alcock, 2002).

According to Alcock (2002), factors influencing water consumption in a Western-style household include: (a) family size and age/sex composition, (b) culture, (c) household income, (d) the cost of water, (e) diet, (f) technological level, such as the number of taps on the property, (g) the availability of alternative supplies such as boreholes, (h) educational standing, (i) social and home business activities (if applicable), (j) water losses through leaks, and (k) the size of the property of land and accordingly the type of garden as well as

plants. Seasonal and climatic factors (such as evapotranspiration) are also important considerations in determining consumption (Alcock, 2002). In the short-term, fluctuations in demand may be caused by variations in prevailing weather conditions (Alcock, 1999). Household water pressure may be another factor influencing consumption, where higher pressures result in higher consumption levels arising in particular through garden watering and plumbing leaks (Gebhardt, 1975, quoted in Alcock, 1999).

By contrast, the daily water consumption in rural and peri-urban areas where water must be fetched from a distant external source and carried back to the household in containers, tends to be at a basic-need level (Alcock, 2002). In this context, several factors will influence the daily per capita or household consumption. These factors include (a) the type of settlement, (b) the number of family members available to collect water, (c) the return of weekly-migrant family members over weekends - resulting in a much higher demand for water, (d) the distance and type of terrain to be walked, and (e) the perceived quality of the water source (Alcock, 2002). Other factors include storage capacity for household water (including provision for rainwater collection and storage), the locations chosen for laundry operations, and the payment/non-payment for water. Whilst there tends to be little seasonal variation in demand, in summer the availability of sufficient rainwater storage facilities usually reduces consumption from external sources (Alcock, 2002). Even within rural and peri-urban areas, a marked reuse of water is evident generally, depending on distance to source or payment for water (Alcock, 2002). Free sources such as springs and rivers for washing of clothes in situations where water has to be purchased in peri-urban and urban areas are often used. The nearest source of water is usually preferred, provided that the water quality is regarded as good (Alcock, 1999).

Presumably as an outcome of the high variability in greywater production and the difficulties involved in quantifying usage especially in those regions for which data may not exist, virtually no reliable data could be sourced for greywater production on a global scale. In general, it has been established that greywater accounts for approximately 46 % of total domestic wastewater discharges (Christova-Boal, 1996). Irrigation for home gardens alone may account for over 50 % of total domestic water consumption (Loh and Coghlan, 2003; Syme *et al.*, 2004), although this is likely to be dependent on the prevailing climate, the area under irrigation and other factors.

Despite much of Africa confronting similar challenges with regards to water and food security, there appears to be a general paucity of greywater reuse-related scientific literature for the continent. However, in South Africa more information on greywater production is

available. A study by Carden *et al*. (2007) attempted to quantify the quality and quantity of greywater produced in the non-sewered areas of South Africa. According to their data, 489 184 m^3 of greywater is produced daily in South Africa. With 117 270 m^3, KwaZulu-Natal accounts for nearly ¼ of all greywater volumes produced in non-sewered areas of South Africa and is the greatest greywater producer by volume among all provinces in the country. Nationally, the average amount of greywater produced on-site per dwelling unit per day is 88 L, while for KwaZulu-Natal this amount is lower, ranging from 60-85 L in the areas surveyed by Carden *et al*. (2007). This study and others however did not source or present data for the eThekwini municipal region, where the greatest numbers of inhabitants in KwaZulu-Natal reside (Stats SA, 2011). Nevertheless, the comparatively high volume of greywater produced in KwaZulu-Natal in other municipalities suggests a strong greywater reuse potential in the province exists.

1.3.2. Greywater Characteristics

In addition to the variability in volumes of greywater production at a global level, the physicochemical characteristics of domestic greywater are also highly variable. This variability in greywater composition is due to several factors associated with its collective production in the household. These include: the quality of the freshwater sourced; the nature of the distribution network of water supply and discharge within the household; behavioural activities relating to water usage within the household; and the different sources of greywater within a household (Eriksson *et al*., 2002). Despite this, significant physicochemical differences have been established and quantified between various domestic greywater effluent streams (Almeida *et al*., 1999; Eriksson *et al.*, 2002; Jefferson *et al*., 2001; Friedler, 2004) and the physical properties and nutrient composition of greywater are now reasonably well understood (Eriksson *et al.*, 2006). In an extensive review study by Eriksson *et al.* (2002) on greywater characteristics, 900 different chemical compounds or compound groups present in greywater sourced from average Danish households were identified. Several of these major, generic constituents occurring in domestic greywater will be discussed in following sections.

- *Detergents*

One of the most significant factors affecting the chemical constituents and characteristics of greywater is the chemical products used in its generation (Eriksson *et al*., 2002). Despite the high variability in the physiochemical properties among various greywater streams in scientific literature, in general the major constituents of all domestic greywater are soaps and detergents (Jefferson *et al.*, 1999; Pinto *et al*., 2010). A typical detergent consists of a surfactant, builder (zeolite, sodium tripolyphosphates), an associated polycarboxylate or 'builder additive' and a bleaching agent (percarbonate or perborate) (Pettersson *et al*., 2000; Carson *et al*., 2006). Other less significant detergent additives may include colourants, alkaline control agents, oxygen bleaches, sud control agents, corrosion inhibitors, antiredeposition agents, perfumes, activators for bleaching agents (tetraacetylethylenediamine or TAED) and enzymes (proteases, lipases and others) (Malmos, 1990; Pettersson *et al*., 2000; Carson *et al*., 2006; Bajpai and Tyagi, 2007; Yangxin *et al*., 2008). Detergents found in greywater are typically environmentally biodegradable (Pickup, 1990) but may biodegrade at different rates (Kolber, 1990; Petterson *et al.*, 2000).

- *Surfactants*

Associated with detergents in greywater are surfactants, which constitute the most abundant source of organic chemicals in greywater (Abu-Zreig *et al.*, 2003; Wiel-Shafran *et al.*, 2006). The term surfactant is a portmanteau for *surface active agent* which, as its name suggests, refers to a substance that is active at surfaces (Jonsson *et al*., 1998). Surfactants are organic molecules which are amphiphilic, since they incorporate both hydrophilic and hydrophobic groups in their molecular structures (Wiel-Shafran *et al.*, 2006; Ying, 2006; Lehrsch *et al*., 2011). The hydrophobic polar head group is either electrically charged or polarised with the ability to form hydrogen bonds, whilst the hydrophobic hydrocarbon tail group has alkyl chain lengths of C_{10} to C_{20} (Wiel-Shafran *et al.*, 2006; Ying, 2006). Surfactants contribute significantly to the chemistry of detergents, constituting between 15 % and 40 % of total detergent formulations (Scheibel, 2004), and therefore are a large chemical constituent of greywater (Wiel-Shafran *et al.*, 2006). There are four major groups of surfactant associated with detergents based on the composition of their polar head groups: cationic, anionic, nonionic and amphoterics (Yangxin, 2008). These can be either synthetically or naturally derived but in all forms surfactants have the common property of lowering the surface tension

of a liquid medium such as water by accumulating at the liquid/gas interface so increasing the molecular distance between water molecules at liquid surfaces (Zajic and Panchel, 1976; Zhang and Miller, 1992; Kuhnt, 1993). In laundry detergents, surfactants have the effect of improving the wettability of textile fibres in the wash (Simončič and Rozman, 2007).

The type of surfactants in greywater are known to vary significantly by both detergent product and greywater source. A study of anionic surfactants present in different greywater streams by Wiel-Shafran *et al.* (2006) found that laundry effluent had the highest mean concentration of anionic surfactant, compared to greywater emanating from either the kitchen or bathroom. In all instances however, anionic surfactants are the most dominant surfactant type used in detergents today, mostly due to their ease and use in manufacturing (Yangxin, 2008). It is likely however that the milder nonionic and amphoteric surfactants will gradually replace anionic surfactants in the future (Bajpai and Tyagi, 2007). At present, differing quantities and types of surfactant may be present in a single laundry detergent product because they differ in their effectiveness with types of fabric soil, fabrics present and their response to water hardness (Bajpai and Tyagi, 2007; Simončič and Rozman, 2007). Softeners also typically consist of surfactants (Petterson *et al.*, 2000) and may be built into laundry powders or combined with water as a stand-alone liquid product (Bajpai and Tyagi, 2007).

- *The Elements*

The concentrations of chemical elements in domestic greywater depend largely on the quality of the water used in its generation (Eriksson *et al.*, 2002), products used (Jenkins, 1998), consumer behaviour and on plumbing fixtures through which all domestic water is channelled (Eriksson and Donner, 2009). However, in general greywater is characterised by high solute levels, particularly sodium. Sodium salt compounds have traditionally been used in powder detergents as a core cation base due to their high water solubility properties, which aid the chemical activities of other chemical components present in detergents (Patterson, 2000). Sodium achieves this role by functioning as a counterion to the anionic surfactants of many laundry detergent powders (Jeppesen, 1996; Saouter and van Hoof, 2002). Sodium chloride is sometimes used in detergents as ion-exchangers (Eriksson *et al.*, 2002). Among sodium salts however, sodium perborate is extensively used for detergents as a peroxy bleaching agent (Firouzabaldi *et al.*, 2001; Carson *et al.*, 2006) as is sodium percarbonate (Wieprecht *et al.*, 2004). Sodium percarbonate is favoured in some countries due to greater efficacy at low

wash temperatures, which are increasingly being favoured by consumers for laundry washing (Bevan, 1997). Sodium perborate found in soaps and detergents is the major sources of boron in greywater (Gross *et al*., 2005). The use of boron in detergents is being limited in some countries (Gross *et al.*, 2005), mostly because it does not biodegrade (Fox *et al*., 2002) and can therefore cause a number of environmental problems. Elevated sodium can be detrimental to plant health when uptake by roots and leaves occurs in excess quantities by altering the nutrient balances of plants (DWAF, 1996). In addition sodium can negatively affect soil structure by inducing soil dispersion and the swelling of clay platelets and aggregates through the disruption of the forces that bind clay particles together (Warrence *et al*., 2003). Through repeated wetting and drying cycles, soils with high Na can reform into a hard, cement-like structure with little or no structure that reduces soil hydraulic conductivity and may lead to waterlogged soils. The resulting anaerobic soils may reduce plant growth and organic matter decomposition rates (Warrence *et al*., 2003).

Phosphorus serves as the primary constituent of many washing detergents, particularly in those countries where phosphate-based washing detergents are still permitted and used (Jeppesen, 1996). Therefore, Phosphorus levels in greywater can be quite variable and dependent significantly on the brand of product(s) used (Patterson, 2000). Globally, P usage in detergents accounts for approximately 12 % of total P demand (Heffer *et al*., 2006). It is however important to also consider that the chemistry of most detergents (and hence greywater) is in continual state of developmental flux to make formulations more efficient, cost-effective and safer with increasing consideration for the natural environment. More holistic attention is being paid toward the environmental risk of detergents, from their manufacture, usage and ultimate disposal (Pettersson *et al*., 2000; Saouter and van Hoof, 2002). In particular there has been a shift in many countries regarding P and B use in hygiene products (Neal *et al*., 2010). Detergent product manufacturers have especially sought to reduce P loading in their products for both environmental and economic considerations (Liu *et al.*, 2008). In particular, phosphates in detergents have largely been blamed for the eutrophication of water bodies in many parts of the world (Schindler, 1974; Lee *et al.*, 1978; Smith *et al*., 1999). Eutrophication is the process whereby water bodies become increasingly eutrophic due to an increase in nutrient loading (Smith *et al*., 1999). As one major limiting nutrient in water, excess phosphates may induce bacterial and algal blooms, resulting in anoxic conditions in water bodies, and potentially causing the death of fish (Schindler, 1974; Smith *et al*., 1999; Yangxin *et al*., 2008). However, Liu *et al*. (2008) argue that a reduction of detergent derived phosphates through bans or limitations in order to reduce eutrophication, is

unlikely to lead to a discernible improvement. Few studies appear to have attempted to evaluate whether P reduction or removal in detergents is wholly beneficial for reuse in environmental applications following the effective disposal of reformulated detergents as greywater.

The concentration of metals in greywater noted in literature (e.g. Eriksson and Donner, 2009) suggest that metals are a fairly insignificant component of greywater. The only exception appears to be in a study of greywater by Christova-Boal (1996) where elevated levels of zinc were found in domestic wastewater. This anomaly had been attributed by Eriksson *et al.* (2002) to acidic chlorine tablets used for water disinfection, which may have caused leaching of Zn from plumbing components. As with P, many powder laundry detergent manufacturers have also sought to reduce heavy metal loadings in their products, resulting in significant reductions in heavy metal elements such as arsenic, zinc, and possibly cadmium (Jenkins, 1998). However, Jenkins (1998) also reported significant increases of copper and possibly silver in reformulated laundry detergents. Chromium, lead, mercury and nickel were found to be comparable between original and reformulated powder detergents (Jenkins, 1998). By comparison, liquid laundry detergents have undergone less extensive reformulation and consequently their heavy metal contents are less likely to have undergone significant change (Jenkins, 1998).

- *pH & EC*

As with the elements, the pH of untreated greywater is often strongly dependent on the pH of the source water used in its generation (Eriksson *et al*., 2002). The pH of greywater has been found to vary significantly by domestic source, but is typically alkaline. Most literature studies cite pH values ranging from 5 to 8.7 for the various individual greywater streams in households (Eriksson *et al*., 2002; Friedler, 2004). For mixed greywater sourced from households, the pH of raw greywater have been shown to vary considerably, with values reported in the ranges of 6.9 to 8.15 (Casanova *et al*., 2001) and 5.1 to 8.1 (Rodda *et al*., 2011b).

The pH of laundry greywater (pH 8 to 10) is known to be generally higher compared with other sources of greywater such as the kitchen or bathroom (pH 5 to 8.7), largely attributable to the high alkalinity associated with laundry detergents generally (Christova-Boal *et al*.,

1996, but see Eriksson *et al*, 2002). Other authors have reported laundry greywater pH in the range 9.3 to 9.5 (Dixon *et al.*, 1999a) and a mean pH of 7.5 (Friedler, 2004).

High electric conductivity (EC) values are typical of greywater irrespective of source due to the elevated levels of dissolved salts, particularly sodium, sourced from detergents. In a physicochemical evaluation of domestic greywater streams, Friedler (2004) found laundry greywater to have the highest EC, with mean values of 2 457 µS cm^{-1}. By contrast, the EC of bath water, shower water, wash basin water and kitchen sink greywater were found to be significantly lower, with values ranging from 1 040 µS cm^{-1} to 1565 µS cm^{-1}. Christova-Boal *et al.* (1996) however reported considerably lower EC values for laundry greywater of 190 µS cm^{-1} to 1400 µS cm^{-1}. Such discrepancies in levels of greywater salinity may be attributed to the different chemical formulations of the laundry detergent products used and the relative concentrations of detergents in water.

- *COD, BOD, Oils & Grease*

Oils and grease are present in all greywater discharge streams from the household (Travis *et al*., 2008). Few studies, however, have evaluated oils and grease contributions from the major domestic greywater streams individually - most have evaluated oils and grease in combined greywater effluent discharges. Exception to this is a study by Friedler (2004), where kitchen greywater was reported to contribute the highest loading of oils and grease to domestic wastewater (greywater and blackwater). Christova-Boal (1996) also established laundry greywater to have 8 mg L^{-1} to 35 mg L^{-1} oil and grease, significantly lower than bathroom generated greywater with values of 37 mg L^{-1} to 78 mg L^{-1}. By comparison, Friedler (2004) reported greywater sourced from washing machines to have significantly lower mean oil and grease concentrations (181 mg L^{-1}) than that from the kitchen (323 mg L^{-1}) but higher than that for bathroom greywater (120.5 mg L^{-1}). According to Friedler (2004), more than 80 % of oils in greywater laundry are derived from the wash and first rinse cycles of washing machines.

The chemical oxygen demand (COD) of greywater is a measure of the oxygen equivalent of organic matter that can be oxidised by a strong chemical oxidant (Standard Methods, 1989). It therefore provides an indirect indication of organic pollution levels (Hu and Yang, 2004) and therefore is a useful indicator of overall water quality. High COD values are suggestive

of poor water quality and, conversely, low COD values are indicative of good water quality. Measured COD among various household greywater fractions indicates that COD of greywater is strongly dependent on the individual source but in general is fairly high, with concentrations up to 1300 mg L^{-1} or more (Friedler, 2004). Surendran and Wheatley (1998) and Laak (1974) both reported mean COD values for laundry greywater of 725 mg L^{-1}, whilst Almeida *et al*. (1999) reported COD values of 1815 mg L^{-1}. By comparison, bathroom greywater had significantly lower COD of < 645 mg L^{-1} (Friedler, 2004) whilst kitchen greywater was found to be similar to laundry greywater with reported values of 936 mg L^{-1} to 1380 mg L^{-1} (Laak, 1974; Surendran and Wheatley, 1998; Friedler, 2004). In laundry greywater, almost all (> 90 %) COD is obtained from the wash and first rinse cycles of machine washing (Friedler, 2004).

- *Physical Characteristics*

Considerable differences among greywater streams have been found with respect to temperature. In a literature review of domestic greywater characteristics, Eriksson *et al*. (2002) found reports of temperature ranging from 18 °C to 38 °C for domestic greywater streams. Water used in personal hygiene is typically heated, producing greywater with temperatures towards the upper end of this temperature range. The temperature of greywater from laundry washing varies according to the cold or warm wash temperature recommendations of laundry articles but also with wash cycle. A study by Siegriest *et al*. (1976) found average laundry wash temperatures of 32 °C and laundry rinse temperatures of 28 °C for rural households. In the developed world, lower laundry wash temperatures are being favoured (Bevan, 1997; Neal *et al*., 2010), which would result in a reduction in temperature differences between wash and rinse cycle greywater.

Turbidity, total solids (TS) and total suspended solids (TSS) influence the reuse potential of greywater by affecting soil clogging. In domestic greywater, most of the particles that affect turbidity, TS and TSS include food particles, soil, hair and laundry fibres (Eriksson *et al*., 2002). Turbidity of laundry greywater have been found to vary significantly during laundry cycles, ranging from 39 to 296 NTU (Nephelometric Turbidity Units) (Rose *et al*., 1991), and also varies with the soiled nature of the laundry itself (Christova-Boal *et al*., 1996). Other domestic greywater streams (excluding kitchen greywater) have been found to have lower

turbidity values of 15.3 NTU to 240 NTU (Gerba *et al.*, 1995; Christova-Boal, 1996; Eriksson *et al.*, 2002).

Laundry greywater has the lowest mean TSS among domestic greywater streams (188 mg L^{-1}), (Friedler, 2004), significantly lower than the mean value of 720 mg L^{-1} reported by Siegrist *et al*. (1976) for kitchen greywater. However, in a study by Friedler (2004), for (TS), laundry greywater was found to have the highest mean value of 2021 mg L^{-1}, significantly higher than other greywater streams where mean TS values ranged from 777 mg L^{-1} to 1272 mg L^{-1}. However, Eriksson *et al.*, (2002) cite various publications which suggest that kitchen and dishwasher greywater have the highest TS and TSS among domestic greywater streams. These differences may be explained by different experimental design approaches in terms of organic and inorganic load representations which possibly vary significantly between studies but also by the washing stage for which laundry water samples were taken; Friedler (2004) reported that approximately 53 % of TS and 71 % of TSS are contributed during the wash and first rinse cycles of machine washing.

- *Greywater in Storage*

For practical considerations greywater often needs to be stored prior to reuse (Tal *et al.*, 2011), since demand in greywater reuse applications is typically significantly lower than greywater production (Friedler, 2004). According to Dixon *et al.* (1999b), depending on household occupancy, the effectiveness of greywater storage in relation to reuse is typically maximised for stored greywater volumes of < 100 L. However, during storage greywater undergoes some significant changes in its physicochemical and biological characteristics. Chemicals present in greywater tend to become more concentrated with storage time due to evaporation (Alcock, 2002). Total dissolved solids and pH of greywater are among the first to parameters to increase following storage (Alcock, 2002). Fresh, untreated greywater is considered unsafe for reuse following 48 hours in storage primarily due to potential proliferation of pathogenic bacteria and hence the associated human health risks (Mustow *et al.*, 1997; Dixon *et al.*, 1999a; Dixon *et al.*, 1999c). Other practitioners of water reuse posit that greywater should not be stored for more than 24 hours for these same concerns (Kourik, 1991; van der Ryn, 1995). The storage of greywater also results in a significant decrease in dissolved oxygen (DO) levels which may render greywater harmful to the natural environment if reused untreated (Schneider, 2009). However, physicochemical changes in

greywater storage may also be largely influenced by factors such as storage temperature, the products used in its generation, and the source water quality, amongst other factors (Tal *et al*., 2011). Higher temperatures and poor source water quality both favour the growth and proliferation of microbial pathogens. Ideally for quality to be maximised during storage, greywater intended for reuse should be sourced from a treated water supply and stored at relatively low ambient temperatures, although in practice this is not always possible.

1.3.3. Greywater for Crop Irrigation: Effects on Soils and Plants

The effects of laundry greywater on soils and plants collectively have received little attention in scientific literature. Only recently, however, more integrated studies examining reuse potential of greywater for plant irrigation have started to emerge. For example, Pinto *et al*. (2010) have performed studies where they have evaluated silverbeet growth and development in relation to the effects on an Australian garden soil treated with greywater. Al-Hamaiedeh and Bino (2010) investigated the effect of treated greywater used for irrigation of olive trees and the vegetables of okra, bean, corn and sunflower grown in a silty clay Jordanian soil. Nevertheless, our scientific understanding of the interaction of laundry greywater with soils and plants remains rather limited, warranting further studies on the environmental effects of laundry greywater used to irrigate plants (Misra and Sivongxay, 2009; Misra *et al*., 2009). Some general effects of greywater application on plants and soils however have been documented and are discussed below.

- *Soil Salinity*

One of the major consequences of the application of greywater (including laundry greywater) to soils is an increase in soil salinity. The rate of salinity increases in soils following greywater application depends on a number of factors, including irrigation water quality, soil transmissivity, organic matter content, irrigation rate, depth to groundwater and land drainage characteristics (WHO, 2006). Since these factors affect soil salinity levels in ways that are not readily predicted, it is more efficient to monitor soils periodically than to estimate salinity from irrigation water quality (WHO, 2006).

Soil salinity may be affected by greywater in one of three ways (WHO, 2006): (i) inducing changes in the osmotic pressures in the root zone due to high salt content; (ii) inducing

specific ion (sodium, boron or chloride) toxicity or; (iii) by breaking down the structure of soil. Greywater application to soils in the long-term invariably leads to increases in salinity because it has a higher concentration of salts than freshwater (WHO, 2006).

- *Soil pH*

For both raw and treated greywater, significant increases in soil pH and EC over experimental periods can occur from as little as 60 days (Pinto *et al*., 2010) to 1 year (Al-Hamaiedeh and Bino, 2010). Effects corresponded with the physicochemical properties of the greywater used for irrigation. Changes in soil solution pH through irrigation may in turn influence the speciation of nutrient ions, and consequently the availability of particular plant nutrients (Grattan and Grieve, 1999). The potential influences of these physicochemically induced changes in soil to plants are discussed further.

- *Soil Water Movement*

The movement of chemicals and water in soils is determined by several factors including the type of added solute, the moisture conditions of soil and soil hydraulic and physical properties (Abu-Zreig *et al*., 2003). According to Minhas *et al*. (1994) changes in hydraulic properties of soils in the field are the outcome of greater probabilities of clay movement and of the re-arrangement of the resultant sub-aggregates, particularly those arising during wet and dry cycles of irrigation, and from seasonal effects of salinization and desalinization. Changes in soils through irrigation (typical of soils with montmorillion/smectite types of clay minerals), as well as slaking, dispersion and movement of clays known to cause pore blockages (typically prevalent among coarser soils), results in reduced soil permeability (Minhas *et al*., 1994). Since all added irrigation water must pass through the top soil layers, it is this horizon of the soil profile that governs infiltration (Minhas *et al.*, 1994). Infiltration has been found by Oster and Schroder (1979) and Agassi *et al.* (1982) to be more sensitive to the quality of the irrigation water than soil hydraulic conductivity. On the other hand, unsaturated hydraulic conductivity has been shown to more sensitive to both soil sodicity and salinity of irrigation waters (Russo and Bresler, 1977; Pal *et al*., 1980; Chawla *et al.*, 1985; Acharya and Abrol, 1991). Another factor affecting soil hydraulic conductivity values is ponding depth due to increased water contents at increased ponding depths (Feng *et al.*, 2002).

Surfactants present in greywater are also known to potentially alter movement of water through soil (Bubenheim *et al*., 1997; Abu-Zreig *et al.*, 2003). When first entering the environment, surfactants present in greywater may adsorb onto soils and cause several changes to soil properties (Rao and He, 2006; Lehrsch *et al*., 2011). These include changes to soil physicochemical and biological properties, the stability of soil aggregates (Piccolo and Mbangwu, 1989), specific soil hydraulic conductivities (Allred and Brown, 1994; Misra and Sivongxay, 2005), soil water retentions (Karagunduz *et al.*, 2001) and metabolism of soil microbes (Kuhnt, 1993; Wang *et al.*, 2005).

These effects of particular surfactants on soils have been exploited to improve the hydro-physical properties of soils, to control soil erosion and to improve the infiltration and structures of particular soils (Abu-Zreig *et al*., 2003). Surfactant sorption onto soil is dependent on a number of factors such as the physicochemical properties of the surfactant, the nature of the sediment and environment parameters (Ying, 2006). Surfactants may either sorb directly onto soil particles or interact with other surfactant molecules already sorbed onto a soil surface (Ying, 2006). The sorption mechanism behind this process depends primarily on the nature of the sorbent as well as the surfactant concentration (Adeel and Luthy, 1995; Brownawell *et al*., 1997). In general however, those soils characterised by higher organic contents enhance the adsorption of hydrophobic materials (Haigh, 1996).

The effect of surfactant-rich water such as greywater on soils has been shown theoretically to result in either increased or decreased infiltration outcomes depending on a number of factors, including soil wettability, the manner in which surfactants are applied, the specific effects of surfactants on surface tension, and on liquid-solid contact angles (Pelishek *et al.*, 1962; Kuhnt, 1993; Feng *et al*., 2002; Lehrsch *et al*., 2011). Because the addition of surfactants to water inherently functions to lower surface tension of the liquid, it holds that applying surfactant-rich water to soils would enhance infiltration in a water-repellent soil, but could result in either a positive or negative effect on infiltration in hydrophilic soils (Feng *et al*., 2002). According to Ma'shum *et al.* (1989), finer-textured soils are less susceptible to water repellency/hydrophobicity than sands due to their larger specific surface area that enables a greater quantity of organics to coat their mineral surfaces. Consequently, for water-repellent soils and sands, the addition of surfactants has proved beneficial in improving soil wettability and turf quality (e.g. Cisar, 2000; Kostka, 2000; Lehrsch *et al.* 2011).

By functioning to lower surface tension of liquid media, surfactants may also affect capillary rise in soils. This is because the property of capillarity is directly proportional to surface tension, Pc, as expressed by the following equation (Brown, 1947; Wiel-Shafran *et al*., 2006):

$$P_c = \frac{2\gamma \cos\alpha}{r} \quad (1.0)$$

where: γ = the surface tension of an imbibing solution

α = represents the contact angle at the interface of a liquid and gas and

r = pore radius.

Therefore, theoretically, for a given imbibing solution in a soil where pore radius remains constant, reductions in surface tension γ would result directly in proportional reductions in capillarity, leading to changes in flow between soil pores (Wiel-Shafran *et al*., 2006). In soils, changes in capillary rise can yield either positive or negative outcomes (Smirnov *et al*., 2003). Capillary rise under conditions of high surface tension can result in the upward movement of soil water which may enhance plant available water supplies near soil surfaces (Raes and Deproost, 2003), but also elevate soil salinity near plant roots (Datta and Jong, 2002). Conversely, it holds that lower surface tension of irrigation water may lead to water deficits at soil surfaces.

The effects of surfactants on soil hydraulic properties may also persist for a considerable period. In studies of the direct land-based application of surfactants, surfactants have been found to persist for 5 to 25 days in summer (Litz *et al*., 1987) and 1.1 to 3.6 days (Knaebel *et al*., 1990) in some American soils. From wastewater sludge applied to soils, some surfactants have been found to persist for between 9 and 117 days (Marcomini *et al*., 1989; Litz *et al*., 1987 but see Scott and Jones, 2000). According to Holt *et al*. (1989), surfactant degradation is primarily microbially driven. Factors such as soil type, agricultural land-use and the application method used were found to have no significant impact on degradation rates. Surfactants have also been found in some studies to be detectable in groundwater long after application to soils (e.g. Field *et al*., 1992). Most of the information on the effects of surfactants on soils appears to be on that found in general domestic wastewater (see review by Scott and Jones, 2000) and as a result only limited information is available on the effects that laundry surfactants in greywater in particular may have on the hydraulic properties of soils (Abu-Zreig *et al*., 2003).

The oils and grease present in laundry greywater may also have a significant effect on water movement through soil. This is due to the non-polar molecular structure and hence hydrophobic characteristics of oils and grease (Travis *et al*., 2008). Travis *et al.* (2008) investigated the accumulation of oil and grease in soil due to greywater irrigation and found that the ability of soils to transmit water became impeded as a result of soils becoming increasingly hydrophobic. Detectable hydrophobicity effects were evident within the upper 0.2 m of soil horizons although similar research by Tarchitzky *et al.* (2007) found that soil hydrophobicity was confined to just below the soil surface. These differences may be explained by the differences in irrigation period used for each study, with longer periods favouring greater accumulations of oils and grease, thereby enhancing soil hydrophobicity along a given soil profile (Travis *et al*., 2008).

Local climate has also been found to play a fundamental role in the response of soils to domestic wastewater irrigation (Minhas *et al.*, 1994). Consequently, laboratory-based studies of soil hydraulic conductances are considered unrepresentative of actual soil behaviour under wastewater irrigation (Minhas *et al.*, 1994). However, no information could be sourced on the effects of climate on the interactions between greywater applications and soils. Nevertheless, given the significant fraction of domestic wastewater that is greywater, the effects of laundry greywater application on soil would be expected to be similar to those noted for wastewater.

- *Sodium & Sodium Adsorption Ratio*

One of the most widely used tools to evaluate potential changes to soil physical properties is the use of Sodium Adsorption Ratio (SAR), defined broadly as the ratio of ionic sodium to the sum of known concentrations of calcium and magnesium of a solution or soil (Robbins, 1984; Al-Hamaiedeh and Bino, 2010; Maimon *et al*., 2010).

$$SAR = [Na^{+}] / [Ca^{2+} + Mg^{2+}] / 2]^{½} \quad (2.0)$$

From the above equation, it is apparent that a high Na content of soils is one of the principal determinants of SAR. It has been reported in several studies that SAR levels relate directly to deteriorations in soil structure, changes in soil permeability as well as changes in crop yields as a result of toxic and osmotic effects (Al-Hamaiedeh and Bino, 2010). Elevated SAR levels of irrigation water has been reported in studies on septic-tank drainage fields and wastewater

to cause the collapse of particular soil aggregates resulting in soil sealing near surfaces as well as the swelling and dispersal of clays leading to the formation of clay skins at particular soil depths, attributed to the loss of soil permeability (Balks *et al*., 1998; Menneer *et al*., 2001; Misra and Sivongxay, 2009). Calcium and magnesium, unlike sodium, do not tend to reduce soil infiltration, hydraulic conductivity and surface crusting (Warrence *et al*., 2003). Their smaller size compared to Na means they cluster closer to clays and compete with Na for clay binding sites and thus can reduce sodium-induced dispersion (Warrence *et al*., 2003). Due to the negative effects of a high SAR on soils, several guidelines have been developed worldwide that define acceptable SAR limits for irrigation water in agriculture in relation to various possible EC values of irrigation waters (see Asano *et al*., 2006; Pedrero *et al*., 2010). In general however, according to Patterson (1994), the internationally accepted maximum upper SAR limit is 6.0, above which soil structural stability and permeability may be compromised. In South Africa, an SAR value of 1.5 has been recommended for irrigation water applied to any local soil type for agricultural purposes, provided irrigation water is applied directly to soil and not on crop foliage (DWAF, 1996). The application of irrigation water with SAR values higher than 1.5 may result in changes to soil infiltration rate, hydraulic conductivity and also induce hardsetting in soils (DWAF, 1996). For example, Patterson (1994) found that a loss of soil permeability occurred at only SAR 3 when EC levels approximated to those typical of domestic wastewater. However, irrigation water SAR values of between 1.5 and 6.0 are also permissible in South African agriculture but depend largely on the soil used, the crop type grown and how it is applied (DWAF, 1996).

- *General Plant Growth*

The response of plants to greywater can be both positive and negative. For example, Rodda *et al*. (2011b) found improved growth and yield of carrot and Swiss chard relative to those irrigated with tap water; Eriksson *et al*. (2006) found toxic effects induced in willow and algae in greywater sourced from both the laundry and kitchen. The variances in response of plants to wastewater irrigation can be partly attributed to differences in their ability to process contaminants present in the irrigation solution; from available literature, the known phytotoxic effects of greywater can vary considerably among species (Pinto *et al*., 2010). This is because plants may affect changes in soil chemical composition through any of five processes: *Phytoextraction*, whereby plants remove soil additives from the rhizosphere through accumulation in biomass; *phytovolatilisation*, whereby soil contaminants are

transported through the plant and then into the atmosphere; *phytostabilisation*, whereby plants prevent the leaching of soil contaminants through soil erosion and water infiltration; *rhizodegradation*, where interactions between plants and soil biota cause the breakdown of pollutants and; *phytodegradation*, where contaminants are metabolised by plants, so reducing their levels (Duggan, 2005). Failure to adequately process contaminants in these ways may cause phytotoxicity in plants. Phytotoxicity may be defined as a delay of seed germination, inhibition of plant growth or any other adverse effect on plants caused by specific substances (phytotoxins) or growing conditions (Romeo, 2000). Surfactants present in greywater may serve to induce phytotoxicity in plants. Bubenheim *et al.* (1997) and Garland *et al*. (2000) have found phytotoxic symptoms in lettuce treated with anionic surfactants grown in hydroponic systems, but not for similarly treated wheat owing to a lack of sufficient surfactant degradation by the microbial community of the rhizosphere (Bubenheim *et al*., 1997). According to Pinto *et al*. (2010) phytoxicity in the greywater reuse context is mostly attributed to the anionic surfactant content which serves to alter the microbial communities associated with the rhizosphere.

Much of the concern around greywater used for the irrigation of crops also arises from the significance of its high salinity as sodium since sodium is known to have an effect on both plants and soils (Rodda *et al*., 2011b). The initial responses of plants to salinity particularly at low to moderate concentrations can be attributed to osmotic effects (Munns and Termaat, 1986; Jacoby, 1994). In general the effect of increased salinity on plants is a reduction in growth rate, resulting in leaves of smaller size and shorter stature, and occasionally fewer leaves (Shannon and Grieve, 1999). The general response of roots to salinity is a reduction in both length and mass, but roots may also become thicker or thinner (Shannon and Grieve, 1999). However, the extent to which growth is reduced by salinity differs considerably by species and to a lesser extent with intra-species varieties (Shannon and Grieve, 1999). The inherent ability of a given plant to tolerate the effect of salts in the root zone or on its leaves without experiencing significant adverse physiological effects is what defines plant salt tolerance (Shannon and Grieve, 1999). Salt tolerance is conferred by a complex, quantitative, genetic make-up, controlled by several genes (Shannon and Noble, 1990; Shannon, 1996) and described in plants quantitatively as a function of yield declines over a range of salt concentration exposures (Maas and Hoffman, 1977; van Genuchten and Hoffman, 1984). Salt tolerance of plants is measured using either of two parameters: threshold EC_t, the electrical conductivity level at which the initial significant reduction in the maximum expected yield and the slope, the latter being the percentage yield likely reduced per additional unit of

salinity above the threshold value (Shannon and Grieve, 1999). According to Shannon and Grieve (1999), the crop salt tolerance threshold, the point at which yield first decreases with increasing concentration, is highly sensitive to environmental interactions, but also depends on growth stage (Lunin *et al*., 1963) and the marketed crop portion of concern (Shannon and Grieve, 1999). For these reasons, classification studies of salinity tolerance among crops have been proposed by Lunin *et al*. (1963) to consider both growth stage of the crop investigated and the particular marketed crop portion. In general however, most vegetable crops are glycophytes (Greenway and Munns, 1980), having evolved in environments characterised by low levels of salinity (Grattan and Grieve, 1999), and therefore are generally considered moderately-salt sensitive to moderately salt-tolerant (Shannon and Grieve, 1999).

The extent to which plants respond to salinity is also governed by environmental factors, such as relative humidity, radiation, temperature and air pollution (Shannon *et al*., 1994). Plant maturation rates may also, depending on the species, be increased or decreased under saline irrigation (Shannon and Grieve, 1999). Ion toxicities or nutrient deficiencies may also occur in plants irrigated with saline solutions, due to a predominance of a particular ion or competition effects among cations or anions (Bernstein *et al*., 1974). It is the osmotic effects of saline water that is responsible for reduced growth rates, leaf colour changes and developmental attributes such as root : shoot ratios and rate of maturation in plants (Shannon and Grieve, 1999). On the other hand, ionic effects generally manifest as either leaf and meristem damage or as nutrient disorder symptoms (Shannon and Grieve, 1999). According to Shannon and Grieve (1999), for example, Na may accumulate in leaf tissues and cause leaf scorch whilst symptoms of nutrient deficiency remain similar to that which occurs in the absence of salinity. However Shannon and Grieve (1999) also note that the effect of salinity on plants is not always negative, and in some cases salinity has had some favourable effects on crop yield, quality and disease resistance.

The evolutionary mechanisms which glycophytes have developed to absorb, transport and utilise mineral nutrients from non-saline substrates may not function as effectively or efficiently under saline conditions (Grattan and Grieve, 1999). Under low saline conditions, the concentrations of Na may grossly exceed that of the macronutrients by one or two orders of magnitude, and by even higher factors for the micronutrients (Grattan and Grieve, 1999). Consequently, according to Grattan and Grieve (1999), further elevated levels of Na may disrupt the activities of nutrient-ions, with the plant becoming increasingly susceptible to

osmotic and specific-ion injury, including nutrient disorders, which may ultimately cause reductions in fruit quality and quantity. The resulting nutrient imbalances caused in salt-stressed plants can manifest in several ways, such as from the effect of salinity on nutrient availability, competitive uptake, transport or partitioning within the plant or may be attributed to a physiological inactivation of a particular nutrient and in turn an increase in a plant's need for an essential element (Grattan and Grieve, 1994). These effects, according to Grattan and Grieve (1994), may also occur simultaneously. Therefore, such individual effects may be difficult to discern, since even in the absence of salinity or other stresses interaction effects concerning nutrient availability, uptake and distribution are complex enough, but are compounded further in complexity by the presence of salinity (Marschner, 1995). Crop species also can differ significantly in terms of both rate of mineral absorption and how elements are distributed spatially within a plant. For example, sodium in irrigation water has been known to have a significant effect on the mobility of calcium and consequently Ca distribution in plant organs; this may also be the case for other plant-essential elements (Grattan and Grieve, 1999). For this reason, Steppuhn *et al*. (2005) for example have proposed the use of standardised salinity-tolerance indices for root-zone salinities of crops.

For plants grown in the absence of elevated salinity, their growth in relation to the concentration of an essential element present in their root media may be described by the "generalised dose response curve" (Berry and Wallace, 1981). On the basis of this graphic representation, there lies a nutrient-concentration window where the growth performances of plants are optimal; concentration levels above this point are regarded as sub-optimal as growth is reduced, whilst above the optimal window, growth may be inhibited owing to either a toxicity or nutrient-deficiency (Grattan and Grieve, 1999). This optimal window could also be narrowed on the basis of human health concerns due to the potential accumulation of harmful compounds in edible plant tissues (Marschner, 1995).

Plant uptake of the macro- and micronutrients may be influenced significantly by salinity depending on the element concerned. Nitrogen, as a major growth-limiting nutrient, functions to improve both plant growth and yield irrespective of whether a crop is salt-stressed or not (Grattan and Grieve, 1999). In general, most salinity and N interaction studies have been conducted on N-deficient soils (Grattan and Grieve, 1999). Some researchers have found that, under conditions where salinity was not severe, the addition of N resulted in improved

growth and/or yield in cowpea, tomato, clover and millet (Papadopoulos and Rending, 1983) as well as for wheat (Soliman *et al*., 1994).

In a review publication of 17 studies on the interactive effects of P and salinity, Champagnol (1979) found when P was added to saline soils, crop growth and yield were improved for 34 crop species out of 37 and with the exception of one study, P did not enhance crop tolerance to salinity (Grattan and Grieve, 1999); generally increased salinity decreases P concentrations in P tissue (Sharpley *et al*., 1992), although according to Grattan and Grieve (1999), other studies have suggested that salinity may either increase or have no effect on P uptake.

Some studies e.g. Francois (1984) and Graifenberg *et al*. (1995), have suggested that on a dry mass basis, K concentrations in leaves declines with increased Na of root media whilst other research has demonstrated the increased acquisition of K may take place with increased Na concentrations (e.g. Meiri *et al*., 1971; Cachorro *et al*., 1993).

Environmental conditions and salinity are also known to potentially affect the uptake of Ca. Adams and Ho (1993), found that Ca uptake in tomato was highly correlated with both solar radiation and temperature. These authors also observed a linear decrease in the rate of Ca uptake with increasing salinity of irrigation media. Similarly, in lettuce, the addition of Ca to various genotypes was found to have no effect on their salt tolerances (Cramer and Spurr, 1986). In other horticultural crops, improved salinity tolerance has been noted with increases in substrate calcium (Grattan and Grieve, 1999). Similarly, salinity induced differences in the elemental uptake of the macronutrients have also been noted (e.g. *see* Grattan and Grieve, 1999).

Sodium-sensitive crops can absorb toxic levels of sodium at SAR level increases above 1.5, such as those induced by greywater application to soils, but is strongly dependent on the crop species concerned (DWAF, 1996). However, an SAR value of 8.0 is generally considered the upper limit of salinity tolerance for most crop types (ANZECC, 1992; DWAF, 1996). For SAR limits above 15.0 all sodium sensitive crops absorb toxic concentrations of sodium via root uptake although some crops can still be irrigated with water of this quality without developing sodium toxicity symptoms (DWAF, 1996).

- *Changes in soil pH*

Soil pH is one of major factors affecting the growth performances of crop species (Islam *et al.*, 1980). The effects of elevated pH on the plant root environment and plant themselves

however can be difficult to discern due to complications arising from the direct effects of excess hydrogen or hydroxyl groups and from those indirect effects related to changes in solubility of mineral elements (Islam *et al*., 1980; Yan *et al*., 1992). For many crop species, maximum or near maximum growth is typically achieved in the mildly acidic soil pH range of 5.5 to 6.5 (Islam *et al*., 1980; Benton-Jones, 1998). At elevated pH values, the availability of particular nutrients, such as Zn, Fe and P may be reduced resulting in symptoms of mineral deficiency in plants (Waskom *et al*., 2007). In particular, plant growth at high soil pH may be limited by the availability of Mo, Ca, P or Mg (Adams, 1981; Noble *et al*., 1988). Similarly, at low pH, the inhibition of nutrient uptake, root growth and nitrogen fixation in plants is also known to occur (Moore, 1974; Munns, 1978; Mahler and McDole, 1987). At low levels of soil pH, elevated aluminium or manganese solubility may occur (Yan *et al*., 1992), and these and other effects may directly inhibit whole plant growth (Andrew, 1976; Mahler and McDole, 1987; Schubert *et al*., 1990a; Schubert *et al*., 1990b; van Beusichem, 1982).

- *Flowering & Fruiting Behaviours*

The abscission of flower buds, flowers and fruit represents a significant yield-limiting factor for many crop species (Wien *et al*., 1989). According to a review on flowering and fruiting behaviours by Stephenson (1981), many plant species produce mature fruits from a comparatively small portion of their female flowers and consequently many of these species abscise both flowers and fruit during their reproductive cycle. This notion is particularly significant since, under a particular abiotic condition(s) or stress, flower production may be reduced or enhanced (e.g. Downton, 1978; Rylski and Spigelman, 1986; Erickson and Markhart, 2001; Erickson and Markhart, 2002; Munns, 2002). A study of flowering and fruiting behaviours in plants may therefore function to provide valuable insights into the general physiological status or stress of a given plant under particular environmental conditions (Pawsey, 1960; Janzen, 1971; Guinn, 1985; Sato *et al.*, 2001).

1.3.4. Management Strategies

The use of greywater for various reuse applications and the treatment of greywater have been the reported for both developed and developing countries (Roesner *et al*., 2006; Morel and Diener, 2006). Despite the growing popularity of greywater reuse and that this practice has been occurring for many years, for most countries there is an absence of formal standards or guidelines informing reuse potential of greywater and related risks (Eriksson and Donner,

2009; Rodda *et al*., 2011a). However, in a few countries where guidelines and policies regarding greywater use have been firmly established and implemented, public health concerns tend to supersede those focused on potential environmental impacts (Gross *et al*., 2003; Vigneswaran and Sundaravadivel, 2004). This appears to be changing with greater availability of information from water authorities, non-govermental organisations, the Internet, manufacturers of greywater treatment systems and from consultants on those precautions that should be taken for greywater reuse for irrigation (Rodda *et al*., 2011a). In South Africa, such guidelines are at a developmental stage and until recently backed by relatively little context-specific research and implementation as policy. DWAF (1996) served as the guidelines that can be applied to greywater reuse in the agricultural context in South Africa, but are being reviewed (N. Rodda, pers. comm.). The guideline limits contained therein are similar to those suggested for Australasian agriculture (ANZECC, 1992).

The National Water Act (NWA) of 1998 serves as the legislation that addresses the use and disposal of water (Rodda *et al*., 2011). Whilst the act makes no direct reference to greywater among the types of wastewater considered among the General Authorisation of the Act, this is the nearest that current South African legislation reaches in terms of providing guidance for the quality of greywater used for irrigation (Rodda *et al*., 2011a). The Department of Water Affairs, the department responsible for the implementation of the NWA, has demonstrated that it supports the single-household use of greywater for purposes of irrigation as a means to save water, provided that no health or pollution hazards are created as a result (Rodda, *et al*., 2011a). Although the NWA does not specify greywater use for irrigation, this practice is considered in accordance with the law, at least when done on a small-scale.

1.3.5. Why Greywater?

- *Water Shortages*

Many regions of the world are confronting unprecedented challenges concerning water security as climate change and a burgeoning human population adversely impact both freshwater quantity and quality. It is predicted that by the year 2050, 67 % of the world's population will experience water stress (defined as < 2000 m^3 of available freshwater $person^{-1}$ $year^{-1}$), from a level of 7 % in the year 2000 (Fischer and Heilig, 1997; Wallace, 2000). The expected changes in regional hydrological cycles as a result of climate change (Ludwig *et al*., 2009) and the logistical increase in growth in the human population of the world (Bongaarts,

2009) has warranted new approaches to water demand management for many countries. Much of the developing world is already beset by chronic water shortages and this problem in these regions is likely to be exasperated by the anticipated increase in drought frequency and severity (Brown *et al*., 2007), and the greater unpredictability of rainfall occurrence (Merrey and Sally, 2006; Ludwig *et al.*, 2009) in coming years. Collectively, these environmental factors may serve to reduce the availability of freshwater, promote desertification and reduce overall soil quality (Kato *et al*., 2009).

Perhaps of greater significance presented by these anticipated threats to freshwater availability is the dependency that exists between water security and food security, since much of the world's demand for freshwater derives from the agricultural production of foodstuffs. By sector, worldwide irrigation demand accounts for approximately 80 % of available freshwater use (Shiklomanov, 1998), making it the single biggest consumer of freshwater globally, although more recent estimates suggest this figure is closer to 70 % (Pearce 2006; Molden *et al.*, 2007). Moreover, as water supplies become increasingly scarce, competition between municipal, industrial and agricultural sectors increases (Mara, 2004). A consequence of this is a decreased allocation of available freshwater to agriculture (Tilman *et al*., 2002). As a result, the agricultural sector in many countries - particularly those of the developing world - are vulnerable to the adverse effects posed by the effects of climate change (Kato *et al.*, 2009). Current and future water scarcity projections by Fischer and Heilig (1997) and Wang (2005) suggest that future water availability will be increasingly inequitable at a global scale over the next few decades, being particularly acute in much of Africa and the Middle East (Wallace, 2000). Moreover, a current or impending water crisis is likely to be felt more directly by the poorest element of the world's population (Rijsberman, 2006) where a considerable dependency exists on rain-fed irrigation water in arid and semi-arid regions and where approximately 52 % of the world's population occurs (UNESCO-WWAP, 2006).

In southern Africa, recent estimates suggest that approximately 80 % of poor people have a direct dependency on agriculture for their livelihoods (Calzadilla *et al*., 2009). This region is also characterised by a high population density (de Wit and Stankiewicz, 2006) and also high current and projected human population growth rates (Vörösmarty *et al*., 2005). Within this region too, access to water is already a concern, with approximately half of the 190 million inhabitants of southern Africa lacking access to safe water. Furthermore, with an annual population growth rate of 3 %, it will became a growing challenge to meet future water needs

and resolve inequality of water supplies (Rothert and Macy, 2001), especially in the backdrop of increasing costs for developing new water supplies (Mwendera *et al*, 2003). Estimates by Nyong (2005) quantify the future extent of the problem, suggesting that by 2050, sub-Saharan Africa may experience a 10 % reduction in rainfall, resulting in major water shortages for the entire region (de Wit and Stankiewicz, 2006). Projected worldwide crop production models created in relation to global climate change scenarios, published by Lobell *et al.* (2008) for the year 2030, indicate that reduced rainfall for certain seasons will likely lead to significant reductions in vegetable and staple crop production for southern Africa. Within southern Africa, various climatological model projections (Ragab and Prudhomme, 2002; Wang, 2005; de Wit and Stankiewicz, 2006) consistently suggest that South Africa will experience a considerable reduction in precipitation and consequently surface run-off leading to crop production declines in coming decades. South Africa is a water-scarce country since approximately 65% of the country receives less than the 500 mm of rainfall annually considered necessary for rain-fed cropping (Schulze, 1997). In addition, the quality of increasingly limited freshwater is progressively threatened by point and non-point source pollution (Rodda *et al*., 2011a). In a country where agriculture demand for freshwater accounts for approximately 72 % of the domestic total available (Gleick, 2003), this remains cause for concern. Such projections of compromised food security under a changing regional climate provide considerable research impetus in new areas of water conservation and reuse in South Africa. Given that many parts of South Africa typically experience some level of water restriction on a seasonal basis, and the prevalence of perennial poverty in low-income settlements in the country, the notion of greywater reuse for irrigation is attractive (Rodda *et al.*, 2011a). Using greywater for the small-scale irrigation for agriculture and in gardens is therefore considered as one means to alleviate water stress (Murphy, 2006). For this, the reuse of laundry greywater for crop irrigation has been considered as one major means to reduce domestic water consumption since laundry greywater represents a considerable proportion of total domestic water consumption (approximately 17 L to 40 L per person per day (Christova-Boal *et al*., 1996). Estimates by Christova-Boal *et al*. (1996) suggest that the proportion of all tap water used by a typical household for laundry is 15 %.

- *Nutrients*

The future sustainability of world agriculture under a changing climate is not only dependent upon the availability of freshwater. Certain nutrient resources are also necessary to ensure

adequate long-term crop production. Sustainable agriculture may be defined as agriculture that is managed toward a greater use-efficiency while sustaining an environment that promotes the evolution of species (Golley *et al*., 1992). The elements phosphorus, nitrogen, potassium and sulphur in particular are all needed by plants in relatively large quantities to ensure their survival and to support growth, and hence are common constituents of many agricultural fertilizers (Benton-Jones, 1998). There are no known substitutes of any of these macronutrients for plant function (USGS, 2011). Of all the mineral resources required for plant growth, P, K and N in particular have been found primarily to limit the growth of both terrestrial and aquatic plants (Schlesinger, 1991; Vitousek and Howarth, 1991). This is due to the critical role that macronutrients collectively perform in higher plants, such as membrane and cell wall synthesis, chlorophyll assembly, pant-water balance, some being key entities of biomolecular components including the composition of proteins, ATP, DNA and RNA amongst others. The extent of these varied roles in supporting plant function are in accordance with Sprengel-Liebig's Law of the Minimum (Liebig, 1855; Smith *et al.*, 1999; van der Ploeg *et al*., 1999). This theory, envisaged by the German agricultural chemist Justus von Liebig and German agronomist Carl Sprengel, states that plant yield is limited by the nutrient that is present in the least quantity in a given environment and which is also essential for plant growth (von Liebig, 1855). This is an over simplistic assertion given that many other factors also govern plant yield, but remains a useful concept. Although many elements other than the macronutrients are necessary for plant growth and survival including B, Cu, Fe, Mn and Zn, these are needed in significantly lower quantities and as such are referred to as micronutrients. The micronutrients perform essential roles in plant metabolism, including photosynthesis, cellular redox reactions and protein assembly. As potentially limiting resources in agriculture, it is therefore the sustainability of the key plant essential elements that also deserves attention. In the context of Sprengel-Liebig's Law, these elements are unlikely to directly constrain agricultural practices in the future. However, it is important to bear in mind that the cost of processing and extraction of these and other increasingly rare resources and low grade supplies from traditional sources will also inevitably escalate in years to come (Fixen, 2009). This aspect, together with the unprecedented human population growth trends mentioned earlier (Bongaarts, 2009), may cause commercial food prices to rise beyond the means of much of the world's poor (Trostle, 2010). According to Fixen (2009) however, the gain in efficiency that can be achieved through the wise stewardship of these non-renewable nutrients used in agriculture, may mitigate the anticipated cost-rise of these commodities. For Sub-Sarahan Africa this is particularly necessary given that it is one of the

poorest regions on Earth, both in terms of living standards and soil fertility (Gilbert, 2012). Average yields of grain crops in the region have stagnated to around 1 tonne per hectare since the 1960s (Gilbert, 2012) due to high input costs of fertilizers and difficulties with distribution (Gilbert, 2012), and mainly for these reasons has failed to achieve yield gains seen in other parts of the world over the past few decades. Although South Africa is one of the few countries in the region with an established fertilizer industry (Ramaru *et al*., 2000), the fertility status of South African soils remains problematic (Scotney and Dijkhuis, 1990; Barnard and du Preez, 2004); a decline in soil organic content, decline in soil N, changes in soil acidity and the expansion of saline and alkaline areas in some regions have been noted as areas of concern (Scotney and Dijkhuis, 1990). Virgin (uncultivated) soils in South Africa have also been found to be generally low in P (van Niekerk, 1989). Nationally, low-levels of naturally occuring trace elements in many soils have been found (Steyn and Herselman, 2000; Herselman and Steyn, 2001). Low micronutrient levels and adverse changes to soil fertility is potentially problematic to human health, particularly the poor, because it is through plants growing in soil, that soil nutrients enter the food chain (Barnard and du Preez, 2004). In South Africa, a number of cases of micronutrient deficiencies causing significant health problems in have been cited. Esophageal cancer has been noted among some Eastern Cape communities (Laker, 1979) and osteoarthritic disease in KwaZulu-Natal (Pooley *et al*., 1997; Ceruti *et al*., 2003). Thus, no longer is sufficient energy from food deemed necessary, but also enough nutrients (Fritschel, 2000).

Since greywater contains N, P and other elements (Eriksson *et al*., 2002; Morel and Diener, 2006), it can function as a potential source of nutrients for plant growth, especially for those poor communities who are unable to afford fertilizer (Rodda *et al*., 2011a). Previous work by Rodda *et al*. (2011b) showed that mixed greywater contains a significant amount of nutrients which was found to enhance the growth of vegetables. Among domestic greywater streams, laundry greywater in particular has potential as a source of nutrients. In some countries of the developed world, annual consumption of laundry detergents is also significant, with average per capita values of 3.7 kg to 7.5 kg (Karlström and Svensson, 1995; Jenkins, 1998) and up to 12.5 kg per capita in some European countries (Fox *et al.*, 2002). The consumption rate of powder laundry detergents is therefore one of the greatest among cleaning products found in the household (Jenkins, 1998). However unlike other domestic greywater streams, laundry greywater has also been shown to be potentially among the least biologically contaminated sources of greywater (Christova-Boal, 1996; Friedler, 2004).

As a consequence of water and nutrient shortages generally, there is a growing need for potential new and innovative means of water use in order to sustain growth in coming years of the world's 'water footprint' (Hoekstra and Hung, 2002) as well as to change the current anthropogenic influenced nutrient cycling flows and dynamics. Towards this end, it appears that the recovery of laundry greywater through reuse may be of substantial benefit for communities wishing to irrigate plants with water that would otherwise be lost through various routes of discharge, and without the additional expense of artificial fertilizers. Essential to this premise is a more detailed appraisal of laundry greywater physicochemical characteristics relative to other various domestic greywater streams and in relation to the potential effects on plants and soils.

CHAPTER II: MATERIALS AND METHODS

2.1. PLANT SPECIES

Two vegetable species formed the focus of this research, namely Swiss chard (*Beta vulgaris subsp. cicla* L., cv. Fordhook Giant – herewith referred to as *Beta vulgaris*) and sweet pepper (*Capsicum annuum* L., cv. California Wonder). These species were specifically chosen on the following premises:

i) They are both fast growing plants and thus suited to a relatively short-term study of this nature.

ii) Are considered sensitive to moderately salt tolerant based on available scientific literature (DWAF, 1996; Shannon and Grieve, 1999). Since most vegetables are classified as such, they are representative vegetable species to use as a potential point of reference in this tolerance category for plants grown under the influence of laundry greywater irrigation.

iii) *B. vulgaris* and *C. annuum* have contrasting leafy and fruiting vegetables growth characteristics respectively, and therefore have different resource allocations to their respective plant organs. This may provide general indications of various vegetable crop-type responses to greywater compositions and also over repeated growth cycles.

iv) Are highly nutritious vegetables commonly grown in both developed and developing countries for subsistence and commercial purposes.

2.2. SOIL

Berea Red is a commonly occurring sandy soil found along coastal regions of the Durban Metropolitan area, and therefore largely representative of local soil-growing conditions of the experimental site locations. For the growth of vegetables in this study, this soil type was also chosen on the premise that it is relatively nutrient poor (Dunlevey, 1997), thereby limiting the potential for findings to be confounded by existing soil nutrients. For both experimental sites, Berea Red soil was sourced from the grounds of the Unilever site and also from the immediate surrounding area. Soils from these different locations were manually mixed by spade to ensure a homogenous mix, with homogeneity being verified from subsequent

laboratory soil sample analysis from random samples taken from various points in the soil mix.

2.3. IRRIGATION TREATMENTS

A total of five irrigation treatments were used, with 9 replicates for each treatment. The treatments were: tap water, greywater produced from a commercial, phosphate-base laundry powder product (PG) suitable for manual top-loader washing machines; greywater produced from a commercial liquid laundry detergent product (LG) suitable for front loader automatics; greywater produced from a carbonate-base laundry powder (CG), a reformulation of the PG product excluding phosphates, also suitable for manual top-loader washing machines (Table 1) and; a hydroponic nutrient solution, which was produced using the commercial macro- and micronutrient balanced powder formulation, Chemicult® (Stark Ayres, RSA), (Table 7). The composition of the powder and liquid formulations used in the generation of greywater are shown in Table 2 and Table 3 respectively. For the first crop growth cycle at the Unilever SA experimental site only four of the five treatments were applied from the beginning of the experiment, namely PG, LG, tap water and the nutrient solution, owing to the unavailability of the CG laundry powder formulation which was in a product testing phase when the experiment at Unilever SA was first initiated. As a result, the CG formulation was only introduced 21 days later as an additional treatment during the first growth cycle at the Unilever site and applied or the same duration, but was used at the same time as other treatments for the second growth cycle. Thus statistical comparisons and interpretations presented for CG during growth cycle 1 are of limited value. The CG treatment was used throughout experimentation at the University site, which was established later. A second growth cycle at the Unilever site was undertaken using the same pots to grow plants for each treatment as before.

Table 1. Attributes of laundry detergents used in the production of greywater (B. Crawford, Unilever UK, pers. comm.).

Product	Form	Type	Major Formulation Base	Other Components	Recommended Machine
PG	Powder	Combination detergent	Phosphate	Added fabric softener	Non-automatic top-loader or twin-tub
LG	Liquid	Surfactant detergent	SLES 3EO- 25 %	None	Automatic front loader
CG	Powder	Combination detergent	Carbonate	Added fabric softener	Non-automatic top-loader or twin tub

Table 2. Percentage compositions of the ingredients used in the production of the two powder formulations used in this study. Reported values are for those commercially available product formulations in South Africa at the time the experiment was initiated (B. Crawford, Unilever UK, pers. comm.).

Ingredient	PG	CG
Surfactants		
LAS	20.00	20.00
Fillers/builders		
Sodium Carbonate Dense	11.00	18.00
Sodium Sulphate	30.00	40.00
STPP	16.00	-
Zeolite	-	4.00
Minors		
Minor Items	5.00	6.50
Sodium Silicate	10.00	9.00
Waste		
Moisture	8.00	2.50
Total Base	**100.00**	**100.00**

Table 3. Percentage composition of the liquid detergent formulation (LG) used in this study. Reported values are for those commercially available product formulations in South Africa at the time the experiment was initiated (B. Crawford, Unilever UK, pers. comm.).

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Ingredient	% Composition
Non-ionic	6.0
Soap	6.0
naLAS	5.5
SLES 3EO-25%	24.0
Propylene Glycol	4.0
Minors	4.5
Water	50.0

Prior to this study, external consumer-based research conducted for Unilever South Africa was used to identify the 16 most prevalent clothing stains occurring in South Africa (Table 4). To ensure both uniformity and representation of the organic and inorganic loading of a typical South African laundry load without faecal inputs, scientifically standardized stains of each of these were produced on 100 mm x 100 mm cotton square swatches and used in every wash. Specifically, prior to every wash, two of every stained swatch type were manually attached to part of the laundry load using an Avery Dennison® Mark III Dennison Tag Fast Pistol Tool (Avery Dennison Corp., USA) to drive nylon textile tags through the linen/stain interface for attachment.

Table 4. Stain types used in each laundry load during the generation of laundry greywater (G. Beukes, Unilever SA, pers. comm.).

Stain Description
Black Tea
Black Coffee
Red Wine
Berocca® Vitamin Drink
Cooking Oil with Dye
Curry
Castrol CWB Grease
Red Soil
Cadbury Milk Chocolate
Grass
Tomato Sauce
Strawberry Jam
White Tea
White Coffee
Black Polish
Rust

The wash conditions used for the generation of the different greywaters used in this study are shown in Table 5 and Table 6. In the case of the top loader, 100 g of laundry powder was then added to the wash drum which had been filled with 38 L of water and agitated into solution by setting the machine into a heavy wash cycle mode for a period of 2 min, in the absence of laundry. Stained swatches that had been attached to part of the laundry load were then placed together inside the washing machine drum. The remaining space in machine drums were filled with a ballast load of clean (pre-washed) white cotton linen and pillow cases, resulting in a total combined load per wash of 2 kg. For the automatic front-loader, 100 mL liquid detergent was added according to manufacturer's recommendations.

Following each of the wash and rinse cycles of both the front- and top loader machines, greywater discharge from every cycle was combined and collected in treatment-separated, 200 L storage drums, which were then lidded shut. For both washing machines, an additional rinse cycle was performed as a precaution to free the load of any remaining soap residue and to mitigate the possibility of treatment contamination in subsequent washes; waste water from this final cycle was discharged to the environment and not added to that greywater already contained in a given storage drum. Filled greywater drums were stored at outside (shade) temperature for both sites.

Laundry greywater was produced and stored on-site in 200 L lidded PVC plastic drums, and used and replaced entirely within 72 h. For both sites, greywater was produced using a Defy Twinmaid 800 top loader washing machine (Defy Appliances (Pty) Ltd., RSA) for the powder detergents (PG, CG) to simulate the more refined conditions associated with manual hand washing, and a Defy Automaid 600 DAW322 front loader washing machine (Defy Appliances (Pty) Ltd., RSA) for the liquid detergent (LG) greywater production. The preset conditions used for each of their respective wash cycles are shown in Tables 4 and 5.

Table 5. Wash conditions used for the automatic front-loader washing machine in the production of 'LG' greywater.

Variable	**Wash conditions**
Load size	2 kg
Dosage	100 mL liquid detergent
Wash temperature	Ambient (no heat)
Wash cycle	Quick (30 min; economy cycle)
Rinse cycle	1 cycle
Volume of greywater per load	51 L

Table 6. Wash conditions used for the top loader washing machine in the production of laundry greywater from powder detergents (PG, CG).

Variable	**Wash conditions**
Wash water volume	38 L
Load size	2 kg
Dosage	100 g powder detergent
Wash temperature	Ambient (no heat)
Wash cycle	15 min. soak, 15 min. wash
Rinse cycle	1 rinse cycle of approximately 6 min. in 38 L water
Volume of greywater per load	76 L

The Chemicult® (Stark Ayres, RSA) nutrient solution (Table 7) was produced on a daily basis in accordance with the manufacturer's general dosage recommendations of 10 g per 5 L by dissolving the nutrient powder thoroughly in tap water with manual mixing. Tap water was sourced daily from a tap connected to a treated municipal water supply.

Table 7. Composition of Chemicult® (Stark Ayres, RSA) hydroponics nutrient fertilizer solution by mass.

Element	Concentration (g kg^{-1})
N	65
P	27
K	130
Ca	70
Mg	22
S	75
Fe	1.5
Mn	0.24
B	0.24
Zn	0.05
Cu	0.02
Mo	0.01

2.4. Water Quality Analyses

Samples of the nutrient solution (n = 3), tap water (n = 3) and greywater formulations were collected periodically during experimentation. Greywater formulations were collected from storage drums (a) immediately following generation (n = 3) and (b) following 72 h of storage (n = 3) to assess any changes in physicochemical quality over time. This reflected the minimum and maximum periods of greywater storage during the experimentation. Since tap water and nutrient solution was always used fresh for crop irrigation (i.e. not stored), only fresh samples were collected and analysed. All samples were sent to an external laboratory (Stewart Inspection and Analysis Pty Ltd., RSA) for analysis of key water quality indicators (Table 8), using Standard Methods (2005) as described in following sections.

2.4.1. COD: Dichromate Reflux Method

Test samples (50 mL) were placed in a refluxing flask to which 1 g $HgSO_4$, several glass beads and 5 mL of a concentrated sulphuric acid reagent H_2SO_4 and $AgSO_4$ were added. While mixing to dissolve $HgSO_4$, the mixture was cooled to prevent the possible loss of volatile compounds. Thereafter, 25 mL 0.250 *N* $K_2Cr_2O_7$ was added to the solution and mixed (for samples where COD was expected to be <50 mL 0.025 *N* $K_2Cr_2O_7$ was used instead for greater test accuracy). The refluxing flask was then attached to a condenser and remaining sulphuric acid reagent (70 mL) added whilst swirling to mix the test solution. A 10:1 ratio of $HgSO_4$:Cl was used to complex sample chloride in 50 mL test samples and the mixture

refluxed for 2 h. The refluxed mixture was then diluted to twice its volume with distilled water, cooled to room temperature and excess $K_2Cr_2O_7$ remaining in mixture titrated with 0.25 *N* Fe $(NH_4)_2(SO_4)_2$ (FAS) prepared by dissolving Fe $(NH_4)_2(SO_4)_2 \cdot 6H_2O$ in distilled water to which 20 mL concentrated H_2SO_4 was added and mixture diluted to 1000 mL; (0.025 *N* FAS used for COD <50 mL). For end-point determination, 0.1 mL to 0.15 mL ferroin indicator solution was used for every titration. A reference blank of distilled water containing reagents was refluxed and titrated in the same manner as described above and used in the calculation of COD as follows:

$$\text{mg COD/L} = \frac{(A - B) \times N \times 8 \times 10^3}{\text{mL of sample}} \quad (3.0)$$

where: A = volume FAS used for blank (mL)
B = volume FAS used for sample (mL) and
N = normality of FAS

2.4.2. pH and Electrical conductivity: pH and EC meter

The pH and EC of water samples was determined using a Hanna® Instruments (Hanna® Instruments Inc., USA) HI 255 combined pH and EC meter.

2.4.3. Ammonia: Titrimetric Method

Water samples were combined with MgO Light which then undergoes Kjeldahl digestion and distilled by Kjeldahl distillation. Thereafter, remaining sample was titrated with boric acid to calculate ammonia present in samples.

2.4.4. Nitrates: Devarda's Alloy Reduction Method

A portion of test sample was diluted to 500 mL with ammonium-free water. Borate buffer (25 mL) was then added and the solution adjusted to pH 9.5 with 6 *N* NaOH with the aid of a pH meter. 250 mL and 300 mL was distilled into a dry receiving flask and subsequently discarded.

Reduction Method: Following the removal of NH_3 as described above, 1 g Devarda's alloy was added to the remaining residue and diluted with sufficient ammonia-free distilled water to a volume of 350 mL. Thereafter, H_3BO_3 absorbent (50 mL) was placed in a receiving flask for every mg NO_3^--N present in a given sample and the condenser end immersed in

absorbent. The mixture was then heated to boiling point after which the heat was reduced and the mixture distilled at a rate of between 5 and 10 mL per minute until 150 mL or more of distillate was collected. Distillate was then titrated with 0.02 *N* H_2SO_4 titrant using a mixture of methyl red and methylene blue as indicator. A blank was used throughout the above procedure for corrective reference. NH_3-N was then computed using the following equation:

$$\text{Mg } NH_3\text{-N/L} = \frac{(A - B) \times 280}{\text{mL sample}} \quad (3.1)$$

where: A = volume of H_2SO_4 titrated for a given sample (mL)
B = volume of H_2SO_4 titrated for a blank (mL)

This value represents the amount of NH_3-N produced that resulted from the total reduction of NO_2^- and NO_3^-. For the determination of NO_3^-, NO_2^- must be determined separately and NO_3^- computed by difference as follows:

Samples were first filtered through 0.45 µm pore diameter membrane filter to remove any suspended solids. Filtrate (50 mL) was then neutralised to pH 7 by reacting for > 2 min with 1 mL sulphanilamide solution prepared previously by dissolving 5 g in a mixture of concentrated HCl (50 mL) and 300 mL nitrite-free water, diluted to 500 mL. To this solution, 1 mL NED-dihydrochloride solution (500 mg N-(1-naphthyl)-ethylenediamine dihydrochloride in 500 mL nitrite-free distilled water) was added, mixed and the solution left to stand for at least 10 min. The absorbance of the solution was then measured using a Genesys 10S UV-VIS spectrophotometer (Thermo Fisher Scientific Inc., USA) at 543 nm. Results were compared with a series of standard NO_2^- solutions of differing volumes diluted to 50 mL with nitrite-free water and to which NED-dihydrochloride reagent was added as above. Comparable colour development in a standard was selected as the representative concentration of NO_2^- for a given test sample and actual NO_2^- calculated as follows:

$$\text{mg } NO_2^-\text{-N/L} = \frac{\mu\text{g } NO_2\text{—N/L (in 52 mL final volume)}}{\text{mL sample}} \quad (3.2)$$

The resulting NO_2^- concentration was then used to calculate NO_3^- by subtraction from the total nitrate concentration established earlier in (3.1).

2.4.5. Calcium, Magnesium, Potassium, Sodium, Aluminium, Boron, Phosphorus and Sulphur: Inductively Coupled Plasma (ICP)

Thoroughly mixed acid-preserved samples (10 mL) were pipetted into polypropylene tubes which had been pre-soaked for 48 h with 2 *N* HNO_3 and rinsed with metal-free water. An appropriate quantity of analyte (< 0.5 mL) for matrix-fortified samples was then added and thereafter concentrated HNO_3 (0.5 mL) was pipetted into samples and quality blanks. Samples were heated at 105 °C in a block heater and allowed to digest for at least 2 h. Where necessary, additional concentrated HNO_3 was added to samples until digestion was complete, indicated by the development of a clear sample solution. Sample tubes were then allowed to cool and subsequently diluted using metal-free water to the original 10 mL volumes. In those cases where particulates were present in sample tubes, samples were centrifuged and the clear supernatant portion decanted into pre-soaked tubes. All completely digested samples were then placed in cold storage at 4 °C until needed for ICP analysis. Samples were analysed for Ca, Mg, Na, Al, B, P and S using a Thermo Scientific Electron iCAP 6300 Duo Inductively Coupled Plasma Optical Emission Spectrometer (Thermo Fisher Scientific Inc., USA).

The determination of $[Ca^{2+}]$, $[MLG^{+}]$ and $[Na^{+}]$ in water effluent by ICP was used to calculate the SAR among treatments.

2.5. EXPERIMENT SITES

Two spatially separate sites differing with respect to a set of environmental conditions, particularly in terms of wind speed, salt exposure from air and shading, were selected for experimental purposes. These two sites were chosen to *independently* evaluate the soil characteristics and growth of vegetables among treatments irrigated with different laundry greywater formulations under the contrasting environmental conditions characterising each site.

2.5.1. Unilever South Africa, La Lucia Ridge

One of the experimental field plot sites was located on the grounds of the Unilever SA Head Office, North Durban, falling under an office park precinct known as La Lucia Ridge (29° 44’ 26’’ S; 31° 03’ 59’’ E; Fig. 1). With this site having an elevation above sea-level of 91 m and a relatively short straight-line distance from the Indian Ocean of approximately 1.34 km (Google™ Earth, 2009), it was susceptible to high onshore land and sea-breezes that typically occur along this entire coastal region. This land-sea breeze geographical phenomenon is due

to localised atmospheric pressure differences between coastal land and adjacent ocean due largely to the diurnally and nocturnally contrasting specific heat characteristics of soil and water. As a result, strong on-shore winds induced during the day can disperse salt spray from breaking waves considerably inland before being deposited as fine droplets and salts. Due to the combined effect of relatively high winds, direct sun exposure and salt deposition, plants grown in these conditions are exposed to wind-induced damage and to high evapotranspirational losses, relatively high levels of salt stress in tissues and organs, and hence increased levels of desiccation.

Fig. 1. Satellite image depicting the location of the experimental site at Unilever South Africa relative to Umhlanga Beach {Site Elevation: 91m; GPS: 29° 44’ 26’’ S; 31° 03’ 59’’ E (Google™ Earth, 2009)}.

2.5.2. UKZN Howard College, Glenwood

An additional experimental site was situated on the grounds of the University of KwaZulu-Natal (Howard College Campus), a location which lies comparatively closer to central Durban. The specific location of the experimental site was selected primarily on the basis that it was largely sheltered from prevailing winds by a brick building approximately 5 m away to the South-East and a row of trees running South-West to North-East, each tree approximately 5.5 m tall (29° 52’ 12’’ S; 30° 58’ 37’’ E; Fig. 2). The site was also located further away from the Indian Ocean compared to the site at Unilever SA, with a straight-line site to shore distance of 2.42 km (Google™ Earth, 2010). Topographically, the University site is also

situated at a higher elevation, being approximately 128 m above sea-level. The general implications of these environmental conditions were reduced exposure to salt and wind-stress and hence conditions more likely to favour the growth and development of most plant types.

Fig. 2. Satellite image showing the location of the experimental site at the UKZN Howard College campus {Site Elevation: 128m; GPS: 29° 52' 12'' S; 30° 58' 37'' E (Google™ Earth, 2009)}.

2.6. PLOT LAYOUTS

For both sites, separate randomised block design layouts for each species that included all individuals from all treatments, were implemented. Pseudo-randomisation of the field plot layouts were achieved using the random number generator function algorithm of Microsoft® Office Excel 2003 (Wichman and Hill, 1982; 1987; Microsoft Corp., USA). However, unlike at the shade-free Unilever experimental site where a randomised *complete* layout could be suitably employed, at the University site an *incompletely* randomised plot was instead implemented. This was done to mitigate any potential confounding effects on plant growth amongst treatments caused by the occurrence of a site-specific shade gradient along a North-West to South-East transect which become particularly acute during the autumn and winter

months. Pots were placed in a 9 replicates × 5 treatment matrix, in rows and columns 500 mm and 350 mm apart from their centres respectively (Figs. 3 and 4). The five treatments were the LG, CG and PG greywaters, tap water and hydroponics solution. At either site, plant spacing exceeded the minimum intra-species space requirements stipulated by the seed supplier.

Fig. 3. Unilever experiment site.

Fig. 4. University experiment site.

2.7. PLANTING

Seedlings were purchased from Tropical Nursery Landscape Studio CC in Durban. Individuals from each of the species had been grown separately direct from commercial seed in partitioned seedling trays that had been prepared with commercial potting soil. Seedlings were carefully transplanted so as to leave the potting soil around their roots relatively intact. In addition, to minimise the impact of transplant shock, all seedlings were transplanted in the morning or evening when daily temperatures and evapotranspiration rates were relatively low. Transplanted seedlings were individually planted in 20 L heavy grade polyethylene plant bags, all of which had a base layer of quarry stone chip (12 mm grade) approximately 50 mm deep to facilitate drainage. This gravel layer was then completely covered with approximately 200 mm of Berea Red soil in which seedlings were planted. Once transplanted, all seedlings from both sites were allowed to establish for a 14 day period and irrigated with municipal tap water before the various irrigation treatments were applied and plant growth measurements taken.

2.8. APPLICATION OF IRRIGATION TREATMENTS

All seedlings were irrigated daily in the mid- to late afternoon, each plant receiving only one treatment for the duration of any given growth cycle. Irrigation treatments were applied below the soil surfaces using a method described by Jackson *et al*. (2006), an adaptation of an inexpensive form of sub-surface irrigation developed by the Durban Metropolitan eThekwini Water and Sanitation Department for food garden plots in semi-rural and peri-urban settlements. In this approach, bottle necks were cut-off from empty plastic confectionary drink bottles (500 mL) just above the bottle shoulder and 5 evenly distributed holes each of 3 mm diameter were drilled into each at the bottom. Bottles were buried upright, roughly ⅔ of their lengths into the soil alongside plants (Figs. 5a and 5b). Water was introduced via the widened neck and left to drain out slowly through the holes in the base.

A daily amount of 500 mL of irrigation water per plant was supplied throughout the experiment, from initial planting of seedlings until whole-plant harvest. In those instances where blockage of drainage holes by soil and other particles impeded the drainage of irrigation water from the bottles into the soil, bottles were either gently shaken or carefully removed and replaced to free holes from blockage(s), thereby ensuring that the full 500 mL irrigation water per plant was supplied daily.

Fig. 5a. A sample of one of the irrigation bottles with neck sawn off used in the experiments. Note drilled holes at the bottle base; Fig. 5b. Irrigation bottles buried alongside experimental *C. annuum* individual plants.

2.9. Soil Analyses

2.9.1. Soil Sampling

Treatment pots from each species were randomly selected for weekly soil sampling (n = 5 per treatment) to assess any irrigation treatment-induced changes in soil EC and pH over time. Pot soil samples were carefully collected using a 12 mL stainless steel spoon from 0 mm to 100 mm below the surface for each of two sampling points in pots: soil immediate to the buried irrigation bottles, and soil at the farthest point away from bottle locations. The rationale for this sampling protocol was twofold: first, sampling soil from the centre of the pots where a given plant was located was considered impractical given the likely damage to plant roots that would ensue during the soil excavation process; second, by sampling from two different loci within pots as opposed to a single point situated away from the pot centre, it would also allow a more representative average to be calculated from those points at which soil EC and pH values would be expected to be greatest and that were it would be expected to be lowest respectively. Due to time and resource limitations, soil EC was only evaluated at the University experiment site.

Soil samples from each pot were sealed in plastic zipper bags and transported to the laboratory for analytical processing. There, individual soil samples were mixed thoroughly with a spatula and placed in food-grade silicone cups for drying in a laboratory oven at 38 °C. The total drying time of soils depended on the moisture content of soil samples, which varied substantially week on week. Once fully dried, soil samples were manually sieved using a 2000 µm stainless steel sieve to remove stone aggregates and organic debris. Sieved material was then used for both EC and pH analyses.

2.10. Soil Physicochemical Properties

2.10.1. pH Analyses

The pH values of soil in pots (n = 9 per treatment) were recorded weekly *in situ* using a soil pH meter (Hadeco, SA). For measurements, the pH measuring probe was inserted in the root zone to a depth of approximately 100 mm and approximately 50 mm from the base of a given plant.

For *ex-situ* measurements, the pH of each soil sample collected was determined using a 1:2.5 soil : solution suspension, produced by weighing 10 g of sieved soil to which 25 mL of 1 *M* KCl was added. Soil suspension samples were then mechanically shaken using a Labnet Orbit™ 1000 (Labnet International Inc., USA) at 20 rpm for 1 h in order to dissolve all soluble salts. A Hanna® Instruments (Hanna® Instruments Inc., USA) HI 221 pH meter coupled with an HI1131 pH glass-calomel electrode and an HI 40688 temperature probe was used to establish temperature-compensated pH values for all samples.

2.10.2. EC Analyses

Soil EC for all soil samples were measured weekly by repeating the same process as described in 2.10.1. pertaining to *ex-situ* measurements, with the exception that a 1:2.5 soil : distilled water suspension was instead used and analysed using a Hanna® Instruments (Hanna® Instruments Inc., USA) EC 215 conductivity meter.

2.10.3. Fertility Analyses

Soil samples (n = 5 per treatment) from both sites were collected from the root zone (0 mm to 100 mm below the soil surface) from plant bags at the beginning and end of each of the growth cycles for each species and submitted to the Soil Fertility and Analytical Services

Laboratory (KwaZulu-Natal Department of Agriculture and Environmental Affairs, Pietermaritzburg, South Africa) for physicochemical assessment. The analytical methods used in the determination of the macro- and micronutrients composition of soil samples are described below.

The Ambic-2 solution (0.25 *M* NH_4CO_3 + 0.01 *M* Na_2EDTA + 0.01 *M* NH_4F + 0.05 g L^{-1} Superfloc) extraction method (Non-Affiliated Soil Analysis Working Committee of South Africa, 1990) was used in the determination of extractable P, K, Zn, Mn and Cu in soils. A soil-solution suspension was first prepared by combining soil samples with Ambic-2 solution in a volumetric ratio of 1:10. For this, a 25 mL aliquot of solution was added to 2.5 mL of soil. This soil suspension was stirred using a multiple stirrer set at 400 rpm for 10 min. Extracts were filtered using Whatman® No.1 filter paper. A 2 mL aliquot of the resulting filtrate was used to determine the concentration of extractable P, using a modification of the Murphy and Riley (1962) molybdenum-blue method (Hunter, 1974; Olsen and Sommers, 1982). A 5 mL aliquot of the filtrate was diluted further using 20 mL de-ionised water, allowing the determination of extractable K by atomic absorption. Extractable Zn, Cu and Mn were then determined by atomic absorption on the remaining undiluted filtrate using Varian Spectra AA-220/280 FS atomic absorption spectrophotometers (Varian Inc., USA).

The determination of total C, N and clay content of soils was outsourced to the Soil Fertility and Analytical Services Laboratory (KwaZulu-Natal Department of Agriculture and Environmental Affairs, Pietermaritzburg, South Africa). Mid infra-red (MIR) spectroscopy of a FT-IR spectrophotometer (Bruker Tensor 27 HTS-XT, Bruker Corp., USA) was used for this purpose.

2.10.4. Salinity

A 1 *N* NH_4OAc ($NH_4C_2H_3O_2$) reagent solution was prepared by adjusting to pH 7 using either acetic acid or ammonium solutions, and mixed with 1.9 g L^{-1} KCl which functioned as an ionic suppressant.

Soil samples were sieved using a 2 mm gauge stainless steel sieve to remove any relatively large (> 2 mm) aggregates and organic debris. Thereafter, 10 mL of sieved soil samples were weighed and placed into centrifuge tubes. To each of these, 50 mL NH_4OAc reagent was added and centrifuge tubes were placed on a reciprocating shaker set to 180 rpm for 30 min. Samples were centrifuged for 10 min at 3000 rpm. The extract was filtered using Whatman®

No.541 filter paper and the resultant filtrate collected. Filtrate was then used to determine Na concentration via spectroscopy for which a Varian Spectra AA-40 atomic absorption spectrophotometer (Varian Inc., USA) was used.

2.10.5. Particle Size

Particle size distribution of soil was determined by the pipette method where soil fractions of clay and fine silt are determined on a percentage mass basis following suspension and dispersion (Day, 1965). A 20 g sample of air-dried soil was initially sieved using a 2 mm sieve, wetted with de-ionised water and reacted with 30 mL 30 % hydrogen peroxide to oxidize organic matter present. To each soil sample, 400 mL of deionised water was then added and soil solutions allowed to stand overnight. Clear supernatant present the following day was collected and discarded. The addition of deionised water and the extraction of supernatant were then repeated the following day for samples in which clay had settled.

A dispersing agent (20 mL sodium hydroxide and 10 mL of sodium hexametaphosphate) was added to each sample and thereafter the soil sample solutions stirred for 10 min on a bench top stirrer. Deionised water (1 L) was then added to samples in a volumetric cylinder. Soil samples were manually agitated into solution for 30 s using a plunger, and allowed to settle for a period dependent on ambient (room) temperature and the particle size investigated. For clay particles (soil fraction <0.002 mm) and fine silt (0.002 mm ≤ fine silt ≤ 0.02 mm), settling times of 5 h to 6 h and 4 min to 5 min were used, respectively. After each respective settling time, soil solution aliquots (20 mL) were sampled at 100 mL below the surface for silt and 75 mL below the solution surface for clays. Extracted samples were then oven-dried at 150 °C for 24 h, weighed, and expressed as percentage clay and percentage silt. The remaining soil fraction, sand (0.05 mm ≤ sand ≤ 2 mm), was then determined by difference.

2.10.6. Hydraulic Conductivity

The Guelph Permeameter (GP) method (Reynolds and Elrick, 1986) was used for the determination of *in-situ* soil hydraulic conductivity of pots for both experimental sites. The Guelph Permeameter apparatus is a constant head permeameter that relies on the Mariotte siphon principle. It is used to measure the *in-situ* hydraulic conductivity of soils by allowing the steady-state rate of water recharge into unsaturated soil from a cylindrical hole, in which a constant head of water is maintained, to be quantified (Eijkelkamp, 2008). From a number of

variables measured in the field, calculations can then be performed to determine field saturated hydraulic conductivity, soil sorptivity and soil matrix flux potential (Eijkelkamp, 2008).

An apparatus analogous to a commercial 2800K1 Guelph Permeameter (Eijkelkamp Agrisearch Equipment BV, Netherlands) was constructed using a 1.32 m long PVC pipe with an inner diameter of 50 mm (Figs. 6a to Fig. 6e).

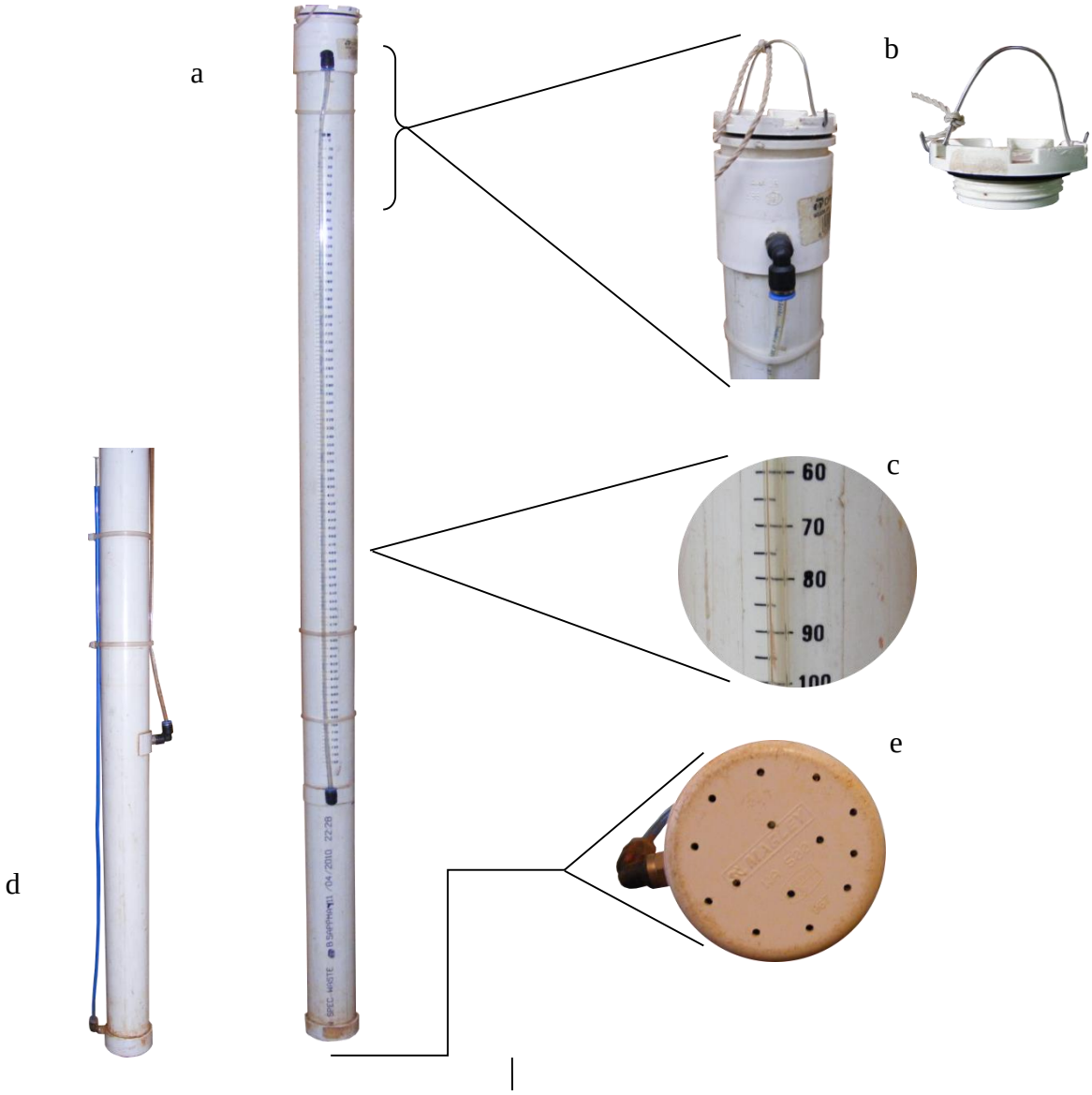

Fig. 8a. The assembled Guelph Permeameter apparatus used for in-situ hydraulic conductivity measurements; Fig. 8b. Top end of the permeameter showing screw cap, rubber seal and hanger; Fig. 8c. Liquid column gauge for polyurethane tube; Fig. 8d. Air-intake tube (blue) connected via hermetic elbow at permeameter bottom; Fig. 8e. Bored bottom end cap of permeameter.

Vertical ends of the pipe were sealed with end caps; a screw on, rubber-washer sealed end cap and a fixed end-cap was utilized for the top and bottom ends respectively, the latter having had several 2 mm holes bored to facilitate the drainage of liquids from within the assembled pipe. Clear, 4 mm (outer diameter) polyurethane tubing was mounted externally along the pipe shaft and hermetically sealed with connecting elbows at either end of the permeameter pipe shaft which opened internally via male fixings to liquid contained in the pipe. Positioned vertically behind the length of polyurethane tube was a self-adhesive linear rule used for flow measurements. A plastic air-intake tube, connected to the permeameter shaft via a connecting elbow, allowed a constant pressure head to be maintained despite changes in liquid level within the permeameter during usage.

In this study, hydraulic conductivity measurements of treatment soils were undertaken using the GP method in response to observations that soil drainage rates of the various treatments appeared to contrast significantly during experimentation. Soil hydraulic characteristic measurements were made at the end of growth cycles for each species grown at experimental sites. All measurements were made prior to whole plant harvest, so as to mitigate potentially confounding changes in soil drainage characteristics as a result of any soil disturbances that may otherwise have been induced.

Cylindrical wells produced from the partially buried irrigation bottles were considered suitable for all soil permeameter-based measurements. Prior to the insertion of the permeameter into the irrigation wells, plastic bottles were carefully removed without disturbing well circumferences. The cylindrical wells so produced were then lightly scarified manually using a soft nylon bristle brush to remove any sealing which may have occurred during bottle removal. Any loose earth and organic debris were then carefully excavated from the base of holes using a stainless steel spoon. Hole depth (H), hole diameter (D), depth to hole base (H_b) and distance to nearest pot side wall (L) (Fig. 7) were measured using a vernier calliper (± 0.02 mm) and linear ruler (± 0.5 mm). The permeameter was filled with one of the five irrigation treatment solutions, depending on the experimental pot/treatment to be tested, and thereafter hermetically sealed at the top using the screw end-cap with a rubber washer. In all cases, the permeameter was suspended vertically using nylon cordage attached to a steel frame positioned above a given pot such that the permeameter outflow cap was 15 mm above the well bases. Permeameter verticality was verified using a reference plumb-bob line suspended from the steel frame. Liquid was prevented from escaping from the

permeameter by generating a complete system vacuum, achieved by closing the air-intake tube of the permeameter. The height of the liquid column within the permeameter was recorded from the meniscus level observed in the clear polyurethane tubing.

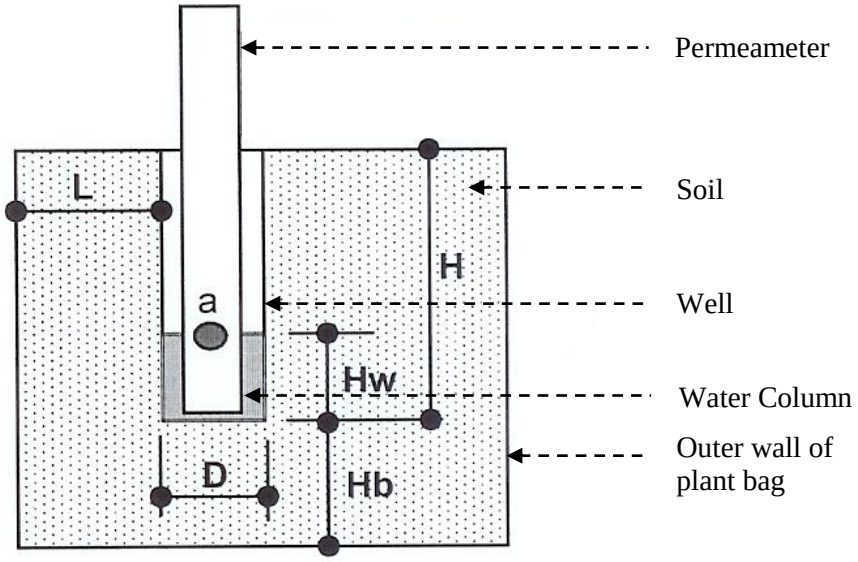

Fig. 7. Cross-section of plant pot showing permeameter inserted into well formed by the careful removal of a buried irrigation bottle. The various parameters of the plant pot and well that were made prior to performing hydraulic conductivity measurements are shown. Where: "L" = length to nearest side-wall of pot relative to well wall; "H" = well depth; "a" = air tube entry into permeameter; "D" = well diameter; "Hw" of water column in well; "Hb" = distance from well base to pot base.

Flow from the permeameter was initiated by opening the air-intake tube. Thereafter, at 60 s intervals, readings of liquid column height were taken, together with readings of soil moisture content. Readings of pot soil moisture were recorded using a Delta-T HH2 Moisture Meter coupled with a ML2X ML Theta Probe (Delta-T Devices Ltd., England). Here the soil meter probe was inserted as close to the centre of the pot (and hence plant) as possible to be representative of soil moisture immediately adjacent to plant roots in relation to hydraulic conductivities. When a steady state rate of flow from the permeameter into experimental soil was achieved, all hydraulic-related measurements were ceased. In instances in which steady state was not achieved before test liquid in the permeameter was exhausted, the abovementioned permeameter recharge procedure and flow-related measurements were repeated until such time as steady state was attained by the system. The change in height of the column of liquid within the permeameter from initial delivery was then expressed as a

function of flow volume, the hydraulic conductivity K_s, using standard cylindrical volumetric calculations in relation to time.

The above process was completed for a total of 3 replicates per treatment, for both species, and for both experimental sites

Flow readings from permeameter replicates were then used to calculate the field-saturated hydraulic conductivity of soils (K_{fs}) in cm s^{-1} as follows (Eijkelkamp, 2008):

$$K_{fs} = \frac{C_1 Q_1}{(2\pi H_1^2 + \pi a^2 C_1 + 2\pi (H_1/\alpha*)} \quad (3.8)$$

Where: $C_1 = [(H/a) / ((1.992+0.091(H/a))]^{0.683}$

Shape factor dependent on H/a ratio of permeameter measurements using empirical equations that were adapted from Zhang *et al*. (1998)

a = well radius, in cm

$\alpha*$= macroscopic capillary length parameter representing ratio of gravity to capillary forces experienced during drainage or infiltration dependent on soil texture-structure. For this study α was determined to be 0.04 cm^{-1} (Simon Lorentz pers. comm.), a constant corresponding to fine textured (clayey or silty), unstructured soil that possibly includes some fine sands

H_1= well height, in cm

Q_1= mathematical gradient describing the steady-state discharge rate of liquid from permeameter into soil, in cm^3 s^{-1}

2.11. Developmental Growth and Yield

2.11.1. Plant Heights

At both sites individual plant heights were measured using a linear rule (± 0.5 mm) on a weekly basis. For *C. annuum*, plant heights were determined by measuring vertical heights from where roots met stems to the central apical buds, i.e. the largest total vertical elongation point of leaves, branches and stems. In the case of *B. vulgaris*, owing to the absence of apical buds, plant heights were instead determined by measuring the lengths of the longest leaf, specifically, from the point where leaf stalks met the main roots, to leaf apexes.

2.11.2. Stem Diameters (C. annuum)

To partially assess any potential differences in biomass resource investment in structural components among treatments, stem diameters were also measured weekly amongst *C. annuum* plants at the University site using a stainless steel vernier calliper (± 0.02 mm). Stem diameters of most plants including bell peppers may vary considerably from a given point on the stem to another, for example at nodes compared to internodes or with height. For standardization, all stem diameter measurements were made of internode segments from the point just below the first node of the cotyledons.

2.11.3. Flowering & Fruiting Behaviours (C. annuum)

As potential treatment-induced stress indicators, flowering and fruiting behaviours of *C. annuum* were assessed from the onset of flowering until whole plant harvest. Flowers present on each *C. annuum* plant undergoing anthesis were counted daily and expressed on a cumulative, per treatment basis, N_{flower} treatment^{-1}. Similarly, the quantity of fully ripe (red) sweet pepper fruit produced per treatment were recorded throughout the growth period, until whole plant harvest, at which point both fully ripe and unripe (green) pepper fruits > 10 mm diameter present on all plants were included in this assessment. On a per treatment basis, the total fraction of harvestable fruit yielded relative to the total number of mature flowers produced throughout each growth cycle was quantified using the following:

$$f_{\text{fruit}} = N_{fruit} \,/\, N_{flower} \tag{3.3}$$

Due to resource and time considerations, this was only possible for the University experiment site.

2.11.4. Fruit Quality

The impact of greywater irrigation on fruit quality was evaluated for *C. annuum* to assess the viability of fruit when grown for commercial agricultural practices and/or subsistence. This assessment encompassed both nutrient content analysis of fruit and physical attribute measurements of random fruit samples pre- and post-harvest. The nutrient analysis assessment was used to evaluate whether any harmful contaminants were present in sufficient quantities in fruits sourced from greywater treatments relative to those from experimental

controls to be of concern to human health, and also to determine nutrient allocation within plants grown under the specified conditions.

The relationship between a given fruit polar axis and fruit equatorial diameter a common measure of commercial fruit quality, may be expressed in terms of a fruit shape ratio (FSR), (González-Real *et al*., 2008):

$$FSR = P_f / E_f \quad (3.4)$$

Where: P_f = fruit polar axis

E_f = fruit equatorial diameter

For this assessment, maximum polar axis lengths and equatorial diameters of *C. annuum* fruit samples were measured using a vernier caliper (± 0.02 mm). Pulp thickness (endocarp and mesocarp) of pepper fruit equatorial diameters were similarly measured for fruit which had been longitudinally sectioned at the mid-lobes.

2.11.5. Physical Leaf Parameters

Post-harvest leaf area measurements of leaves from *C. annuum* and *B. vulgaris* individuals were determined using a CI-202 portable leaf area meter (CID Inc., USA).

Mean leaf thickness (LT) of a leaf lamina was determined according to Roderick *et al.* (1990) from the ratio of its volume (V_L) to its projected area (A):

$$LT = V_L/A \quad (3.5)$$

A universal estimate of LT was calculated from Specific Leaf Area (SLA) and Leaf Dry Matter Content (LDMC) using the following equation from Vile *et al*. (2005):

$$LT = (SLA \times LDMC)^{-1} \quad (3.6)$$

Estimates of LT were calculated for both *B. vulgaris* and *C. annuum* as described in equation 3.6. For *B. vulgaris* this estimate was the mean derived from both leaf and stem thickness, so estimates of leaf thickness should *not* be considered as absolute.

2.11.6. Biomass

Following harvest, fresh mass (FM) and dry mass (DM) of the above-ground and below-ground components of each plant were established. For *C. annuum*, this first entailed excision by secateurs of each plant into its major anatomical organs (roots, stems, leaves and fruit). On the other hand, in the case of *B. vulgaris*, for practical considerations, whole plants were separated into leaves (encompassing the fleshy stems) and roots only. For both species, root biomass assessment included both roots and burls from below the cotyledon nodes.

Once the fresh mass of each plant organ was established after weighing material on a mass balance, dry masses were recorded by first drying the fresh plant material components in an oven at 80 ˚C for 72 h, and thereafter weighing dried material. This oven drying time was established as suitable to ensure for the complete drying of all vegetative material on the basis of a drying curve initially undertaken (results not shown). That fraction of dry matter of each organ contributing towards the total DM of each plant, f_{organ}, was then calculated from González-Real *et al*. (2008):

$$f_{organ} = DM_{organ} / DM_{plant} \quad (3.7)$$

Where: DM_{organ} = Dry mass of organ of interest

DM_{plant} = Total dry mass of plant

2.12. PLANT TISSUE ANALYSES

2.12.1. Phosphorus, Calcium, Magnesium, Potassium, Sodium, Zinc, Copper, Iron, Manganese: ICP

All plant tissue elemental analyses were performed by the Cedara Plant Laboratory (Cedara Soil Fertility and Analytical Services, KwaZulu-Natal Department of Agriculture, Pietermaritzburg, South Africa). Leaf (*B. vulgaris* and *C. annuum*) and fruit tissue samples (*C. annuum*) each of 0.5 g mass were dried at 105 °C for 2 h and weighed before undergoing ashing overnight in a furnace at 450 °C. The resulting dried ash samples were wetted with a few drops of distilled water, following which 2 mL of concentrated HCl was added to each

sample. Wetted samples were then allowed to evaporate to dryness in a fume cupboard. Once dried, 25 mL of 1*M* HCl was added to each sample. Each of these samples were then stirred using a rubber policeman. The acid-digested samples were filtered through Advantec 5B 90 mm diameter filter papers (Advantec MFS Inc., USA/Japan). The resulting filtrate was collected and analysed by ICP for the abovementioned elements using a Varian Vista-MPX CCD Simultaneous ICP-OES machine (Varian Inc., USA).

2.12.2. Boron: Spectrophotometer

An azomethine-H reagent was prepared by dissolving 0.9 g azomethine-H and 2 g ascorbic acid with distilled water and diluting to 100 mL. A buffer-masking solution was prepared by dissolving 280 g ammonium acetate, 20 g potassium acetate, 8 g nitrilotriacetic acid and 20 g tetrasodium salt of EDTA in 400 mL of distilled water. Undissolved precipitate present in stock buffer solution was removed by passing through Whatman® No.4 filter paper (Whatman PLC, UK). A 100 mg L^{-1} boron stock solution was prepared using boric acid dissolved in distilled water. Different concentrations of boron standard solutions of 5, 10, 20 and 30 mg L^{-1} were prepared separately by dilution with 1 *N* H_2SO_4, made up to 100 mL. Calcium oxide (0.1 g g^{-1}) was added to either 0.5 g leaf (*B. vulgaris* and *C. annuum*) or fruit (*C. annuum*) tissue as prepared as in 2.6.1 above, and samples were placed overnight in a furnace at 450 °C. Thereafter, 4 drops of deionised water and 10 mL 1 *N* H_2SO_4 were added to samples. Ash solutions were allowed to stand for 1 h and stirred occasionally. Solutions were filtered using Whatman® No.1 filter paper (Whatman PLC, UK) and 4 mL of the resulting filtrate added to a test tube. Azomethine-H solution (1 mL) and 4 mL buffer-masking solution were added to the filtrate, mixed thoroughly on a test tube shaker and allowed to stand for 30 min. The filtrate solution was again mixed on a test tube shaker and allowed to stand for 1 h to allow for the development of colour. Absorbance was measured using a Varian Spectra AA-220/280 FS spectrophotometer (Varian Inc., USA) at 420 nm. Boron concentrations of samples were calculated by plotting absorbency against standard concentrations.

2.13. EXPERIMENTAL DESIGN OVERVIEW

A summary of the experimental design processes followed at each site are shown in Figures 8 and 9.

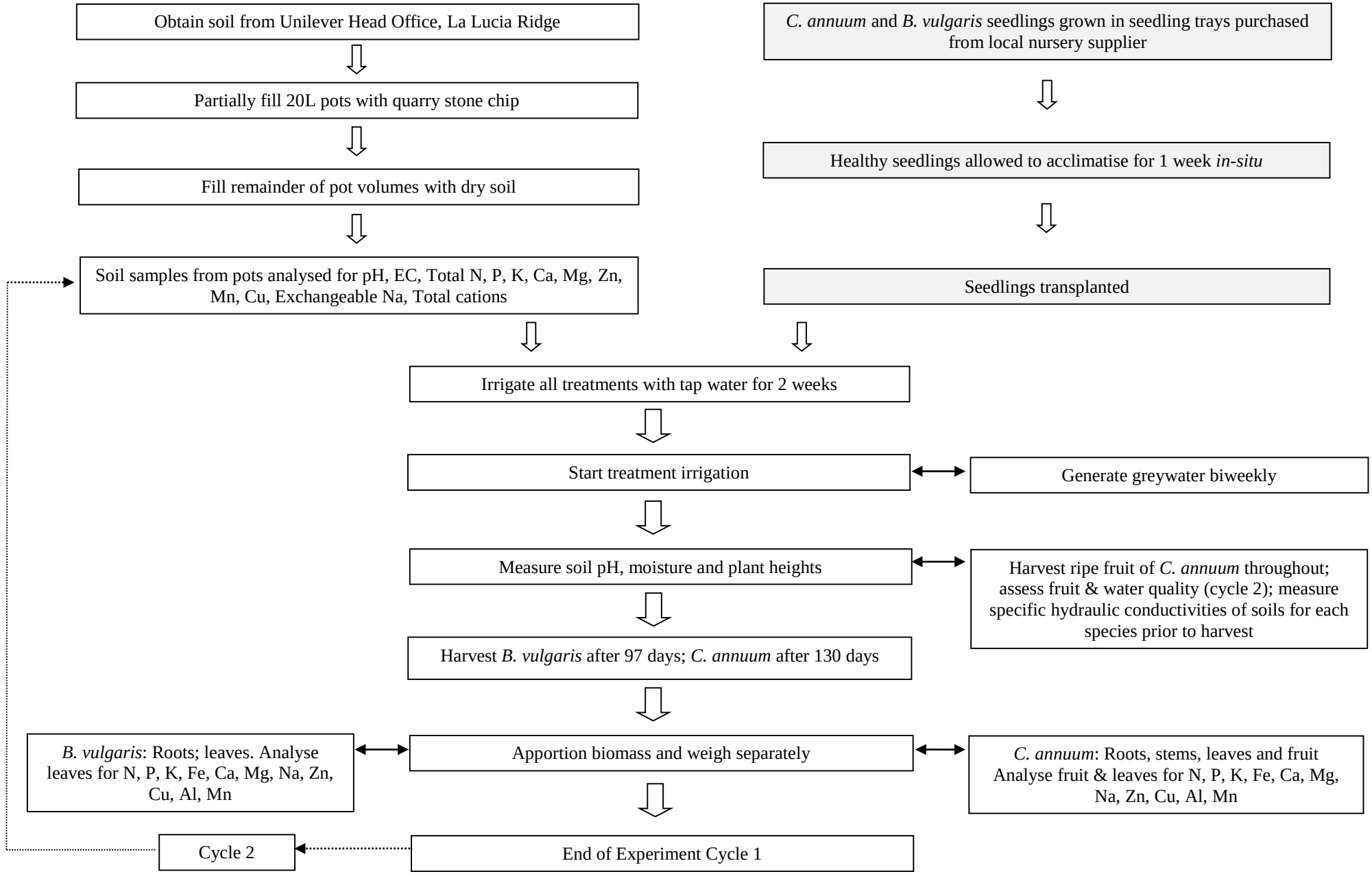

Fig. 8. Flowchart summarising key steps taken for the experiment at the Unilever experimental site.

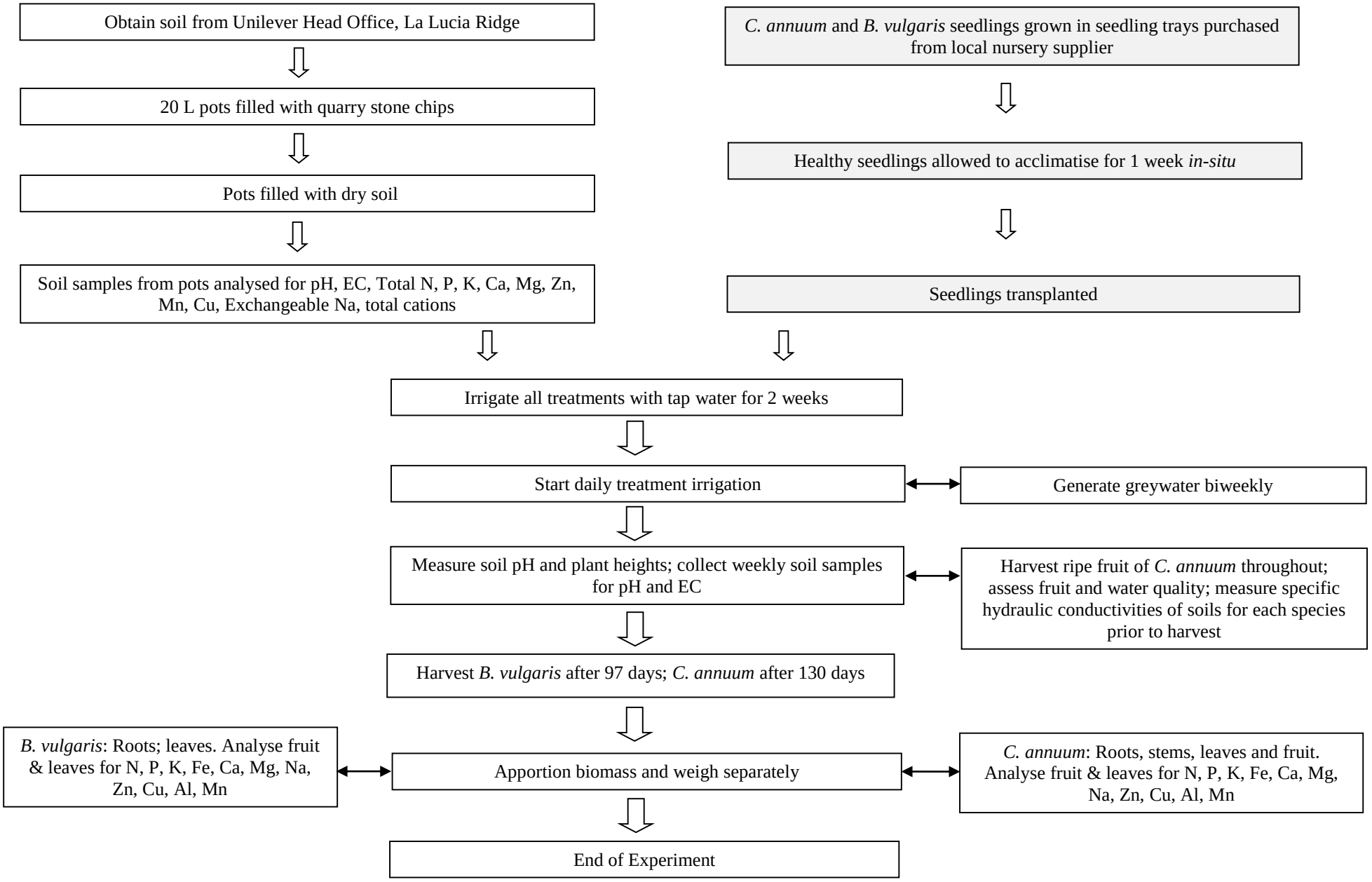

Fig. 9. Flowchart summarising key steps taken for the experiment at the University experimental site.

2.14. METEOROLOGICAL CONDITIONS

2.14.1. Weather Stations

A Kestrel 4500 Portable Weather Station (Nielson-Kellerman, Inc., USA) stationed at each site was used to record weather-related parameters, including temperature, relative humidity, wind speed, wind direction and atmospheric pressure (Fig. 10). The weather stations were positioned centrally at elevations of 0.7 m to 1 m within each experimental site to obtain an average of environmental conditions characterising each site by accommodating for any localised variations in ambient conditions, for example, shade versus no shade.

The data logging intervals of each was programmed so that all weather data would be recorded continuously, stored in on-board flash memory every 20 min and downloaded later for analysis.

Fig. 10. A Kestrel 4500 Portable Weather Station (Nielson-Kellerman Inc., USA) stationed at the University experimental site.

2.14.2. Vapour Pressure Deficits

The saturation vapour pressure of air (VP_{sat}) for each experimental site was calculated according to Prenger and Ling (2001):

$$VP_{sat} = e^{(A/T + B + CT + DT\text{^}2 + ET\text{^}3 + FlnT)} \quad (3.9)$$

Where:

$A = -1.044\times10^{4}$ $D = 1.289\times10^{-5}$

$B = -1.129\times10^{1}$ $E = -2.478\times10^{-9}$

$C = -2.702\times10^{-2}$ $F = 6.456$

and T = temperature of ambient air in °R; $\{T(°R) = {}^{9}/_{5} \cdot [T(°C) + 273.15]\}$ (4.0)

Daytime VPD (VPD_{dt}) was calculated from temperature and relative humidity values recorded every 20 min from 06h00 until 18h00 using the following formula adapted from Howell and Dusek (1995):

$$VPD_{dt} = \frac{\sum_{i}^{37} [e^*(T_i)\,(1 - RH_i)]}{37} \quad (4.1)$$

This inclusive VPD temporal range used in equation (4.1) was established from sunrise and sunset times for Durban using software developed by Daniels (2000). This 12 h period was found to approximate most closely to the average daylight h experienced over the experimental period.

2.15. Statistical Analyses

2.15.1. Tests

Statistical comparisons among all treatments for above and below ground dry plant biomass and proportion contributions thereof to overall dry plant biomass, were performed using one-way ANOVA with Scheffe's post hoc test. The beginning and end harvest cycle soil pH and EC values were statistically compared using parametric paired sample t-tests. Cycle-on-cycle comparisons of biomass and nutrient uptake by plant tissues were evaluated statistically using independent samples t-tests. All analysis- specific assumptions were statistically verified. Where data failed to satisfy these assumptions, logarithmic, square root or arcsine transformations of data were performed. In those instances where these and other data transformation functions did not achieve resolution of assumption violations, associated non-parametric statistical analyses were used instead. For statistical evaluations, *P* values less than 0.05 were considered to indicate statistical significance.

2.15.2. Software Packages

For all statistical analyses, SPSS® 15.0 (SPSS Inc., USA) and PASW Statistics 18 Release 18.0.0 (IBM Corp., USA), for Windows, were utilized.

CHAPTER III: RESULTS I

3.1. METEOROLOGICAL DATA

3.1.1. Wind

Mean daily wind speed at the Unilever experiment site was found to fluctuate between values of 0 m s^{-1} and 13 m s^{-1}, considerably more than that of the University experimental site where average wind speeds recorded for the period was only intermittently above 0 m s^{-1} (Fig. 11). Consequently, mean wind speeds for each site contrasted significantly, with values of 4.2 m s^{-1} and 0.42 m s^{-1} recorded respectively.

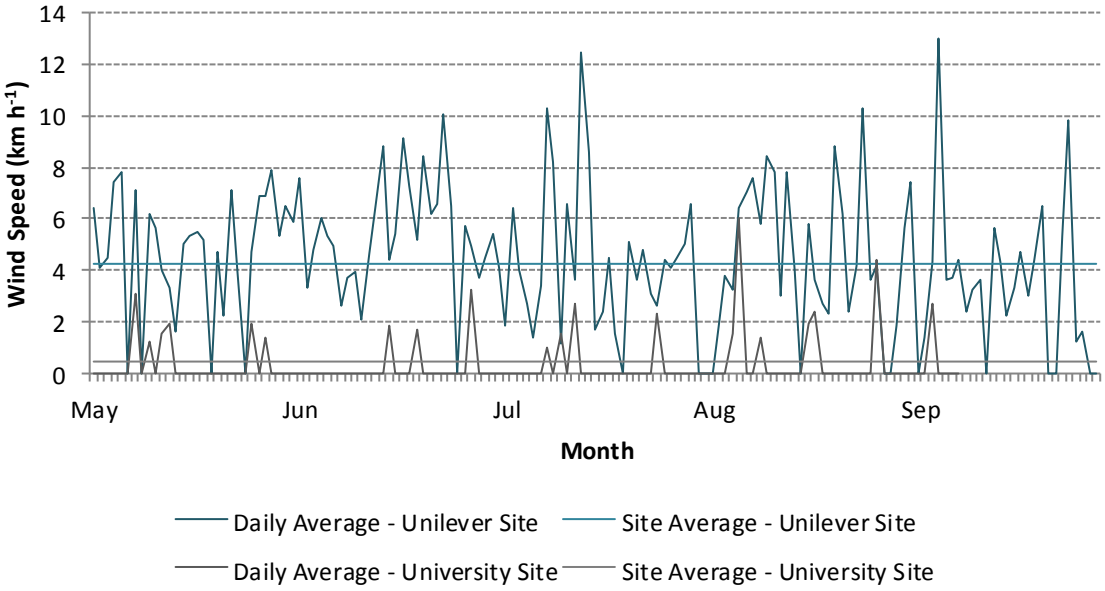

Fig. 11. Mean daily wind speed recorded at experimental sites for the period during which plants were grown (n = 72 recordings per day).

3.1.2. Relative Humidity

Relative humidity values, recorded at each site and averaged on a monthly basis, are shown in Fig. 12. The University experimental site was found to have significantly higher mean RH values relative to the Unilever experiment site for the months of May, June and July, but was found to be statistically similar for the months of August and September. Seasonal effects on relative humidity were also apparent for both sites with reductions in monthly RH noted over the winter. However, mean monthly relative humidity values remained considerably high throughout the experimental period for both sites, with values typically above 70 %.

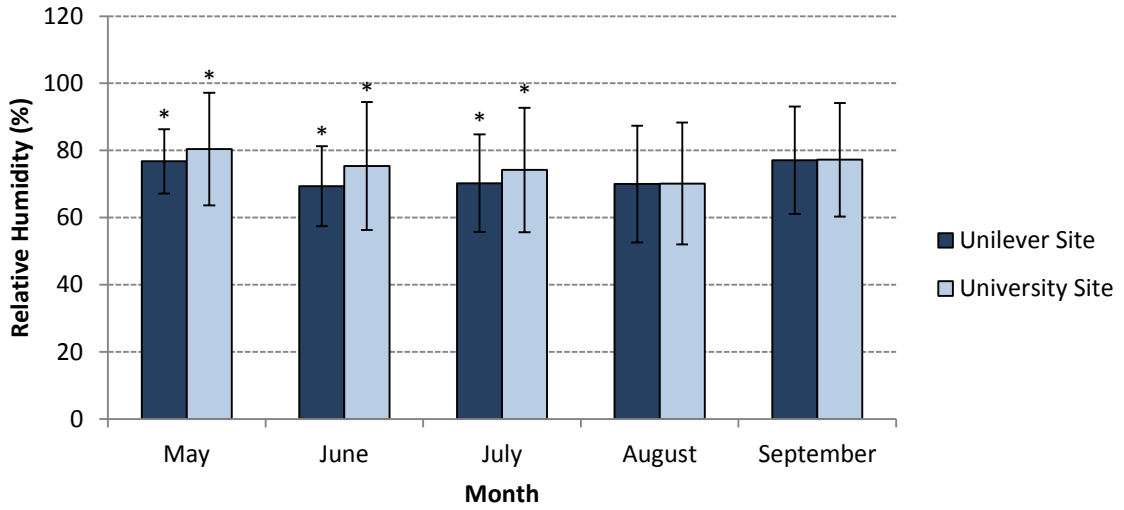

Fig. 12. Mean monthly relative humidity recorded at experiment sites for the period May to September 2010. Vertical bars represent standard deviations. Asterisks denote mean separation between sites by independent samples t-test for a given month ($P < 0.05$; $n = 72$ recordings per day).

3.1.3. Temperature

As shown in Fig. 13, temperature recordings at each experimental site were indicative of mild shifts in temperatures across the autumn, winter and spring seasons when plants were grown. Slight differences in temperature trends between the experimental sites were however noted, with the University experiment site cooler on average (18.8 °C) than the Unilever experiment site (19.3 °C), with statistically significant differences apparent among the two sites for the months in which plants were grown. Monthly temperature means however were found to be mild for both sites, with temperatures range-bound between 17.2 °C and 21.3 °C.

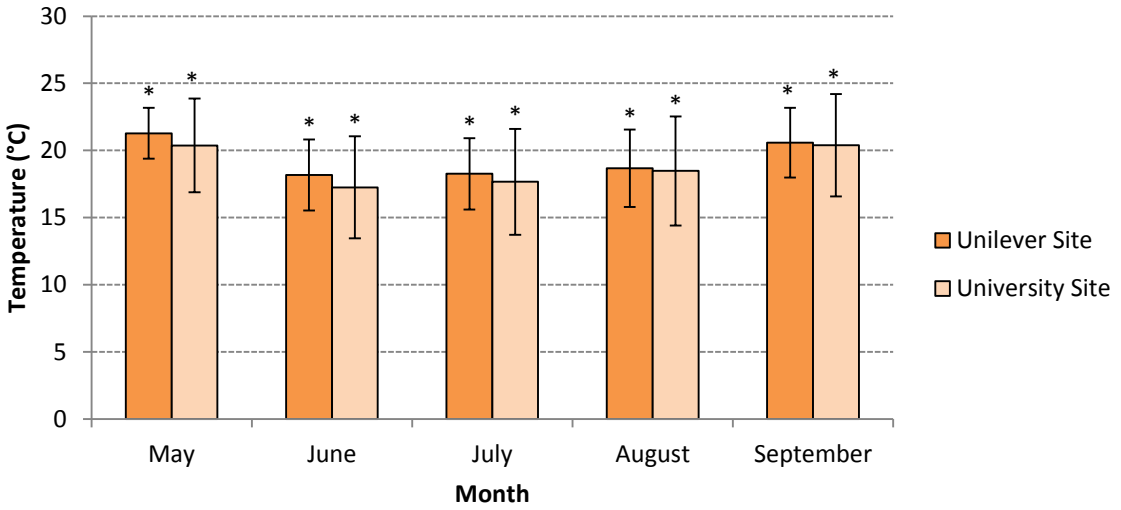

Fig. 13. Mean monthly temperatures recorded at experiment sites for the period May to September 2010. Vertical bars represent standard deviations. Asterisks denote mean separation between sites by independent samples t-test for a given month ($P< 0.05$; $n = 72$ recordings per day).

3.1.4. Vapour Pressure Deficits

Vapour Pressure Deficits (VPD) for both experimental locations, calculated from diurnal temperature and relative humidity data (as described in section 2.13), are shown in Fig. 14.

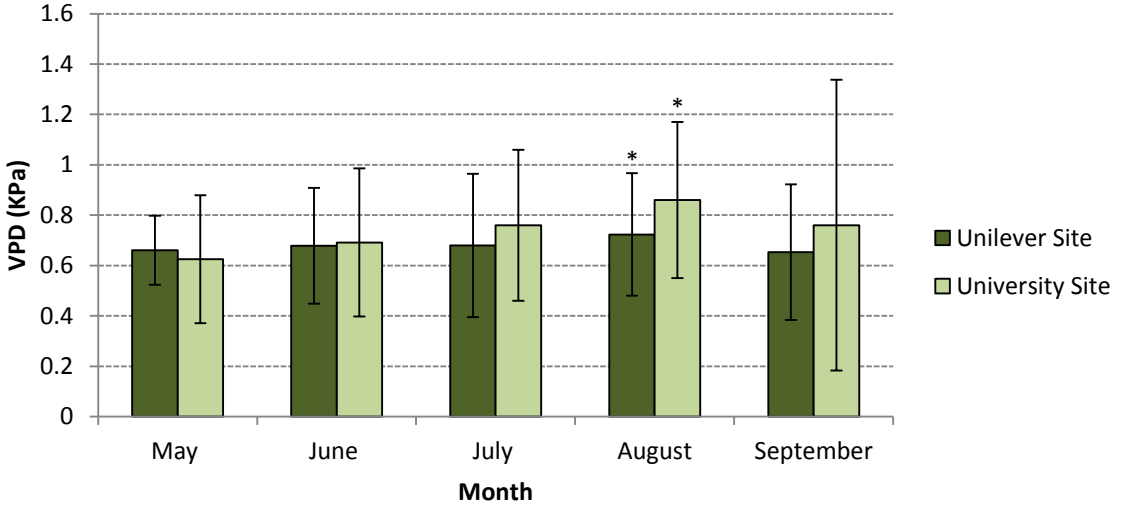

Fig. 14. Mean diurnal VPD (VPD_{dt}) established from meteorological data of experiment sites for the months during which plants were grown. Vertical bars represent standard deviations. Asterisks denote mean separation between sites by independent samples t-test for a given month ($P< 0.05$; $n = 37$ recordings per day; only diurnal recordings were used).

Although inter-specific site differences were apparent for both temperature and relative humidities, this did not generally translate into VPD differences between sites for those corresponding months encompassing the experimental period. The only exception occurred in late winter/early spring when comparative VPD site differences were found to be significantly higher at the University experiment site, with calculated VPD values there found to exceed 0.85 KPa. For most months however, VPD values were typically high for both experimental sites, falling between 0.65 KPa and 0.76 KPa.

3.1.5. Rainfall

As shown in Figs. 15 and 16, winter months are typically dry periods for both experimental sites, with much of the annual rainfall contribution occurring during the spring and summer months, particularly in December and January.

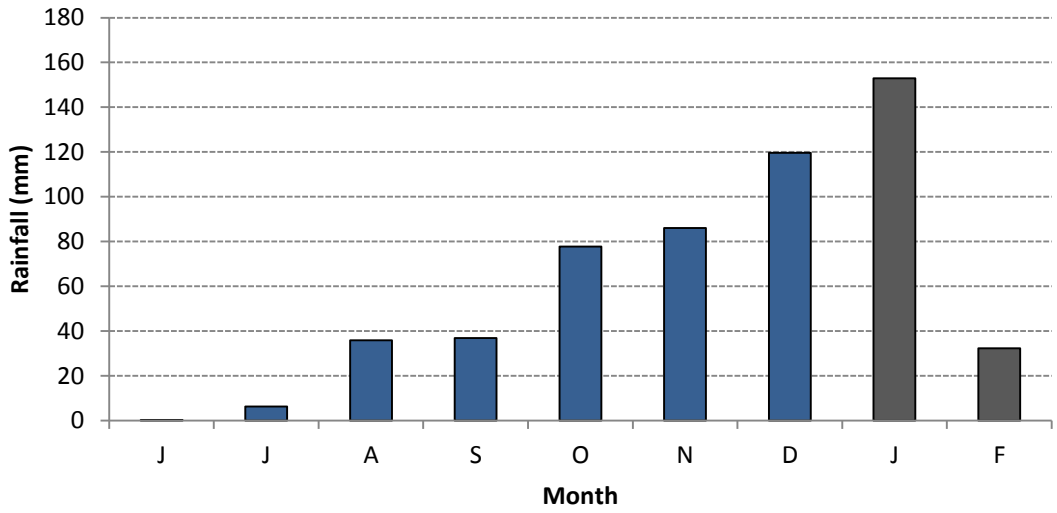

Fig. 15. Monthly precipitation recorded from June 2009 to February 2010, the period over which plants were grown at the Unilever experiment site for growth cycle 1. Blue bars indicate rainfall data for the months of 2009, grey bars rainfall data for the months of 2010.

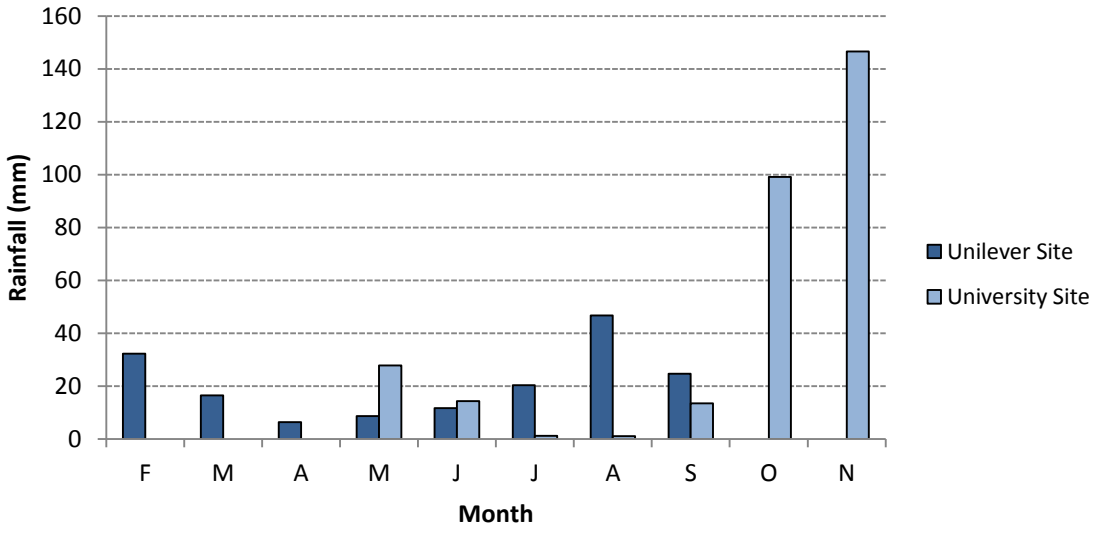

Fig. 16. Monthly precipitation data for both experimental sites recorded over the period in 2010 during which plants were grown at each site (second growth cycle for Unilever experiment site).

3.2. WATER QUALITY

The physicochemical characteristics of irrigation media were found to vary considerably (Table 8). Differences were particularly evident among treatments with respect to COD, EC, and pH. The use of the powder laundry detergents PG and CG resulted in greywater effluent with mean COD values of 365 mg L^{-1} to 464 mg L^{-1}. These were significantly lower than COD of greywater generated from liquid laundry detergent (LG), which reflected the highest mean COD values among all treatments of 1006 mg L^{-1}. Conversely, greywater generated from the washing of laundry with powder detergents resulted in significantly higher pH values of (9.5 to 9.9) relative to greywater effluent resulting from the use of the liquid detergent product, for which a mean pH value of 7.95 was recorded. Among treatments, the nutrient solution was found to have the greatest EC values (216 mS m^{-1}), roughly one and a half times higher than that of the powder detergent-generated greywater. Interestingly, EC values recorded among LG-generated greywater were not significantly different from those measured for tap water. For select macro- and micronutrients evaluated among greywater treatments, most were found to occur in similar concentrations in the three greywaters. The notable exceptions were for Na, which was found to be approximately four times higher in concentration than greywater generated from liquid detergent, and the macronutrients P and K which were also found to be significantly higher in PG powder derived-greywater relative to the other greywater treatments used. The value of the balanced plant nutrition formulation of the nutrient solution was demonstrated by a fairly even ratio of N-P-K together with

significantly higher levels of nitrates, total N, ammonia, Ca, Mg, P and S. By comparison the concentration of P found in PG effluent laundry (52 mg L^{-1}) was found to be statistically similar to that of the nutrient formulation (45 mg L^{-1}) and also significantly higher than any of the other greywater formulations (0.5 mg L^{-1} to 5.2 mg L^{-1}). Established values of Total N, S and B however in the nutrient solution were higher than would have been expected from theoretical calculations (data not shown).

Table 8. Mean chemical attributes of greywater samples analysed immediately following generation (fresh) and following 72 hours in storage (aged). Nutrient and tap water solutions were analysed only fresh, as was used for crop irrigation throughout experimentation**.**

Chemical Attribute mg L^{-1} unless otherwise stated	**Nutrient**	**PG**		**LG**		**CG**		**Tap Water**
	Fresh	Fresh	Aged	Fresh	Aged	Fresh	Aged	Fresh
COD	10^{a}	365^{*b}	380^{*d}	1006^{*c}	866^{*d}	464^{*b}	416^{*d}	0.5^{a}
pH [$-\log_{10}(a_{H+})$]	6.53^{a}	9.50^{c}	9.60^{d}	7.95^{b}	7.47^{e}	9.93^{c}	10.03^{d}	$7.33^{a/b}$
EC (mS m^{-1})	216^{a}	129^{c}	135^{a}	33^{d}	35^{b}	147^{b}	151^{c}	21^{d}
Nitrates	39^{a}	0.5^{b}	0.5^{c}	0.5^{b}	0.5^{c}	0.5^{b}	0.5^{c}	0.5^{b}
Ammonia	5^{a}	0.5^{b}	0.5^{c}	0.5^{b}	0.5^{c}	0.5^{b}	0.5^{c}	0.5^{b}
Total N	61^{a}	1.5^{a}	1.6^{b}	2.6^{a}	2.4^{b}	3.1^{a}	0.8^{b}	1.6^{a}
P	45^{a}	52^{a}	45^{c}	2.8^{*b}	2.1^{*d}	5.2^{b}	3.7^{d}	0.5^{b}
K	270^{a}	4.4^{*b}	3.6^{*c}	5.0^{*a}	3.8^{*c}	5.5^{a}	4.1^{c}	2.2^{a}
N-P-K	1: 1: 6	1: 35: 3	1: 28: 2	1: 1: 2	1: 1: 2	1 : 2 : 2	1 : 5 : 5	1 : 0.3 : 1.4
B	0.5^{a}	0.7^{a}	0.7^{c}	3.8^{b}	3.6^{d}	0.8^{a}	0.8^{c}	0.5^{a}
S	75^{a}	$57^{a/b}$	40^{c}	5.4^{b}	8.3^{d}	78^{a}	50^{c}	8.9^{b}
Al	0.5^{a}	1.5^{a}	0.7^{b}	0.7^{a}	0.5^{b}	3.0^{a}	3.4^{b}	0.5^{a}
Ca	110^{a}	16^{b}	16^{c}	14^{b}	14^{c}	14^{b}	14^{c}	12^{b}
Mg	55^{a}	6.1^{b}	6.0^{c}	5.2^{b}	5.4^{c}	6.2^{b}	5.3^{c}	5.2^{b}
Na	26^{a}	250^{b}	241^{c}	71^{a}	75^{d}	273^{b}	237^{c}	20.8^{a}
SAR	2.86	75.21	72.66	22.92	24.08	85.90	76.29	7.09

*Denotes statistically significant differences between fresh and aged samples for a given treatment. Different letters denote statistically significant differences between treatments for a given attribute.

Sodium adsorption ratios calculated among treatments were generally found to be very high, with mean values for both powder detergent generated greywater exceeding tenfold the recommended SAR guideline value of 8 (DWAF, 1996). Mean SAR of greywater generated from liquid detergent LG was found to be considerably less by comparison, but remained approximately four-fold higher than the upper limit of recommended guidelines (DWAF, 1996). Even tap water, envisaged to be fairly chemically inert, was found to have an elevated

mean SAR. Among treatments, the nutrient solution was found to have the lowest mean SAR with a value of 2.86, the only irrigation media used that fell well below the upper SAR limit suggested suitable for crop irrigation (DWAF, 1996)

The effect of greywater storage for 72 h appeared to generally have a negligible effect on greywater chemistry for all formulations used. However, greywater generated using CG- and LG-derived detergents experienced a significant decrease in COD over the period of storage whilst PG had a significant increase over the same period. Potassium and P also experienced a significant decrease in concentration over 72 h for the PG- and LG-, and LG-derived greywater respectively.

3.3. Plants: Unilever Site, La Lucia Ridge

3.3.1. Plant Heights: B. vulgaris

Growth trends among treatments during both growth cycles at the Unilever experiment site reflected sigmoidal growth distributions (Figs. 17 and 18).

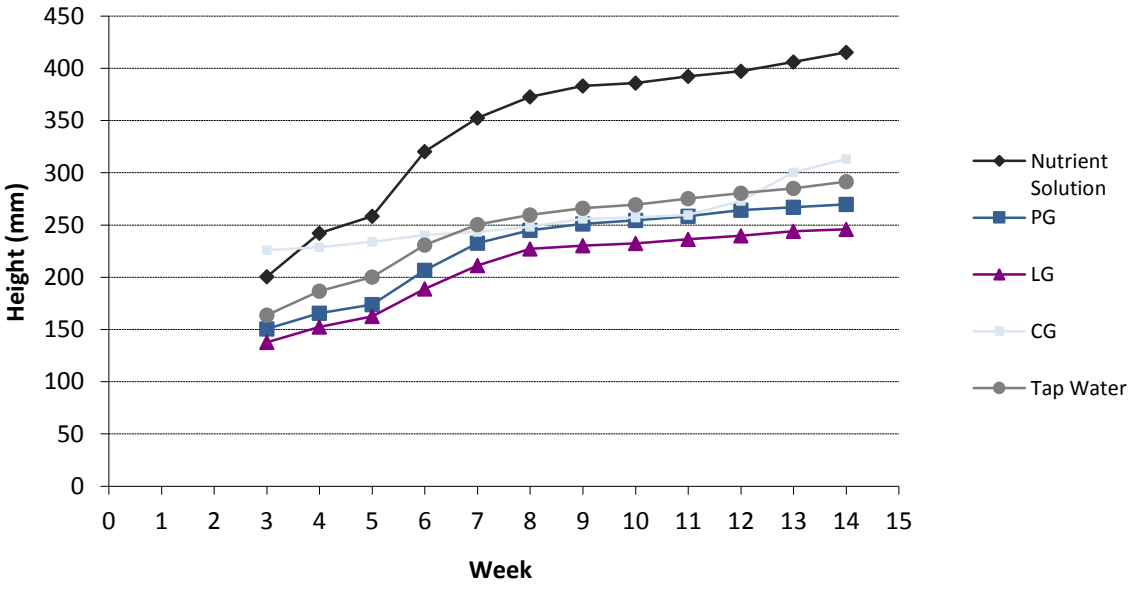

Fig. 17. Weekly mean heights (n = 9 per treatment) of *B. vulgaris* plants from the irrigation treatments tested, recorded during growth cycle 1 at the Unilever experiment site. Treatments: "Nutrient solution" = irrigated with nutrient solution; "PG" = irrigated with phosphate base powder-derived greywater; "LG" = irrigated with liquid laundry detergent-derived greywater; "CG" = irrigated with carbonate base powder-derived greywater; "Tap water" = irrigated with municipal tap water supply. Vertical bars represent standard errors about means.

Nutrient-irrigated treatments grew both taller and faster, attaining greater mean harvest heights relative to all other treatments. Greywater- and tap water-irrigated treatments reflected similar absolute mean growth and growth rates, terminating apical growth at similar mean leaf lengths. Trends among treatments for a given growth cycle were found to be similar.

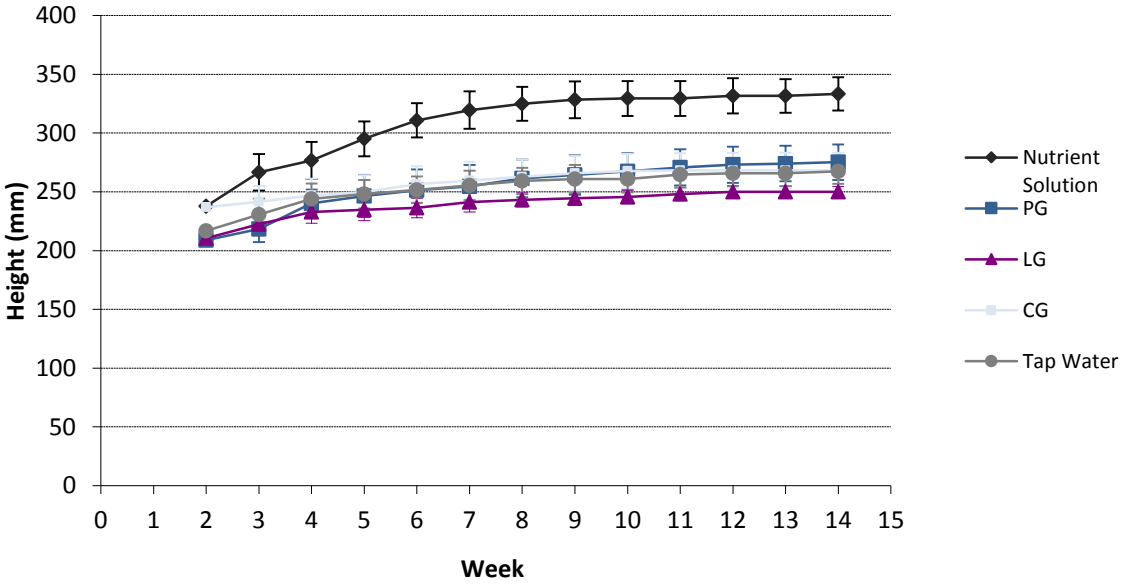

Fig. 18. Weekly mean heights (n = 9 per treatment) of *B. vulgaris* plants from the irrigation treatments tested, recorded during growth cycle 2 at the Unilever experiment site. Treatments: "Nutrient solution" = irrigated with nutrient solution; "PG" = irrigated with phosphate base powder-derived greywater; "LG" = irrigated with liquid laundry detergent-derived greywater; "CG" = irrigated with carbonate base powder-derived greywater; "Tap water" = irrigated with municipal tap water supply. Vertical bars represent standard errors about means.

3.3.2. Plant Heights: C. annuum

Weekly mean heights of *C. annuum* individuals recorded during growth cycles 1 and 2 at the Unilever experiment site, are shown in Fig. 19 and Fig. 20 respectively. A Gampertzian growth trend was followed by all experimental treatments during growth cycle 1 and 2, and was most pronounced for growth cycle 1. With the exception of the carbonate-base laundry powder CG-irrigated treatment, the irrigation of *C. annuum* with greywater during cycle 1 and 2 did not influence mean heights significantly over the experimental period and was comparable to results found for both tap-water irrigated and nutrient-irrigated *C. annuum*. However, as mentioned earlier, it should be noted that the planting of the CG-irrigated treatment was only initiated approximately 3 weeks after planting of the other treatment individuals took place, resulting in the anomalous recordings of *C. annuum* height noted during cycle 1. As a result, height recordings pertaining to CG-irrigated treatments are

excluded from further scientific interpretation and evaluation for growth cycle 1 at the Unilever site.

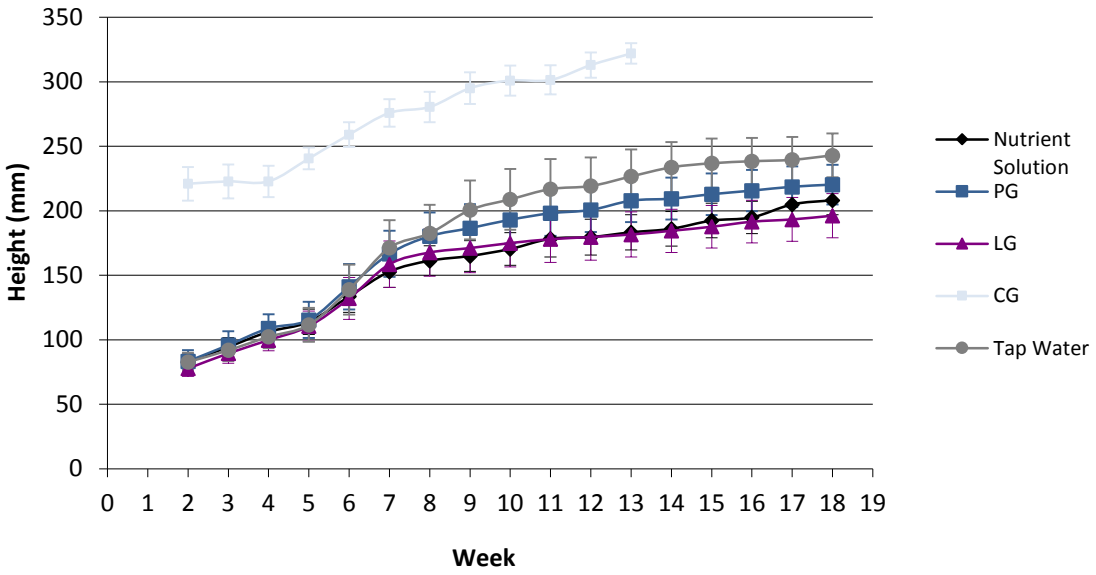

Fig. 19. Weekly mean heights of *C. annuum* plants from the irrigation treatments tested, recorded during growth cycle 1 at the Unilever experiment site (n = 9 plants per treatment except CG; for CG, n = 11). Treatments: "Nutrient solution" = irrigated with nutrient solution; "PG" = irrigated with phosphate base powder-derived greywater; "LG" = irrigated with liquid laundry detergent-derived greywater; "CG" = irrigated with carbonate base powder-derived greywater; "Tap water" = irrigated with municipal tap water supply. Vertical bars represent standard errors about means.

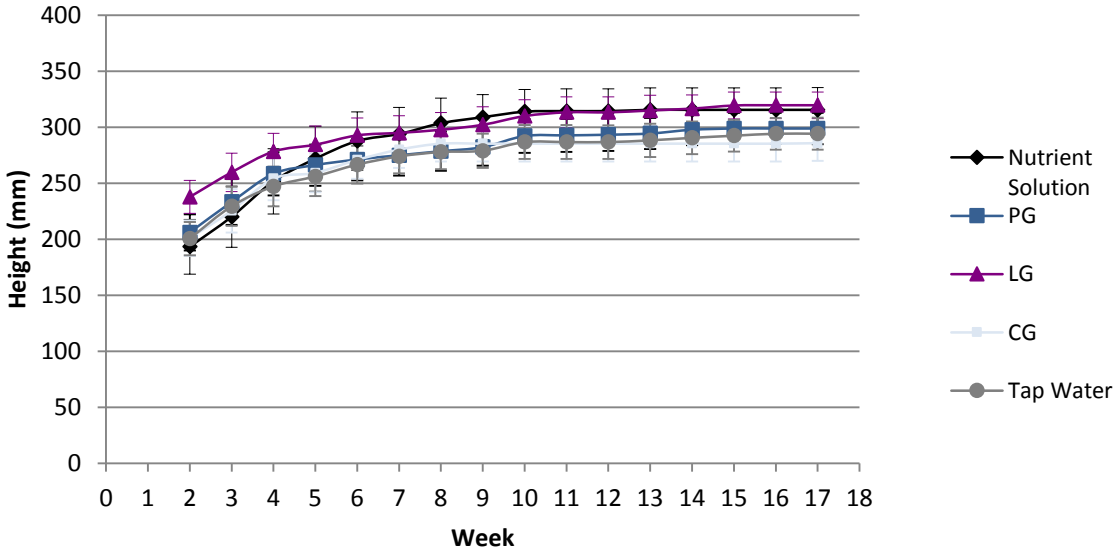

Fig. 20. Weekly mean heights of *C. annuum* plants from the irrigation treatments tested, recorded during growth cycle 2 at the Unilever experiment site (n = 9 plants per treatment except CG; for CG, n = 11). Treatments: "Nutrient solution" = irrigated with nutrient solution; "PG" = irrigated with phosphate base powder-derived greywater; "LG" = irrigated with liquid laundry detergent-derived greywater; "CG" = irrigated with carbonate base powder-derived greywater; "Tap water" = irrigated with municipal tap water supply. Vertical bars represent standard errors about means.

3.3.3. Phenotypic Plasticity: B. vulgaris

Supporting visual impressions of the abovementioned growth trends and crop health changes over time in *B. vulgaris* are depicted in Fig. 21. Individuals from all treatments appeared to be affected negatively by prevailing environmental conditions as evidenced by stunted growth and structural damage to exposed leaf laminas. The manifestation of leaf chlorosis was symptomatic of likely nutrient disorders among greywater and tap water treatments which became more pronounced over time.

Fig. 21. Time snapshots of representative B. vulgaris individuals from each experimental treatment taken during growth cycle 2 (111 days after planting) at the Unilever experiment site. Treatments: "Nutrient solution" = irrigated with nutrient solution; "PG" = irrigated with phosphate base powder-derived greywater; "LG" = irrigated with liquid laundry detergent-derived greywater; "CG" = irrigated with carbonate base powder-derived greywater; "Tap water" = irrigated with municipal tap water supply. Note emergence of leaf chlorosis in greywater and tap water irrigated treatments.

3.3.4. Biomass: B. vulgaris

Statistically significant differences for both leaf and root biomass were found between the nutrient-irrigated *B. vulgaris* and all other treatments (Table 9 and Table 10) where values were found to be at least several times higher than other treatments for either growth cycle. However, no statistically significant differences were established between the tap water-irrigated treatment and those of greywater treatments. Second growth cycle mean leaf and root biomass values trends among treatments were found to be identical to those established among treatments for the first growth cycle.

Table 9. Leaf and root biomass attributes measured among treatments in harvested *B. vulgaris* at the end of growth cycle 1 at the Unilever experiment site (mean ± SD). Letters represent mean separation among treatments by Scheffe's multiple range test ($P< 0.05$; $n = 9$ per treatment).

Attribute	Unit	Nutrient Solution	Phosphate Powder	Liquid Detergent	Carbonate Powder	Tap Water
Dry Leaf Mass	**g**	**78.9 ± 18.4^{a}**	**10.7 ± 2.46^{b}**	**5.93 ± 2.27^{b}**	**2.09 ± 0.92^{b}**	**10.5 ± 2.77^{b}**
Dry Root Mass	**g**	**29.3 ± 19.2^{f}**	**9.31 ± 3.35^{g}**	**4.21 ± 1.10^{g}**	**1.27 ± 0.66^{g}**	**9.49 ± 2.97^{g}**

Table 10. Leaf and root biomass attributes measured among treatments in harvested *B. vulgaris* at the end of growth cycle 2 at the Unilever experiment site (mean ± SD). Letters represent mean separation among treatments by Scheffe's multiple range test ($P< 0.05$; $n = 9$ per treatment).

Attribute	Unit	Nutrient Solution	Phosphate Powder	Liquid Detergent	Carbonate Powder	Tap Water
Dry Leaf Mass	**g**	**21.9 ± 4.76^{d}**	**3.02 ± 1.70^{e}**	**3.13 ± 1.29^{e}**	**3.25 ± 1.38^{e}**	**4.70 ± 1.38^{e}**
Dry Root Mass	**g**	**17.9 ± 3.10^{h}**	**3.28 ± 1.89^{i}**	**2.80 ± 0.88^{i}**	**3.86 ± 2.19^{i}**	**3.92 ± 1.30^{i}**

An unexpected observation of numerous root nodules primarily on *B. vulgaris* irrigated with greywater and tap water treatments, was made (Fig. 22a and Fig. 22b). Subsequent longitudinal sectioning of root nodules examined under a light microscope suggested root nodules were merely growth extensions of the root cortex, consisting mainly of dense parenchyma tissue. Higher nutrient loading in soils appeared to promote fine root development as evidenced by the finer root development in the nutrient-irrigated *B. vulgaris*

whilst low nutrient availability typical of the tap water and greywaters tested appeared to promote coarse root development (Fig. 23).

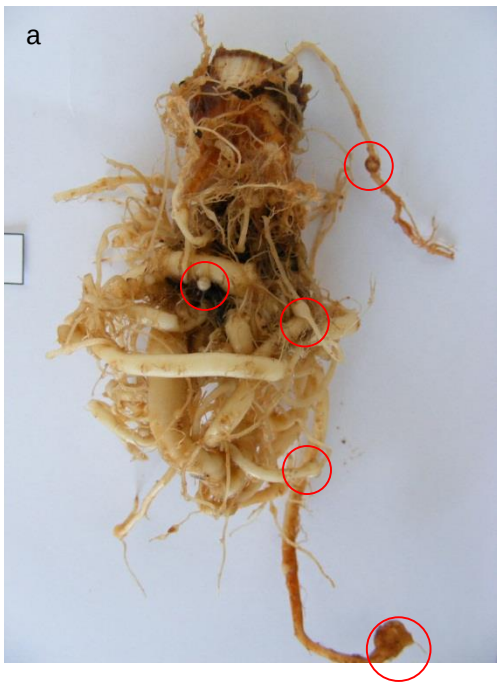

Fig. 22a and Fig. 22b. Typical root nodules (encircled in red) observed among greywater and tap water-irrigated *B. vulgaris*.

Fig. 23. Representative *B. vulgaris* roots from each treatment at the end of growth cycle 1 at the Unilever experiment site. Note differences in fine root and coarse root investments among treatments. Trends in root growth observed in growth cycle 2 were similar and hence are not shown. Figures are not to scale among treatments. Treatments: "Nutrient solution" = irrigated with nutrient solution; "PG" = irrigated with phosphate base powder-derived greywater; "LG" = irrigated with liquid laundry detergent-derived greywater; "CG" = irrigated with carbonate base powder-derived greywater; "Tap water" = irrigated with municipal tap water supply.

The irrigation of *B. vulgaris* with different media resulted in no significant differences among treatments in the resource allocations to leaves or roots for either growth cycle (Fig. 24).

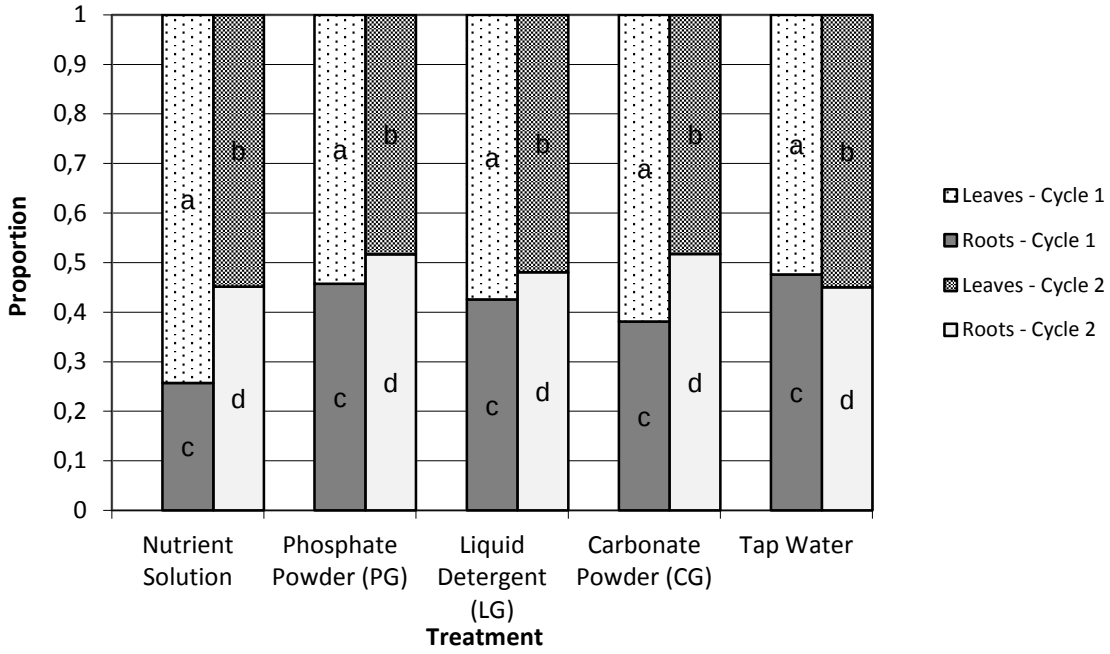

Fig. 24. Mean proportional dry biomass allocation to *B. vulgaris* leaves and roots among the five irrigation treatments for growth cycles 1 and 2 at the Unilever experiment site. Letters represent mean separation among treatments for a given growth cycle by Scheffe's multiple range test ($P < 0.05$; $n = 9$ per treatment).

3.3.5. Phenotypic Plasticity: C. annuum

Fig. 25 provides a visual impression of changes in *C. annuum* individuals among treatments that occurred during the second growth cycle following 111 days after planting. The slow growth response of *C. annuum* noted, together with the manifestation of leaf curl and leaf damage among all treatments, were suggestive of environmental conditions detrimental to plant health and hence overall growth. The emergence of leaf chlorosis as evidenced by increasingly lutescent foliage among greywater and tap water-irrigated *C. annuum* was also suggestive of phytoxicity. As a result of the higher prevalence of leaf necrosis in LG-treated *C. annuum* during experimentation, leaf senescence was also found to be most acute for this treatment towards the end of the experimental growth cycle.

Fig. 25. Time snapshots of representative *C. annuum* individuals from each experimental treatment taken during growth cycle 2 (111 days after planting) at the Unilever experiment site. Treatments: "Nutrient solution" = irrigated with nutrient solution; "PG" = irrigated with phosphate base powder-derived greywater; "LG" = irrigated with liquid laundry detergent-derived greywater; "CG" = irrigated with carbonate base powder-derived greywater; "Tap water" = irrigated with municipal tap water supply. Note emergence of leaf chlorosis in greywater and tap water irrigated treatments.

3.3.6. Biomass: C. annuum

Irrigation with either laundry greywater or tap water resulted in significantly lower leaf investment in *C. annuum* for growth cycle 1 or 2 relative to *C. annuum* irrigated with nutrient solution (Table 11 and Table 12). Dry mass resource allocation to stems and branches for PG- or CG-irrigated *C. annuum* were significantly lower than other treatments in growth cycle 1. However, in growth cycle 2 investment trends among treatments in stems and branches changed such that greywater and tap water-irrigated *C. annuum* were statistically similar to each other, but significantly lower than those irrigated with nutrient solution. Trends among treatments for dry biomass allocations to roots were statistically similar for growth cycle 1 and these trends were maintained in growth cycle 2. Among treatments, overall dry plant

biomass remained lowest among LG- and CG-irrigated treatments, and highest among nutrient-irrigated *C. annuum*.

Table 11. Leaf and root biomass attributes measured among treatments in harvested *B. vulgaris* at the end of growth cycle 1 at the Unilever experiment site (mean ± SD). Letters represent mean separation among treatments by Scheffe's multiple range test ($P < 0.05$; $n = 9$ per treatment).

Attribute	Unit	Nutrient Solution	Phosphate Powder	Liquid Detergent	Carbonate Powder	Tap Water
Dry Leaf Mass	**g**	**11.9 ± 4.89^{a}**	**2.28 ± 1.30^{b}**	**1.52 ± 0.82^{b}**	**0.96 ± 0.44^{b}**	**4.09 ± 2.41^{b}**
Dry Stems + Branches	**g**	**3.01 ± 2.39^{f}**	**2.57 ± 1.42$^{f/g}$**	**1.27 ± 0.62^{h}**	**1.08 ± 0.54^{h}**	**2.90 ± 2.79$^{f/g}$**
Dry Root Mass	**g**	**3.01 ± 2.39^{k}**	**2.57 ± 1.42^{k}**	**1.27 ± 0.62^{k}**	**1.08 ± 0.54^{k}**	**2.9 ± 2.79^{k}**

Table 12. Leaf and root biomass attributes measured among treatments in harvested *B. vulgaris* at the end of growth cycle 2 at the Unilever experiment site (mean ± SD). Letters represent mean separation among treatments by Scheffe's multiple range test ($P < 0.05$; $n = 9$ per treatment).

Attribute	Unit	Nutrient Solution	Phosphate Powder	Liquid Detergent	Carbonate Powder	Tap Water
Dry Leaf Mass	**g**	**11.69 ± 2.78^{d}**	**1.09 ± 0.51^{e}**	**0.83 ± 0.47^{e}**	**1.04 ± 0.61^{e}**	**2.12 ± 0.83^{e}**
Dry Stems + Branches	**g**	**6.89 ± 2.80^{i}**	**1.57 ± 0.53^{j}**	**1.69 ± 0.31^{j}**	**1.08 ± 0.48^{j}**	**1.67 ± 0.60^{j}**
Dry Root Mass	**g**	**1.29 ± 1.03^{l}**	**0.44 ± 0.25^{l}**	**0.41 ± 0.16^{l}**	**0.27 ± 0.09^{l}**	**0.39 ± 0.19^{l}**

Representative images of *C. annuum* roots from each treatment are shown in Fig. 26. Although differing in overall size, roots were found to be fairly similar in appearance among treatments for each growth cycle.

Fig. 26. Representative *C. annuum* roots from each treatment at the end of growth cycle 1 at the Unilever experiment site. Note differences in fine root and coarse root investments among treatments. Trends in root growth observed in growth cycle 2 were similar and hence are not shown. Figures are not to scale among treatments. Treatments: "Nutrient solution" = irrigated with nutrient solution; "PG" = irrigated with phosphate base powder-derived greywater; "LG" = irrigated with liquid laundry detergent-derived greywater; "CG" = irrigated with carbonate base powder-derived greywater; "Tap water" = irrigated with municipal tap water supply.

The influence of irrigation treatment on the proportional allocation to total biomass of the major anatomical components of *C. annuum* among treatments were found to be generally minor (Fig. 27). Only the proportion of leaves contributing to total plant biomass were found to be higher in the nutrient solution-irrigated treatment, marginally lower in the tap water irrigated treatments, and lowest among greywater treatments.

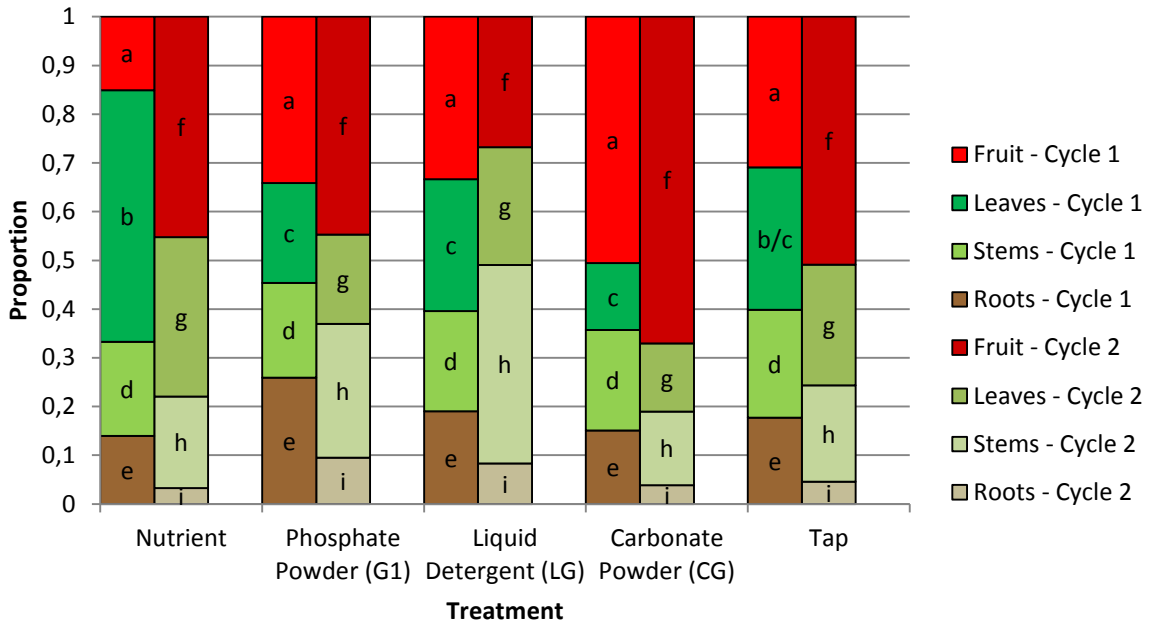

Fig. 27. Mean proportional dry biomass allocation to the major *C. annuum* anatomical components among the five irrigation treatments for growth cycles 1 and 2 at the Unilever experiment site. Letters represent mean separation among treatments for a given growth cycle by Scheffe's multiple range test ($P < 0.05$; $n = 9$ per treatment).

Unilever Site

3.3.7. Leaf Morphology: B. vulgaris

Leaf parameters measured in *B. vulgaris* for each treatment are shown in Table 13 below. Leaf area differences among the three greywater treatments and tap water treatment were found to be statistically non-significant, with values averaging between 9.4×10^{-3} m^2 and 14×10^{-3} m^2. By contrast, irrigation with the nutrient solution, acting as a positive control, yielded mean harvestable *B. vulgaris* leaf areas of approximately one order of magnitude greater. Despite differences in leaf areas, the influence of irrigation treatment on SLW and SLA values were not statistically significant among treatments, suggesting differences in leaf area and leaf mass among treatments ultimately conformed to a relatively uniform leaf area to leaf mass ratio in *B. vulgaris* irrespective of treatment. The effect of irrigation treatment on the dry matter content of leaves (LDMC) was found to be negligible with values ranging between 1.3×10^{-4} mg g^{-1} and 1.5×10^{-4} mg g^{-1} for all treatments. The calculated theoretical average leaf thickness – an assessment which encompassed both leaf and stem thicknesses for *B. vulgaris* – are also shown for treatments in Table 13. By this assessment, although mean differences in leaf thickness among treatments were apparent, with values ranging between 1.55 mm and 2.55 mm, statistically these were not significant.

Table 13. Leaf characteristics measured for individual *B. vulgaris* leaves post-harvest among the five irrigation treatments for growth cycle 2 at the Unilever experiment site (mean ± SD). Letters represent mean separation among treatments by Scheffe's multiple range test ($P < 0.05$; $n = 9$ per treatment).

Parameter	Unit	Nutrient Solution	Phosphate Powder	Liquid Detergent	Carbonate Powder	Tap Water
Area	$\mathbf{m^2}$	0.11 ± 0.03^{a}	0.01 ± 0.01^{b}	0.009 ± 0.004^{b}	0.09 ± 0.004^{b}	0.01 ± 0.005^{b}
SLW	$\mathbf{g_{DM} \cdot m^{-2}}$	211 ± 56^{c}	237 ± 85^{c}	351 ± 126^{c}	368 ± 140^{c}	362 ± 137^{c}
SLA	$\mathbf{m^2 \cdot (mg_{DM})^{-1}}$	5.02 ± 1.25^{d}	4.76 ± 1.84^{d}	3.09 ± 1.1^{d}	3.13 ± 1.25^{d}	3.2 ± 1.4^{d}
LDMC	$\mathbf{10^4 \times mg\ g^{-1})}$	1.35 ± 0.21^{d}	1.41 ± 0.21^{e}	1.53 ± 0.39^{e}	1.52 ± 0.33^{e}	1.4 ± 0.2^{e}
LT	**µm**	1549 ± 203^{f}	1701 ± 584^{f}	2373 ± 603^{f}	2458 ± 961^{f}	2590 ± 957^{f}

3.3.8. Harvestable Yields: B. vulgaris

On a hectare basis, harvestable yields of *B. vulgaris* (stems and leaves) were considerably higher for crops fertilized with nutrient solution (119 t ha^{-1} and 36 t ha^{-1} for the first and second growth cycles respectively) than for crops irrigated with greywater or tap water (< 18 t ha^{-1} in either cycle; Fig. 28). Yields among greywater- and tap water-irrigated *B. vulgaris* were found to be similar for each growth cycle.

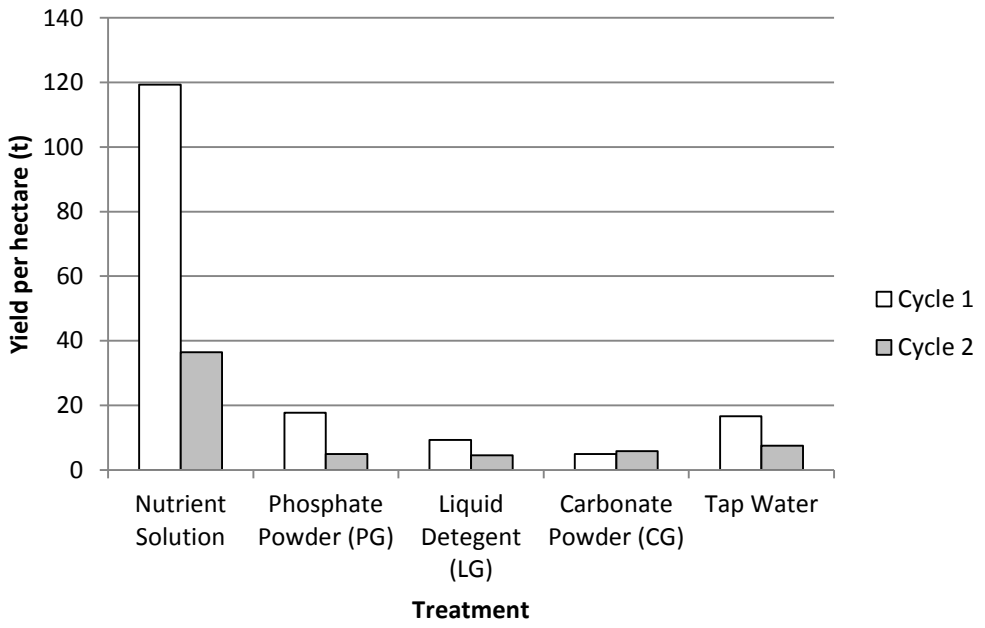

Fig. 28. Theoretical yield per hectare of fresh edible biomass of *B. vulgaris* (leaves and stems) for the five irrigation treatments at the Unilever experiment site for growth cycles 1 and 2 ($n = 9$ per treatment).

3.3.9. Leaf Morphology: C. annuum

Greywater treatment resulted in reduced leaf areas of *C. annuum*, and these values were comparable to those individuals irrigated with tap water, the latter functioning as a negative control (Table 14). By contrast, irrigation with nutrient fertilizer solution yielded leaves with a mean area six-fold greater (6×10^{-2} m^2). The effect of irrigation treatment on the SLW and SLA of *C. annuum* (Table 14) were found to be statistically non-significant.

Despite statistically significant differences among treatments noted earlier for leaf biomass, these did not translate into statistically significant differences for LDMC recorded among treatments (Table 14).

The calculated leaf thickness for *C. annuum* individuals from the five irrigation treatments are shown in Table 14. For *C. annuum*, this estimate is based upon leaf mass and area pertaining to the leaf lamina and petioles. By this estimation, no statistically significant differences were apparent among greywater treatments or controls for leaf thickness estimates, suggesting the influence of irrigation treatment on leaf thickness for *C. annuum* was negligible.

Table 14. Leaf characteristics measured for individual *C. annuum* leaves post-harvest among the five irrigation treatments for growth cycle 2 at the Unilever experiment site (mean ± SD). Letters represent mean separation among treatments by Scheffe's multiple range test ($P< 0.05$; $n = 9$ per treatment).

Parameter	Unit	Nutrient Solution	Phosphate Powder	Liquid Detergent	Carbonate Powder	Tap Water
Area	**m^2**	0.062 ± 0.022^a	0.007 ± 0.004^b	0.003 ± 0.0012^b	0.005 ± 0.002^b	0.01 ± 0.009^b
SLW	**$g_{DM} \cdot m^{-2}$**	199 ± 63^a	174 ± 78^a	252 ± 72^a	258 ± 202^a	299 ± 214^a
SLA	**$m^2 \cdot (mg_{DM})^{-1}$**	5.4 ± 1.4^b	6.6 ± 2.8^b	4.2 ± 1.4^b	6.4 ± 4.1^b	5.0 ± 2.9^b
LDMC	**$10^4 \times mg\ g^{-1}$)**	2.3 ± 0.3^c	2.3 ± 1.0^c	3.8 ± 0.9^c	3.1 ± 1.8^c	2.6 ± 0.9^c
LT	**µm**	864 ± 186^d	758 ± 319^d	672 ± 122^d	1021 ± 1015^d	1154 ± 885^d

3.3.10. Fruit Morphology: C. annuum

Irrigation treatment was found not to have a significant influence on fruit morphology on the basis of the general fruit quality indices accessed (Table 15). Fruit were found to generally be of a small stature among treatments (ca. 55 mm in length with equatorial diameters of

ca. 45 mm). Among treatments, fruit were marginally oblong along the longitudinal axis (FSR > 1).

Table 15. Morphological characteristics of ripe *C. annuum* from the five irrigation treatments at harvest during growth cycle 2 at the Unilever experiment site (mean ± SD). Letters represent mean separation among treatments by Scheffe's multiple range test ($P< 0.05$).

Parameter	Unit	Nutrient Solution	Phosphate Powder	Liquid Detergent	Carbonate Powder	Tap Water
Polar Axis Length	mm	59.4 ± 13.1[a]	49.7 ± 14.6[a]	50.5 ± 18.7[a]	55.6 ± 10.3[a]	56.8 ± 7.5[a]
Equatorial Ø	mm	57.8 ± 6.8[b]	41.4 ± 11.4[b]	39.8 ± 11.6[b]	45.3 ± 10.7[b]	52.3 ± 15.4[b]
Polar Axis : Equatorial Ø (FSR)	-	1.0 ± 0.3[c]	1.2 ± 0.1[c]	1.3 ± 0.2[c]	1.3 ± 0.2[c]	1.1 ± 0.3[c]
Pericarp Thickness	mm	3.5 ± 0.7[d]	3.0 ± 0.9[d]	2.7 ± 0.7[d]	3.3 ± 0.8[d]	3.1 ± 0.8[d]

3.3.11. Harvestable Yields: C. annuum

Fruit production appeared to be influenced considerably by irrigation treatment for both cycles of growth (Fig. 29). Nutrient-irrigated individuals yielded more harvestable fruit by count than any other irrigation treatment, whilst PG-, CG- and tap water-irrigated treatments yielded similar fruit quantities. For either growth cycle, irrigation with LG-generated greywater resulted in the lowest total fruit number yielded among treatments. In the first growth cycle, irrigation treatment had no influence on the total dry biomass yield of fruit among treatments. In the second cycle however, some differences were apparent, with the nutrient solution having the highest dry fruit biomass yields (Fig. 30).

On a yield per hectare basis, contrasting fresh fruit yields were evident among treatments (Fig. 31); the nutrient treatment was highest among treatments in the second growth cycle, with fruit yields of ca. 40 t ha^{-1}. This was in contrast to the first cycle where the nutrient solution irrigated plants returned the lowest yields (ca. 6 t ha^{-1}) among treatments in the first growth period. Yield trends among treatments were maintained across both growth cycles - greywater-treated *C. annuum* yielded less total fruit biomass than tap water-irrigated plants for both growth cycles.

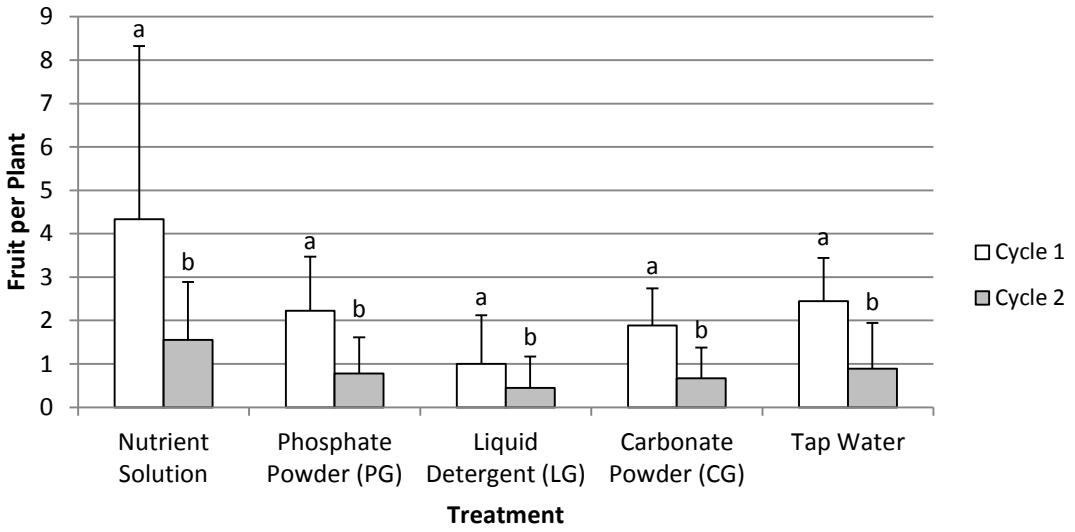

Fig. 29. Mean number of *C. annuum* fruit per plant from the five irrigation treatments following 135 days of irrigation (Cycle 1: CG = 97 days) for each growth cycle at the Unilever experiment site. Vertical bars represent standard deviations. Letters represent mean separation among treatments for a given growth cycle by Scheffe's multiple range test ($P < 0.05$; $n = 9$ per treatment).

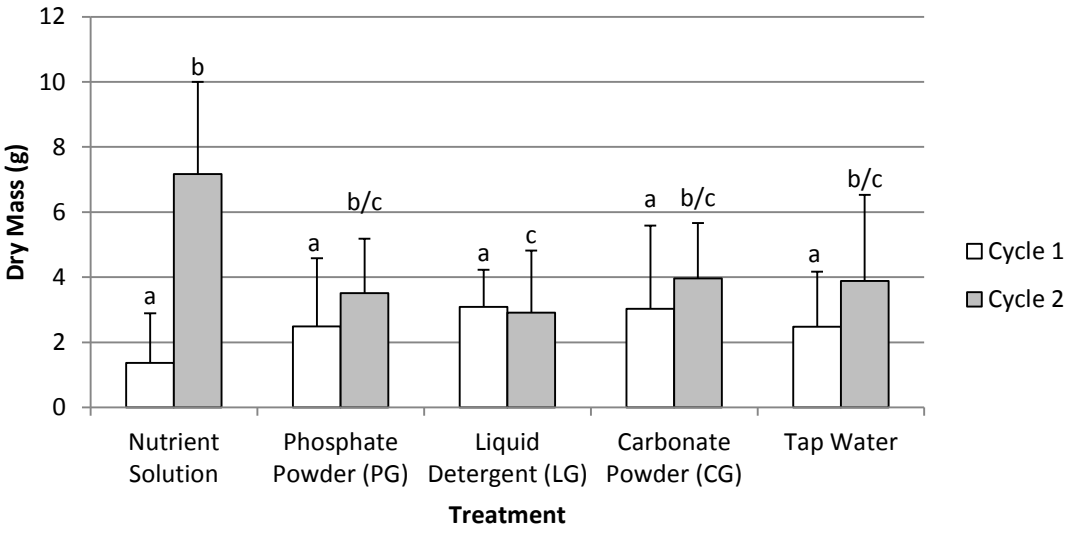

Fig. 30. Mean dry mass of individual *C. annuum* fruit per plant harvested during growth cycles 1 and 2 from the five irrigation treatments following 135 days of irrigation (Cycle 1: CG = 97 days) for each growth cycle at the Unilever experiment site. Vertical bars represent standard deviations. Letters represent mean separation among treatments for a given growth cycle by Scheffe's multiple range test ($P < 0.05$; $n = 9$ per treatment).

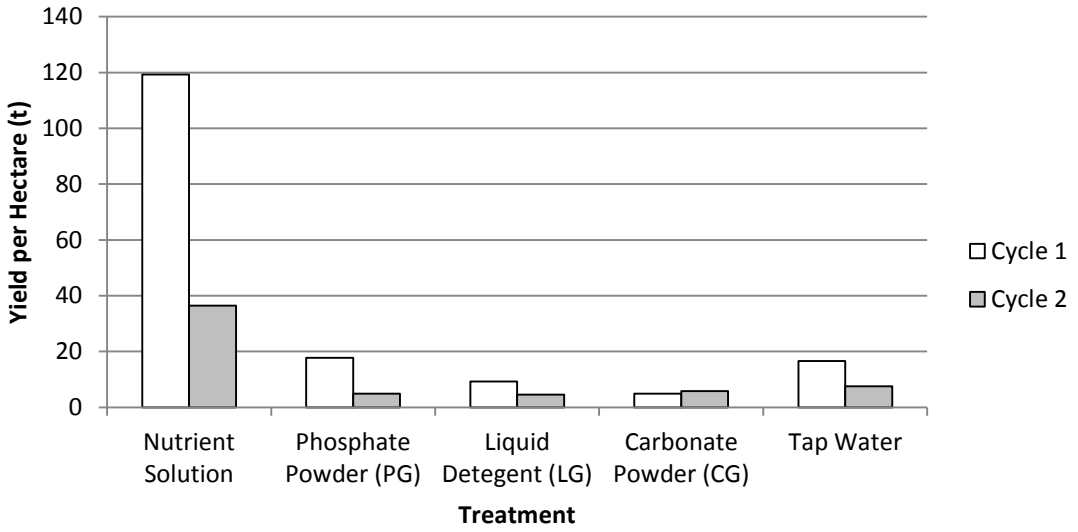

Fig. 31. Theoretical yield per hectare of fresh fruit in *C. annuum* for the five irrigation treatments at the Unilever experiment site for growth cycles 1 and 2.

3.3.12. Leaf Tissue Nutrients: B. vulgaris

Irrigation treatment appeared to exert some influence on macronutrient uptake in leaf and stem tissue of *B. vulgaris* grown during cycles 1 and 2 (Table 16 and Table 17); elevated levels of both N and K were found in nutrient-irrigated treatments, levels which were found to be significantly higher than that of either greywater or tap water treatments. The uptake of P by leaf and stem tissue however was found to be significantly higher in *B. vulgaris* irrigated with the phosphate-base laundry powder product PG, whilst uptake of P remained similar across the remaining treatments for both growth cycles. It appeared that P availability was sufficiently low in certain individuals from the greywater and tap water treatments to induce the possible deficiency symptoms observed in these leaves (Figs. 32a and 32b). The long-term addition of greywater did not result in increased assimilation of either N or P in leaf tissues. Nutrient-irrigated *B. vulgaris* had the highest uptake of K relative to other treatments for both growth cycles. No significant differences in K uptake were apparent between greywater and tap water-irrigated *B. vulgaris* for either growth cycle.

The uptake of Ca among treatments was found to be highly variable (Table 16 and Table 17); although plants irrigated with liquid detergent-generated greywater (LG) for both cycles were shown to have a statistically similar uptake of Ca to that of tap water-irrigated *B. vulgaris*, uptake of Ca among the remaining treatments, being statistically similar to each other, were all found to be significantly less by comparison.

Greywater treatment was not found to influence Mg uptake in leaf and stems any more significantly than that of tap water for either growth cycle (Table 16 and Table 17). However, the irrigation of *B. vulgaris* with nutrient solution resulted in a significantly greater uptake of Mg in leaf and stem tissues relative to all other treatments although this was only the case for growth cycle 1.

Of all the elements investigated in the leaf and stem tissue of *B. vulgaris*, Na was found to constitute the highest concentration among treatments for both growth cycles. Contrasting levels of Na prevalent in leaf and stem tissue of *B. vulgaris* was noted, with both PG-greywater and CG greywater reflecting a significantly higher concentration of Na relative to either the liquid detergent-derived greywater (LG), nutrient- or tap water-irrigated treatments for both growth cycles (Table 16 and Table 17). No statistically significant differences between tissue concentrations of Na were noted among nutrient solution, laundry greywater product LG, or tap water-irrigated *B. vulgaris* for either growth cycle.
The concentrations of Zn in leaves and stems of *B. vulgaris* were found to be statistically similar among all treatments for growth cycle 1, whilst in cycle 2 Zn concentrations were found to be significantly higher for LG among all treatments investigated (Table 16 and Table 17). Significant differences among some treatments were established for Cu concentrations in leaf and stem tissues (Table 16 and Table 17); interestingly, in cycle 1 the Cu uptake in these tissues was found to be significantly higher for both phosphate-powder derived greywater (PG) and tap-water irrigated *B. vulgaris*, whilst uptake was supressed in other treatments, most notably for the nutrient-irrigated treatment; in cycle 2 however no significant differences in Cu concentrations were found among treatments.

The uptake of Mn was found to be statistically similar among treatments (Table 16 and Table 17), with the notable exception of phosphate-base laundry powder derived greywater (PG) and liquid detergent (LG) irrigated-treatments in cycles 1 and 2 respectively.

The uptake of Fe and Al within treatments in leaf and stem tissues were found to be remarkably similar in general for both growth cycles (Table 16 and Table 17). The notable exception was for growth cycle 1 where both greywater and tap water-irrigated treatments reflected significantly higher uptake of Fe and Al in tissues than nutrient-irrigated individuals.

With the exception of the LG greywater, which had the highest significant B uptake among treatments, the influence of irrigation treatment on B uptake in leaf and stem tissues of *B. vulgaris* was found to be uniform among the remaining treatments in growth cycle 1 (Table 16 and Table 17). In growth cycle 2, there was no significant difference in B uptake among treatments.

Table 16. Nutrient concentrations established for *B. vulgaris* leaves post-harvest among the five irrigation treatments at the Unilever experiment site at the end of growth cycle 1 (mean ± SD). Letters represent mean separation among treatments by Scheffe's multiple range test ($P< 0.05$; $n = 5$ per treatment).

Element	Unit	Nutrient Solution	Phosphate Powder	Liquid Detergent	Carbonate Powder	Tap Water
N	mg kg^{-1}	**55.1 ± 2.31^{a}**	**17.3 ± 2.05^{b}**	**14.3 ± 1.21^{b}**	**17.8 ± 0.53^{b}**	**17.5 ± 2.29^{b}**
P	mg kg^{-1}	**5.20 ± 1.02^{d}**	**14.6 ± 2.74^{c}**	**4.75 ± 0.80$^{d/e}$**	**6.11 ± 1.96^{e}**	**4.20 ± 0.82$^{d/e}$**
K	mg kg^{-1}	**100 ± 10.3^{f}**	**39.6 ± 10.0^{g}**	**42.5 ± 4.59^{g}**	**30.1 ± 8.25^{g}**	**41.7 ± 7.37^{g}**
Ca	mg kg^{-1}	**6.47 ± 1.16^{h}**	**8.39 ± 2.74^{h}**	**13.5 ± 0.99^{i}**	**8.70 ± 1.09^{h}**	**12.9 ± 1.49^{i}**
Mg	mg kg^{-1}	**11.1 ± 2.72^{j}**	**4.14 ± 1.15^{k}**	**3.56 ± 0.64^{k}**	**2.97 ± 1.39^{k}**	**4.51 ± 1.02^{k}**
Na	mg kg^{-1} × 10^{3}	**8.64 ± 1.45^{l}**	**27.8 ± 5.36^{m}**	**16.0 ± 2.88^{l}**	**31.9 ± 4.67^{m}**	**16.6 ± 4.07^{l}**
Zn	mg kg^{-1}	26.3 ± 6.90^{n}	26.9 ± 5.32^{n}	29.9 ± 8.54^{n}	23.0 ± 4.76^{n}	27.2 ± 5.44^{n}
Cu	mg kg^{-1}	**2.22 ± 0.69^{o}**	**9.94 ± 1.96^{q}**	**7.51 ± 1.53$^{p/q}$**	**4.54 ± 2.28$^{o/p}$**	**9.98 ± 1.08^{q}**
Mn	mg kg^{-1}	**124 ± 31.0^{r}**	**261 ± 74.7^{s}**	**206 ± 68.9$^{r/s}$**	**136 ± 24.5^{r}**	**214 ± 66.1$^{r/s}$**
Fe	mg kg^{-1}	**437 ± 52.0^{t}**	**1155 ± 303^{u}**	**1180 ± 503^{u}**	**1126 ± 166^{u}**	**1026 ± 187$^{t/u}$**
Al	mg kg^{-1}	**556 ± 53.9^{v}**	**1186 ± 287^{w}**	**1187 ± 518^{w}**	**1123 ± 171^{w}**	**993 ± 184$^{v/w}$**
B	mg kg^{-1}	**46.8 ± 1.10^{x}**	**44.0 ± 3.16^{x}**	**81.6 ± 19.2^{y}**	**44.4 ± 7.54^{y}**	**39.2 ± 6.10^{y}**

Table 17. Nutrient concentrations established for *B. vulgaris* leaves post-harvest among the five irrigation treatments at the Unilever experiment site at the end of growth cycle 2 (mean ± SD). Letters represent mean separation among treatments by Scheffe's multiple range test ($P < 0.05$; $n = 5$ per treatment).

Element	Unit	Nutrient Solution	Phosphate Powder	Liquid Detergent	Carbonate Powder	Tap Water
N	**mg kg^{-1}**	**31.4 ± 5.44^{a}**	**16.0 ± 3.60^{b}**	**16.0 ± 1.64^{b}**	**20.4 ± 7.44^{b}**	**18.7 ± 4.03^{b}**
P	**mg kg^{-1}**	**8.63 ± 2.19$^{c/d}$**	**13.9 ± 4.36^{d}**	**7.27 ± 1.79^{c}**	**7.07 ± 2.36^{c}**	**4.07 ± 1.04^{c}**
K	**mg kg^{-1}**	**71.3 ± 7.40^{f}**	**24.7 ± 6.39^{g}**	**29.9 ± 6.04^{g}**	**27.5 ± 8.84^{g}**	**36.3 ± 5.85^{g}**
Ca	**mg kg^{-1}**	18.9 ± 3.05^{g}	11.2 ± 1.44^{g}	25.5 ± 3.88^{g}	8.69 ± 1.89^{g}	24.1 ± 3.17^{g}
Mg	**mg kg^{-1}**	8.90 ± 1.30^{h}	4.61 ± 1.08^{h}	8.49 ± 2.68^{h}	5.92 ± 2.56^{h}	7.88 ± 1.10^{h}
Na	**mg kg^{-1} × 10^{3}**	**10.8 ± 1.99^{i}**	**34.8 ± 3.88^{j}**	**21.7 ± 2.09^{i}**	**36.9 ± 12.4^{j}**	**14.8 ± 2.49$^{i/j}$**
Zn	**mg kg^{-1}**	**43.3 ± 3.21^{k}**	**25.5 ± 5.96^{m}**	**68.1 ± 19.2^{k}**	**32.6 ± 17.8$^{k/l}$**	**33.3 ± 6.75$^{l/m}$**
Cu	**mg kg^{-1}**	**18.9 ± 3.05^{n}**	**11.2 ± 1.44$^{n/o}$**	**25.5 ± 3.88^{o}**	**8.69 ± 1.90$^{n/o}$**	**24.1 ± 3.17^{o}**
Mn	**mg kg^{-1}**	**191 ± 51.9^{p}**	**167 ± 34.7^{p}**	**417 ± 110^{q}**	**169 ± 30.2^{p}**	**224 ± 22.7^{p}**
Fe	**mg kg^{-1}**	**839 ± 396^{r}**	**435 ± 99.8^{r}**	**548 ± 221^{s}**	**535 ± 151^{r}**	**749 ± 267^{r}**
Al	**mg kg^{-1}**	**838 ± 344^{t}**	**463 ± 111^{t}**	**561 ± 231^{u}**	**625 ± 184^{t}**	**733 ± 272^{t}**
B	**mg kg^{-1}**	**58.4 ± 6.39^{v}**	**63.2 ± 22.9$^{v/w}$**	**76.0 ± 8.12^{w}**	**56.8 ± 8.32^{v}**	**54.4 ± 11.4$^{v/w}$**

Fig. 32a and Fig. 32b. Possible P deficiency symptoms as evidenced by interveinal purpurescent patches and purpurescent leaf margins observed among some *B. vulgaris* individuals from greywater and tap water treatments.

3.3.13. Leaf Tissue Nutrients: C. annuum

As shown in Table 18 and Table 19, the uptake of macronutrients by *C. annuum* leaves reflected a varied response among treatments. The concentration of N in leaves of individuals irrigated with nutrient solution (63 mg kg^{-1}) was significantly higher than all other treatments in cycle 1. Uptake of N in cycle 1 among LG and PG greywater treatments (38 mg kg^{-1} to 42 mg kg^{-1}) and tap water (40 mg kg^{-1}) were found to be statistically similar, whilst N uptake in the CG treatment was found to be significantly lower than for any other treatment. However, this trend was not continued in cycle 2 since all treatments were uniform. In cycle 1, the concentration of P leaves for nutrient-irrigated *C. annuum* of 5 mg kg^{-1} was found to be significantly lower than greywater irrigated individuals with the exception of CG-irrigated *C. annuum* which was found to be statistically similar. Uptake in PG and LG treatments (15 mg kg^{-1}) was significantly higher than either the nutrient or CG treatments, and marginally higher than for tap water-irrigated *C. annuum*. In cycle 2, these trends in P uptake were generally continued.

The uptake of K in leaves by tap water-irrigated *C. annuum* (57 mg kg^{-1}) was found to be marginally higher than the PG and LG treatments in cycle 1, but lower than for the nutrient treatment, which reflected the highest significant uptake of K among treatments (114 mg kg^{-1}). The trend in cycle 2 among treatments were found to be similar with the notable exception that CG-irrigated *C. annuum* was statistically similar to those irrigated with nutrient solution. Similarly, for the other macronutrients mentioned above, the uptake of K by the CG-irrigated *C. annuum* was found to be significantly the lowest among treatments (22 mg kg^{-1}).

The irrigation of *C. annuum* with greywater and tap water resulted in a similar response to the uptake by leaves of Ca (Table 18 and Table 19). Values recorded were highest for LG- and tap water-irrigated *C. annuum* (36 mg kg^{-1} to 40 mg kg^{-1}) whilst marginally lower for either the PG and CG treatments. Nutrient-irrigated *C. annuum* had the lowest significant uptake of Ca among treatments. Leaf tissue concentrations of Mg were found to be similar among treatments (Table 18 and Table 19).

Table 18. Nutrient concentrations established for *C. annuum* leaves post-harvest among the five irrigation treatments at the Unilever experiment site at the end of growth cycle 1 (mean ± SD). Letters represent mean separation among treatments by Scheffe's multiple range test ($P< 0.05$; $n = 5$ per treatment).

Element	Unit	Nutrient Solution	Phosphate Powder	Liquid Detergent	Carbonate Powder	Tap Water
N	mg kg^{-1}	**63.5 ± 3.17^{a}**	**38.2 ± 1.98^{b}**	**42.3 ± 2.39^{b}**	**22.7 ± 4.69^{c}**	**40.2 ± 5.24^{b}**
P	mg kg^{-1}	**5.20 ± 0.56^{d}**	**15.4 ± 5.54^{e}**	**14.5 ± 3.60^{e}**	**4.70 ± 2.02^{d}**	**10.2 ± 2.41$^{d/e}$**
K	mg kg^{-1}	**114 ± 8.25^{f}**	**54.8 ± 13.4$^{g/h}$**	**54.6 ± 10.9$^{g/h}$**	**22.0 ± 11.7^{h}**	**56.6 ± 26.3^{g}**
Ca	mg kg^{-1}	**20.7 ± 2.52^{i}**	**35.9 ± 8.07$^{i/j}$**	**41.3 ± 4.52^{j}**	**27.1 ± 14.6$^{i/j}$**	**43.4 ± 6.46^{j}**
Mg	mg kg^{-1}	**12.9 ± 1.67^{k}**	**11.8 ± 2.67$^{k/l}$**	**11.8 ± 1.04$^{k/l}$**	**7.12 ± 3.34^{l}**	**13.9 ± 2.04^{k}**
Na	mg kg^{-1} × 10^{3}	**5.96 ± 1.09^{m}**	**14.3 ± 1.49^{n}**	**12.3 ± 2.68$^{m/n}$**	**11.5 ± 5.81$^{m/n}$**	**12.4 ± 1.99$^{m/n}$**
Zn	mg kg^{-1}	**78.7 ± 5.07^{o}**	**236 ± 48.3^{q}**	**281 ± 38.5^{q}**	**130 ± 65.7$^{o/p}$**	**198 ± 26.6$^{p/q}$**
Cu	mg kg^{-1}	**4.89 ± 1.07^{r}**	**24.0 ± 4.56^{s}**	**43.3 ± 8.62^{t}**	**11.8 ± 3.74^{r}**	**24.9 ± 3.56^{s}**
Mn	mg kg^{-1}	**171 ± 38.7$^{u/v}$**	**215 ± 18.5^{u}**	**231 ± 23.7^{u}**	**115 ± 60.7^{v}**	**180 ± 10.3$^{u/v}$**
Fe	mg kg^{-1}	**1251 ± 280^{w}**	**2193 ± 408$^{w/x}$**	**2729 ± 574^{x}**	**1760 ± 1114$^{w/x}$**	**2653 ± 664$^{w/x}$**
Al	mg kg^{-1}	**1339 ± 217^{y}**	**2278 ± 403$^{y/z}$**	**2817 ± 580^{z}**	**1452 ± 898^{y}**	**2684 ± 521^{z}**
B	mg kg^{-1}	**97.2 ± 5.93^{α}**	**102 ± 14.7^{α}**	**184 ± 62.6^{β}**	**90.5 ± 52.8^{α}**	**94.8 ± 13.3^{α}**

Leaf uptake of Na was found to be very high compared to all other elements investigated. Statistically, Na leaf concentrations were similar across the LG, CG and tap water treatments with values of between 11×10^{3} mg kg^{-1} and 13×10^{3} mg kg^{-1} recorded, but were marginally lower than for the PG treatment, which had the highest leaf uptake of Na among the experimental treatments (> 14×10^{3} mg kg^{-1}; Table 18 and Table 19). Relative to all treatments, mean Na uptake by leaves was found to be significantly lowest for *C. annuum* irrigated with nutrient solution.

In Table 18 and Table 19, the uptake of both Zn and Cu by leaves in *C. annuum* in response to treatment was found to be highly variable. Among treatments, Zn uptake was found to be significantly higher in PG- and LG-irrigated *C. annuum* in cycle 1 (236 mg kg^{-1} to

281 mg kg^{-1}), which were marginally higher than for the tap water. Among greywater and tap water treatments, CG-irrigated *C. annuum* had the lowest significant mean uptake of Zn (130 mg kg^{-1}), but was significantly higher than the nutrient treatment, which had the lowest mean uptake of Zn among treatments (64 mg kg^{-1}). Similarly, among treatments Cu uptake was found to be lowest for the nutrient solution- and CG-irrigated *C. annuum* and highest for the LG treatment (43 mg kg^{-1}). Irrigation of *C. annuum* with PG and LG greywater resulted in significantly greater levels of uptake of Mn by leaves relative to either the nutrient, tap-water or CG treatments, with CG reflecting the lowest significant level among treatments.

Fe and Al uptake by leaves within treatments were found to be similar (Table 18 and Table 19). LG- irrigated *C. annuum* reflected the highest significant uptake of Fe (2.7×10^3 mg kg^{-1}) and Al (2.8×10^3 mg kg^{-1}), marginally higher than either the PG- and tap water-irrigated *C. annuum* and also significantly higher than the nutrient and CG treatments for both Fe and Al uptake.

Boron tissue uptake by *C. annuum* leaves over growth cycles 1 and 2 is shown for all treatments in Table 18 and Table 19 respectively. In cycle 1, uptake in individuals irrigated with the liquid detergent (LG) generated greywater was found to be significantly higher than all other treatments attaining leaf tissue concentrations of 184 mg kg^{-1}.

Table 19. Nutrient concentrations established for *C. annuum* leaves post-harvest among the five irrigation treatments at the Unilever experiment site at the end of growth cycle 2 (mean ± SD). Letters represent mean separation among treatments by Scheffe's multiple range test ($P< 0.05$; $n = 5$ per treatment).

Element	Unit	Nutrient Solution	Phosphate Powder	Liquid Detergent	Carbonate Powder	Tap Water
N	mg kg^{-1}	37.3 ± 4.88^{a}	37.2 ± 3.20^{a}	30.2 ± 0.47^{a}	33.8 ± 6.10^{a}	33.6 ± 3.51^{a}
P	mg kg^{-1}	**5.05 ± 0.75^{b}**	**10.9 ± 0.81^{d}**	**5.97 ± 1.87$^{b/c}$**	**9.46 ± 0.64$^{c/d}$**	**9.04 ± 1.70$^{c/d}$**
K	mg kg^{-1}	**44.8 ± 7.43^{e}**	**19.8 ± 0.89^{f}**	**13.0 ± 7.13^{f}**	**36.9 ± 2.45^{e}**	**21.3 ± 3.84^{f}**
Ca	mg kg^{-1}	36.8 ± 5.37^{g}	33.0 ± 5.15^{g}	43.3 ± 7.07^{g}	31.3 ± 2.97^{g}	36.9 ± 3.78^{g}
Mg	mg kg^{-1}	9.97 ± 1.41^{h}	13.0 ± 1.97^{h}	10.4 ± 2.11^{h}	10.5 ± 0.97^{h}	10.3 ± 0.93^{h}
Na	mg kg^{-1} × 10^{3}	**6.16 ± 1.24^{i}**	**10.2 ± 0.98^{j}**	**6.00 ± 1.95^{i}**	**11.1 ± 1.27^{j}**	**7.95 ± 0.56$^{i/j}$**
Zn	mg kg^{-1}	72.5 ± 14.4^{k}	136 ± 10.7^{m}	84.6 ± 12.2^{k}	99.6 ± 14.4$^{k/l}$	124 ± 10.4$^{l/k}$
Cu	mg kg^{-1}	8.19 ± 3.60^{m}	20.3 ± 4.46$^{m/n}$	27.7 ± 1.43^{n}	18.0 ± 3.56$^{m/n}$	25.3 ± 9.25^{n}
Mn	mg kg^{-1}	86.0 ± 5.93^{o}	106 ± 36.6^{o}	166 ± 11.2^{p}	119 ± 8.98$^{o/p}$	118 ± 20.2$^{o/p}$
Fe	mg kg^{-1}	**850 ± 178^{q}**	**976 ± 162$^{q/r}$**	**2497 ± 208^{r}**	**989 ± 463^{q}**	**907 ± 287^{r}**
Al	mg kg^{-1}	**847 ± 180^{s}**	**962 ± 113^{s}**	**2185 ± 198^{t}**	**1030 ± 535^{s}**	**896 ± 314^{s}**
B	mg kg^{-1}	**80.7 ± 3.06^{u}**	**129 ± 24.1$^{u/v}$**	**191 ± 50.8^{v}**	**103 ± 44.1^{u}**	**149 ± 14.0$^{u/v}$**

3.3.14. Fruit Tissue Nutrients: C. annuum

Mean macronutrient concentrations established for ripe fruit tissue of *C. annuum* at the end of each growth cycle are shown in Table 20 and Table 21. Among treatments, statistically identical trends were found among treatments for both N and K uptake in fruits; nutrient-irrigated *C. annuum* had a significantly higher uptake of both N and K in fruit tissue during growth cycle 1 (45 mg kg^{-1} and 50 mg kg^{-1} respectively), whilst no statistically significant differences in fruit tissue uptake were noted for either of these elements among the greywater and tap water treatments. P uptake in fruit tissue among treatments reflected no statistically significant differences, with concentrations ranging between 5.5 mg kg^{-1} and 7.6 mg kg^{-1}.

No significant differences were noted among treatments for Ca, Mg and Na uptake in fruit tissue (Table 20 and Table 21). Similarly, as shown in Table 20 and Table 21, irrigation treatment was found to exert no statistically significant influence on Zn and Cu uptake in *C. annuum* fruit.

Relative to greywater, uptake of Mn in fruit tissue of tap water-irrigated *C. annuum* was found to be statistically similar (14 mg kg^{-1} to 19 mg kg^{-1}), although among treatments the nutrient-irrigated *C. annuum* had the highest uptake (42 mg kg^{-1}; Table 20 and Table 21). A similar trend was found for the uptake of Fe (Table 20 and Table 21), where greywater and tap water treatments had statistically similar concentrations (118 mg kg^{-1} to 160 mg kg^{-1}), but significantly less than for the nutrient treatment which had a fruit tissue concentration of 361 mg kg^{-1}. However, no statistically significant differences were found among treatments for Al uptake in fruit (Table 20 and Table 21). In contrast to the leaf uptake of Al and Fe, in fruit the tissue concentrations of Al and Fe differed markedly.

Statistical analysis of B concentrations in fruit harvested during cycle 1 found no significant differences among treatments (Table 20 and Table 21). However the long-term irrigation of *C. annuum* over two growth cycles resulted in an additive effect for the LG treatment with LG fruit tissue having a significantly higher concentration of B (31 mg kg^{-1}) than all the other experimental treatments for which values ranged from 12 mg kg^{-1} to 14 mg kg^{-1}. The latter were all found to be statistically similar. Inter-cycle comparisons for each treatment found a statistically significant decline in boron allocation to fruit for the nutrient treatment, whilst the remaining greywater and tap water treatments remained statistically similar.

Table 20. Nutrient concentrations established for *C. annuum* fruit post-harvest among the five irrigation treatments at the Unilever experiment site at the end of growth cycle 1 (mean ± SD). Letters represent mean separation among treatments by Scheffe's multiple range test ($P < 0.05$; $n = 5$ per treatment).

Element	Unit	Nutrient Solution	Phosphate Powder	Liquid Detergent	Carbonate Powder	Tap Water
N	mg kg^{-1}	**45.0 ± 10.2^{a}**	**24.9 ± 6.98^{b}**	**19.5 ± 1.72^{b}**	**19.5 ± 6.42^{b}**	**21.0 ± 0.99^{b}**
P	mg kg^{-1}	7.56 ± 1.18^{c}	7.25 ± 1.27^{c}	5.75 ± 1.03^{c}	6.33 ± 1.66^{c}	6.08 ± 0.69^{c}
K	mg kg^{-1}	**49.7 ± 9.44^{d}**	**33.1 ± 5.92^{e}**	**29.7 ± 1.40^{e}**	**30.9 ± 6.20^{e}**	**28.6 ± 3.19^{e}**
Ca	mg kg^{-1}	3.25 ± 1.62^{f}	1.79 ± 0.31^{f}	1.99 ± 0.75^{f}	4.00 ± 4.55^{f}	2.42 ± 0.41^{f}
Mg	mg kg^{-1}	2.69 ± 0.82^{g}	2.08 ± 0.61^{g}	1.69 ± 0.20^{g}	2.45 ± 1.46^{g}	1.55 ± 0.18^{g}
Na	mg kg^{-1} × 10^{3}	3.11 ± 1.47^{h}	2.80 ± 0.83^{h}	1.83 ± 0.30^{h}	2.67 ± 1.65^{h}	1.95 ± 0.29^{h}
Zn	mg kg^{-1}	**26.4 ± 6.41^{i}**	**22.2 ± 5.39^{k}**	**18.3 ± 3.96^{k}**	**13.3 ± 9.97$^{i/j}$**	**29.0 ± 8.79$^{j/k}$**
Cu	mg kg^{-1}	**5.46 ± 4.28^{l}**	**11.8 ± 3.71^{m}**	**8.81 ± 3.48^{n}**	**7.74 ± 4.50^{l}**	**9.67 ± 1.36^{m}**
Mn	mg kg^{-1}	**42.0 ± 15.1$^{o/p}$**	**18.9 ± 5.18^{o}**	**14.6 ± 3.24^{o}**	**22.2 ± 17.4^{p}**	**14.2 ± 1.84$^{o/p}$**
Fe	mg kg^{-1}	**361 ± 176^{q}**	**137 ± 29.0^{r}**	**118 ± 20.7^{r}**	**147 ± 65.6^{r}**	**160 ± 20.0^{r}**
Al	mg kg^{-1}	224 ± 185^{s}	77 ± 25^{s}	69 ± 14^{s}	126 ± 139^{s}	119 ± 30^{s}
B	mg kg^{-1}	24.0 ± 8.64^{t}	16.5 ± 3.79^{t}	22.4 ± 4.56^{t}	18.7 ± 5.03^{t}	14.4 ± 1.67^{t}

Table 21. Nutrient concentrations established for *C. annuum* fruit post-harvest among the five irrigation treatments at the Unilever experiment site at the end of growth cycle 2 (mean ± SD). Letters represent mean separation among treatments by Scheffe's multiple range test ($P< 0.05$; $n = 5$ per treatment).

Element	Unit	Nutrient Solution	Phosphate Powder	Liquid Detergent	Carbonate Powder	Tap Water
N	mg kg^{-1}	**23.5 ± 5.19^{a}**	**17.7 ± 0.59$^{a/b}$**	**19.2 ± 0.88$^{a/b}$**	**16.5 ± 1.71^{b}**	**18.7 ± 1.55$^{a/b}$**
P	mg kg^{-1}	4.20 ± 0.55^{c}	4.67 ± 0.18^{c}	4.09 ± 0.72^{c}	3.83 ± 0.95^{c}	5.00 ± 0.39^{c}
K	mg kg^{-1}	30.4 ± 5.07^{d}	24.3 ± 1.41^{d}	30.8 ± 4.59^{d}	27.0 ± 4.70^{d}	25.7 ± 3.13^{d}
Ca	mg kg^{-1}	1.49 ± 0.40^{e}	1.55 ± 0.30^{e}	1.77 ± 0.35^{e}	1.58 ± 0.62^{e}	2.38 ± 0.61^{e}
Mg	mg kg^{-1}	1.53 ± 0.22^{f}	1.65 ± 0.15^{f}	1.83 ± 0.20^{f}	1.62 ± 0.43^{f}	1.77 ± 0.12^{f}
Na	mg kg^{-1} × 10^{3}	**1.19 ± 0.34^{g}**	**1.55 ± 0.22^{h}**	**1.32 ± 0.15^{g}**	**2.57 ± 1.46^{h}**	**1.31 ± 0.42$^{g/h}$**
Zn	mg kg^{-1}	**11.4 ± 1.73^{i}**	**12.4 ± 1.02^{k}**	**13.5 ± 3.59^{i}**	**11.8 ± 4.24$^{i/j}$**	**11.6 ± 3.12$^{j/k}$**
Cu	mg kg^{-1}	**3.71 ± 1.33^{l}**	**7.21 ± 1.69$^{l/m}$**	**3.48 ± 4.47^{m}**	**3.11 ± 2.40$^{l/m}$**	**9.23 ± 0.36^{m}**
Mn	mg kg^{-1}	**10.1 ± 3.89^{n}**	**11.6 ± 1.18^{n}**	**13.6 ± 1.76^{o}**	**14.0 ± 4.66$^{n/o}$**	**12.5 ± 1.82$^{n/o}$**
Fe	mg kg^{-1}	65.5 ± 23.2^{p}	64.2 ± 18.8^{p}	52.2 ± 29.6^{p}	70.9± 39.7^{p}	73.4 ± 13.2^{p}
Al	mg kg^{-1}	60 ± 20^{q}	210 ± 190^{q}	38 ± 13^{q}	499 ± 918^{q}	365 ± 627^{q}
B	mg kg^{-1}	**12.0 ± 3.74^{r}**	**13.6 ± 1.67^{r}**	**30.7 ± 6.11^{s}**	**14.4 ± 2.61^{r}**	**14.0 ± 1.41^{r}**

3.4. Soils: Unilever Site, La Lucia Ridge

3.4.1. pH: B. vulgaris & C. annuum

As shown in Fig. 33, soil pH values recorded weekly were found to increase amongst all *B. vulgaris* treatments over the entire experimental period, changing from weakly acidic (± 6.4) to neutral (± 7). Interestingly treatments followed very similar trend oscillations relative to each other, suggesting that factors other than treatment were influencing soil pH.

Changes in mean soil pH of *C. annuum* treatments, shown in Fig. 34, were found to approximate closely to those changes in soil pH recorded among *B. vulgaris* treatments; soil pH was found to generally increase over the experimental period from weakly acidic (± 6.6) to neutral (± 7) among all treatment groups.

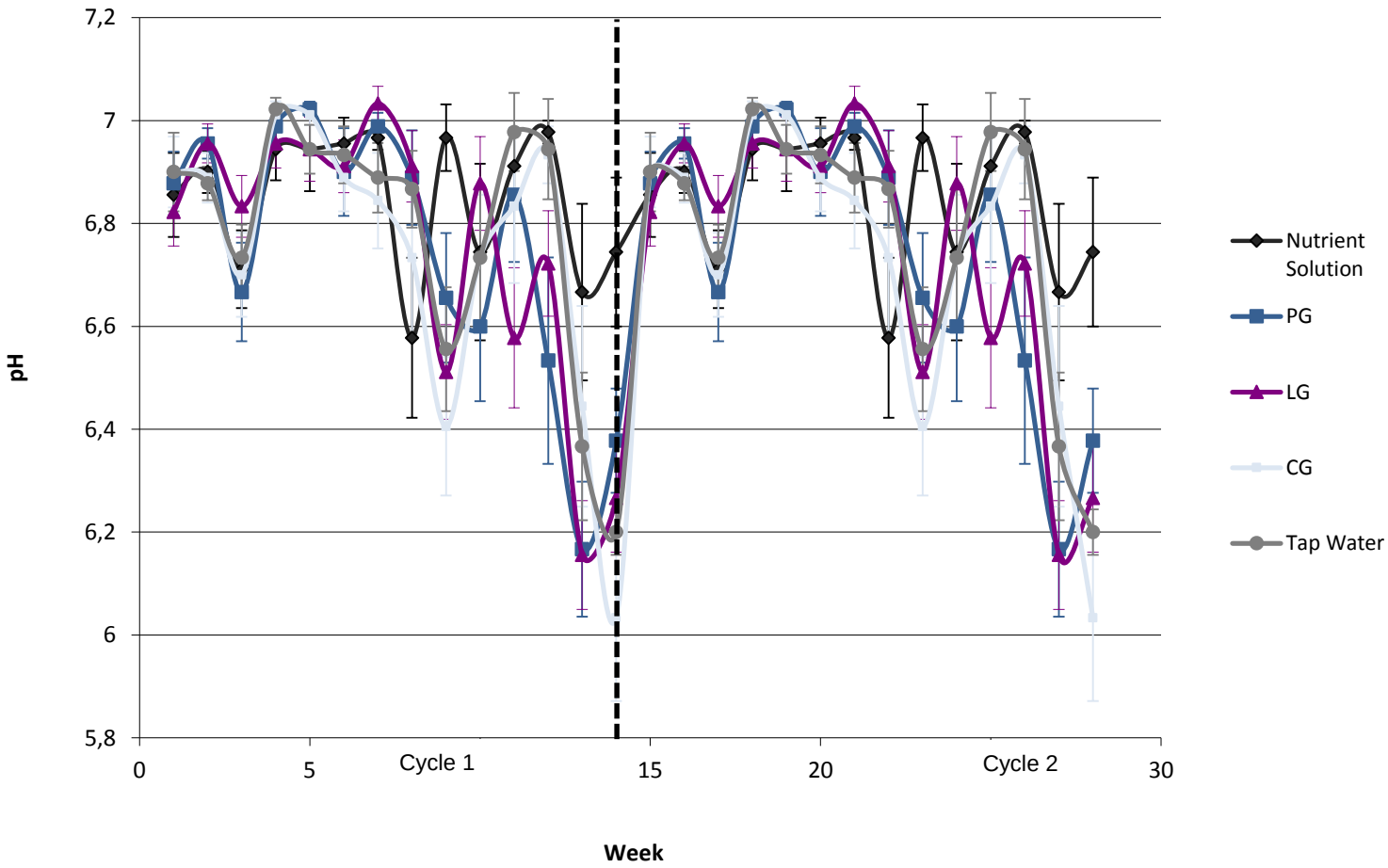

Fig. 33. Weekly mean soil pH values among *B. vulgaris* treatments measured in the field for growth cycles 1 and 2 at the Unilever experiment site. Dashed vertical line represents growth cycle temporal separation. Treatments: "Nutrient solution" = irrigated with nutrient solution; "PG" = irrigated with phosphate base powder-derived greywater; "LG" = irrigated with liquid laundry detergent-derived greywater; "CG" = irrigated with carbonate base powder-derived greywater; "Tap water" = irrigated with municipal tap water supply. Vertical bars represent standard errors about means.

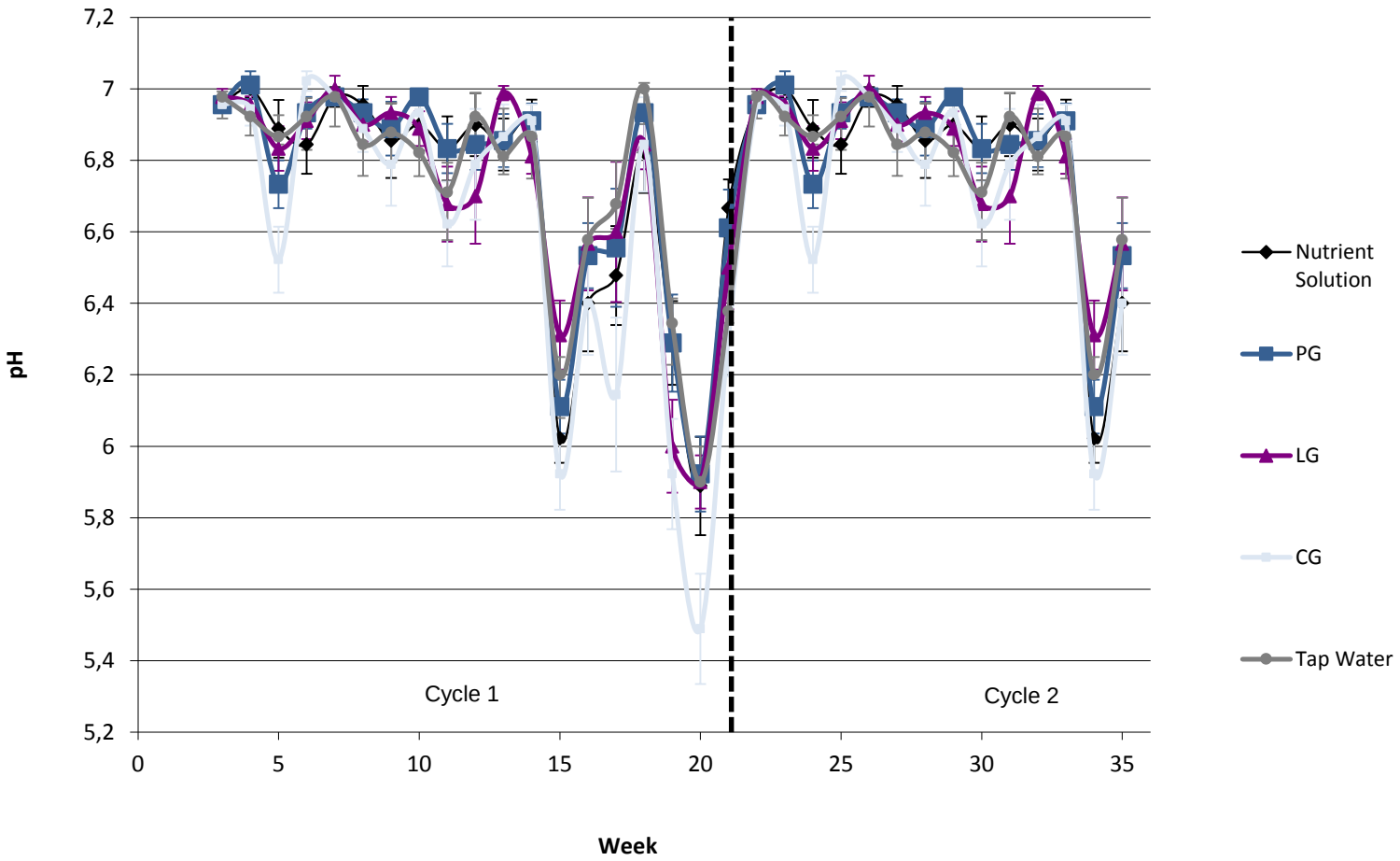

Fig. 34. Weekly mean soil pH values among *C. annuum* treatments measured in the field for growth cycles 1 and 2 at the Unilever experiment site. Dashed vertical line represents growth cycle temporal separation. Treatments: "Nutrient solution" = irrigated with nutrient solution; "PG" = irrigated with phosphate base powder-derived greywater; "LG" = irrigated with liquid laundry detergent-derived greywater; "CG" = irrigated with carbonate base powder-derived greywater; "Tap water" = irrigated with municipal tap water supply. Vertical bars represent standard errors about means.

3.4.2. Fertility: B. vulgaris

Macro- and micronutrient fertility of soils in which *B. vulgaris* were grown were investigated at the end of growth cycles 1 and 2 at the Unilever experiment site.

A significantly higher concentration of P was noted among treatments for the nutrient solution in cycle 1, whilst both the powder-based detergents PG and CG greywaters had intermediary values relative to all treatments and were found to be statistically similar in this respect, despite the different formulation bases of each (Table 22 and Table 23). In cycle 2 however, no differences in soil P were found for any of the treatments investigated.

Relative to the nutrient treatment, suppressed soil K values were found for all greywater treatments and the tap water treatment during growth cycle 1, with a similar statistical trend following in cycle 2 (Table 22 and Table 23).

In growth cycles 1 and 2, soil Ca was found to be significantly higher among nutrient-treated soils when compared with remaining treatments, the latter which were found to be statistically similar to each other. As shown in Table 22 and Table 23, the effects of greywater addition on soil Ca over repeated growth cycles was found to be insignificant with the exception of LG, where a small but significant increase in Ca concentration was found. Similarly, higher concentrations of Mg were found for nutrient-treated soils following growth cycle 1 relative to other treatments although over a longer irrigation term no significant differences were noted among treatments for soil Mg concentrations.

As with the elements noted earlier, nutrient treated soils reflected significantly higher concentrations of Zn when compared statistically with either that of the tap water and greywater treatments for both cycles 1 and 2 (Table 22 and Table 23). The addition of nutrient solution and PG-generated greywater to soils resulted in significant increases in Zn concentration, but remained statistically non-significant for the other treatments investigated. Statistical evaluations of Mn and Cu concentrations among treatment soils found no statistically significant differences among treatments for either growth cycle.

Table 22. Soil concentrations of nutrients established post-harvest in *B. vulgaris* pots among the five irrigation treatments following growth cycle 1 at the Unilever experiment site (mean ± SD). Presence of letters denotes mean separation among treatments by Scheffe's multiple range test ($P < 0.05$; $n = 5$ per treatment).

Element	Unit	Nutrient Solution	Phosphate Powder	Liquid Detergent	Carbonate Powder	Tap Water
P	mg kg^{-1}	**179 ± 1.53^{a}**	**76.3 ± 43.8$^{c/b}$**	**23.3 ± 3.21^{c}**	**90.0 ± 7.00^{b}**	**20.0 ± 2.00^{c}**
K	mg kg^{-1}	**526 ± 169^{d}**	**39.7 ± 3.06^{e}**	**48.3 ± 3.61^{e}**	**64.0 ± 3.61^{e}**	**27.7 ± 6.66^{e}**
Ca	mg kg^{-1}	**1920 ± 930^{f}**	**660 ± 157$^{f/g}$**	**739 ± 185$^{f/g}$**	**574 ± 185^{g}**	**734 ± 94.7$^{f/g}$**
Mg	mg kg^{-1}	**163 ± 36.8^{h}**	**60.3 ± 11.7^{i}**	**65.3 ± 7.02^{i}**	**46.0 ± 17.1^{i}**	**58.0 ± 14.8^{i}**
Zn	mg kg^{-1}	**6.13 ± 1.10^{j}**	**4.83 ± 0.45$^{j/k}$**	**4.60 ± 0.70$^{j/k}$**	**3.67 ± 0.06^{k}**	**4.50 ± 0.26^{k}**
Mn	mg kg^{-1}	13.3 ± 11.2^{l}	4.67 ± 1.53^{l}	32.7 ± 49.7^{l}	4.33 ± 0.58^{l}	3.33 ± 1.15^{l}
Cu	mg kg^{-1}	2.70 ± 0.72^{m}	2.83 ± 0.57^{m}	2.93 ± 0.35^{m}	2.73 ± 0.12^{m}	3.60 ± 0.87^{m}

Table 23. Soil concentrations of nutrients established post-harvest in *B. vulgaris* pots among the five irrigation treatments following growth cycle 2 at the Unilever experiment site (mean ± SD). Presence of letters denotes mean separation among treatments by Scheffe's multiple range test ($P < 0.05$; $n = 5$ per treatment).

Element	Unit	Nutrient Solution	Phosphate Powder	Liquid Detergent	Carbonate Powder	Tap Water
P	mg kg^{-1}	155 ± 126^{a}	148 ± 16.6^{a}	41.3 ± 4.04^{a}	121 ± 12.2^{a}	31.3 ± 9.71^{a}
K	mg kg^{-1}	**135 ± 40.3^{b}**	**47.3 ± 9.45^{c}**	**43.3 ± 7.23^{c}**	**72.0 ± 14.2^{c}**	**37.0 ± 6.56^{c}**
Ca	mg kg^{-1}	**1214 ± 127^{d}**	**620 ± 32.4^{e}**	**798 ± 32.4^{e}**	**674 ± 69.6^{e}**	**789 ± 113^{e}**
Mg	mg kg^{-1}	68.0 ± 7.94^{f}	70.0 ± 5.20^{f}	63.0 ± 17.6^{f}	66.3 ± 20.6^{f}	77.0 ± 7.00^{f}
Zn	mg kg^{-1}	**10.3 ± 1.51^{g}**	**6.13 ± 1.01^{h}**	**6.07 ± 0.67^{h}**	**4.67 ± 0.64^{h}**	**5.83 ± 1.07^{h}**
Mn	mg kg^{-1}	10.3 ± 1.15^{i}	11.7 ± 3.79^{i}	9.67 ± 1.53^{i}	8.33 ± 2.08^{i}	8.67 ± 2.52^{i}
Cu	mg kg^{-1}	4.87 ± 0.64^{j}	4.03 ± 0.75^{j}	5.17 ± 0.64^{j}	4.17 ± 0.21^{j}	5.53 ± 1.00^{j}

3.4.3. Fertility: C. annuum

As shown in Table 24, the addition of PG-generated greywater to soil resulted in a statistically similar effect as that of the nutrient treatment, with the latter reference soil P concentration found to be 192 mg kg^{-1} and that of the former 191 mg kg^{-1}. These values were both significantly higher than either of the remaining greywater and tap water irrigation treatments, for which P values were < 69 mg kg^{-1}. The effect of greywater irrigation on the concentration of K in soils was found to be negligible and statistically similar to that obtained in soils treated with tap water. Among treatments, the addition of the nutrient solution resulted in a significant increase of soil K relative to other treatments, with a value of > 100 mg kg^{-1}.

None of the irrigation treatments had a significant effect on soil concentrations of Ca (Table 24). However, for Mg, nutrient-treated soils had significantly lower mean concentrations of 44 mg kg^{-1} compared with the remaining treatments for which mean values ranged from 71 mg kg^{-1} to 78 mg kg^{-1} and were found to be statistically similar.

The addition of the greywater treatments and tap water to soils had a negligible effect on the soil concentrations of Zn, Cu and Mn over the two experimental growth cycles (Table 24). The addition of the nutrient fertilizer solution to soils did however result in a significantly elevated mean level of Zn in soils of 9.9 mg kg^{-1} relative to all remaining treatments for which mean concentrations ranged from 4.9 mg kg^{-1} to 6.3 mg kg^{-1}.

Table 24. Soil concentrations of nutrients established for *C. annuum* post-harvest among the five irrigation treatments following growth cycle 2 at the Unilever experiment site (mean ± SD). Different letters denote mean separation among treatments for a given growth cycle by Scheffe's multiple range test.

Element	Unit	Nutrient Solution	Phosphate Powder	Liquid Detergent	Carbonate Powder	Tap Water
P	**mg kg^{-1}**	**192 ± 82.3^{a}**	**191 ± 42.9^{a}**	**37.3 ± 5.03^{b}**	**68 ± 28.8$^{a/b}$**	**28.3 ± 1.53^{b}**
K	**mg kg^{-1}**	**105 ± 27.5^{c}**	**45.0 ± 4.36^{d}**	**46.3 ± 12.6^{d}**	**53.7 ± 13.7^{d}**	**53.3 ± 13.3^{d}**
Ca	mg kg^{-1}	1018 ± 453^{e}	646 ± 36^{e}	764 ± 44^{e}	726 ± 75^{e}	818 ± 20^{e}
Mg	**mg kg^{-1}**	**44.0 ± 8.72^{f}**	**78.0 ± 2.65^{g}**	**70.7 ± 7.37^{g}**	**71.7 ± 10.4^{g}**	**75.3 ± 2.08^{g}**
Zn	**mg kg^{-1}**	**9.90 ± 2.51^{h}**	**6.33 ± 0.25$^{h/i}$**	**5.67 ± 1.00^{i}**	**5.00 ± 0.35^{i}**	**4.87 ± 0.21^{i}**
Cu	mg kg^{-1}	4.83 ± 1.79^{j}	4.03 ± 0.61^{j}	3.83 ± 0.91^{j}	3.90 ± 0.10^{j}	4.43 ± 0.64^{j}
Mn	mg kg^{-1}	11.3 ± 1.53^{k}	10.7 ± 2.89^{k}	10.3 ± 4.04^{k}	10.7 ± 2.08^{k}	9.3 ± 2.52^{k}

3.4.4. Salinity: B. vulgaris

Soil salinity properties among treatments, measured at the end of growth cycle 1 and 2 are shown in Table 25 and Table 26 respectively. Greywater generated from laundry powders had a significant additive effect on soil salinity measured as exchangeable Na, with both PG and CG treatments having significantly higher mean soil exchangeable salinity values for both cycles that were several times higher than both LG-generated greywater and the experimental controls.

For both growth cycles, the addition of greywater treatments to soils resulted in no statistically significant higher cation totals compared with tap water, suggesting the additive effect of powder formulations to water did not translate directly into total soil cation increases.

Relative to other treatments evaluated, significantly higher ESP values for both growth cycles were found for those soils to which powder detergents product PG and CG were added. For these treatments, ESP values were typically above 19 % for both growth cycles. Among greywater treatments, addition of liquid laundry detergent-generated greywater (LG) resulted in the lowest soil ESP values (< 6.5 %) which were statistically similar to that of tap water for both growth cycles. Among treatments and for both growth cycles, soils treated with nutrient solution fertilizer had the lowest ESP values (< 0.8 %).

The elevated levels of Na reported for soils treated with powder laundry detergents translated into higher SAR values reported for these treatments for both cycles. Sodium absorption ratios of soils treated with the liquid detergent were found to be intermediate among treatments, whilst for nutrient fertilizer and tap water treated soils, SAR values were found to be similar to each other and lowest among treatments.

Table 25. Soil salinity characteristics measured among treatments at the Unilever experiment site, recorded in *B. vulgaris* pots at the end of growth cycle 1 (mean ± SD). Different letters denote mean separation among treatments for a given growth cycle by Scheffe's multiple range test ($P < 0.05$; $n = 5$ per treatment).

Parameter	Unit	Nutrient Solution	Phosphate Powder	Liquid Detergent	Carbonate Powder	Tap Water
Total Cations	-	**12.4 ± 4.86^{a}**	**3.79 ± 0.96^{b}**	**4.39 ± 0.24^{b}**	**3.44 ± 1.07^{b}**	**4.25 ± 0.61^{b}**
Exchangeable Na	**mg kg^{-1}**	**6.00 ± 2.79^{c}**	**149 ± 45.1^{e}**	**45.6 ± 43.3$^{c/d}$**	**133 ± 26.1$^{d/e}$**	**10.2 ± 1.05^{c}**
ESP	%	**0.30 ± 0.07^{f}**	**19.0 ± 5.44^{g}**	**5.60 ± 4.96$^{f/g}$**	**20.2 ± 8.34^{g}**	**1.40 ± 0.11^{f}**
SAR	-	**0.25 ± 0.12^{h}**	**10.6 ± 2.78^{i}**	**3.10 ± 2.94$^{h/i}$**	**11.00 ± 3.29^{i}**	**0.69 ± 0.02^{h}**

Table 26. Soil salinity characteristics measured among treatments at the Unilever experiment site, recorded in *B. vulgaris* pots at the end of growth cycle 2 (mean ± SD). Different letters denote mean separation among treatments for a given growth cycle by Scheffe's multiple range test ($P < 0.05$; $n = 5$ per treatment).

Parameter	Unit	Nutrient Solution	Phosphate Powder	Liquid Detergent	Carbonate Powder	Tap Water
Total Cations	-	**7.07 ± 0.82^{a}**	**3.86 ± 0.25^{b}**	**4.68 ± 0.19^{b}**	**4.16 ± 0.50^{b}**	**4.74 ± 0.62^{b}**
Exchangeable Na	**mg kg^{-1}**	**7.71 ± 0.00^{c}**	**221 ± 205$^{c/d}$**	**62.3 ± 17.3$^{c/d}$**	**396 ± 110^{d}**	**39.3 ± 17.7$^{d/e}$**
ESP	%	**0.77 ± 0.00^{f}**	**22.3 ± 17.2$^{f/g}$**	**6.51 ± 2.04^{f}**	**34.8 ± 5.40^{g}**	**4.73 ± 1.84^{f}**
SAR	-	**0.47 ± 1.00^{h}**	**16.1 ± 13.8$^{i/j}$**	**4.04 ± 1.14^{i}**	**27.0 ± 7.07^{j}**	**2.67 ± 1.22^{i}**

3.4.5. Salinity: C. annuum

Soil salinity characteristics of *C. annuum* pots were established at the end of growth cycle 2 (Table 27). Differences in exchangeable sodium of soils were noted but these were found to be statistically non-significant.

By contrast, the total cation count for the nutrient treated soils was - depending on the stringency of the statistical test applied - significantly higher (6.6) than either of the greywater or tap water treatments where values ranged from 3.9 to 4.9. Among greywater treated soils, PG was found to be marginally the lowest.

A more accurate representation of exchangeable Na in soil, the exchangeable sodium percentages (ESP), are shown for each treatment in Table 27. Relative to nutrient solution and tap water irrigation media, the addition of greywater to soils had no significant effect on soil ESP although data variability was high.

No statistically significant differences among treatments were apparent for soil SAR (Table 27).

Repeated irrigation of Berea Red soils with PG- and CG-derived greywater resulted in the occurrence of a white powdery substance on soil surfaces after only a few weeks of treatment (Fig. 35). This powder persisted on soil surfaces for the rest of experimentation (cycles 1 and 2).

Table 27. Soil salinity characteristics measured among treatments at the Unilever experiment site, recorded in *C. annuum* pots at the end of growth cycle 1 (mean ± SD). Letters represent mean separation among treatments by Scheffe's multiple range test ($P < 0.05$; $n = 3$ per treatment).

Parameter	Unit	Nutrient Solution	Phosphate Powder	Liquid Detergent	Carbonate Powder	Tap Water
Total Cations	-	**6.59 ± 2.23^{a}**	**3.92 ± 0.19^{b}**	**4.57 ± 0.28$^{a/b}$**	**4.43 ± 0.43$^{a/b}$**	**4.92 ± 0.04$^{a/b}$**
Exchangeable Na	mg kg^{-1}	5.70 ± 2.61^{c}	122 ± 105^{c}	17.4 ± 13.7^{c}	54.1 ± 29.0^{c}	10.1 ± 2.26^{c}
ESP	%	0.61 ± 0.44^{d}	15.3 ± 11.3^{d}	2.30 ± 1.77^{d}	6.89 ± 3.30^{d}	1.25 ± 0.27^{d}
SAR	-	0.35 ± 0.21^{e}	9.20 ± 7.96^{e}	1.22 ± 0.97^{e}	3.81 ± 1.95^{e}	0.68 ± 0.15^{e}

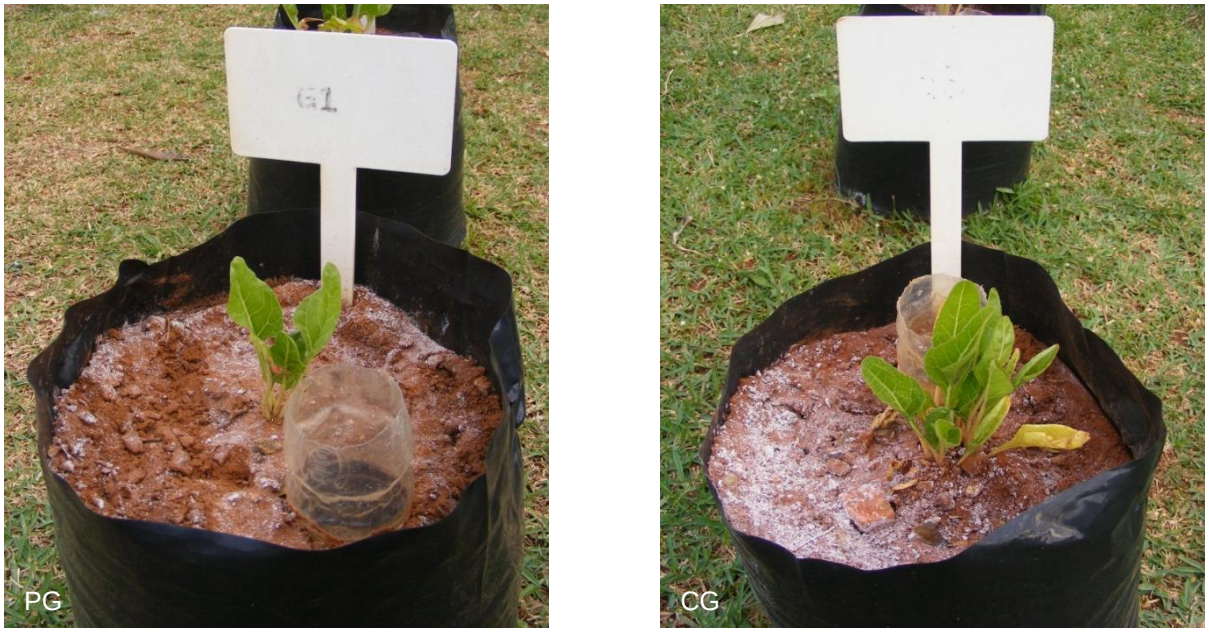

Fig. 35. Representative pots illustrating the occurrence of a white powder on soil surfaces in all pots irrigated with PG and CG greywater at the Unilever experiment site. Treatments: "PG" = irrigated with phosphate base powder-derived greywater; "CG" = irrigated with carbonate base powder-derived greywater.

3.4.6. Specific Hydraulic Conductivity: B. vulgaris & C. annuum

The long-term addition of greywater to *B. vulgaris* experimental soils appeared to impact considerably on hydraulic flow through soils (Fig. 36). Greywater irrigation was typically

found to reduce mean K_s of experimental soils, resulting in lower mean K_s values relative to either the nutrient-irrigated or tap water irrigated treatments. The long-term application of nutrient solution to soils was found to have comparable influence on K_s to that of tap water irrigated soils.

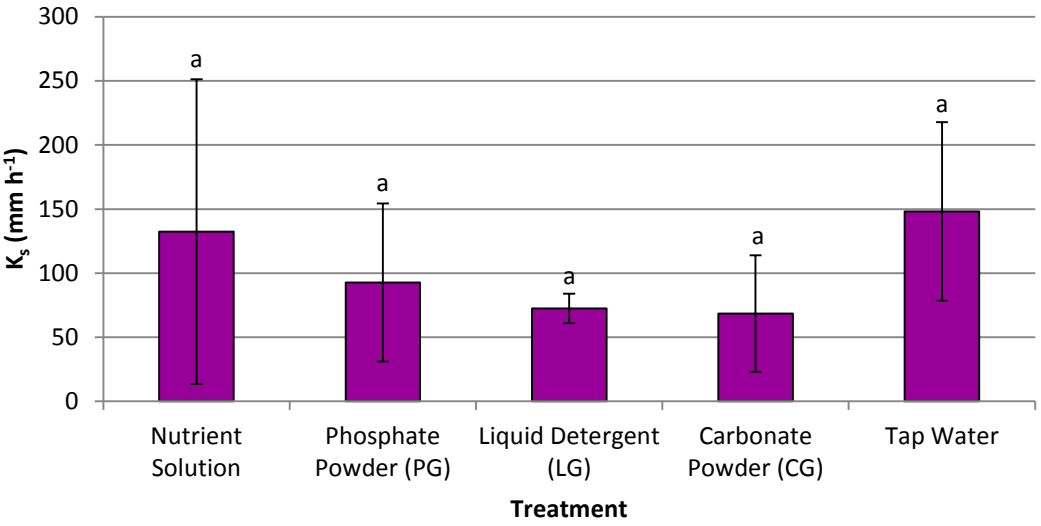

Fig. 36. Mean specific hydraulic conductivities of soils in which *B. vulgaris* were grown, recorded after growth cycle 2 at the Unilever experiment site. Vertical bars about means represent standard deviations. Letters represent mean separation among treatments by Scheffe's multiple range test ($P< 0.05$; $n = 3$ per treatment).

The application of greywater to *C. annuum* soils over two growth cycles appeared to reduce mean K_s of treatment soils, with the lowest mean soil K_s noted for CG-irrigated soils (Fig. 37).

Although the application of irrigation treatments to soils for *C. annuum* occurred over a considerably longer period than that for *B. vulgaris*, no considerable differences in K_s trends were found among soils in which these species were grown. Both nutrient- and tap water-irrigated soils were found to have similar soil K_s values, reflecting higher mean values than either of the greywater irrigated treatments.

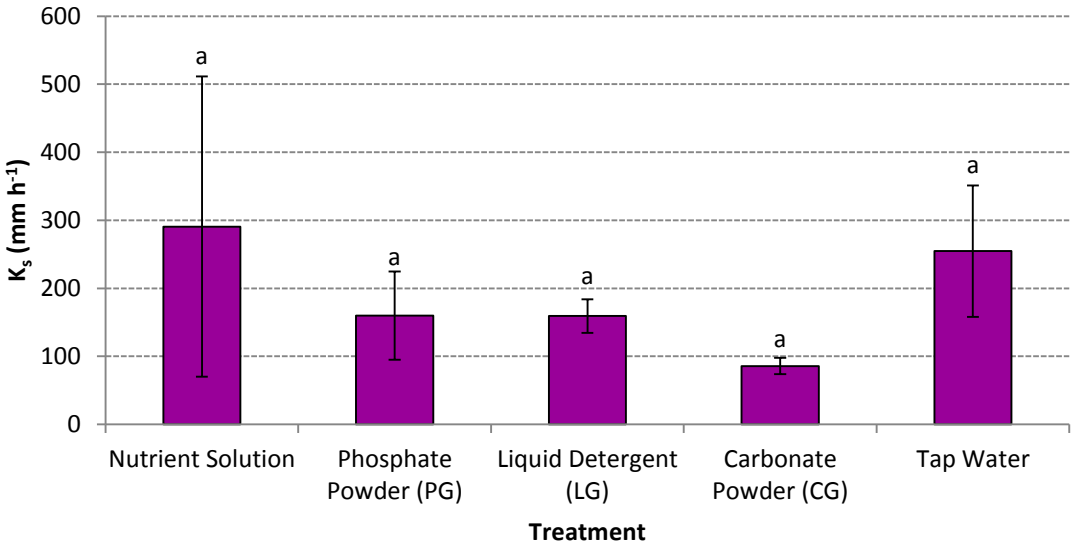

Fig. 37. Mean specific hydraulic conductivities of soils in which *C. annuum* were grown, recorded after growth cycle 2 at the Unilever experiment site. Vertical bars about means represent standard deviations. Letters represent mean separation among treatments by Scheffe's multiple range test ($P< 0.05$; $n = 3$ per treatment).

CHAPTER III: RESULTS II

3.5. Plants: University Site, Glenwood

3.5.1. Plant Heights: B. vulgaris

Vertical growth trends of all experimental *B. vulgaris* plants reflected asymmetric sigmoidal (Gompertzian) distributions, most apparent for the nutrient-irrigated treatment where the overall growth rate attained was greatest (Fig. 38). After 14 weeks of growth, mean heights obtained from nutrient-irrigated individuals were in excess of 590 mm. By contrast, depressed asymmetric sigmoidal (Gompertzian) distributions of weekly mean heights among the greywater- and tap water-irrigated treatments were established, with these treatments all attaining final mean heights at harvest in the range 350 mm to 380 mm. Statistical comparisons of mean heights among treatments at harvest (14 weeks) showed that mean height of the nutrient-irrigated *B. vulgaris* was significantly larger than that of all other treatments. However, mean plant heights of greywater and tap water treatments were found to be statistically similar at the same stage.

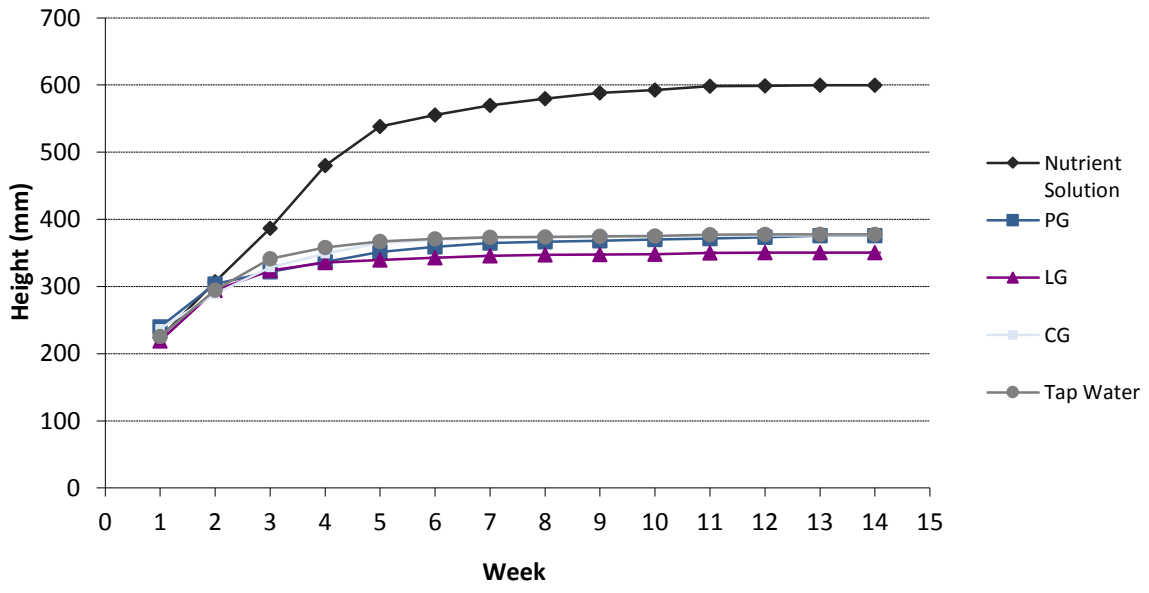

Fig. 38. Weekly mean heights of *B. vulgaris* plants from the irrigation treatments tested, recorded during growth cycle 1 at the University experiment site (n = 9). Treatments: "Nutrient solution" = irrigated with nutrient solution; "PG" = irrigated with phosphate base powder-derived greywater; "LG" = irrigated with liquid laundry detergent-derived greywater; "CG" = irrigated with carbonate base powder-derived greywater; "Tap water" = irrigated with municipal tap water supply. Vertical bars depict standard errors about sample means.

3.5.2. Plant Heights & Stem Diameters: *C. annuum*

Temporal change in *C. annuum* plant heights are shown in Fig. 39. An asymmetric sigmoidal (Gompertzian) growth trend was followed by all treatments over the 14 week experimental growth period. Plant growth response to nutrient-irrigated treatments was found to be most favourable as the overall rate of change in vertical growth was greatest with final mean height of plants over 360 mm. As with *B. vulgaris*, final plant heights attained among the greywater- and tap water-irrigated treatments of *C. annuum* were similar ($P > 0.05$), but significantly different from the nutrient-irrigated treatment. Similarly, for stem diameters of *C. annuum*, an asymmetric sigmoidal (Gompertzian) growth trend was followed by all greywater- and tap water irrigated-treatments (Fig. 40). However, growth trends of stem diameters of nutrient irrigated treatments approximated more closely to a linear increase over the experimental period. As a result, stem diameters measured just before harvest were significantly larger for the nutrient-irrigated treatments than that of the other treatments.

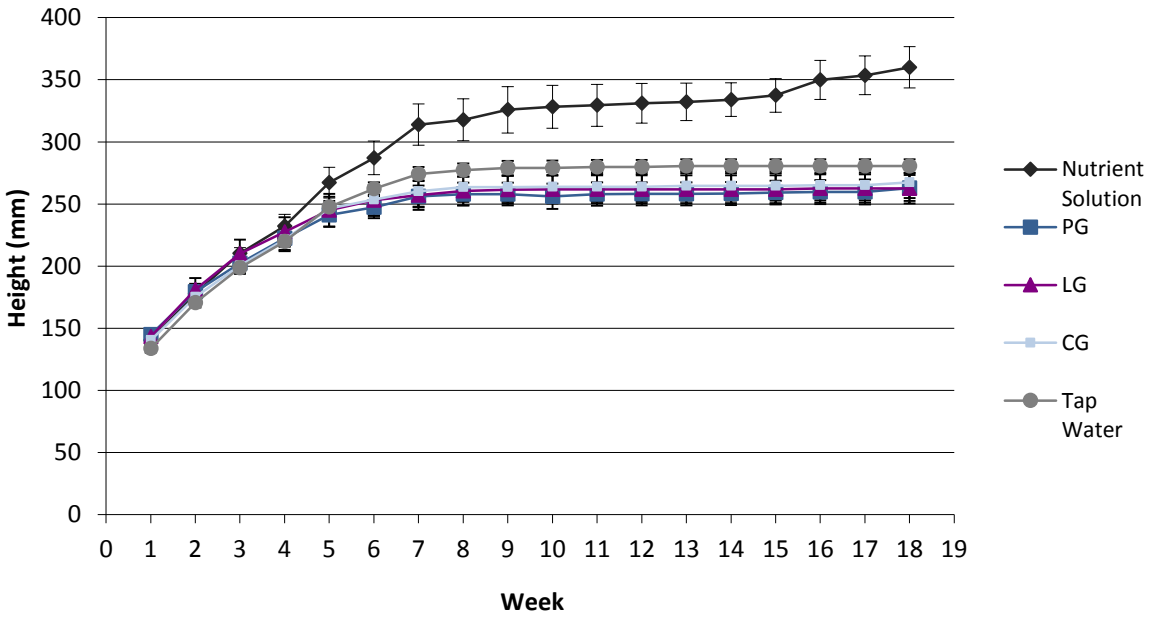

Fig. 39. Weekly mean heights of *C. annuum* individuals from the irrigation treatments tested, recorded during growth cycle 1 at the University experimental site (n = 9). Treatments: "Nutrient solution" = irrigated with nutrient solution; "PG" = irrigated with phosphate base powder-derived greywater; "LG" = irrigated with liquid laundry detergent-derived greywater; "CG" = irrigated with carbonate base powder-derived greywater; "Tap water" = irrigated with municipal tap water supply. Vertical bars about means represent standard errors about means.

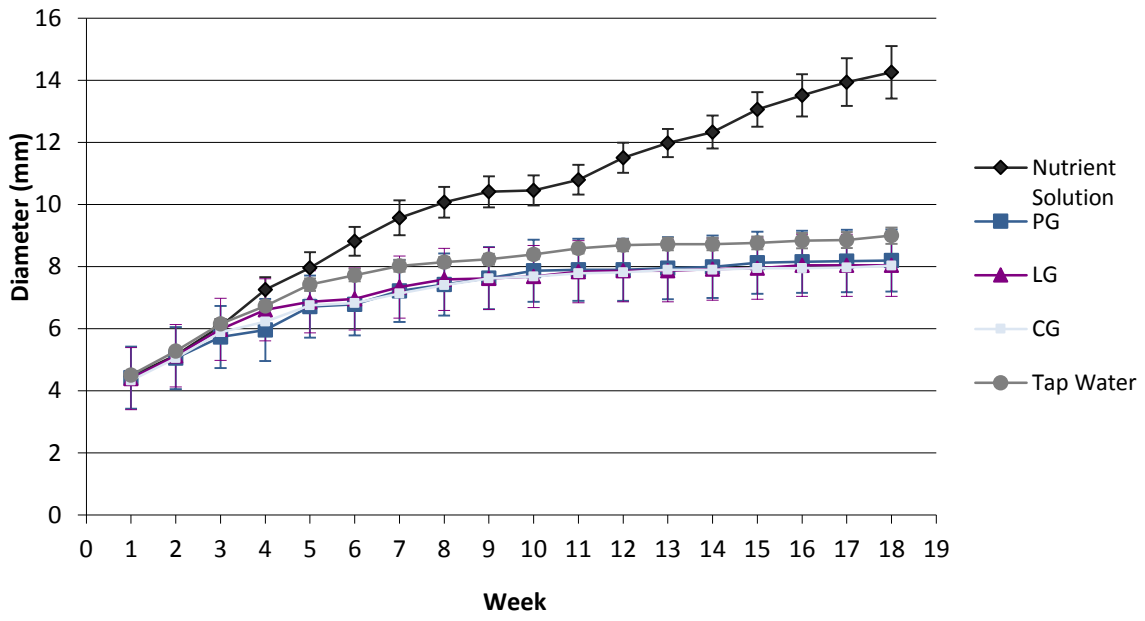

Fig. 40. Weekly mean stem diameters of *C. annuum* individuals from the irrigation treatments tested, recorded during growth cycle 1 at the University experimental site (*n* = 9). Treatments: "Nutrient solution" = irrigated with nutrient solution; "PG" = irrigated with phosphate base powder-derived greywater; "LG" = irrigated with liquid laundry detergent-derived greywater; "CG" = irrigated with carbonate base powder-derived greywater; "Tap water" = irrigated with municipal tap water supply. Vertical bars about means represent standard errors about means.

3.5.3. Phenotypic Plasticity: B. vulgaris

Visual impressions of physical changes to representative *B. vulgaris* individuals grown during growth cycle 1 at the University experimental site following 116 days after planting, are shown in Fig. 41. Nutrient deficiency symptoms and chlorosis become evident among greywater- and tap water-irrigated treatments. Nutrient solution-irrigated *B. vulgaris* remained healthy throughout the experimental period, producing leaves which were both considerably larger and of a visibly darker hue of green relative to the other treatments, the latter becoming particularly evident shortly before harvest. Neither leaf curl nor environmental leaf damage among *B. vulgaris* treatments for the experimental period was observed.

Fig. 41. Time snapshots (116 days after planting) depicting physical changes in representative *B. vulgaris* individuals from each experimental treatment at the University experiment site. Note emergence of leaf chlorosis in greywater and tap water irrigated treatments. Treatments: "Nutrient solution" = irrigated with nutrient solution; "PG" = irrigated with phosphate base powder-derived greywater; "LG" = irrigated with liquid laundry detergent-derived greywater; "CG" = irrigated with carbonate base powder-derived greywater; "Tap water" = irrigated with municipal tap water supply. Note emergence of leaf chlorosis in greywater and tap water irrigated treatments.

3.5.4. Biomass: B. vulgaris

Biomass parameters measured in harvested *B. vulgaris* are shown in Table 28. Total dry leaf biomass of *B. vulgaris* individuals among greywater- and tap water-irrigated treatments were established to be statistically similar, with less than 20 g of dry biomass allocated to leaves for these treatments on a mean basis. In contrast, nutrient- irrigated *B. vulgaris* individuals had significantly greater total mean leaf biomass, with more than 120 g of dry biomass invested in leaves per plant.

No statistically significant differences for either root biomass or volume were found between the tap water-irrigated treatments and greywater treatments. However, for nutrient-irrigated *B.*

vulgaris, significantly higher mean dry biomass allocation to roots relative to other treatments was found, positively corroborated by the higher mean root volumes established for this treatment.

Apportioned leaf and root dry biomass among treatments were found to be statistically similar with the exception of the liquid laundry detergent greywater-irrigated *B. vulgaris*, which was found to have a significantly greater proportion of investment in roots as opposed to leaves compared to other treatments.

Table 28. Leaf and root biomass attributes measured among treatments in harvested *B. vulgaris* at the end of the University experiment site (mean ± SD). Letters represent mean separation among treatments by Scheffe's multiple range test ($P < 0.05$; $n = 9$ per treatment).

Attribute	Unit	Nutrient Solution	Phosphate Powder	Liquid Detergent	Carbonate Powder	Tap Water
Dry Leaf Mass	**g**	**126 ± 56.6^{a}**	**15.4 ± 4.2^{b}**	**10.3 ± 1.9^{b}**	**12.0 ± 4.3^{b}**	**14.0 ± 3.3^{b}**
Dry Root Mass	**g**	**109 ± 10.1^{c}**	**5.2 ± 2.0^{d}**	**4.2 ± 1.0^{d}**	**4.3 ± 2.1^{d}**	**4.8 ± 1.7^{d}**
Root Volume	**cm^3**	**107 ± 50.1^{e}**	**32.9 ± 8.1^{f}**	**21.8 ± 6.0^{f}**	**26.9 ± 8.6^{f}**	**27.2 ± 8.6^{f}**

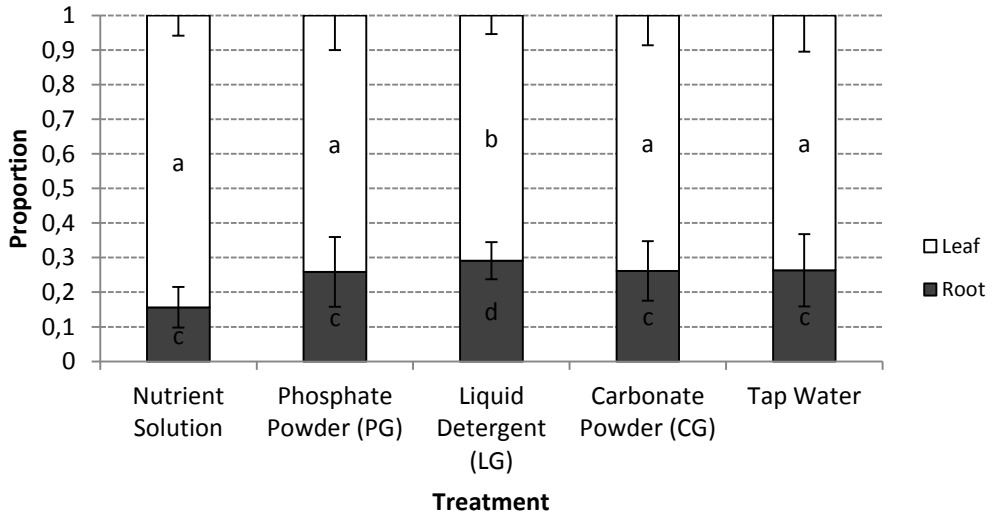

Fig. 42. Mean proportional dry biomass allocation to *B. vulgaris* leaves and roots among the five irrigation treatments at the University experiment site. Vertical bars represent standard deviations. Letters represent mean separation among treatments by Scheffe's multiple range test ($P < 0.05$; $n = 9$ per treatment).

3.5.5. *Phenotypic Plasticity: C. annuum*

A visual illustration of changes in representative *C. annuum* individuals as influenced by irrigation treatment following 116 days after planting, are shown in Fig. 43. At this stage, symptoms of leaf chlorosis among both greywater and tap water treatments was apparent. By comparison, nutrient solution-irrigated *C. annuum* exhibited a higher order of stem branching, supporting a greater number of leaves which were also of a significantly darker green hue. Leaf senescence was found to be most rapid in LG-irrigated *C. annuum* and also reflected the greatest prevalence of leaf necrosis among treatments towards the end of experimentation. Pepper fruit production was maintained among all treatments until whole-plant harvest.

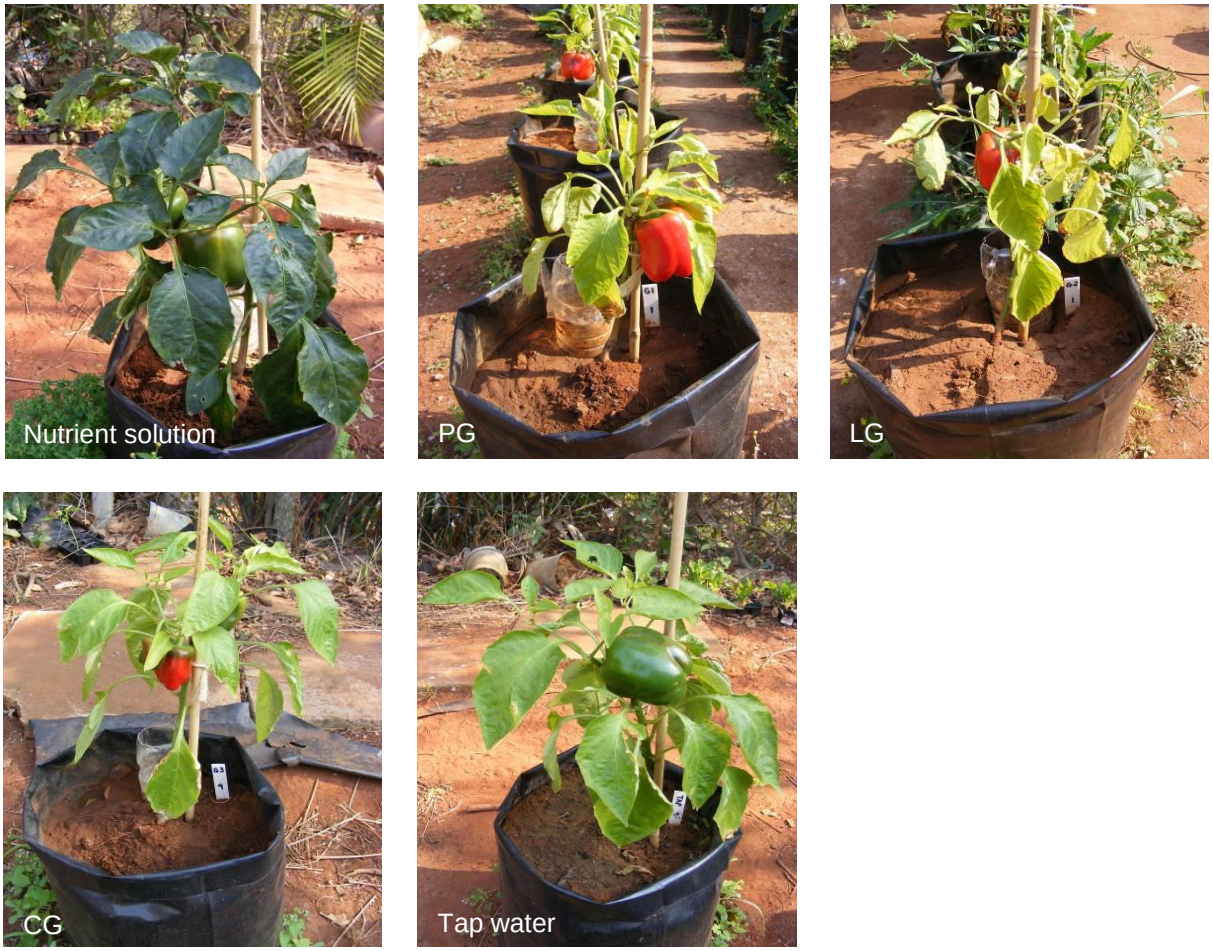

Fig. 43. Time snapshot (116 days after planting) depicting physical changes in representative *C. annuum* individuals from each experimental treatment. Note emergence of leaf chlorosis in greywater- and tap water-irrigated treatments. Treatments: "Nutrient solution" = irrigated with nutrient solution; "PG" = irrigated with phosphate base powder-derived greywater; "LG" = irrigated with liquid laundry detergent-derived greywater; "CG" = irrigated with carbonate base powder-derived greywater; "Tap water" = irrigated with municipal tap water supply.

3.5.6. Biomass: C. annuum

Irrigation treatment exerted considerable influence over biomass allocation to anatomical components and differences in investment in leaves, stems and roots among treatments were noted (Table 29). Biomass allocations to any of these components were found to be highest in nutrient-irrigated *C. annuum* and these differences were found to be significantly different for all anatomical cases. Among greywater-irrigated treatments, individuals irrigated with the liquid laundry detergent greywater LG invested the least biomass as roots, stems or leaves and these differences were statistically significant. Mean biomass allocations to stems and roots among *C. annuum* irrigated with tap water, phosphate power PG and phosphate-free greywater CG were found to be marginal. For *C. annuum* leaf biomass, no statistically significant differences were found between CG- and tap water-irrigated treatments.

Statistical analysis of *C. annuum* root volumes recorded for fresh root samples among treatments post-harvest showed highest root volumes were found with the nutrient-irrigated treatment whilst differences noted amongst greywater- and tap water-irrigated treatments were not significant.

Table 29. Leaf, stem, branch and root biomass attributes measured among treatments in harvested *C annuum* at the end of the University experiment site (mean ± SD). Letters represent mean separation among treatments by Scheffe's multiple range test ($P < 0.05$; $n = 9$ per treatment).

Attribute	Unit	Nutrient Solution	Phosphate Powder	Liquid Detergent	Carbonate Powder	Tap Water
Dry Leaf Mass	**g**	**10.4 ± 4.28^{a}**	**$1.41 \pm 0.72^{b/c}$**	**0.86 ± 0.61^{c}**	**1.96 ± 0.50^{b}**	**2.15 ± 0.55^{b}**
Dry Stem + Branches	**g**	**8.37 ± 3.21^{d}**	**$2.29 \pm 0.75^{e/f}$**	**1.89 ± 0.58^{d}**	**$2.37 \pm 0.59^{e/f}$**	**2.95 ± 0.67^{e}**
Dry Root Mass	**g**	**2.54 ± 1.18^{g}**	**$0.88 \pm 0.35^{h/i}$**	**0.67 ± 0.21^{i}**	**$0.90 \pm 0.14^{h/i}$**	**1.25 ± 0.35^{h}**
Root Volume	**cm^3**	**13.89 ± 7.57^{j}**	**4.01 ± 2.06^{k}**	**3.18 ± 1.29^{k}**	**4.21 ± 0.91^{k}**	**6.04 ± 2.47^{k}**

Treatments were found to differ significantly only in the proportional allocation of dry biomass to leaves (Fig. 44); nutrient solution-irrigated *C. annuum* invested a significantly larger proportion (25 %) in dry leaf biomass relative to other treatments among which PG-irrigated *C. annuum* was found to be significantly lowest with a proportional value of 12 %.

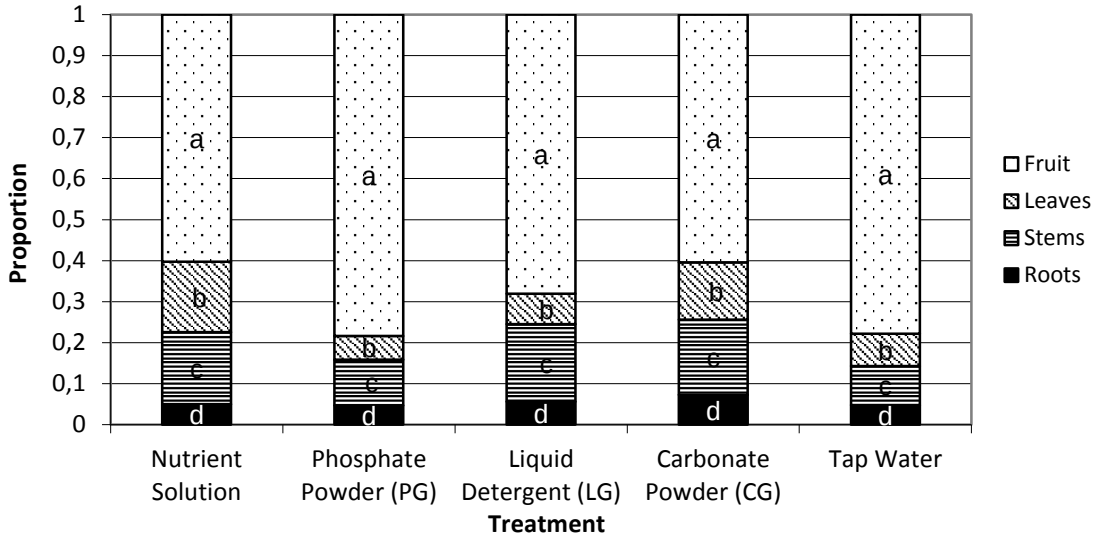

Fig. 44. Mean proportional dry biomass allocations to various anatomical components of *C. annuum* among the five irrigation treatments at the University experiment site. Letters represent mean separation among treatments by Scheffe's multiple range test ($P< 0.05$; $n = 9$ per treatment). For clarity in illustration, error bars are not shown.

3.5.7. Leaf Morphology: B. vulgaris

Leaf morphological parameters measured in *B. vulgaris* leaves are shown in Table 30. Mean leaf areas established among treatments post-harvest reflected similar trends as those measured for dry leaf biomass, with the leaf-areas of nutrient solution-irrigated *B. vulgaris* significantly higher than remaining treatments. There were no statistically significant differences among all treatments with respect to specific leaf area or specific leaf weight.

Among treatments, mean leaf dry matter content was found to be similar, with no significant differences in LDMC.

Leaf thicknesses, an assessment which for *B. vulgaris* was derived from the arithmetic mean encompassing stem and leaf lamina thickness from biomass, found no statistically significant differences among treatments, with mean leaf/stem thicknesses ranging from 1.5 µm to 2×10^{-3} µm.

Table 30. Leaf characteristics measured for individual *B. vulgaris* leaves post-harvest among the five irrigation treatments at the University experiment site (mean ± SD). Letters represent mean separation among treatments by Scheffe's multiple range test ($P < 0.05$; $n = 9$ per treatment).

Parameter	Unit	Nutrient Solution	Phosphate Powder	Liquid Detergent	Carbonate Powder	Tap Water
Area	**m^2**	**0.32 ± 0.1^a**	**0.05 ± 0.03^b**	**0.04 ± 0.01^b**	**0.04 ± 0.01^b**	**0.06 ± 0.021^b**
SLW	**$g_{DM} \cdot m^{-2}$**	409 ± 144^c	372 ± 170^c	323 ± 133^c	340 ± 146^c	255 ± 48^c
SLA	**$m^2 \cdot (mg_{DM})^{-1}$**	2.86 ± 1.49^d	3.09 ± 1.07^d	3.52 ± 1.17^d	3.39 ± 1.24^d	4.04 ± 0.74^d
LDMC	**$10^4 \times mg\ g^{-1}$**	1.63 ± 0.51^e	1.68 ± 0.22^e	1.76 ± 0.24^e	1.66 ± 0.14^e	1.58 ± 0.21^e
LT	**µm**	2509 ± 560^f	2155 ± 760^f	1821 ± 656^f	2042 ± 811^f	1613 ± 200^f

3.5.8. Leaf Morphology: C. annuum

Contrasting leaf morphological parameters of *C. annuum* were found among treatments (Table 31). Mean differences in leaf area among greywater and tap water-irrigated treatments were found to be marginal and dependant on the stringency associated with the type of post-hoc test applied ($P \leq 0.05$). Differences in mean leaf area of the nutrient-irrigated treatment relative to other treatments were found to be statistically significant. Despite this, mean specific leaf weight and specific leaf area among treatments were not statistically different (Table 31). Similarly, for all treatments, one-way ANOVA reflected no statistically significant differences with respect to leaf dry matter content for *C. annuum*. Estimations of leaf thickness from the reciprocal product of leaf area and LDMC among all treatments were found to range from approximately 438 µm to 572 µm. However, a one-way ANOVA on these data found no statistically significant differences in mean LT among treatments.

Table 31. Leaf characteristics measured for individual *C. annuum* leaves post-harvest among the five irrigation treatments at the University experiment site (mean ± SD). Letters represent mean separation among treatments by Scheffe's multiple range test ($P < 0.05$; $n = 9$ per treatment).

Parameter	Unit	Nutrient Solution	Phosphate Powder	Liquid Detergent	Carbonate Powder	Tap Water
Area	**m^2**	0.11 ± 0.05^{a}	0.008 ± 0.006^{b}	0.007 ± 0.005^{b}	0.016 ± 0.005^{b}	0.02 ± 0.009^{b}
SLW	**$g_{DM} \cdot m^{-2}$**	104 ± 26^{c}	192 ± 157^{c}	130 ± 31^{c}	134 ± 41^{c}	123 ± 35^{c}
SLA	**$m^2 \cdot (mg_{DM})^{-1}$**	10.1 ± 2.4^{d}	7.5 ± 3.8^{d}	8.13 ± 2.13^{d}	8.04 ± 2.21^{d}	8.71 ± 2.58^{d}
LDMC	**$10^4 \times mg\ g^{-1}$**	2.06 ± 0.52^{e}	3.30 ± 1.88^{e}	3.03 ± 0.76^{e}	2.91 ± 0.81^{e}	2.51 ± 0.77^{e}
LT	**µm**	513 ± 78^{f}	572 ± 284^{f}	438 ± 93^{f}	464 ± 84^{f}	504 ± 108^{f}

3.5.9. Harvestable Yields: B. vulgaris

Extrapolation of total yield among treatments from the area of pots used in this study to hectare was used to calculate the total edible crop yield per hectare by treatment (Fig. 45). For *B. vulgaris*, this theoretical yield assessment encompassed both fresh leaf and stem biomass as these collectively constitute the edible portion of the plant. A significantly greater total theoretical yield could be expected from the nutrient-irrigated treatment as compared to all other treatments. However, theoretical differences in edible yield produced per hectare among the greywater- and tap water-irrigated treatments were found to be marginal.

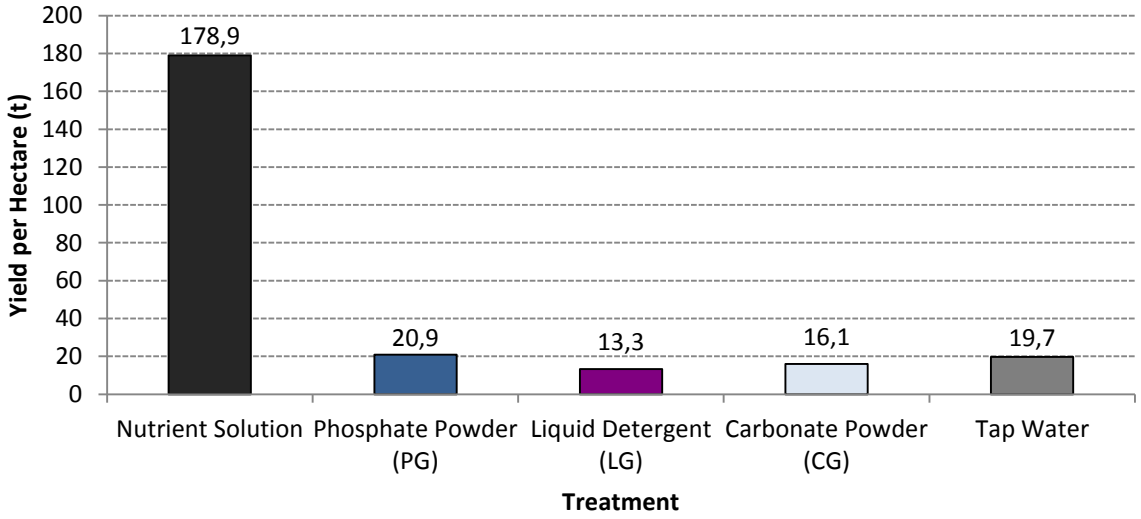

Fig. 45. Theoretical yield per hectare of fresh edible biomass of *B. vulgaris* (leaves and stems) for the five irrigation treatments at the University experiment site ($n = 9$ per treatment).

3.5.10. Leaf Tissue Nutrients: B. vulgaris

The uptake of nutrients by *B. vulgaris* in leaf and stem tissue was found to be variable among treatments for certain elements. Mean uptake of N and K was higher in nutrient solution-irrigated treatments (35 mg kg^{-1} and 90 mg kg^{-1} respectively) than either greywater or tap water- irrigated treatments which reflected a statistically similar uptake of N (50 mg kg^{-1} to 63 mg kg^{-1}) but contrasting P tissue concentrations of 6 mg kg^{-1} to 15 mg kg^{-1}. K uptake in leaf and stem tissue was found to differ significantly between nutrient solution- and greywater treatments collectively, but relative to tap water, irrigation with greywater did not result in significant changes in the *B. vulgaris* leaf tissue concentrations of K.

Irrigation treatment was found to have no significant effect on leaf uptake of Ca. Mg uptake, which was found to be statistically similar for both tap water and greywater treatments with values of 4.4 mg kg^{-1} to 7 mg kg^{-1}, was significantly higher for the nutrient treatment which had mean Mg tissue concentrations of 14 mg kg^{-1}.

Na uptake in leaf and stem tissue of *B. vulgaris* was found to be highest among the laundry powder generated-greywater treatments PG and CG, with concentration values exceeding 20×10^3 mg kg^{-1} dry mass. By comparison, mean uptake was found to be significant lower (11×10^3 mg kg^{-1} to 15×10^3 mg kg^{-1} dry mass) for nutrient solution-, liquid detergent-generated greywater- and tap water-irrigated treatments, among which Na uptake was found to be statistically similar.

Mean Zn uptake by greywater and tap water irrigated *B. vulgaris* (25 mg kg^{-1} to 38 mg kg^{-1}) were found to be significantly lower than for the nutrient solution irrigated treatment (±69 mg kg^{-1}). The influence of treatment on the uptake of Cu was found to be negligible, with all treatments attaining Cu tissue concentrations of < 10 mg kg^{-1}.

The concentration of Mn in *B. vulgaris* leaf tissue was found to vary among treatments; significantly higher tissue uptake was noted for individuals from the nutrient treatment (589 mg kg^{-1}) relative to either of the laundry powder-derived greywater treatments (251 mg kg^{-1} to 278 mg kg^{-1}) and was also marginally higher than that of LG- or tap water-irrigated *B. vulgaris*. Leaf tissue concentrations of Fe and Al within treatments were again found to be similar in trends. Among treatments, no statistically significant differences in leaf tissue uptake were noted for either of these micronutrients.

Despite differences noted in the concentration of B in the chemistry of irrigation treatments, B uptake in *B. vulgaris* leaf tissue was found not to differ significantly among treatments.

Table 32. Nutrient concentrations established for *B. vulgaris* leaves post-harvest among the five irrigation treatments at the University experiment site (mean ± SD). Letters represent mean separation among treatments by Scheffe's multiple range test ($P < 0.05$; $n = 5$ per treatment).

Element	Unit	Nutrient Solution	Phosphate Powder	Liquid Detergent	Carbonate Powder	Tap Water
N	mg kg^{-1}	**35.5 ± 6.56^{a}**	**11.9 ± 1.93^{b}**	**13.5 ± 2.01^{b}**	**17.4 ± 7.11^{b}**	**13.2 ± 1.63^{b}**
P	mg kg^{-1}	7.61 ± 1.33^{d}	11.1 ± 1.92^{c}	6.35 ± 0.56$^{d/e}$	4.48 ± 1.14^{e}	6.58 ± 1.17$^{d/e}$
K	mg kg^{-1}	89.7 ± 14.2^{f}	49.0 ± 5.52^{g}	51.6 ± 6.42^{g}	48.5 ± 14.1^{g}	64.2 ± 7.11^{g}
Ca	mg kg^{-1}	10.1 ± 1.25^{h}	10.2 ± 1.38^{h}	19.2 ± 5.5^{h}	18.7 ± 16.4^{h}	18.7 ± 2.24^{h}
Mg	mg kg^{-1}	**14.3 ± 2.43^{i}**	**4.48 ± 1.27^{j}**	**6.10 ± 2.18^{j}**	**6.93 ± 3.89^{j}**	**7.08 ± 0.99^{j}**
Na	mg kg^{-1} $\times 10^{3}$	**14.6 ± 1.68^{k}**	**21.0 ± 1.17^{l}**	**14.3 ± 3.30^{k}**	**21.1 ± 4.71^{l}**	**11.1 ± 1.67^{k}**
Zn	mg kg^{-1}	**69.3 ± 18.1^{m}**	**25.0 ± 5.99^{n}**	**32.9 ± 9.56^{n}**	**38.0 ± 28.3$^{m/n}$**	**31.7 ± 10.6^{n}**
Mn	mg kg^{-1}	**588 ± 163^{o}**	**251 ± 88.3^{p}**	**490 ± 84.3$^{o/p}$**	**278 ± 99.5^{p}**	**449 ± 126$^{o/p}$**
Cu	mg kg^{-1}	3.87 ± 2.10^{q}	3.04 ± 1.98^{q}	6.96 ± 1.77^{q}	6.54 ± 3.83^{q}	6.97 ± 1.90^{q}
Fe	mg kg^{-1}	444 ± 117^{r}	425 ± 133^{r}	1243 ± 733^{r}	692 ± 486^{r}	571 ± 202^{r}
Al	mg kg^{-1}	432 ± 109^{s}	393 ± 103^{s}	1032 ± 618^{s}	685 ± 497^{s}	453 ± 167^{s}
B	mg kg^{-1}	74 ± 3.7^{t}	47 ± 7.1^{lt}	71 ± 16.9^{t}	16.9 ± 21.3^{t}	50 ± 3.85^{t}

3.5.11. Leaf Tissue Nutrients: C. annuum

Mean concentrations of the nutrient uptake among *C. annuum* leaves are shown in Table 33. The uptake of the elements P, K, Ca, Zn and Cu by leaves was found to be statistically similar among all treatments investigated. However, mean N concentration of 50.6 mg kg^{-1} among nutrient solution-irrigated *C. annuum* leaves was found to be significantly highest among treatments; mean values of greywater and tap water treatments ranged from 21.4 mg kg^{-1} to 31.1 mg kg^{-1} and were found to be statistically similar.

Among greywater treatments, Mg leaf tissue concentrations were significantly lower than that found for nutrient and tap water treatments. By contrast, mean leaf tissue concentrations of Na

was found to be significantly highest among the PG and CG irrigation treatments, with mean values of 7.1×10^3 mg kg^{-1} and 7.2×10^3 mg kg^{-1} respectively. Significantly lower mean Na concentrations were established for both nutrient and tap water treatments, with respective concentrations of 3.2×10^3 mg kg^{-1} and 3.8×10^3 mg kg^{-1}. Liquid detergent greywater treated (LG) soils had Na values intermediate among treatments.

The influence of treatment on Zn uptake in leaf tissue was negligible. However, relative to greywater-irrigated *C. annuum*, mean Cu concentrations in individuals from the nutrient solution treatment were significantly lower (7.0 mg kg^{-1}) but significantly higher for individuals irrigated with tap water (20.6 mg kg^{-1}). No statistically significant differences were found in leaf tissue concentrations of either Mn or Fe among treatments post-harvest. Leaf tissue concentrations of Al however were found to vary among treatments. LG- and CG-irrigated *C. annuum* reflected the highest significant leaf tissue concentrations of Al, with mean values of 2.3×10^3 mg kg^{-1} and 2.4×10^3 mg kg^{-1} respectively, and were marginally higher than those of the PG and tap water treatments. Treatment with nutrient solution resulted in the lowest Al leaf tissue concentrations, attaining a mean value of 4.8×10^2 mg kg^{-1}.

The concentrations of leaf B in *C. annuum* among treatments were found to be quite variable. Individuals irrigated with the liquid detergent generated greywater LG had the highest concentration of B in leaves, with a mean value of 171 mg kg^{-1}, significantly higher than for individuals irrigated with the PG and CG powder detergent greywater formulations (80 mg kg^{-1} and 84 mg kg^{-1} respectively) and experimental controls. Statistical differences in leaf B concentrations between individuals irrigated with tap water and powder formulations were found to be marginal.

Table 33. Nutrient concentrations of established for *C. annuum* leaves post-harvest among the five irrigation treatments at the University experiment site (mean ± SD). Letters represent mean separation among treatments by Scheffe's multiple range test ($P < 0.05$; $n = 5$ per treatment).

Element	Unit	Nutrient Solution	Phosphate Powder	Liquid Detergent	Carbonate Powder	Tap Water
N	**mg kg^{-1}**	**50.6 ± 4.36^{a}**	**25.3 ± 5.23^{b}**	**26.0 ± 6.69^{b}**	**21.4 ± 1.73^{b}**	**31.1 ± 3.54^{b}**
P	mg kg^{-1}	4.88 ± 1.11^{c}	8.30 ± 2.50^{c}	5.49 ± 3.06^{c}	5.29 ± 0.66^{c}	7.99 ± 0.50^{c}
K	mg kg^{-1}	61.5 ± 45.3^{d}	45.3 ± 11.2^{d}	36.1 ± 13.2^{d}	40.1 ± 16.0^{d}	46.7 ± 17.4^{d}
Ca	mg kg^{-1}	36.6 ± 6.64^{e}	38.5 ± 6.73^{e}	40.9 ± 5.63^{e}	38.0 ± 9.43^{e}	48.2 ± 3.72^{e}
Mg	**mg kg^{-1}**	**11.5 ± 0.62^{f}**	**8.31 ± 1.64^{g}**	**8.21 ± 1.18^{g}**	**7.20 ± 2.13^{g}**	**9.49 ± 1.04$^{f/g}$**
Na	**mg kg^{-1}**	**3188 ± 719^{h}**	**7100 ± 1334^{i}**	**4473 ± 1008$^{h/i}$**	**7225 ± 2249^{i}**	**3848 ± 647^{h}**
Zn	mg kg^{-1}	50.2 ± 8.03^{j}	64.4 ± 23.1^{j}	66.0 ± 21.5^{j}	62.9 ± 11.9^{j}	82.8 ± 14.5^{j}
Cu	mg kg^{-1}	7.01 ± 2.13^{k}	13.6 ± 4.02$^{k/l}$	15.1 ± 3.46$^{k/l}$	12.1 ± 2.58$^{k/l}$	20.6 ± 6.39^{k}
Mn	mg kg^{-1}	192 ± 96.2^{m}	187 ± 29.9^{m}	245 ± 76.1^{m}	184 ± 51.4^{m}	120 ± 7.48^{m}
Fe	mg kg^{-1}	637 ± 243^{n}	1739 ± 931^{n}	3097 ± 1617^{n}	3430 ± 2343^{n}	1399 ± 560^{n}
Al	mg kg^{-1}	478 ± 217^{o}	1223 ± 585$^{o/p}$	2341 ± 1066^{p}	2440 ± 1441^{p}	1148 ± 411$^{o/p}$
B	mg kg^{-1}	56.8 ± 11.5^{q}	86.6 ± 13.7$^{q/r}$	171 ± 25.0^{s}	80.4 ± 24.4$^{q/r}$	104 ± 4.34^{r}

3.5.12. Flowers: C. annuum

Cumulative numbers of flowers at anthesis recorded daily among treatments are shown in Fig. 46. All treatments had flowers entering anthesis at a similar stage of 32 to 38 days after planting (DAP) and increased similarly until 53 DAP at which point flowering production behaviour was found to differ significantly among treatments. Initially, CG-irrigated *C. annuum* produced mature flowers at the greatest rate until 71 DAP at which point production of flowers reaching anthesis ceased. Among greywater treatments, LG-irrigated *C. annuum* produced the least flowers, and did not produce any flowers reaching anthesis from 71 DAP onwards. By comparison, mature flower production for the PG and tap water-irrigated *C. annuum* was more rapid and continued slightly longer, ceasing at 73 DAP. Nutrient-irrigated individuals followed a similar rate of mature flower production relative to the other

treatments, but resumed a second phase of robust flower production beyond 91 DAP with a logistical increase in flower numbers attaining anthesis. Although less pronounced by comparison, a phase of resumed flower production in PG-irrigated treatments also occurred towards the end of experimentation.

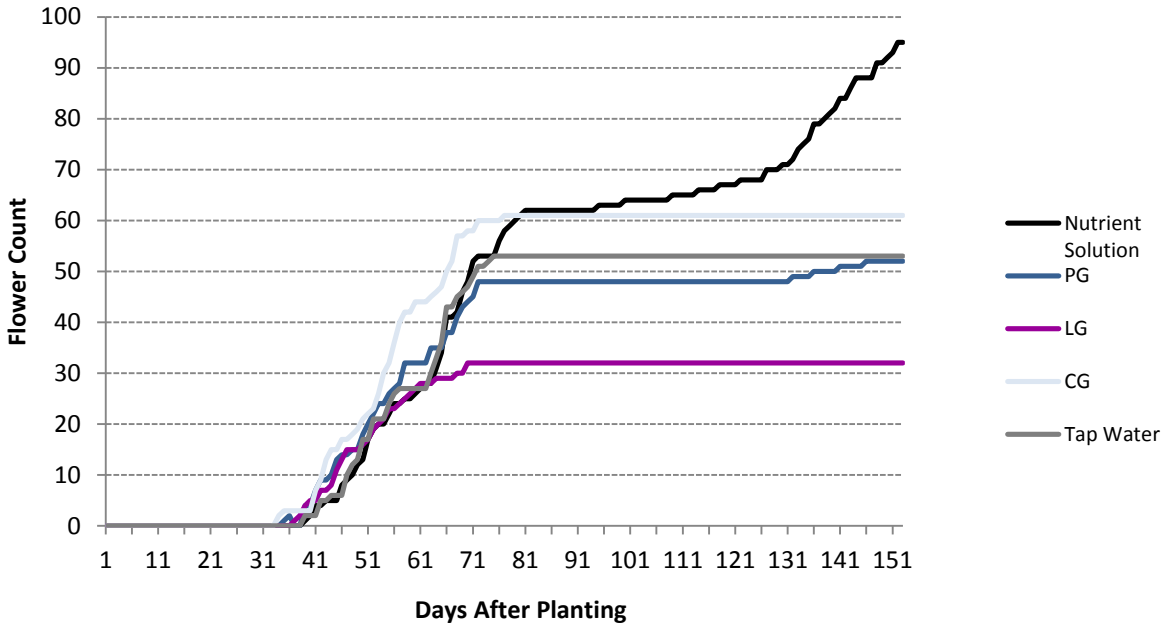

Fig. 46. Cumulative number of mature flowers observed from planting to harvest among *C. annuum* treatments at the University experiment site (*n* = 9 individuals per treatment). Treatments: "Nutrient solution" = irrigated with nutrient solution; "PG" = irrigated with phosphate base powder-derived greywater; "LG" = irrigated with liquid laundry detergent-derived greywater; "CG" = irrigated with carbonate base powder-derived greywater; "Tap water" = irrigated with municipal tap water supply.

Individuals from all irrigation treatments initiated fruit ripening all within a narrow band of 117 to 127 DAP and increasing in a step-wise fashion until 146 DAP when cumulative fruit production generally remained static between 10 and 16 fruit per treatment (Fig. 47). Differences among treatments were not marked by this assessment, except for individuals irrigated with nutrient solution which had a very strong second phase of fruit production shortly before experimentation was terminated.

A general index of plant stress, the ratio between the number of mature flowers produced and harvestable fruit yielded, is shown for all treatments in Fig. 48. A high ratio of flowers to fruit is generally suggestive of physiologically stressed individuals, whilst the converse typically holds for healthy plants. Considerably higher numbers of mature flowers for CG-irrigated *C. annuum* were produced relative to fruit resulting in a significantly higher mean flower : fruit

ratio of 5.8 : 1.0 among treatments. Flowers reaching anthesis on nutrient solution-irrigated *C. annuum* were statistically more likely to produce viable fruit than other treatments, with a mean flower : fruit ratio of 2.7 : 1.0. Intermediate mean ratio values were found for the remaining treatments which were found to be statistically similar.

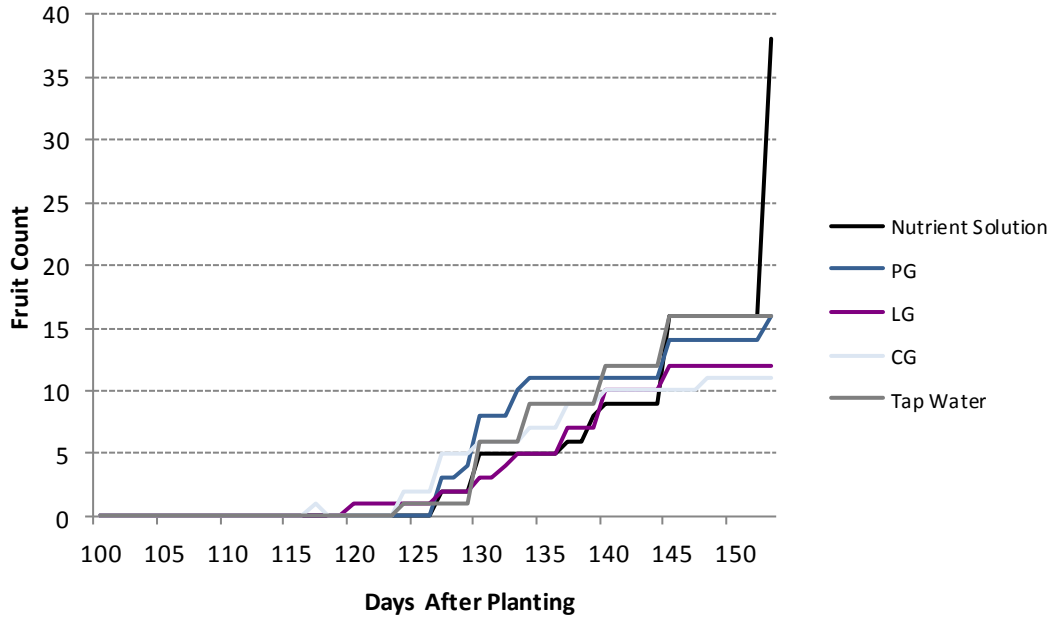

Fig. 47. Cumulative number of ripe *C. annuum* fruit harvested from experimental treatments at the University experiment site (*n* = 9 individuals per treatment). Treatments: "Nutrient solution" = irrigated with nutrient solution; "PG" = irrigated with phosphate base powder-derived greywater; "LG" = irrigated with liquid laundry detergent-derived greywater; "CG" = irrigated with carbonate base powder-derived greywater; "Tap water" = irrigated with municipal tap water supply.

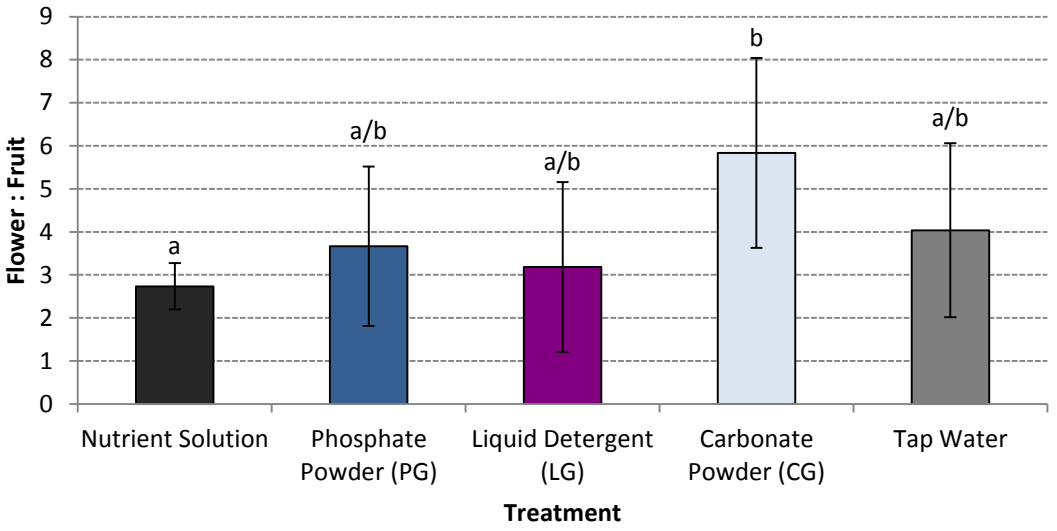

Fig. 48. Mean ratio of mature flowers to harvested fruit recorded for *C. annuum* among treatments at the University experiment site. Vertical bars represent standard deviations. Letters represent mean separation among treatments by Scheffe's multiple range test ($P < 0.05$; $n = 9$ plants per treatment).

As shown in Fig. 49, relative to other treatments, significantly higher flowers counts at anthesis were found for nutrient solution-irrigated *C. annuum* with mean flower numbers per individual of 12.6. By contrast, greywater and tap water treatments were found to have statistically similar mean values of 3.6 to 6.8 flowers per individual. Higher flower numbers recorded per individual for nutrient solution-irrigated *C. annuum* translated in the greatest fruit numbers produced among treatments, averaging 4.7 fruit per plant. Consistent with fewer flower buds reaching anthesis among greywater and tap water-irrigated *C. annuum,* fruit production was also found to be statistically similar, with mean fruit produced per plant between 1.2 and 1.8 for these treatments.

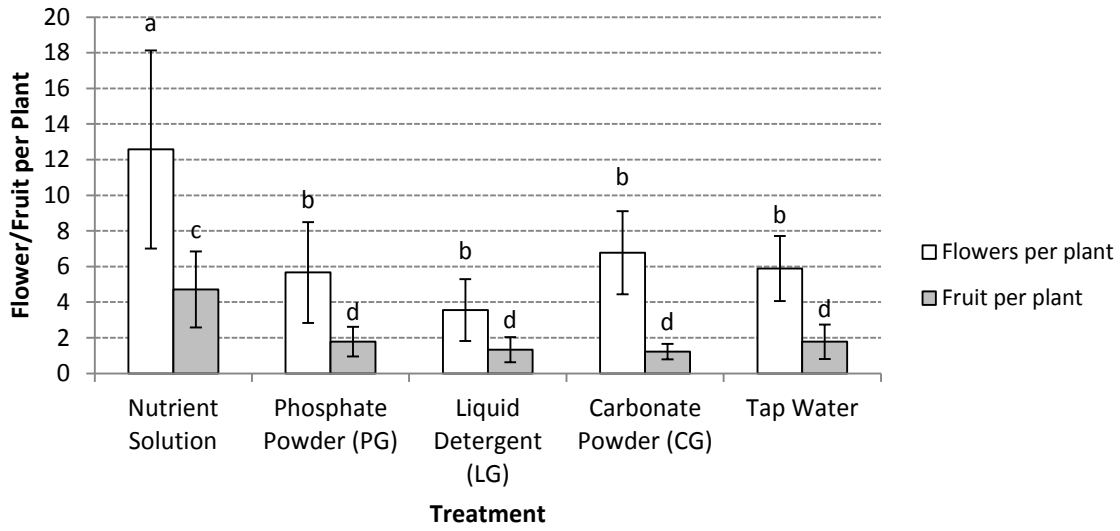

Fig. 49. Mean number of flowers and fruit produced by *C. annuum* individuals among treatments at the University experiment site. Vertical bars represent standard deviations. Letters represent mean separation among treatments by Scheffe's multiple range test ($P < 0.05$; $n = 9$ per treatment).

3.5.13. Fruit Morphology: C. annuum

Irrigation of *C. annuum* with either greywater or tap water resulted in statistically similar yields of dry fruit biomass produced per individual with mean production ranging from 4.7 g per plant to 8.1 g per plant (Fig. 50). By contrast, nutrient solution-irrigated *C. annuum* responded more positively to irrigation treatment, resulting in significantly higher mean dry fruit biomass production of 24.6 g per individual.

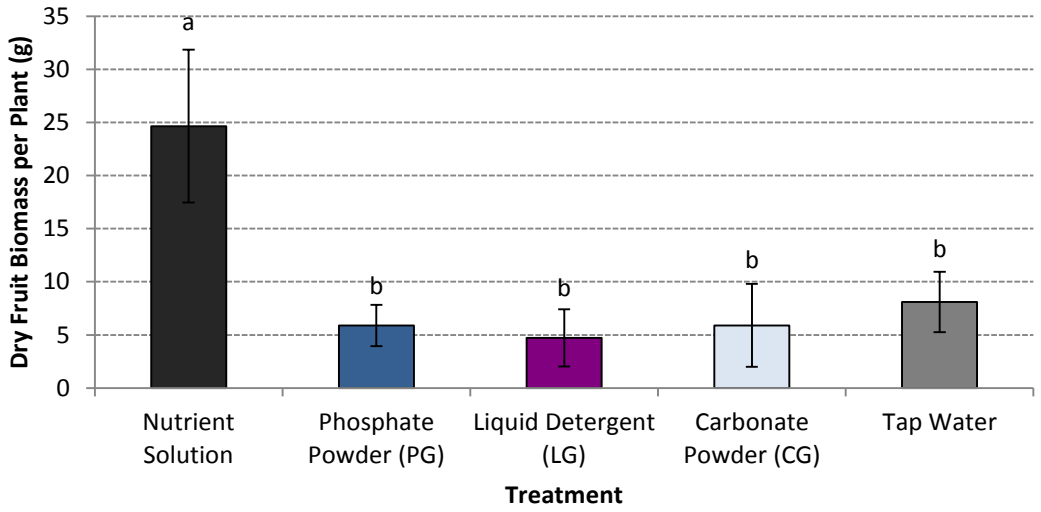

Fig. 50. Mean dry fruit biomass of *C. annuum* individuals recorded from the five irrigation treatments at the University experiment site. Vertical bars represent standard deviations. Letters represent mean separation among treatments by Scheffe's multiple range test ($P < 0.05$; $n = 9$ plants per treatment).

The nutrient-irrigated treatment produced *C. annuum* fruits significantly greater in length, whilst the irrigation with the phosphate based-powder (PG) greywater and liquid detergent-derived (LG) greywater were not conducive to the development of comparatively elongated fruit (Table 34). Amongst treatments however, carbonate powder (CG) greywater-irrigated plants produced fruits that were only marginally intermediate in length from either nutrient- or tap water-irrigated treatments and other greywater treatments.

The influence of irrigation treatment on equatorial diameters of harvested fruit are shown in Table 34. Greywater-irrigated *C. annuum* supported the development of narrower diameter fruit, whilst nutrient solution promoted the development of broader fruit. Fruit produced from tap water treatments were neither larger nor smaller in diameter relative to other treatments.

The influence of treatment on fruit shape as a general measure of fruit quality was evaluated by exploring the relationship between the polar axis lengths and related equatorial diameters of harvested fruit among treatments (Table 34). Despite the apparent differences among treatments with respect to polar axis and equatorial lengths noted previously, an overall mean ratio factor of > 1 for fruits was found among all treatment groups, suggesting fruits to be marginally oblong in shape. However, variability in individual fruit shapes meant fruits ranged from longitudinally oblong to latitudinally oblong as reflected by SDs for each treatment extended both above and below 1.

As shown in Table 34, nutrient solution irrigation was found to positively influence the development of fruit pericarp, with the highest overall mean among treatments of 5.75 mm. By comparison, a significant mean reduction (36.2 %) in pericarp thicknesses were found among fruit irrigated with phosphate-based laundry powder PG, with a mean value of 4.22 mm. Tap water, liquid detergent LG- and carbonate-based powder CG-irrigated treatments yielded similar pericarp thickness, intermediate between those from the PG and nutrient treatment.

Table 34. Morphological characteristics of ripe *C. annuum* from the five irrigation treatments at the University experiment site (mean ± SD). Letters represent mean separation among treatments by Scheffe's multiple range test ($P< 0.05$).

Parameter	Unit	Nutrient Solution	Phosphate Powder	Liquid Detergent	Carbonate Powder	Tap Water
Polar Axis Length	mm	70.5 ± 13.1^{a}	54.6 ± 14.9^{b}	52.2 ± 16.1^{b}	$58.2 \pm 7.71^{a/b}$	$54.8 \pm 15.1^{a/b}$
Equatorial Ø	mm	61.8 ± 5.4^{c}	48.5 ± 11.3^{d}	47.6 ± 8.2^{d}	48.4 ± 7.3^{d}	$50.2 \pm 10.8^{c/d}$
Polar Axis: Equatorial Ø (FSR)	-	1.15 ± 0.24^{e}	1.16 ± 0.31^{e}	1.08 ± 0.25^{e}	1.22 ± 0.17^{e}	1.09 ± 0.17^{e}
Pericarp Thickness	mm	5.8 ± 1.4^{f}	4.2 ± 1.2^{g}	$4.5 \pm 1.0^{f/g}$	$4.7 \pm 1.3^{f/g}$	$4.6 \pm 1.3^{f/g}$

3.5.14. Harvestable Yields: C. annuum

Projected yields on a hectare basis for greywater-irrigated treatments were similar, with yields of *C. annuum* fruits ranging from 13.3 and 12.3 metric tons per hectare (Fig. 51). By comparison, the tap water-irrigated treatment, functioning as the experimental negative control, would be expected to produce an improved yield of 20.9 metric tons per hectare under the experimental conditions. A more than two-fold superior yield could be expected from the nutrient-irrigated treatment, with over 46 metric tons of fruit produced per hectare.

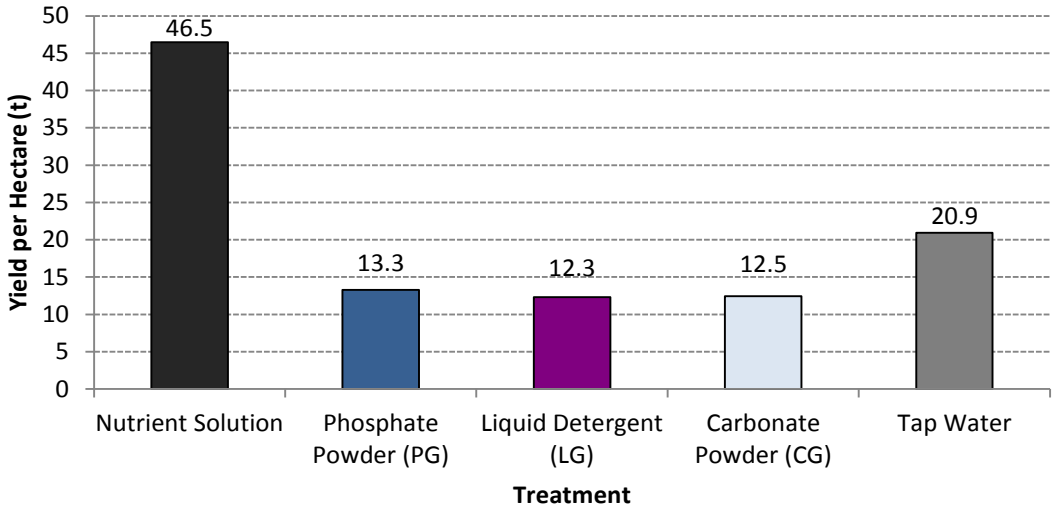

Fig. 51. Theoretical mean yield per hectare of fresh edible biomass of *C. annuum* (ripe fruit) for the five irrigation treatments at the University experiment site (n = 9 plants per treatment).

3.5.15. Fruit Tissue Nutrients: C. annuum

The uptake of nutrients in C. annuum fruit among treatments are shown in Table 35. Irrigation treatment had no significant effect on the fruit tissue concentrations of the macronutrients P and K. However, *C. annuum* individuals fertilized with the nutrient solution had mean fruit N concentrations of 29.8 mg kg^{-1}, a mean value more than six fold greater than that of the remaining treatments, the latter which were found to be statistically similar.

Irrigation with the greywater had no significant effect among these treatments on the fruit tissue concentrations of either Ca or Mg.

The Na response to greywater treatment in *C. annuum* fruit was found to vary significantly. Relative to other treatments, the powder-based detergents PG- and CG- generated greywater caused a significant increase in Na recorded with mean values of > 2100 mg kg^{-1} found for each. By contrast, *C. annuum* irrigated with the liquid detergent-derived greywater, LG, yielded fruit for which tissue concentrations of Na were statistically similar to that found for both the tap water and nutrient solution treatments.

The fruit tissues concentrations of Cu and Mn were not affected by any of the irrigation treatments evaluated. Similarly, all greywater treatments had a negligible effect relative to irrigation with tap water on the fruit tissue concentrations of Zn for which the means ranged from 10.1 mg kg^{-1} to 12.6 mg kg^{-1}, whilst the irrigation of *C. annuum* with the nutrient solution resulted in a significantly elevated mean Zn concentration of 16.2 mg kg^{-1}.

The irrigation of *C. annuum* with the different greywater treatments yielded Fe and Al concentrations in fruit that were statistically comparable to those fruit tissue concentrations of Fe and Al obtained for individuals irrigated with the nutrient solution and tap water.

Greywater type was found to result in varied fruit tissue concentrations of B in *C. annuum*. Most notably, among greywater and the experimental controls, individuals irrigated with the liquid detergent LG had significantly higher levels of B recorded, with a mean value of 21.6 mg kg^{-1}. Values obtained for the CG greywater treatment were found to be statistically similar to those reported for fruit harvested from the tap water treatment and were intermediate among greywater treatments. Fruit tissue concentrations of B for the PG treatment was the lowest among greywater treatments with a mean value of 14.8 mg kg^{-1} and statistically similar to B concentrations in fruit tissue found for fruit harvested from the nutrient solution treatment.

Table 35. Fruit tissues concentrations among the five irrigation treatments established for *C. annuum* fruit collected post-harvest at the University experiment site (mean ± SD). Letters represent mean separation among treatments by Scheffe's multiple range test ($P < 0.05$; $n = 5$ per treatment).

Element	Unit	Nutrient Solution	Phosphate Powder	Liquid Detergent	Carbonate Powder	Tap Water
N	**mg kg^{-1}**	**29.9 ± 3.85^{a}**	**15.2 ± 0.45^{b}**	**16.9 ± 1.72^{b}**	**15.8 ± 0.50^{b}**	**14.9 ± 1.99^{b}**
P	mg kg^{-1}	4.51 ± 0.49^{c}	3.68 ± 0.16^{c}	3.14 ± 1.05^{c}	3.49 ± 0.29^{c}	3.67 ± 0.55^{c}
K	mg kg^{-1}	32.7 ± 4.31^{d}	30.6 ± 2.16^{d}	30.9 ± 2.00^{d}	33.0 ± 0.51^{d}	29.4 ± 3.59^{d}
Ca	mg kg^{-1}	1.36 ± 0.36^{e}	1.08 ± 0.24^{e}	1.25 ± 0.48^{e}	1.29 ± 0.30^{e}	1.84 ± 0.51^{e}
Mg	mg kg^{-1}	1.75 ± 0.18^{f}	1.57 ± 0.09^{f}	1.53 ± 0.25^{f}	1.61 ± 0.19^{f}	1.59 ± 0.22^{f}
Na	mg kg^{-1}	1315 ± 173^{g}	2137 ± 389^{h}	877 ± 117^{g}	2249 ± 281^{h}	823 ± 72.9^{g}
Zn	mg kg^{-1}	**16.2 ± 4.53^{i}**	**10.4 ± 1.11$^{i/j}$**	**10.1 ± 3.11^{j}**	**11.5 ± 2.11$^{i/j}$**	**12.6 ± 2.49$^{i/j}$**
Cu	mg kg^{-1}	4.98 ± 3.24^{k}	1.05 ± 0.70^{k}	1.77 ± 2.19^{k}	2.28 ± 1.76^{k}	3.39 ± 2.04^{k}
Mn	mg kg^{-1}	18.3 ± 6.91^{l}	13.9 ± 0.96^{l}	13.1 ± 2.50^{l}	14.7 ± 1.97^{l}	11.7 ± 2.46^{l}
Fe	mg kg^{-1}	83.9 ± 52.0^{m}	11.4 ± 8.63^{m}	33.9 ± 36.1^{m}	24.4 ± 18.7^{m}	41.0 ± 18.3^{m}
Al	mg kg^{-1}	88.2 ± 40.8^{n}	307 ± 572^{n}	202 ± 327^{n}	41.1 ± 28.7^{n}	48.2 ± 13.1^{n}
B	**mg kg^{-1}**	**14.0 ± 2.00^{o}**	**14.80 ± 2.28^{o}**	**21.6 ± 2.97^{p}**	**16.5 ± 1.91$^{o/p}$**	**18.4 ± 4.34$^{o/p}$**

3.6. Soils: University Site, Glenwood

3.6.1. Particle Size: B. vulgaris

Proportional soil structural composition among *B. vulgaris* treatments following the completion of one growth cycle, is shown in Fig. 52 below. Among all treatments, Berea red soils were found to constitute more than 80 % of coarse silt and sand, approximately 15 % of clay and the remainder fine silt.

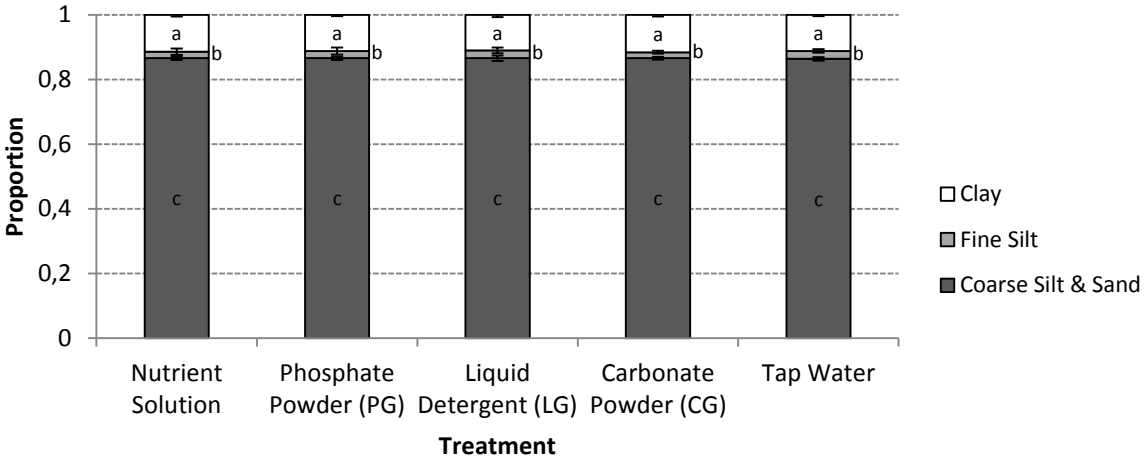

Fig. 52. Mean soil particle distributions among experimental soils in which *B. vulgaris* were grown at the University experiment site. Vertical bars represent standard deviations. Letters represent mean separation among treatments by Scheffe's multiple range test ($P < 0.05$; $n = 5$ per treatment).

3.6.2. Particle Size: C. annuum

As shown in Fig. 53, Berea Red soils in which *C. annuum* were grown were characterised by high proportions of coarse silt and sand, and relatively low levels of clay and fine silt. The clay and fine silt compositions of experimental soils were found to be statistically similar but differed significantly in the proportion of coarse silt and sand contents. Significantly higher proportion of coarse silt and sand was found for LG treated soils relative to other treatments, but was significantly lowest for nutrient solution treated soils. PG, CG and tap water-treated soils were found to have intermediary proportions of coarse silt and sand relative to other treatments.

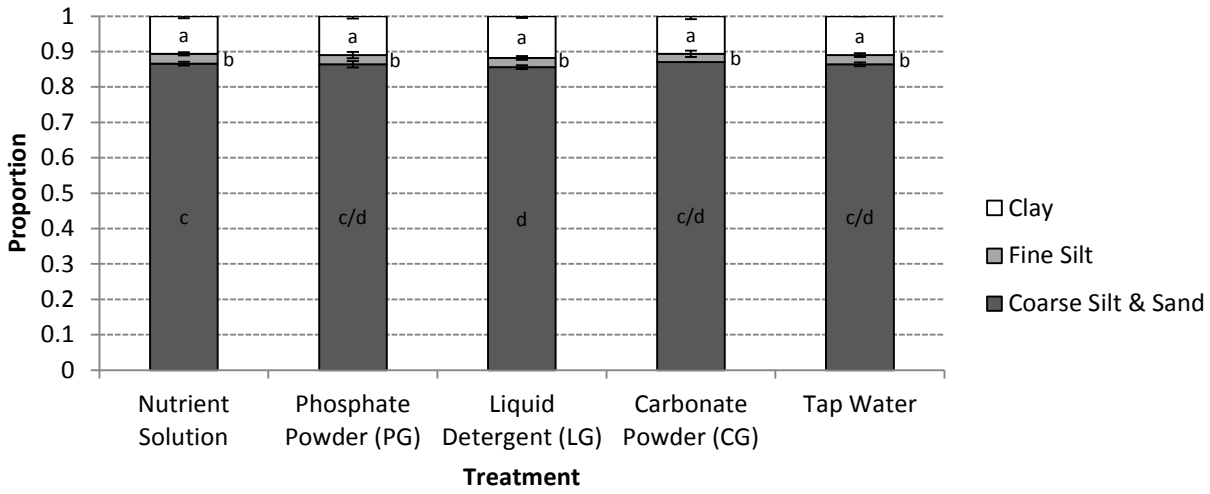

Fig. 53. Mean soil particle distributions among experimental soils in which *C. annuum* were grown at the University experiment site. Vertical bars represent standard deviations. Letters represent mean separation among treatments by Scheffe's multiple range test ($P < 0.05$; $n = 5$ per treatment).

3.6.3. pH: B. vulgaris

Mean soil pH values of *B. vulgaris* soils recorded weekly in the field were found to generally decrease over the 14 week experimental period for all treatments (Fig. 54). As with soil pH trends noted for *B. vulgaris* at the Unilever experimental site, soil pH values among treatments were found to follow similar trend oscillations relative to each other; tap water and greywater irrigated-treatments in particular followed very close trend oscillations in pH throughout the experimental period whilst nutrient-irrigated soil pH values deviated slightly from this central trend after 8 weeks.

Soil pH among all treatments measured *ex-situ* found soils ranged from weakly to mildly alkaline over the experimental period (Fig. 55). Oscillation trends of soil pH values among treatments were found to generally fluctuate in phase, although 10 weeks into the irrigation regime, pH values appeared to deviate with a slight increase in soil acidity occurring in LG- and nutrient- irrigated treatments and an increase in soil alkalinity among tap water-, CG- and PG-irrigated treatments; one-way ANOVA among treatments at the end of experimentation reflected the order: CG > Tap water; PG; LG > Nutrient.

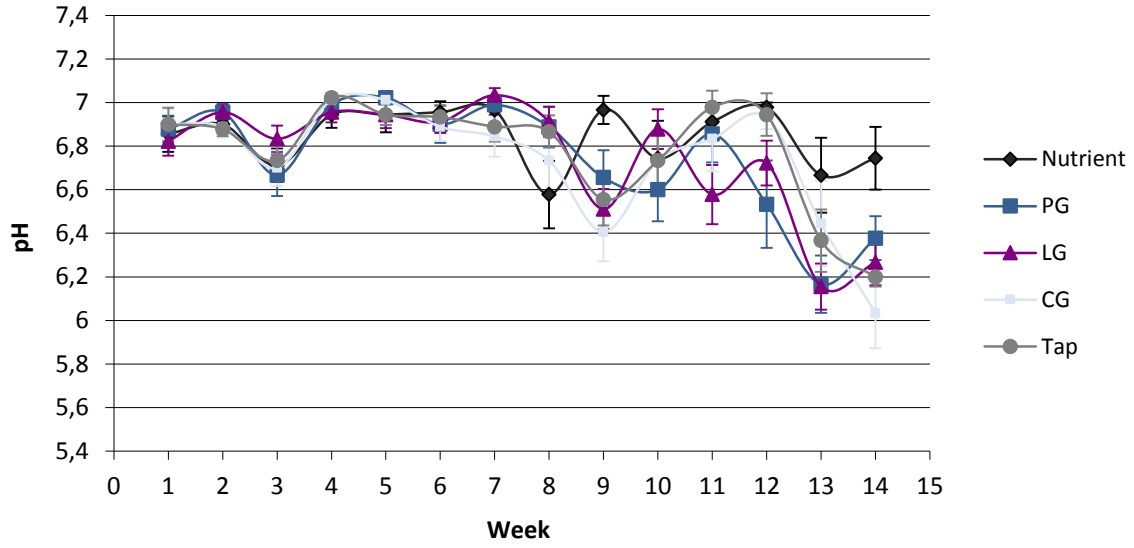

Fig. 54. Weekly mean soil pH values among *B. vulgaris* treatments measured in the field for growth cycle 1 at the University experiment site (n = 9). Treatments: "Nutrient solution" = irrigated with nutrient solution; "PG" = irrigated with phosphate base powder-derived greywater; "LG" = irrigated with liquid laundry detergent-derived greywater; "CG" = irrigated with carbonate base powder-derived greywater; "Tap water" = irrigated with municipal tap water supply. Vertical bars depict standard errors about sample means

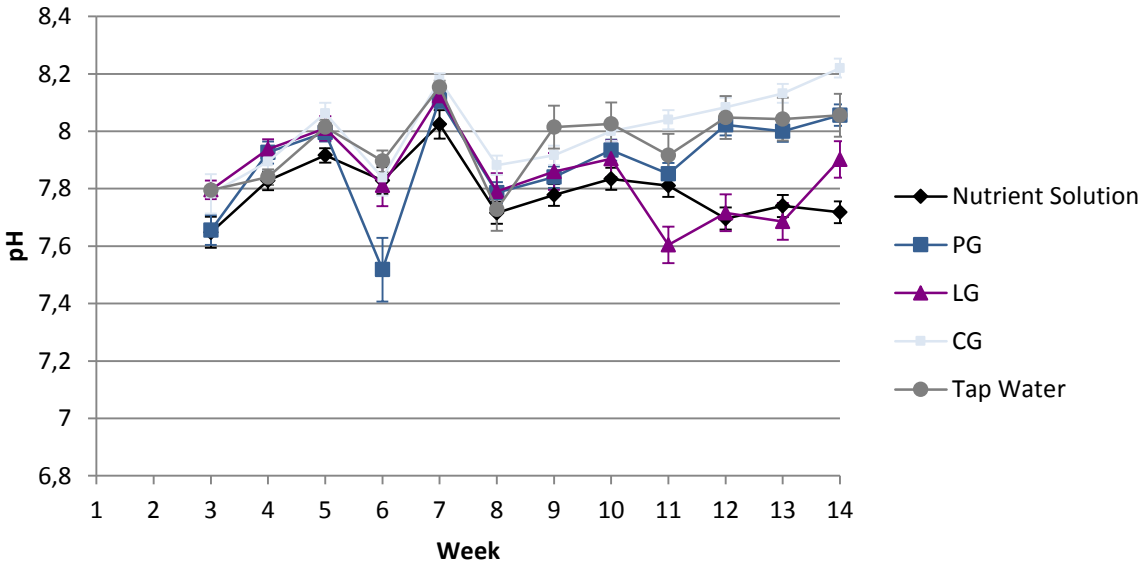

Fig. 55. Weekly mean soil pH values among *B. vulgaris* treatments recorded *ex-situ* for growth cycle 1 at the University experimental site (n = 5). Treatments: "Nutrient solution" = irrigated with nutrient solution; "PG" = irrigated with phosphate base powder-derived greywater; "LG" = irrigated with liquid laundry detergent-derived greywater; "CG" = irrigated with carbonate base powder-derived greywater; "Tap water" = irrigated with municipal tap water supply. Vertical bars depict standard errors about sample means.

3.6.4. pH: C. annuum

Remarkable similarities in soil pH trends recorded the field for *C. annuum* pots were noted among treatments throughout the experimental duration (Fig. 56), suggesting factors influencing soil pH other than treatment. Soil pH values recorded weekly were range-bound between 6.8 and 7 during the first 14 weeks of experimentation but in subsequent weeks experienced large fluctuations in value. At 15 weeks a rapid decrease in soil pH was observed for all treatments, where soils attained pH values of 5.9 to 6.2. This decrease was immediately followed by a rapid increase in soil pH for all treatment soils until original pH values were re-established at 17 weeks. Thereafter, pH values showed a rapid decrease to final values of 5.5 to 5.9 recorded at the time of harvest. However, trends recorded *in-situ* were found to contrast significantly with those established *ex-situ* (Fig. 57) where original soil pH values were found to be significantly higher and trends were found to vary considerably among treatment soils. The pH values of untreated soil samples were found to range from 7.9 to 8.0, reflecting a logarithmic value difference of ± 1 pH unit from that recorded *in-situ* on the same treatment soils. Values of soil pH among treatments recorded *ex-situ* showed a general decrease in pH for those soils to which LG greywater or nutrient solution were applied, with these soils attaining pH values of 7.4 to 8.5. By contrast, soil pH values measured *ex-situ* for soils to which PG, CG and tap water was applied reflected a general increase in soil pH, attaining final pH values of 7.9 to 8.5.

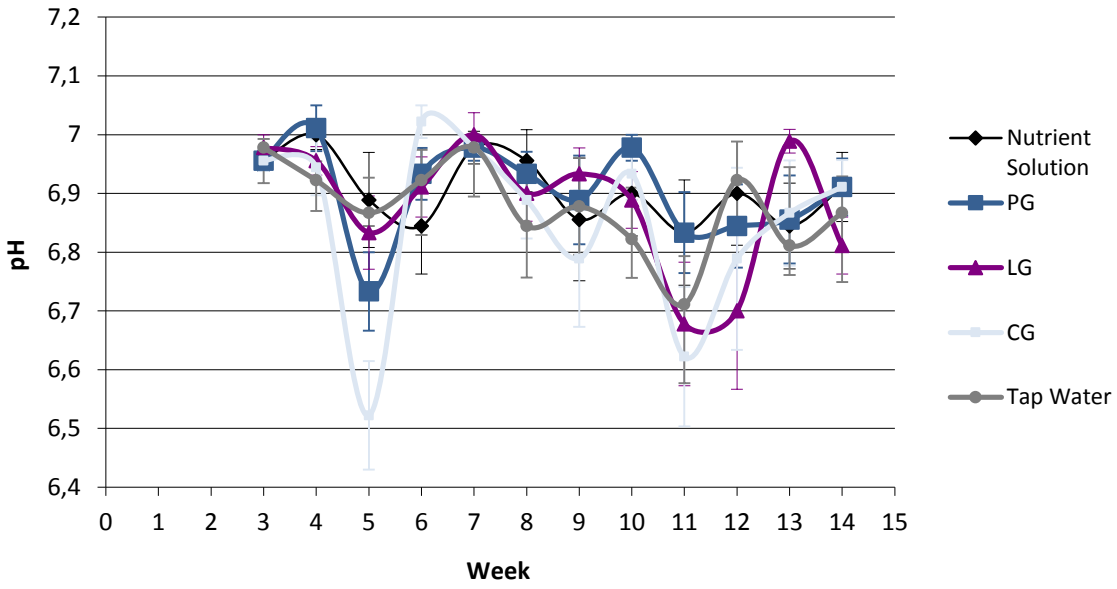

Fig. 56. Weekly mean soil pH values among *C. annuum* treatments measured in the field for growth cycle 1 at the University experimental site (n = 9). Treatments: "Nutrient solution" = irrigated with nutrient solution; "PG" = irrigated with phosphate base powder-derived greywater; "LG" = irrigated with liquid laundry detergent-derived greywater; "CG" = irrigated with carbonate base powder-derived greywater; "Tap water" = irrigated with municipal tap water supply. Vertical bars represent standard errors about sample means.

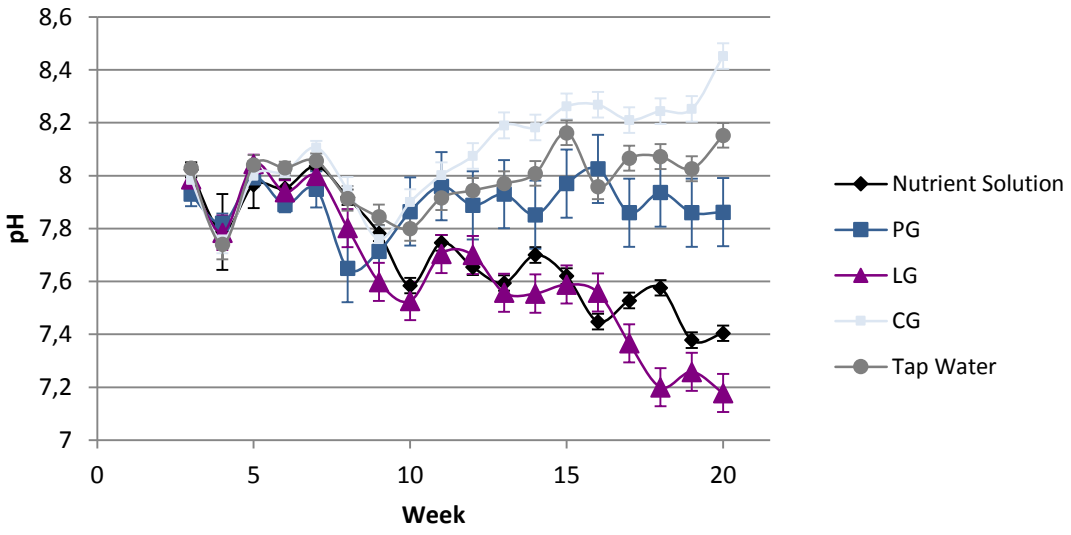

Fig. 57. Weekly mean soil pH values among *C. annuum* treatments recorded *ex-situ* for growth cycle 1 at the University experimental site (n = 5). Treatments: "Nutrient solution" = irrigated with nutrient solution; "PG" = irrigated with phosphate base powder-derived greywater; "LG" = irrigated with liquid laundry detergent-derived greywater; "CG" = irrigated with carbonate base powder-derived greywater; "Tap water" = irrigated with municipal tap water supply. Vertical bars represent standard errors about sample means.

3.6.5. EC: B. vulgaris

Soil EC values recorded weekly for *B. vulgaris* experimental pots (Fig. 58) were suggestive of irrigation treatment exerting significant influence on soil EC, and which become exaggerated with time as total treatment loading increased. Soil EC among treatments reflected similar oscillations for a given week but differed considerably in magnitude. Soils irrigated with nutrient solution maintained the highest EC values throughout the experiment and after 14 weeks the mean EC value of 1.03 mS m^{-1} attained was significantly higher than that of other treatments where values were < 0.5 mS m^{-1}. The application of laundry powder-derived greywater PG and CG to soils resulted in similar EC values throughout the experiment and which were intermediary relative to other treatments (0.34 mS m^{-1} after 14 weeks). Interestingly, EC values recorded for tap water and LG-irrigated soils remained very similar throughout the experimental period, and were both significantly lower than for other treatments after 14 weeks with values attained of 0.23 mS m^{-1} to 0.34 mS m^{-1} respectively.

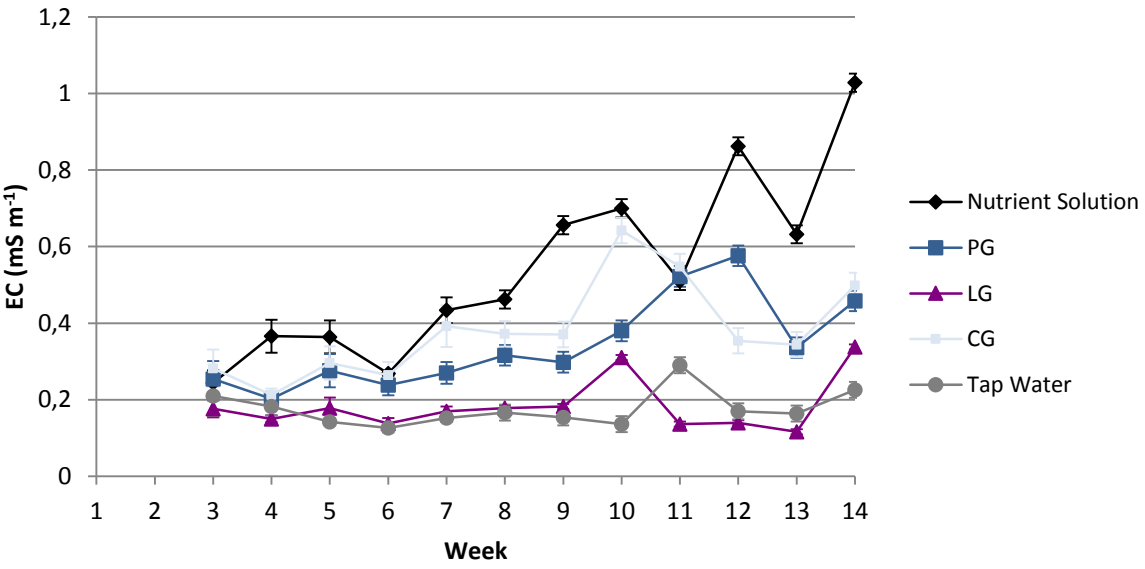

Fig. 58. Weekly mean soil EC values recorded among *B. vulgaris* treatments *ex-situ* for growth cycle 1 at the University experimental site (n = 5). Treatments: "Nutrient solution" = irrigated with nutrient solution; "PG" = irrigated with phosphate base powder-derived greywater; "LG" = irrigated with liquid laundry detergent-derived greywater; "CG" = irrigated with carbonate base powder-derived greywater; "Tap water" = irrigated with municipal tap water supply. Vertical bars represent standard errors about sample means.

3.6.6. EC: C. annuum

Soil EC was strongly influenced by irrigation treatment, resulting in relatively large variations in EC values noted generally among treatments (Fig. 59). Weekly soil EC values recorded

among tap water- and LG-treated soils were found to be remarkably similar, and interestingly both also reflected a slight decrease in soil EC measured over the experimental period despite cumulative treatment loadings. Soil EC values recorded for the nutrient treatment were found to increase significantly and remained highest among all treatments soils throughout experimentation. Similarly, a considerable increase in soil EC was also noted for CG-treated soils, and to a lesser extent a general increase was also noted for soils to which PG-greywater was applied.

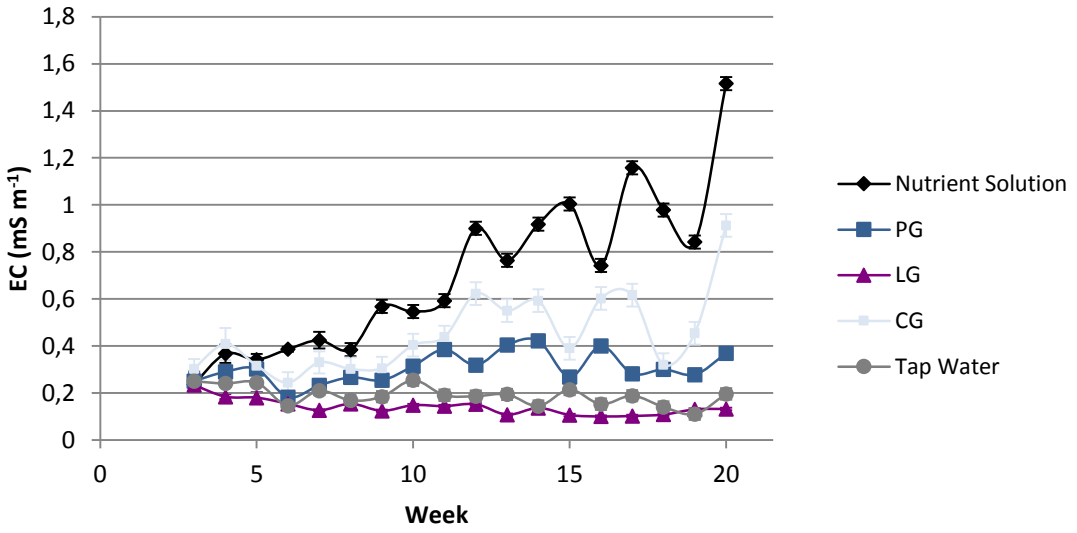

Fig. 59. Weekly mean soil EC values among *C. annuum* treatments recorded *ex-situ* for growth cycle 1 at the University experimental site (n = 5). Treatments: "Nutrient solution" = irrigated with nutrient solution; "PG" = irrigated with phosphate base powder-derived greywater; "LG" = irrigated with liquid laundry detergent-derived greywater; "CG" = irrigated with carbonate base powder-derived greywater; "Tap water" = irrigated with municipal tap water supply. Vertical bars represent standard errors about sample means.

3.6.7. Fertility: B. vulgaris

The soil concentrations of select macro- and micro- nutrients for *B. vulgaris* pots are shown in Table 36. It is particularly noteworthy that P levels in experimental soils were found to be statistically similar in soils irrigated with PG greywater and nutrient solution (75 mg kg^{-1} and 102 mg kg^{-1} respectively). By comparison, the remaining treatment soils all reflected significantly lower P values. The application of greywater to soil was found to result in soils with statistically similar exchangeable K values of 60 mg kg^{-1} to 77 mg kg^{-1} as compared to those soils to which only tap water was applied (62 mg kg^{-1}). Soils treated with nutrient solution had significantly elevated levels of exchangeable K (172 mg kg^{-1}) relative to all other treatments.

The concentration of Ca in soils was found to vary significantly among treatments. Soils treated with nutrient solution resulted in the highest significant levels (909 mg kg^{-1}) of Ca among treatments and lowest significant levels for PG-irrigated soils (735 mg kg^{-1}). Among the remaining treatments, the Ca concentration of soils was found to be intermediary. By contrast, irrigation treatment was found to have no significant effect on the concentration of Mg, Zn, Mn and Cu in soils.

Table 36. Soil element concentrations established for *B. vulgaris* soils post-harvest among the five irrigation treatments at the University experiment site. Vertical bars represent standard deviations. Letters represent mean separation among treatments by Scheffe's multiple range test ($P< 0.05$; $n = 5$ per treatment).

Element	Unit	Nutrient Solution	Phosphate Powder	Liquid Detergent	Carbonate Powder	Tap Water
P	mg kg^{-1}	75.2 ± 32.5^{a}	102 ± 11.0^{a}	32.2 ± 1.92^{b}	41.6 ± 3.78^{b}	35.2 ± 2.59^{b}
K	mg kg^{-1}	172 ± 38.8^{c}	59.8 ± 8.61^{d}	76.6 ± 12.3^{d}	67.2 ± 19.2^{d}	62.8 ± 11.7^{d}
Ca	mg kg^{-1}	909 ± 58.3^{e}	735 ± 57.7^{g}	$794 \pm 12.6^{f/g}$	$751 \pm 44.4^{f/g}$	$847 \pm 40.3^{e/f}$
Mg	mg kg^{-1}	72.4 ± 15.0^{h}	52.2 ± 14.2^{h}	47.6 ± 1.52^{h}	51.2 ± 21.0^{h}	49.6 ± 4.34^{h}
Zn	mg kg^{-1}	3.80 ± 0.58^{i}	4.80 ± 2.63^{j}	3.68 ± 0.18^{j}	$3.06 \pm 0.82^{i/j}$	3.66 ± 0.29^{j}
Cu	mg kg^{-1}	2.44 ± 0.55^{k}	2.20 ± 0.31^{k}	2.18 ± 0.08^{k}	2.16 ± 0.19^{k}	2.20 ± 0.07^{k}
Mn	mg kg^{-1}	7.60 ± 0.89^{l}	11.0 ± 3.94^{m}	$8.20 \pm 1.10^{l/m}$	7.40 ± 1.14^{l}	$7.00 \pm 0.00^{l/m}$

Statistical evaluation of the organic C and Total N values established from MIR spectroscopy for experimental treatment soils, found no significant differences (Table 37).

Table 37. Percentage organic C and N (mean ± SD) among *B. vulgaris* treatment soils at the University experiment site as determined by MIR spectroscopy. Letters represent mean separation among treatments by Scheffe's multiple range test ($P< 0.05$; $n = 5$ per treatment).

Element	No Treatment	Nutrient Solution	Phosphate Powder	Liquid Detergent	Carbonate Powder	Tap Water
Organic C (%)*	$< 0.5^{a}$	$< 0.5^{a}$	$< 0.5^{a}$	$< 0.5^{a}$	$< 0.5^{a}$	$< 0.5^{a}$
Total N (%)	0.058 ± 0.005^{b}	0.056 ± 0.005^{b}	0.063 ± 0.012^{b}	0.065 ± 0.01^{b}	0.055 ± 0.01^{b}	0.052 ± 0.004^{b}

* MIR organic C values for all treatments were beyond detection limit of instrumentation.

As shown in Table 38, soils in which *B. vulgaris* were grown to which the various treatments were added were nutrient poor, with almost all samples tested found to be deficient in the essential macronutrients of P and K relative to the known nutrient requirements of *B. vulgaris.* No PG-treated soils were found to be deficient in the macronutrient P, the only treatment for which this was found to be the case. In general, treatment soils investigated were also found to be severely deficient in K, with 80 % or more of the samples tested for each found to be in deficit.

Table 38. Percentage of soil samples from each treatment in which *B. vulgaris* were grown at the University experiment site found to be deficient in P and K (n = 5 per treatment).

% Deficit	No Treatment	Nutrient Solution	Phosphate Powder	Liquid Detergent	Carbonate Powder	Tap Water
P	100	40	0	80	100	100
K	100	80	100	80	100	100

3.6.8. Fertility: C. annuum

The macro- and micronutrient concentrations established for experimental soils in which *C. annuum* were grown are shown in Table 39. Among greywater treated soils, P concentration was found to be significantly higher in soils to which PG greywater was applied, attaining mean values of 82.4 mg kg^{-1} that was statistically similar to nutrient fertilized soils. Soils irrigated with tap water were found to have P concentrations statistically similar to that of LG and CG treated soils where means ranged from 33.2 mg kg^{-1} to 35.2 mg kg^{-1}. Greywater- and tap water-treated soils differ significantly in K concentration, with mean K concentrations of 53 mg kg^{-1} to 69 mg kg^{-1}, whereas nutrient solution-treated soils had a significantly higher soil concentration of K (165 mg kg^{-1}) among treatments.

Soils concentrations of either Ca or Mg were found not to differ significantly among treatments. Similarly, soil concentrations of Mn or Cu were found not to be influenced significantly by irrigation treatment. However, significant differences in concentrations of Zn among treatments was evident (Table 39). Soils to which CG greywater was applied had significantly lower mean Zn concentrations (3.50 mg kg^{-1}) relative to all other treatments, and nutrient-treated soils reflected the highest Zn concentration among treatments (4.32 mg kg^{-1}). By comparison, PG-, LG- and tap water-treated soils were found not to differ significantly amongst each other and had intermediate Zn concentrations of 3.7 mg kg^{-1} to 4.0 mg kg^{-1}.

Table 39. Soil element concentrations established for *C. annuum* soils post-harvest among the five irrigation treatments at the University experiment site. Vertical bars represent standard deviations. Letters represent mean separation among treatments by Scheffe's multiple range test ($P< 0.05$; $n = 5$ per treatment).

Element	Unit	Nutrient Solution	Phosphate Powder	Liquid Detergent	Carbonate Powder	Tap Water
P	**mg kg^{-1}**	**78.2 ± 7.50^{a}**	**82.4 ± 7.02^{a}**	**35.2 ± 2.39^{b}**	**33.2 ± 4.55^{b}**	**35.0 ± 2.92^{b}**
K	**mg kg^{-1}**	**165 ± 36.6^{c}**	**64.2 ± 3.83^{d}**	**57.6 ± 12.8^{d}**	**53.0 ± 5.15^{d}**	**69.0 ± 8.12^{d}**
Ca	mg kg^{-1}	678 ± 136^{e}	731 ± 73.6^{e}	759 ± 48.4^{e}	773 ± 33.1^{e}	820 ± 11.9^{e}
Mg	mg kg^{-1}	89.8 ± 28.4^{f}	60.0 ± 19.5^{f}	54.2 ± 18.4^{f}	49.6 ± 5.73^{f}	69.6 ± 19.8^{f}
Zn	**mg kg^{-1}**	**4.32 ± 0.59^{g}**	**3.96 ± 0.25$^{g/h}$**	**3.70 ± 0.26$^{g/h}$**	**3.50 ± 0.20^{h}**	**4.00 ± 0.38$^{g/h}$**
Cu	mg kg^{-1}	2.34 ± 0.30^{i}	2.14 ± 0.22^{i}	2.26 ± 0.23^{i}	2.08 ± 0.31^{i}	2.08 ± 0.08^{i}
Mn	mg kg^{-1}	5.80 ± 1.09^{j}	7.60 ± 1.14^{j}	6.0 ± 1.22^{j}	5.80 ± 0.84^{j}	6.20 ± 0.45^{j}

Organic C and total N established for treatment soils, shown in Table 40, were found not to differ significantly among treatments.

Table 40. Percentage organic carbon and nitrogen (mean ± SD) among *C. annuum* treatment soils at the University experiment site as determined by MIR spectroscopy ($n = 5$). Letters represent mean separation among treatments by Scheffe's multiple range test ($P< 0.05$; $n = 5$ per treatment).

Element	No Treatment	Nutrient Solution	Phosphate Powder	Liquid Detergent	Carbonate Powder	Tap Water
Organic C (%)*	< 0.5^{a}	< 0.5^{a}	< 0.5^{a}	< 0.5^{a}	< 0.5^{a}	< 0.5^{a}
Total N (%)	0.058 ± 0.058^{b}	0.065 ± 0.013^{b}	0.062 ± 0.011^{b}	0.068 ± 0.011^{b}	0.06 ± 0.008^{b}	0.066 ± 0.015^{b}

Note: * MIR organic C values for all treatments were below detection limit of instrumentation.

As shown in Table 41, untreated soils were found to be nutrient poor with all samples calculated to be deficient in the plant essential macronutrients of P and K. Soils treated with PG greywater were found not to be deficit in P, and represented the only greywater treated soil not requiring the addition of P fertilizers to support the optimal growth of *C. annuum*. By contrast, LG- and tap water-treated soils were found to contribute insufficient quantities of P to satisfy the nutrient demands of *C. annuum*. Interestingly, following 135 days of irrigation,

nearly all treated soils, including those to which nutrient solution were added, were found to be deficient in K.

Table 41. Percentage of soil samples from each treatment in which *C. annuum* were grown at the University experiment site found to be deficient in P and K (n = 5 per treatment).

Element	No Treatment	Nutrient Solution	Phosphate Powder	Liquid Detergent	Carbonate Powder	Tap Water
P	100	0	0	100	80	100
K	100	80	100	100	100	100

3.6.9. Salinity: B. vulgaris

Soils to which the detergent powder-based greywater were added reflected a significant increase in the exchangeable Na levels relative to the soils to which only tap water was added (110 mg kg^{-1} to 140 mg kg^{-1}, and 11 mg kg^{-1} respectively; Table 42). By comparison, the addition of the liquid detergent-derived greywater, LG, to soils over the experiment period resulted in significantly lower exchangeable sodium levels which were found to be statistically similar to those soils treated with tap water.

Nutrient-treated soils had the highest mean concentration of cations recorded among all evaluated treatments with a count value of 5.6. Despite the additive effect of adding the various detergent formulations to tap water to produce greywater, this had no significant effect on the total cation composition of treatment soils relative to tap water. For the measured soil exchangeable sodium percentages (ESP), statistical trends in the results were found to mirror very closely those established for exchangeable Na (Table 42). Soils to which powder detergent-generated greywaters were applied had significantly higher SAR values than remaining treatments which were found to be statistically similar relative to each other.

Table 42. Soil salinity characteristics measured among treatments at the University experiment site, recorded in *B. vulgaris* pots. Letters represent mean separation among treatments by Scheffe's multiple range test (P< 0.05; n = 5 per treatment).

Parameter	Unit	Nutrient Solution	Phosphate Powder	Liquid Detergent	Carbonate Powder	Tap Water
Total Cations	-	**5.62 ± 0.35^{a}**	**4.29 ± 0.31^{b}**	**4.60 ± 0.07^{b}**	**4.39 ± 0.37^{b}**	**4.83 ± 0.20^{b}**
Exchangeable Na	**mg kg^{-1}**	**2.42 ± 0.41^{c}**	**140 ± 53.8^{e}**	**9.87 ± 4.74^{d}**	**110 ± 31.9^{e}**	**11.4 ± 2.87^{d}**
ESP	%	**0.26 ± 0.04^{f}**	**16.4 ± 5.03^{h}**	**1.29 ± 0.62^{g}**	**13.2 ± 3.32^{h}**	**1.42 ± 0.31^{g}**
SAR	-	**0.15 ± 0.02^{i}**	**9.88 ± 3.71^{j}**	**0.67 ± 0.33^{i}**	**7.69 ± 2.24^{j}**	**0.76 ± 0.18^{i}**

3.6.10. Salinity: C. annuum

Total cations recorded among treatment soils were not found to be significantly different, with mean cation count values narrowly range-bound between 4.3 and 4.9 (Table 43). The application of powder-based laundry greywater formulations to soils over one growth cycle appeared to induce considerable change to the exchangeable sodium levels recorded in *C. annuum* pots (Table 43). Mean values of several times higher were found for both PG and CG greywater treatments (67 mg kg^{-1} and 42 mg kg^{-1} respectively) compared to those values of remaining treatments, for which exchangeable sodium concentrations were less than 2.6 mg kg^{-1}. A statistically similar trend to the above was found for the calculated exchangeable sodium percentage of treatment soils, as shown in Table 43. The SAR of soils was found to be significantly higher for laundry powder generated greywater compared to all other treatments which were found to be statistically similar compared to one another.

Table 43. Soil salinity characteristics measured among treatments at the University experiment site, recorded in *C. annuum* pots. Letters represent mean separation among treatments by Scheffe's multiple range test ($P < 0.05$; $n = 5$ per treatment).

Parameter	Unit	Nutrient Solution	Phosphate Powder	Liquid Detergent	Carbonate Powder	Tap Water
Total Cations	-	4.59 ± 0.45^{a}	4.35 ± 0.42^{a}	4.42 ± 0.34^{a}	4.44 ± 0.21^{a}	4.89 ± 0.19^{a}
Exchangeable Na	**mg kg^{-1}**	**1.78 ± 0.29^{b}**	**46.9 ± 36.9^{c}**	**2.53 ± 1.00^{b}**	**29.7 ± 32.9^{c}**	**1.80 ± 0.35^{b}**
ESP	%	**0.24 ± 0.05^{d}**	**6.40 ± 5.10^{e}**	**0.34 ± 0.10^{d}**	**3.86 ± 4.20^{e}**	**0.22 ± 0.05^{d}**
SAR	-	**0.09 ± 0.02^{f}**	**2.42 ± 1.94^{g}**	**0.12 ± 0.05^{f}**	**1.49 ± 1.65^{g}**	**0.09 ± 0.02^{f}**

3.6.11. Specific Hydraulic Conductivities: B. vulgaris & C. annuum

The specific hydraulic conductivities of experimental Berea Red soils in which *B. vulgaris* were not significantly influenced by irrigation treatment due to large variances in K_s about means (Fig. 60). Among treatments, the lowest mean K_s value of 0.82 mm h^{-1} was determined for LG-irrigated soils and was highest for nutrient solution treated soils where a mean K_s value among replicates of 87 mm h^{-1} was established. By comparison, CG- and tap water-irrigated treatments mean K_s values were found to be intermediary among the treatments investigated.

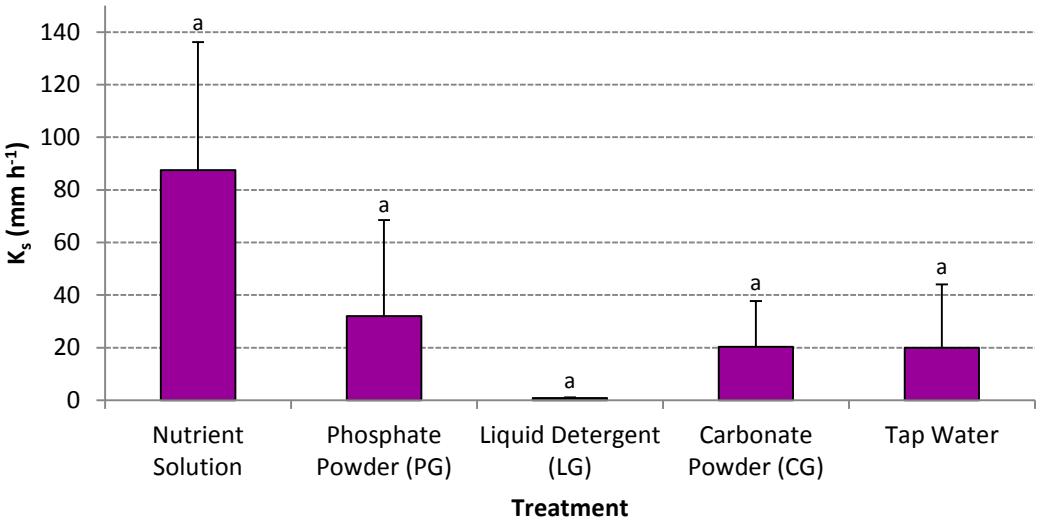

Fig. 60. Mean specific hydraulic conductivities of soils in which *B. vulgaris* were grown, recorded after growth cycle 1 at the University experiment site. Vertical bars about means represent standard deviations. Letters represent mean separation among treatments by Scheffe's multiple range test ($P<$ 0.05; n = 3 per treatment).

As shown in Fig. 61, in *C. annuum* pots the application of greywater to soils induced lower soil K_s values as compared to either nutrient fertilizer or tap water treated soils. For greywater treated soils, the application of LG greywater in particular resulted in the lowest mean recorded K_s among replicates. A supporting illustration of the typical surface ponding observed among greywater treatments is shown in Fig. 62.

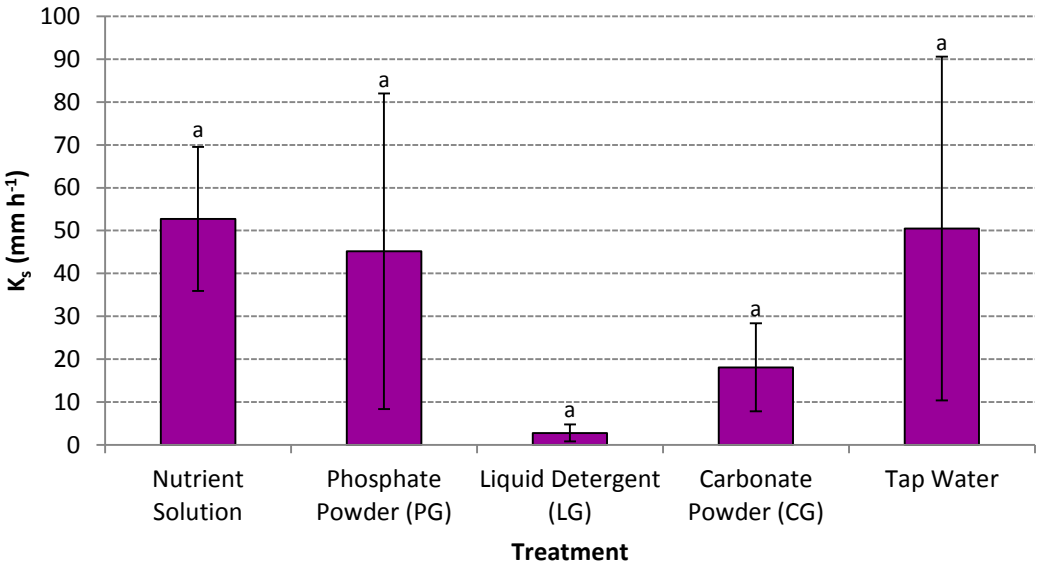

Fig. 61. Mean specific hydraulic conductivities of soils in which *C. annuum* were grown, recorded after growth cycle 1 at the University experiment site. Vertical bars about means represent standard deviations. Letters represent mean separation among treatments by Scheffe's multiple range test ($P<$ 0.05; n = 3 per treatment).

Fig. 62. A typical example of the surface ponding observed among greywater treatments. In this figure a LG-treated soil is shown approximately 15 minutes after irrigation.

CHAPTER IV: DISCUSSION

4.1. WATER QUALITY

4.1.1. pH

The highly alkaline levels of greywater generated from the two powder formulations of 9.5 to 9.9 used were in agreement with some values reported in the literature. Christova-Boal *et al*., (1996) reported laundry water pH values in the range of 9.3 to 10.0. Surendran and Wheatley (1998) and Friedler (2004) however, reported lower mean pH values of 8.1 and 7.5 for laundry greywater respectively. For the pH of greywater produced from liquid detergents, Misra and Sivongxay (2009) found levels of pH 9.2, levels comparable to those found in this study for greywater produced from powder detergents. This was in contrast with the lower pH values of 8.0 established for greywater produced from the liquid detergent product used in the study. The above pH differences may be attributed to differences in the form of laundry detergents used in each study. In a study that encompassed greywater quality of laundry effluent generated from 60 commercial Australian laundry products assessed individually (20 liquid detergents; 40 powder based detergents), Patterson (2000) found only 18 % produced greywater with a pH of less than 9 – of these, all were liquid detergents.

Alkaline pH values are typically associated with laundry detergents in solution (Christova-Boal *et al.*, 1996) and are caused by alkaline agents in detergent formulations of which sodium silicates and sodium carbonates are typical examples of those commonly used (Bajpai and Tyagi, 2007). For the powder detergents assessed in this study, the sodium carbonate and sodium sulphate components were the likely building agents responsible for the alkalinisation of these greywater solutions. An elevated pH for detergents in solution is especially desired in the detergent industry since it directly affects the ability of detergents to remove fabric soils (Patterson, 2000). According to Bajpai and Tyagi (2007), oily fabric soils containing fatty acids in particular can be more efficiently removed in alkaline solutions from the formation of soaps in dirt than in solutions of lower pH.

In this study, the pH values of laundry powder generated greywater exceeded the irrigation quality guidelines pH range of 6.5 to 8.4 recommended for South African agriculture by DWAF (1996). However, since soil pH is more strongly buffered against pH changes than water, the effects of irrigation with alkaline water is unlikely to present a problem except in

extreme cases and in long-term applications (DWAF, 1996). Some studies have shown that the buffering effect of other sandy soils is particularly high at irrigation water pH values above 7 and below 4, and less effective at pH values between these (Magdoff and Bartlett, 1985). The adverse effects of using irrigation water of high or low pH for irrigation is most pronounced when irrigation water is applied directly to crop foliage as opposed to the application directly to soil, with the latter the case in this study (DWAF, 1996; Jordan *et al*., 2001). It is therefore unlikely that the apparent phytotoxic symptoms of plants irrigated with the laundry powder generated greywater observed in this study were due solely to the effects of the alkaline irrigation media.

4.1.2. EC

Higher EC values of 129 mS m^{-1} to 151 mS m^{-1} recorded among greywater produced from powder detergents could be explained in part by the high Na concentrations present, sourced from the detergents used. However, EC values for these were offset slightly by the relatively low presence of other ion-forming elements such as Ca and Mg. Christova-Boal *et al*. (1996) reported an EC range of 19 mS m^{-1} to 140 mS m^{-1} for laundry greywater, the upper bound of which was still lower than that which was measured among greywater produced in this study for powder detergents. Other researchers e.g. Friedel (2004), have reported similarly high EC values to this study of 245 mS m^{-1}. However in both of these previous studies, laundry greywater from several homes was collected and the laundry detergents types used by owners were not specified. Few studies appear to have assessed greywater EC on a more specific and standardised basis as was adopted in this study. The low EC values found for the liquid detergent-generated greywater were caused by a combination of comparatively low level of Na (for reasons to be elaborated upon in section *4.1.5*) and the low presence of other metal cations. The very high EC values reported for the nutrient solution were also expected, given the stronger presence and more uniform concentrations of ionizing elements particularly those of the alkali earth metals, such as Ca and Mg, closely associated with EC. However, whereas the major source of the high EC levels measured in greywater was Na, in the nutrient solution the major sources were Ca and Mg.

4.1.3. COD

Of particular concern in the agricultural reuse context were the high levels of COD of greywater generated from liquid laundry detergent, suggesting the pollution potential of this product as applied to soil was significant. The observed differences in COD among generated

greywater however could not be explained simply on the basis of organic loading or storage time, since these variables were standardised in this study. Although not directly tested, it likely that differences with respect to the surfactants used and concentrations thereof in each product contributed significantly towards changes in greywater COD. The percentage composition by mass of surfactant in the liquid detergent constituted approximately 30 % of the product tested, 10 % higher than the surfactant presence in either of the powder formulations (Bob Crawford, pers. communication). However, actual COD values established among treatments appeared to be closely related to the molecular weight of the organic fraction of their compositions. SLES, the primary surfactant constituent of the liquid detergent, has a higher organic molecular mass fraction than that of NaLAS which may have contributed to the comparatively elevated COD in liquid detergent generated greywater relative to greywater generated from powder detergents.

4.1.4. Fertilizer Value

The balanced nutrient fertilizer solution used in this study represented the optimum set of nutrients and should in the context of this study be viewed as the sum of both nutrient fertilizer additive *and* tap water. Similarly, results obtained for greywater treatments should be assessed in terms of whether laundry detergent addition to tap water had a positive or negative relative effect on the plant-soil system. The physicochemical attributes of laundry greywater found in this study suggested laundry greywater was generally a poor fertilizer for the irrigation of the crops investigated. Greywater and tap water had similarly low levels of macronutrients and when compared to the nutrient solution was likely the major cause of growth differences observed between the nutrient treatment and the other treatments. The high N levels in particular reported for the nutrient fertilizer treatment compared to greywater and tap water treatments when viewed in relation to growth performance outcomes among treatments, suggested that N was likely limiting crop growth. Although P values for the PG greywater medium was comparable with those values established for commercial nutrient fertilizer, in the context of Sprengel-Liebig's Law of the Minimum (von Liebig, 1855; van der Ploeg *et al*., 1999), relative shortages of the other essential macronutrients of N and K would likely severely limit plant growth and productivity once initial soil supplies became exhausted. For the liquid (LG) and carbonate powder (CG) generated greywater, the ratio of N-P-K appeared favourable for crop production on a proportional basis; however on an absolute quantitative basis, values of N, P and K were all highly deficient when compared

with the nutrient solution. The high P values found in this study for the PG-powder generated greywater were likely derived from the sodium tripolyphosphate (STPP, $Na_6P_3O_{10}$) builder base present in the PG powder detergent. The powder laundry greywaters used in this study were found to be sufficient in the essential macroelement S compared to the nutrient solution, but remained deficient in both Ca and Mg. The concentration of Al among greywater treatments was found to be comparable to the nutrient solution. Overall, laundry greywater was found to contribute positively as a source of nutrients when compared with tap water – for every essential plant macro- and micro-element evaluated in this study, greywater outperformed tap water. This was anticipated, given that greywater is essentially the sum of a detergent and tap water. Additionally, this also provided verification that the resolution of analyses were generally sufficient accurate with the possible exception being the testing of B, Total N and S for the nutrient solution where values where higher than would have been expected theoretically. The extra nutrients contained in greywater may not always be biologically available to plants under particular physicochemical conditions however. Whilst the said differences in nutrient concentrations between laundry greywater in this study and tap water are likely trivial in the broader agricultural context, it nevertheless highlights theoretically the potential value of laundry greywater as an additional source of nutrients for plants as compared with tap water. In this study it is also important to note that organic loading was kept fairly minimal for the generation of laundry greywater relative to other greywater streams such as that typically sourced from kitchens (see comparative review by Eriksson *et al*., 2002). In this study no heavily soiled or faecal-contaminated laundry loads were used in the generation of synthetic laundry greywater, which functioned to reduce the quantity of some plant essential nutrients in the model greywater. In a true-to-life scenario, it is possible that the organic fraction of laundry greywater may be higher than was the case in the current study (e.g. Ottoson and Stenström, 2003). A higher organic loading in laundry detergent would likely contribute significantly to the nutrient pool available.

4.1.5. Sodium & SAR

The comparatively high Na levels found for greywater in this study were not unexpected, given the widespread use of Na in the chemical formulation foundations typically used in the production of many laundry detergents (Patterson, 2000). In this study, higher levels of Na in compounds were used in the formulations of PG and CG greywater. Na levels recorded among greywater samples in addition to the stipulated manufacturer formulations of each, for which Na-based compounds in builders accounted for more than half of their respective

formulations. By contrast, the reduced levels of Na found for the liquid detergent generated greywater compared with the powder detergent-generated greywaters also suggested that laundry detergent formulation compositions - and hence laundry products - may vary significantly in the absolute amount of Na present.

This finding was in agreement with a study by Patterson (2000), where analysis of the contributions of several laundry powder detergents and liquid detergent products to Na loading in laundry greywater were investigated. Patterson (2000) found that most of the contribution of Na to domestic wastewater as laundry effluent arose when powder detergents were used. By comparison liquid detergents generally contributed significantly less Na. The specific source of sodium in the powder formulations used in the generation of greywater for this study was found to be sodium carbonate and sodium sulphate, both functioning as builders (Firouzabaldi *et al.,* 2001; Wieprecht *et al.,* 2004; Carson *et al.,* 2006). The absolute Na level of 71 $mg.L^{-1}$ to 273 $mg.L^{-1}$ recorded among greywater treatments were also in agreement with those reported in a study of greywater by Christova-Boal *et al*. (1996), where values of 49 $mg.L^{-1}$ to 480 $mg.L^{-1}$ were reported for laundry greywater. Friedler (2004) reported even higher sodium concentrations of 530 $mg.L^{-1}$ for laundry greywater. It therefore holds that differences in detergent formulations would primarily account for the discrepancies in the Na concentrations of laundry greywater among studies.

The high SAR values obtained for greywater produced from laundry powder detergents were largely expected but far exceeded the magnitude envisaged, with values approximately 8 fold greater than the permissible values stipulated in many standard agricultural guidelines worldwide (e.g. ANZECC, 1992; DWAF, 1996). Because SAR is directly proportional to ionic Na concentrations and inversely proportional to the sum of ionic Ca and Mg, it holds that elevated SAR can arise in one of two ways: increase in sodium and/or decreases in Ca and Mg. In the case of the laundry powder detergents, the former was true, with the contribution of the Na-based compounds to these formulations found to be high. Higher detergent compositions were reported by Al-Jayyousi (2003) to correspond with higher SAR levels in greywater, suggesting high Na in detergents contributed to elevated SAR. Misra and Sivongxay (2009) found SAR values for liquid laundry detergent generated greywater of 12.3, considerably lower than the SAR value of 22.9 to 24.0 established in this study for greywater generated from liquid laundry detergent.

Unexpectantly, the SAR for tap water sourced from the municipal water supply had levels well above the recommended guideline value of 1.5 recommended for purposes of agricultural irrigation (DWAF, 1996). However, in a closely related study by Finley *et al.* (2009) that included an assessment of greywater characteristics relative to tap water, similar SAR values of 7.7 to 7.8 for tap water sourced in a major Canadian city were found. On the other hand, Al-Jayyousi (2004) found a baseline mean SAR value of 0.83 for tap water. Such considerable variations in SAR values among studies suggest other environmental factors may play a significant role in local water quality (Hem, 1986) and hence SAR values pertaining to greywater from various studies should be viewed on a relative basis to that of tap water used in its generation.

4.1.6. Greywater in Storage

Contrary to expectation and findings by other researchers, greywater storage over a 72 h period at ambient temperature did not have a negligible effect on the organic quality of greywater for all the formulations investigated. Although bacteriological and viral assessments of greywater in storage did not form part of this study, changes in biological quality can be indirectly inferred by changes in certain physicochemical parameters such as COD, since a fairly positive correlation between BOD and COD has been reported for greywater in other studies. Jefferson *et al.* (2004) reported a BOD to COD ratio for domestic greywater of between 2.8 and 3.6. According to Eriksson *et al.* (2002), approximately 95 % of the organic constituents in greywater effluent was found to be derived from detergents; of these 60 % were found to contribute towards measured COD (Santala *et al*., 1998). Since all greywater was stored under identical conditions and generated from identical organic loadings in the form of pre-prepared wash-load stain swatches which were standardised for every treatment, differences noted among treatments with respect to COD appear to be attributed to differences noted in detergent formulations.

COD levels were not maintained over the three day storage period for each of the greywater treatments. A previous study showed that stored washing machine greywater generated from a powder detergent experienced a rapid decrease in COD on the first day of storage and then an increase after 8 to 10 days (Dixon *et al*., 1999a). The increases in COD during the shorter storage time that was used in this study did not fully corroborate these trends. Dixon *et al.* (1999a) attributed their findings of dynamic COD concentrations over this time to the release of soluble COD as a result of anaerobic activity together with different settling depths of

organic matter with time that occurred whilst sampling depth was kept constant. In this study, sampling depth was consistent among sample collections and stored greywater was mixed manually prior to sampling, but this mixing may have been insufficient.

4.2. PLANTS

4.2.1. Growth Rate & Biomass

Nutrient solution-fertilized plants reflected the highest vertical growth rates throughout experimentation at both sites and for both species, as measured by the length of the longest leaf (*B. vulgaris*) and the height of the highest apical bud (*C. annuum*). Greywater- and tap water- irrigated plants, significantly less by comparison, were found to have similar growth rates. Differences in growth rates and final heights attained among *C. annuum* individuals across cycles at the Unilever experiment site could be explained by the higher initial heights of *C. annuum* seedlings used in the second growth cycle since the age of seedlings provided were only approximate. Higher growth rates in the nutrient solution treatment observed for both crop species were likely due to greater macronutrient availability, which enabled improved photosynthetic activity and carbon fixation to biomass (Medina, 1971; Oxman *et al.*, 1977; Ryle and Hesketh, 1969; Longstreth and Nobel, 1980; Terry and Ulrich, 1973a; Thyok *et al*., 1973; Bershtein *et al*., 1971; Peoples and Koch, 1979; Terry and Ulrich, 1973b; Wolf *et al*., 1976). Greywater type had little influence on the biomass accumulations to plant anatomical components for both *C. annuum* and *B. vulgaris*, and biomass attainment was found to be similar to those plants irrigated with tap water. An experimental reduction in mineral nutrient supply and availability has been shown to reduce leaf area expansion and whole plant growth rates in small birch (McDonald *et al*., 1986). Similarly, Binkley *et al*. (1994) found reduced growth and leaf areas in plants under decreased nutrient supply. The intact potting soil compost maintained around seedlings following may have contributed to a higher nutrient pool environment immediate to plant roots than possibly would have been measured in surrounding soil and masked some of the effects of nutrient limitation. However this effect would have been similar for all treatments.

The high edible yields obtained in nutrient solution-irrigated crops suggested that crops responded positively to high fertilizer application rates in biomass terms. In the case of *B. vulgaris*, harvested yields of ca. 179 metric tons per hectare were more than four-fold higher than typical yields expected for this growing region of South Africa (DAEA, 1995). From an economical perspective, irrigation with PG greywater and tap water were encouraging, both

producing yields - albeit towards the lower estimates reported by DAEA (1995) - of ca. 20 tons per hectare for *B. vulgaris*. However, irrigation of *B. vulgaris* and *C. annuum* with CG and LG greywater had a negative effect on edible crop yields compared with crop yields typically expected for the region (DAEA, 1995). Since tap water-irrigated treatments produced higher edible biomass yields, these findings suggest that phytotoxic effects occurred in crops irrigated with these greywater formulations. Plant growth attributes measured in this study for *B. vulgaris* did not compare favourably with those measured previously or the improved yields noted for *B. vulgaris* irrigated with mixed domestic greywater under similar experimental conditions in a study by Rodda *et al.* (2011b). In the study by Rodda *et al*. (2011b), *B. vulgaris* irrigated with mixed greywater showed improved growth and yield relative to those crops irrigated with tap water only whilst crops irrigated with the same nutrient fertilizer attained the fastest growth rate and the greatest biomass. Whilst the growth attributes measured for *B. vulgaris* irrigated with nutrient solution were similar to findings reported by (Rodda *et al*., 2011b), the poorer performances of greywater relative to tap water noted in the current study suggest further that the nutrient compositions of laundry greywater were insufficient. This was confirmed by the similar nutrient uptakes in crops irrigated with laundry greywater and tap water in contrast to the improved nutritional values relative to tap water reported by Rodda *et al.* (2011b) for mixed greywater-irrigated crops.

The uniform decline in growth parameters and biomass trends among all treatments generally on a cycle-on-cycle basis, suggested these changes could not be explained on the basis of the cumulative effects of irrigation treatment on soils. Instead declines observed may be more closely attributed to seasonal effects such as rainfall which declined considerably in the second growth cycle. Increased soil compaction over time may also have impaired plant growth among treatments (Wolkowski and Lowery, 2008**)**. Together these (and other unknown) factors may have masked absolute changes in biomass accumulations over the long-term, although these conditions were the same for all treatments and thus still provide a valid indication of the relative long-term changes among treatments. Moreover, the approach of using closely spaced consecutive growth cycles was done to simulate a true-to-life scenario where a food garden from a low-income household would ideally need to be made productive for as long as possible throughout a year.

4.2.2. Roots

The effect of greywater irrigation on root development and growth of *B. vulgaris* and *C. annuum* were similar for all greywater- and tap-water irrigated treatments. The response of roots irrigated with greywater appeared to relate in part to the effects of salinity, which is known to cause reductions in root length, root mass, and fine root development (Shannon and Grieve, 1999). The relatively low contributions to the soil nutrient pool offered by greywater and tap-water treatments also appeared to have a significant effect on the developmental responses of roots. Plant roots have been shown to exhibit phenotypic plasticity in their nutrient acquisition capacity by adjusting their physiological, longevity, morphological and/or architectural characteristics to meet changes in shoot nutrient demand (Chapin, 1980; Clarkson and Hanson, 1980; Clarkson, 1985; Bassirirad, 2000; Forde and Lorenzo, 2001). Nutrients such as nitrates, phosphate, sulphate and iron can act as signalling mechanisms that modify cellular division and differentiation processes in roots which can have a significant influence on root architecture (López-Bucio *et al*., 2003). In particular, root-hair formation, lateral root formation and primary root growth are influenced by changes in the external and internal nutrient concentrations (López-Bucio *et al*., 2003). Differences in treatment concentrations of N, P and S among treatments may therefore have had a role in influencing root development. The lower root biomass in greywater and tap-water treatments suggested that the cost-benefit relationship under low nutrient conditions favoured small root mass with less fine root development. The benefit of fine root development is a root system that is allowed to explore the soil volume more efficiently by reducing the construction and maintenance cost to the root system (Forde and Lorenzo, 2001). The coarser roots associated with the nutrient irrigated treatments, whilst more costly to produce, have an enhanced transport capacity and are less vulnerable to pathogenic attack, physical damage, desiccation and grazing by soil-borne microarthropods and so are generally longer-lived (Fitter, 1987). Consequently, roots with high specific root lengths (root length per unit weight), fine roots, are typically found in plants grown under nutrient deficient conditions (Fitter, 1985), a notion consistent with findings reported here.

The root nodules observed in *B. vulgaris* may be attributed to the influence of certain rhizobial species inhabiting the soil environment in which the plants were grown. Due to their endophytic nature these species are more likely found in the root than soils (Rivas *et al*., 2004). Rhizobia typically enter roots via intracellular infection where infection is temporarily

possible through a deformed root hair in a process known as *root hair entry*, occurring in a similar manner to endocytosis. Alternatively, rhizobia may enter through another mechanism known as *crack entry* where no root deformation occurs and access is instead permitted via cellular cracks produced by lateral root emergence (Perrine-Walker *et al*., 2007). The consequence of this infection is cellular division in the root cortex where a root nodule appears (Caetano-Anollés and Gresshoff, 1991). In infected nodules, rhizobia differentiate morphologically into bacteroids which in turn fix elemental N_2 into a form useful to plants, ammonium, via the enzyme nitrogenase (Tubb, 1976; Markmann *et al*., 2011). Supply of this reduced form of nitrogen provides benefit to plants under conditions where soil nitrogen is limiting (Markmann *et al*., 2011). In *Beta vulgaris* (sugar beet), a very closely related species to Swiss chard grown in this study, the rhizobium *Bradyrhizobium betae* was first isolated from root nodules (Rivas *et al*., 2004). On this basis, this species or at least a candidate species from this genus look likely causative of these root nodules observed in Swiss chard. Further evidence for a nitrogen fixing rhizobia presence in root nodules was the finding of lower nitrate and ammonium levels in greywater and tap water irrigation media in contrast with the significantly higher nitrate and ammonium levels found in the nutrient solution. Since root nodules were only noted for greywater and tap water-irrigated *B. vulgaris*, it suggested a greater need for symbiosis between rhizobia and plants of benefit to the plant through a greater supply of the limiting nitrogen.

4.2.3. Nutrient Uptake & Yields

The uptake of nutrients by the nutrient solution treated crops may be viewed as the model uptake in the context of this experiment for purposes in assessing the possible occurrence of nutrient excess and deficiency among treatments. The uptake of the macronutrients by leaf tissues showed some consistent trends for both study species and at both experimental sites. The lower N and K generally found among greywater and tap water treatments in *B. vulgaris* and *C. annuum* tissues compared to nutrient solution treated plants and viewed in relation to the plant growth trends noted in the study, provided further evidence of macronutrient limitations in these media. The limitation of N especially would likely have impaired photosynthetic function in both species given its important role in protein synthesis, especially the key enzyme involved in photosynthesis carbon dioxide fixation, Ribulose-1,5-bisphosphate carboxylase-oxygenase (Rubisco). In C_3 plants Rubisco accounts for up to 50 % of soluble leaf proteins (Spreitzer and Salvucci, 2002) and 20 % to 30 % of total leaf nitrogen

(Evans and Seeman, 1989; Makino, 2003; Kumar *et al*., 2002). A lower N level would consequently reduce the activity of Rubisco and leaf photosynthetic capacity, leading to reduced leaf carbon gain (Evans, 2004). In the fruit tissues of *C. annuum*, N concentration trends among treatments were identical to those established for leaves, providing further evidence of limitations in N availability among greywater and tap water treatments. Concentration values of N reported in this study were considerably lower than the 8.0 mmol L^{-1} to 9.0 mmol L^{-1} N tissue levels target range for optimal dry mass production reported for *Capsicum annuum* (Bar-Tal *et al*., 2001). For the nutrient treatment, the possible excess N fertilization may have favoured greater investment for the development of leafy crop canopies as opposed to fruits (Kharbanda and Tewari, 1996). A further consequence of excess nitrogen fertilizer in crops can be a delay in fruit set and reduced yields (Fanggong and Yinyan, 2004). Possible evidence for this was a strong second phase of fruit production in nutrient-irrigated *C. annuum* which became apparent shortly before experimentation was terminated. Nitrogen availability has been shown to relate strongly to crop yield per hectare and fruit numbers per plant in *Capsicum annuum*, with a positive correlation between N supply and these yield measures (Khan *et al*., 1982). These effects were found when N was used alone or in combination with P. These findings support findings in the current study, with increased N uptake by leaves allowing greater resource partitioning to fruit, resulting in the higher per hectare yields and fruit numbers per plant noted in this study. Similarly, in the study by Khan *et al.* (1983), the effects of N availability on fruit size was negligible, a finding consistent with those reported in this study.

Among greywater treatments and tap water treatments the uptake of K to plant tissues appeared to be low compared to the nutrient treatment, likely a result of low concentration of K in these irrigation media. Despite its low and highly variable concentration in soils, plants typically accumulate large quantities of K, resulting in K constituting between 2×10^4 mg kg^{-1} and 1×10^5 mg kg^{-1} of the dry matter of plant tissues (Leigh and Wyn-Jones, 1984; Tisdale *et al*., 1993; Ashley *et al*., 2006). The low tissue uptake of K among treatments relative to the abovementioned range could have induced an adverse ionic environment for metabolic processes in these treatments (Leigh and Wyn-Jones, 2006). Beyond a certain quantity, a decline in K can lead to changes in vacuole biochemistry as other solutes are accumulated in the vacuole. The consequence of this is decreased metabolic rates leading to declines in plant growth (Leigh and Wyn Jones, 2006). The decreased N and K contents in leaf tissues therefore provides further evidence for macronutrient limitations independent of study species

or set of abiotic conditions characterising each site. At mild levels, visible symptoms of K deficiency in crops are not easily discernible owing to the high rates of redistribution between mature and developing plant tissues (Römheld and Kirkby, 2010). Initially, only a reduction in growth rate followed by chlorosis and leaf necrosis in mature leaves are observed, occurring particularly in leaf margins and tips (Mengel and Kirby, 2001; Römheld and Kirkby, 2010). Unlike for P and N however, no significant changes in biomass allocation or root architecture are known to occur under K deficiency (Römheld and Kirkby, 2010) and hence a K deficiency could not explain the differences in root biomass noted among treatments.

Phosphorus did not appear to be primarily limiting for plant growth for either *B. vulgaris* or *C. annuum*. Evidence for this was the highest tissue concentration of P generally in leaves of plants irrigated with PG greywater (with the highest P-level among irrigation media comparable only with the nutrient solution) and low P-levels in leaf tissues of other treatments. P availability to plants in soils is generally very low because most of it remains bound in soils (Vance, 2001); concentration gradient differentials between soil water and plant cells typically are more than 2000 fold, with the concentration of free P in soil solutions averaging only 1 µmol (Bieleski, 1973, Ragothma, 1999). This suggests that the additional P was to some extent biologically available in PG-irrigated plants. A P deficiency in plants would otherwise suppress growth, resulting in the development of weak and stunted crops (Benton-Jones, 1998). Since those plants irrigated with PG greywater were similar in growth rate and biomass at harvest to tap water-irrigated individuals, other factors such as soil salinity differences, the availability of other elements and/or interactive effects in the presence of other element(s) may have offset gains in P availability.

No clear trend in uptake of Ca was found among the study species or at either experimental site. Despite the very high Ca concentration of the nutrient solution media relative to the other treatments, leaf uptake of Ca was found to be similar among treatments and no example existed where nutrient solution-treated plants had significantly higher Ca uptake rates to leaves. This also occurred despite higher Ca concentrations noted in soils for *B. vulgaris*; in *C. annuum* the Ca concentrations were somewhat lower presumably as a result of the higher uptake rates of Ca to *C. annuum* leaf tissues. This could be due to differing nutrient requirements of these species. In fruits, Ca concentrations were found to be similar, suggesting that irrigation treatment had a negligible influence on fruit Ca concentrations and

that soil Ca levels were sufficient. This is perhaps not surprising given that deficiencies of Ca in the natural environment are typically rare since it is one of more abundant cations present in a given soil solution (Simon, 1978; White and Broadley, 2003). The Ca concentrations found in this study, though higher in *C. annuum* tissues than *B. vulgaris*, were within the range of 3×10^3 mg kg^{-1} to 3×10^4 mg kg^{-1} on a leaf dry weight basis. This is considered for most crops to be typically sufficient (Benton-Jones, 1998). It is therefore unlikely in this case that calcium, in its divalent form, would be sufficiently low so as to impair the structural roles it performs in the cell, intracellular messaging in the cytosol or its role as a counterion for several organic and inorganic anions in the vacuole (Marschner, 1995).

Irrigation treatment had a strong determining influence on the uptake of Na to leaf tissues for both species investigated and was found to be independent of experiment site. The highest Na uptake rates were consistently found in leaves of plants irrigated with either PG and CG greywater, the likely source of which were the sodium powder bases of the PG and CG detergents as discussed earlier. Similarly, the uptake of Na to fruit tissue was generally highest in PG- and CG-treated *C. annuum.*

In many higher plants, the primary cause of ion-specific damage is Na^+ (Tester and Davenport, 2003). Although certain halophytes and C_4 plants need Na for growth, many crop plants experience toxic effects at high millimolar concentrations (Mäser *et al*., 2002; Flowers and Colmer, 2008; Zhang *et al*., 2010). The effects of Na toxicity on plants is varied, with osmotic stress (Tarczynski *et al*., 1993), competition with K (Mäser *et al*., 2002) and the inhibition of key enzymes (Munns, 1993; Murguia *et al.*, 1995) being among these. Generally, at exposure to high Na concentrations, whole plant growth may be inhibited, development accelerated, senescence occur and potentially plant death when exposure is prolonged (Zhu *et al*., 2007). Direct evidence in this study for the general growth inhibition in C_3 plants (Zhang *et al*., 2010) as a consequence of increased salinity that significantly higher internal Na^+ would contribute towards, was also lacking. Growth inhibitions due to Na^+ specific damage is related to the accumulation of Na^+ in leaf tissues, causing leaf necrosis in older leaves that start at leaf tips and migrate towards leaf bases (Zhang *et al*.., 2010). In this way, the lifespan of individual leaves are reduced so reducing net productivity as growth and yield reductions (Munns, 1993). This was in agreement with the faster rates of leaf necrosis and leaf fall visually apparent in greywater-irrigated treatments. At high uptake levels, Na is known to cause nutrient deficiencies of other nutrients (Silberbush and Ben-Asher, 2001) such as P, Fe and Zn by disrupting their uptake via interference with root plasma membrane transporters;

and inhibiting root growth due to the osmotic effects of Na and the effects of Na on soil structure (Tester and Davenport, 2003). While it is difficult to isolate the effects of Na^+ on these factors directly, firm evidence for impaired uptake of P, Fe or Zn or lower root biomass investments in this study among LG- and CG-treated plants of either species relative to the other treatments were not found. However, the overall similar growth trends and biomass accumulations among greywater and tap-water irrigated plants in relation to internal Na concentrations suggested inhibitory effects on growth were either insignificant at the reported concentration levels or were confounded by other factors.

Differences in leaf uptake of the elements Zn, Mn and Cu were marginal and consequently irrigation treatment had little effect on uptake. Although not measured directly in irrigation water analyses, concentrations can be inferred from the elemental concentrations in soils. However Zn, Mn or Cu concentrations in soils were generally found to be similar suggesting the composition of irrigation media treatments were similar for these elements.

Tissue concentrations of Zn among all treatments were considerably higher than typical values considered sufficient for most crops. At these high levels Zn can be toxic (Benton-Jones, 1998), but visible symptoms of excess (spatially heterogeneous or interveinal chlorosis and leaf bronzing, auxin deficiency related responses and inward curling of leaf lamina, internodal shortening and a reduction in leaf size (Broadley *et al*., 2007)) in either of the study species were not apparent.

Mn concentrations in tissues though high and in some cases approaching the minimum of the excess toxicity range reported for some species (Edwards and Asher, 1982), were found to be generally similar among treatments. No associated visible symptoms of brown spots with chlorotic regions were evident on plants, suggesting any induced toxic effects could not fully account for the growth trends observed.

For most crops, dry matter leaf tissue concentrations of Cu in the range of 3 mg kg^{-1} to 7 mg kg^{-1} are considered adequate (Benton-Jones, 1998). Whilst Cu levels in *B. vulgaris* were generally within this range (Benton-Jones, 1998), in *C. annuum* tissue concentration in plants irrigated with LG greywater at the Unilever site were in excess of the toxic range 20 mg kg^{-1} to 30 mg kg^{-1} for leaf tissues defined by Benton-Jones (1998); an excess of Cu may have contributed in part to the relatively low biomass noted for these plants.

Overall, it is unlikely that at these concentrations for Zn, Mn or Cu environmental problems to soils would be posed, given the similarities of tissues concentrations among treatments relative to those plants irrigated with the comparatively inert tap water treatment.

Leaf uptake of Al and Fe were not consistent overall but followed very similar trends for a given species at either site, suggesting the contrasting set of abiotic conditions were less influential on uptake than genotype. For *C. annuum* Al was highest in the leaves of plants irrigated with LG- greywater despite similar Al concentrations among treatments. The similar general trends of Al and Fe uptake for each species may be attributed to the similar residual charges on chelating molecules that has been noted to govern uptake, given the similar ionic behaviour associated with their trivalent charges (Jones, 1961).

In crops, leaf Fe concentrations can range broadly, with values from 10 mg kg^{-1} to 1×10^{3} mg kg^{-1}. Given this range, in general, leaf tissue concentrations of the essential micronutrient Fe were high among treatments for both species. For *C. annuum* in particular, Fe leaf tissue concentrations were in most cases higher than considered toxic for some plant species, but the typical bronzing associated with excess symptoms of several hundred mg kg^{-1} in tissues, leaves may assume a bronzing appearance with brown spots (Benton-Jones, 1998). However since this trend of high Fe was found across treatments with the exception of the nutrient solution-irrigated *C. annuum*, Fe toxicity could not explain the growth trend differences found among treatments. A possible source of Fe that could account for the generally high leaf tissue concentrations among treatments however may be related to the soil chemistry in which plants were grown; high iron oxide contents are characteristic of Berea Red Soil (Okonta and Manciya, 2010).

Trends in B concentrations in leaves among treatments were generally independent of species and site. Boron concentrations were not measured in soils since it is difficult to utilise soil analysis to precisely predict plant growth on high B soils as B availability is influenced by many abiotic factors associated with the soil environment (Goldberg, 1993; Nable *et al.*, 1997). Differences were instead inferred by water quality analysis and corroborated by leaf tissue concentrations measured. Leaf tissue concentrations of B were in most cases highest among LG-irrigated individuals from either of the study species and similar among the remaining treatments. This uptake trend can be attributed to the higher B composition of the LG-based detergent formulation relative to the other treatments. Visible symptoms of B toxicity such as leaf burn that occurs in most species or fruit disorders (Nable *et al.*, 1997) however were not evident in LG-irrigated plants. The concentrations in LG-treated *B. vulgaris* and *C. annuum* leaf tissues were ca. 80 mg kg^{-1} and 200 mg kg^{-1} respectively. While leaf tissue concentrations of 40 mg kg^{-1} to 100 mg kg^{-1} (dry weight basis) is typical for species that normally accumulate B in leaves, leaves can contain up to 250 mg kg^{-1} dry weight under

conditions of B toxicity (Nable *et al*., 1997). These suggest that B toxicity may have arisen in *C. annuum* plants irrigated with LG greywater. However, it is difficult to firmly establish B toxicity due to widely varying toxicity ranges among and even within species, and the markedly contrasting regions of B concentration that can exist on the leaf lamina (Nable *et al*, 2007). In this study, for *C. annuum* whole leaf laminas were used in tissue analyses whilst for *B. vulgaris* only partial lamina tissues of leaves were used due to their large size which may explain lower B concentration estimates noted for this species. Future B analyses may wish to compare levels among different regions within the leaf lamina of a given plant where it is known to be lower and higher, as suggested by Shorrocks (1995).

Uptake of the microelements Ca, K, S, Mn, Cu, Zn, B have also been shown to be influenced by the ESP content of soil (Bains and Fireman, 1964). In this study however, no clear evidence for this was found since contrasts in ESP were not always consistent with variations in uptake to plant tissues of these elements.

4.2.4. Flowering & Fruiting Behaviours

Flower and fruiting behaviour expressed in absolute and relative terms were found to be a useful discriminator of physiological stress among *C. annuum* individuals from the various experimental treatments. Drivers of change in the flowering and fruiting behaviours of plants have been suggested to be caused by several environmental factors, including temperature, relative humidity, soil moisture, photoperiod duration and intensity, plant nutrition status, insects, disease and through mechanical forces such as wind and rain (van Schaik and Probst, 1958). Nutrient stress conditions have been shown to induce flowering in many plant species (Wada and Takeno, 2010). The interspecific differences among treatments at each site with respect to flowering and fruiting that normally follows could therefore be explained on the basis of plant nutrition status, the only variable amongst these with the known variability most likely to have been causative given the spatial scale of this study.

In a study on the flowering and fruiting behaviours of *C. annuum* by Marcelis *et al*. (2004), it was also found that most of the abortion of flowers and fruits could be related to contrasting vegetative growth rates, where the latter was considered to be an indicator of the plant source-sink ratio. In this study, contrasting vegetative growth rates and lower dry weight productions of plant tissue due to decreased source strength among treatments, viewed in relation to flower and fruiting behaviours, appeared to corroborate their findings. In this study, generally

higher flower abortion rates were found in greywater and tap water treatments for which nutrient supply was limiting. Fruit size expressed in terms of either weight or diameter, may be determined by both environmental and genetic factors (Nesbitt and Tanksley, 2001). Many environmental effects may influence competition for photosynthates within plants, resulting in changes in fruit size measures. According to van Ravenstijn and Molhoek (1978), increases in the total numbers of flowers has been shown to intensify competition for photosynthates, with the consequence of reduced fruit size; this effect may be the consequence of either competition among inflorescences (Fisher, 1977) or between fruit on a single inflorescence (Veliath and Ferguson, 1972). This effect however was not realized in this study possibly because source strength was sufficiently high in nutrient-irrigation treatments to partly offset intraspecific competition among fruit and inflorescences. Considerable effects of irrigation treatment on fruit size were generally found. Although the fruit shape factor index expressed did not reflect any significant differences among treatments, differences were evident in absolute biomass terms. Fruit yield in *C. annuum* appeared to relate strongly to the availability of nutrients, with the higher presence of nutrients in the nutrient fertilizer soils yielding fruits of greater size and overall mass relative to the other treatments investigated. O'Sullivan (1979) found that yield increases in *C. annuum* occurred with increasing N application to soils. Interestingly, in this same study, the effect of N application on the wall thickness of peppers and fruit thickness was found to be unaffected by N application rate, and was found to be more strongly affected by irrigation frequency. Among greywater and tap water-irrigated treatments – with the exception of a second phase of fruit production in the most latter part of the Unilever site study – fruit counts among treatments were similar but were typically smaller in size than fruits from those plants treated with nutrient solution. (Rubio *et al.*, 2011) found a similar trend in peppers treated with NaCl where fruit yields were reduced due to a reduction in fruit size and biomass rather than through a decrease in fruit numbers produced. Whilst edible biomass yields and quality among tap-water and greywater treatments were similar, it is not known whether taste-related compounds in edible portions are altered perceptively to the human palate as a result of the different treatment applications. This is possible given that crop nutrition and fertilizer treatment are known to influence the flavour of fruit and vegetables by altering volatile compound compositions in edible tissues (Mattheis and Fellman, 1999).

4.3. Soil

4.3.1. pH

Increases in soil pH for the treatments investigated were found to be generally consistent for both *C. annuum* and *B. vulgaris* soils at the Unilever experiment site. For both species and for all treatments, soil pH was found to generally increase from weakly acidic to neutral over the experimental period. A significantly rapid decline in pH corresponding with the end of the first growth cycle at the Unilever experiment site was however observed for both treatments. It is thought that for the Unilever site this may have been due to soil leaching of base cations (Ca, Mg, K and Na) as a result of the high rainfall experienced in the month of January 2010, leading to acidification of soils (Haynes and Goh, 1980). However, the higher rainfall at the University in November 2010 did not completely coincide with a decline in pH suggesting other factors may have been responsible.

The longer growth period for which *C. annuum* were grown compared with that of the *B. vulgaris* appeared to have a negligible effect on the soil pH, since at the end of each growth cycle for each, soil pH values appeared to remain fairly neutrally bound. For soils in which *C. annuum* and *B. vulgaris* were grown, irrigation treatment was found to have an insignificant effect on soil pH values recorded since values were similar to tap water and nutrient solution treatments. These assessments were based on two experimental methods that were employed to evaluate changes in soil pH at the University experimental site due to the considerable inconsistencies observed between expected values and those values recorded. Significant discrepancies between methodologies utilized were found for pH of soils in which *B. vulgaris* and *C. annuum* were grown. For field-based measurements, soil pH values were found to decrease over the experimental period at both sites and for both plant species investigated. By contrast, when the *ex-situ* laboratory method was adopted, soil pH was found to generally increase during the experimental period. Soil pH is influenced by a number of factors which can include temperature (Hornick, 1992; Conyers *et al*., 1995), soil water potential (Conyers *et al*., 1995), organic loading and activity from fungi and bacteria (e.g. Kourtev *et al*., 2003). It is thought that field-based soil measurements may be particularly susceptible to prevailing soil physical conditions, such as temperature and soil water content, which may explain differences in the results measured by each approach given the relatively rapid changes in these variables on a daily basis. However, soils samples analysed *ex-situ* may also have undergone changes in pH between sampling and analysis, although the more alkaline values measured for greywater soils this way were expected, given the more alkaline properties of

the greywater media noted earlier. An indirect consequence of these shifts is that soil pH may, in turn, affect the biological characteristics and activities of certain fungi and bacteria (Rosenzweig and Stotzky, 1979; Rousk *et al*., 2009). Bacteria have been reported by Azcon *et al.* (1976) to potentially contribute to the growth of plants due to the ability to synthesise plant hormones, which may lead to greater exploratory area explored by roots, and in turn, result in higher uptake of particular nutrients. Soil pH has also been shown to exert a significant influence over the acquisition of the essential elements, such as manganese, zinc and iron-oxides (Sims, 1986), phosphorus (Schachtman *et al*., 1998) as well as lead, copper, zinc and nickel (Harter, 1983). This may have contributed in part towards some of the nutrient deficiencies in the species investigated but given the similar pH trends followed among treatments, any effect would likely have been similar for all treatments.

In this study plant type also appeared to influence soil pH; considerable differences in soil pH changes over time were noted among *C. annuum* and *B. vulgaris* pots and were apparent for both methods adopted at the University site. A study on the effect of certain plants on soil properties by Kourtev *et al*., (2003) found plants affected soil pH and nitrogen concentration through differential uptake rates of nitrogen as well as from changing interactions with microbiological communities of the soil in which they were grown. Since the rate of nitrogen uptake among the two crop species investigated was found not to be similar, it is possible that these differences may have had an indirect effect by altering soil pH.

A soil pH between 6 and 7 is considered optimum for the growth of *B. vulgaris* (DAEA, 1995). In this study, field-based measurements found pH values to be within this optimum range during the experimental period, but was in conflict with the higher pH values measured *ex-situ.* It is therefore uncertain whether the edaphic pH environment was conducive to optimal growth in this species. By contrast a soil pH of 5.5 to 6.0 is recommended for the optimum growth of *C. annuum* in the climatic region of this study (DA, 2001). On the basis of either method used to measure soil pH, values were above this range suggesting the pH range for all treatments were sub-optimal. This may provide further plausible evidence for some of the apparent deficiency symptoms in the tissues of *C. annuum* plant organs which may have been enhanced by treatment-induced changes in soil pH.

4.3.2. EC

Relative to tap water, the addition of greywater to soils had the effect of raising EC levels of soils over the experimental period, a development which was found for soils in which both *B. vulgaris* and *C. annuum* were grown. These findings were expected, given the high solute loadings of ionizing elements noted earlier, particularly Na, associated with the chemistry of greywater products used. Fluctuations of EC values among treatments, generally found to occur in unison over the experimental period, suggested irrigation treatment was not the only factor affecting soil EC. These similar trends, together with the slight decreases noted for LG and tap water treatments from original soil levels for both species could possibly be explained by the seasonal effects of higher rainfall towards the latter stages of the growth cycles. According to Suarez *et al.* (2006), rainfall events on a sodic soil can lead to a reduction in soil electrical conductivity. This is particularly pronounced in the upper soil horizons for many classifications of soils (Suarez *et al*., 2006), including those as sampled in this study. Since the growth period of plants took place across seasons with contrasting wet and dry rainfall spells, rainfall occurrence may therefore have induced soil EC phase shifts in the short-term; never-the-less the overall effect of increased soil EC with time remained suggesting EC to be resilient and persistent.

4.3.3. Hydraulic Conductivity

Differences in soil specific hydraulic conductivities among treatments, although corroborating the observed differences in soil drainage characteristics of soils, were not significant. With greater replicates than that used in the study, the coefficient of variation within treatment groups could be reduced, and differences among treatments become significant at the $P< 0.05$ level. However, the lower mean soil specific hydraulic conductivities established among greywater treatments were expected considering the relatively high SAR values recorded for greywater treated soils. At the Unilever experiment site, most of the initial reductions in soil K_s were maintained beyond the first growth cycle, extending into the second despite the fallow period between growth cycles. In almost all cases, either tap water- or nutrient solution-treated soils had higher mean K_s values, with greywater treatments typically lower for both experiment sites. Among greywater treatments, soils to which LG and CG treatments had been added reflected particularly low K_s values; PG however generally reflected comparatively higher values.

Based on observations, the apparent reductions in K_s among all treatments appeared to occur within a few weeks of irrigation applications to soils – this may be partly explained by soil compaction which may have reduced the initial soil K_s by reducing the number of soil pores (Taylor and Brar, 1991) – but could not fully explain K_s differences among treatments in the long-term. The generally lower K_s among greywater treatments compared to either the nutrient solution- or tap water- treated soils may however be partly explained by greater salinity induced dispersion and displacement of clays in greywater treated soils, which consequently may have clogged soil pores and disturbed their continuity (Minhas *et al.*, 1994). Reduced K_s in the PG treatment relative to the nutrient and tap-water treatment may have been enhanced by the presence of sodium tripolyphosphate which has been previously shown to reduce the permeability in a sandy loam soil and disrupt soil crumb integrity (Morgan and Watkinson, 1992). In the case of STPP, the magnitude of this effect on reducing soil permeability was perhaps offset somewhat by the relatively lower induced precipitation of insoluble salts known to cause pore blockages compared to soils to which other phosphates are added (Morgan and Watkinson, 1992). The white powdery substance found on pots to which powder-derived laundry greywater were added may therefore have been caused by precipitates of insoluble salts on soil surfaces. Mean differences in K_s among greywater treatments may also be explained by differences in surfactant-induced hydrophobicity. The lowest K_s and SAR was found in LG greywater but also the highest COD. By contrast PG greywater had a higher SAR and a higher K_s. The high ESP values reported in this study for the powder based-greywater formulations were generally higher than critical ESP values of 6 and 15 defined for soil sodicity in Australia and the United States respectively (Rengasamy and Olsson, 1991). Contrasting ESP recorded among treatments however did not have the expected influence on K_s and the basis for this is not fully understood. Excessively high ESP levels is known to reverse the process of soil aggregation in soils, causing soil aggregates to disperse into their individual constituent soil particles through flocculation (Davis *et al.*, 2012). This is due to the monovalent properties of Na, whereby Na can only absorb onto negatively charged soil particles at one end. Polyvalent cations such as Mg, Al or Ca can counteract this effect and induce soil aggregation (Marchuk and Rengasamy, 2010). The consequence of aggregation is changes in the physical properties of soils, including a reduction in soil macroporosity leading to low K_s (Chaudhari, 2001). However, in this study, whilst the relative concentrations of Mg, Al and Ca were similar across greywater treatments, the concentration of Na was lowest for LG greywater and LG-treated soils had the lowest K_s. Low K_s associated with greywater irrigation in this instance is therefore more likely related to

the high surfactant levels contributing toward increased soil hydrophobicity, rather than the effects of increased salinity on soil.

4.4. Conclusions and Recommendations for Future Research

Considerable inter-treatment variation in the physicochemical characteristics of irrigation media were found in this study and was explained by differences in the formulations of the laundry detergent products used to generate the different greywater formulations. Findings concerning trends in laundry greywater compositions were generally found to be congruent with other related studies, but comparisons among studies are inherently difficult. Laundry greywater is highly variable; wash load-size, wash-water volumes, wash-load, fabric soil type and nature, detergent choice and usage, and the wash cycles included in physicochemical assessment all can result in the production of laundry greywater of markedly different qualities. Therefore comparisons of greywater compositions among studies are dependent on whether actual laundry greywater productions are used from several households (which may encompass a plethora of laundry products and hence an effectively mixed laundry greywater combination) or whether greywater production was simulated artificially using a single laundry detergent product. Thus the marked differences in physicochemical ranges generally reported for greywater could also be explained largely by the experimental design adopted. Consequently, caution should be exercised in the broad interpretation of findings concerning the physicochemical characteristics of greywater. As a stand-alone study however, results presented here suggest that the nutrient compositions of laundry greywater were generally poor, particularly in terms of macronutrient availability. The exception was the PG greywater, which was found to have a P concentration comparable to the nutrient fertilizer solution. The higher P in the PG greywater formulation contributed to a greater P nutrient pool that was readily taken up by plant tissues and may be considered beneficial on this basis by contributing to plant growth. This benefit however was likely offset by N and K values that were significantly lower than the nutrient fertilizer, which meant that as key macroelements, these would ultimately constrain plant growth and function. The additional P may also prove unfavourable in the broader environmental context due the potential contribution of this element to eutrophication when used in sufficient quantities. Given the increasing cost of using P in detergent manufacturing and perceived environmental risk associated with P containing detergents, it is also likely that P containing detergents will become increasingly rare in coming years.

This study showed that the storage of greywater, at least for period of up to 72 hours, was practical for the formulations tested since the physicochemical quality of greywater remained largely unchanged. This finding is particularly positive for households where storage is possible, especially at times where rainfall is unpredictable or scarce such as during times of drought. Biological changes to greywater which may occur under the experimental conditions used were beyond the scope of this study and were not directly investigated. However since any adverse biological changes could be a decisive factor in determining the viability and risk associated with laundry greywater in storage, this could form part of future investigations on laundry greywater stored for reuse purposes.

Despite the differences in compositions associated with the laundry greywater formulations tested, the findings presented in this study do not suggest a particular detergent formulation to be notably superior compared to other detergent formulation for reuse in crop irrigation applications. On the basis of the physiological measures used in this study, trends among treatments were also found to be largely independent of species or conditions at each experimental site. The generally similar productivity of tap water-irrigated *C. annuum* and *B. vulgaris* relative to greywater-irrigated treatments supports the indicated use of certain laundry greywater formulations as alternative irrigation mediums for crop production at least in the short-term. In most cases, greywater was either similar or marginally detrimental to the use of tap water irrigation as assessed by crop growth rates (stem diameters and/or heights depending on the species), biomass (absolute and partitioned), nutrient uptake to leaves and fruit and edible biomass yields. Fruit quality in *C. annuum*, as assessed by pulp thickness and fruit size and shape were adversely affected by greywater irrigation compared to nutrient solution fertilizer, but was similar to tap water. This finding is positive from a socio-economic perspective, since the ultimate goal of reusing greywater for crop irrigation is to produce edible yields. For impoverished communities especially, this study demonstrates that greywater may be used as a substitute for tap water whilst still maintaining crop yields and yields of reasonable quality.

More distinction concerning the viability of treatments for crop irrigation could be made based on differences in the soil physicochemical characteristics among greywater treatments that were generally found. The high pH, SAR and EC values measured among the powder detergent-based greywaters were found to be problematic to soil structure and plants over the

experimental period. The tentatively lower mean K_s found among greywaters treated soils generally, but particularly for the LG-based greywater, suggested that the liquid laundry detergent product may be the least desirable choice since the potential for altering the integrity of the soil tested appeared to be greatest among the treatments investigated. In the long-term, irrigation with greywater generally resulted in increasing SAR and EC values, with concurrent declines in plant leaf and root biomass in most cases. This may be due in part to the relatively small seasonal shifts over which the two growth cycles were performed at the Unilever experiment site, but may also reveal that the effects on soils and plants were more chronic in nature.

The uptake of elements by either species were found to mirror closely the chemistry of irrigation media and soil to which they were applied. In instances where elemental uptake by plants was at levels considered toxic, deficiency symptoms were not readily apparent among treatments suggesting the additional influence of potentially confounding factors in shaping plant development. These findings highlight that it is inherently difficult to distinguish precisely the effect(s) of a particular element or physicochemical property measured in an integrated plant-soil system where interactive effects are likely. To isolate the effects of a single element contributing to a particular trend in plant development with near certainty would require a considerable time investment and was beyond the original aim of this study.

Considering the findings related to irrigation water quality, plants and soil collectively, irrigation with laundry greywater derived from the formulations tested would not be advisable if treated tap water were readily available primarily because of its adverse effects found on the soil tested. In times of acute water shortages however, greywater-irrigated crops can still produce viable quantities of edible crop portions. When the intended application is relatively short such as during an acute drought, laundry greywater can function to augment existing water supplies for agricultural practices.

An additional study considering the effects of laundry greywater irrigation on the perceived taste of edible crop portions to determine palatability, may provide conclusive information on whether the practice of crop irrigation with laundry greywater is advisable from a human consumption perspective. Overall, if the adoption of laundry greywater for crop irrigation is to be viewed as a comprehensively viable means for the alleviation of water scarcity and enhancement of food security, further research is warranted which would need to examine the same research premise but in different contexts. For example, in this study, laundry was

generated artificially with relatively minimal organic loadings which may be higher in some true-to-life scenarios. Additional organic loadings would likely enhance the fertilizer value of laundry greywater for crop irrigation based on performances of crops irrigated with mixed greywater in previous studies. Another approach could be to establish the influence of the addition of fertilizer to the greywater formulations on soils and plants. Study objectives were also confined to examination of soil property changes to greywater for a single soil type. However, soil type may prove critical to changes in plant growth and development under the influence of greywater irrigation. The chronic effects of repeated laundry grey water application on these and other soils, and on soil aggregate stability under the influence of markedly different climatic conditions, remain largely unknown, warranting further research. A study of long-term greywater applications to soils and plant may also wish to consider the effects on soil biota which were not part of this study but may be an important aspect, potentially influencing soil characteristics and plant growth.

The environmental and socio-economical aspects of using laundry greywater for crop production were also not addressed in this study. These would need to be examined in greater detail to explore the potential tensions between these facets.

CHAPTER V: REFERENCES

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CHAPTER VI: APPENDICES

6.1. APPENDIX A: UNILEVER EXPERIMENT SITE

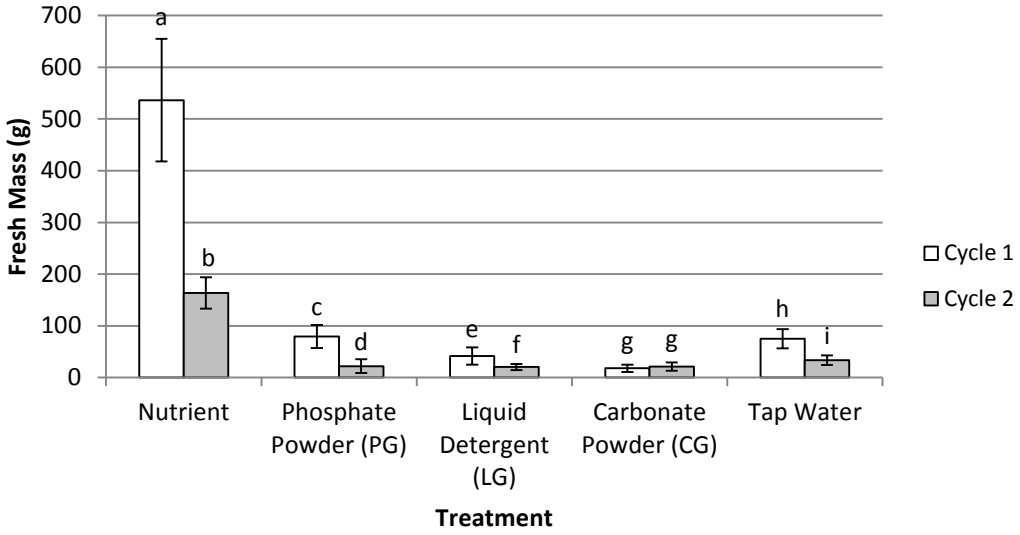

Fig. A1. Mean fresh mass of *B. vulgaris* leaves from the five irrigation treatments following 96 days of irrigation for each growth cycle. Vertical bars represent standard deviations. Letters represent mean separation between cycles by Scheffe's multiple range test (P <0.05; n = 9 per treatment).

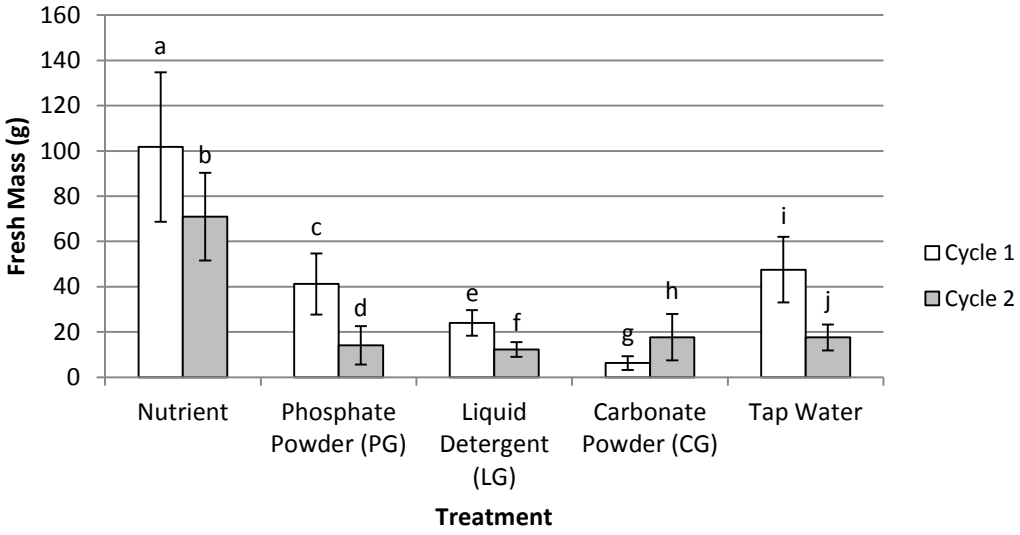

Fig. A2. Mean fresh mass of *B. vulgaris* roots from the five irrigation treatments following 96 days of irrigation for each growth cycle. Vertical bars represent standard deviations. Letters represent mean separation between cycles by Scheffe's multiple range test (P <0.05; n = 9 per treatment).

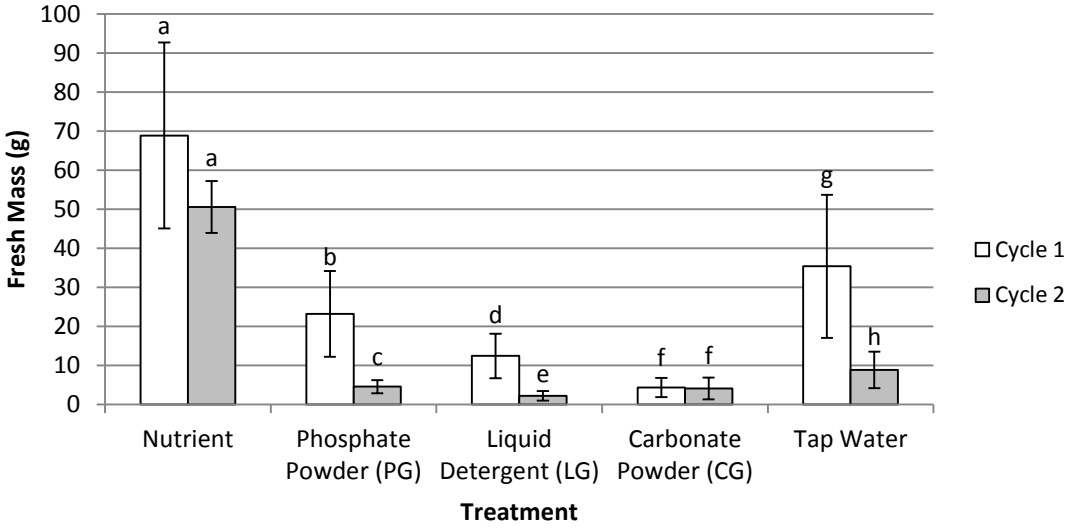

Fig. A3. Mean fresh mass of *C. annuum* leaves from the five irrigation treatments following 135 days of irrigation (Cycle 1 G3 = 97 days) for each growth cycle. Vertical bars represent standard deviations. Letters represent mean separation between cycles by Scheffe's multiple range test (P <0.05; n = 9 per treatment).

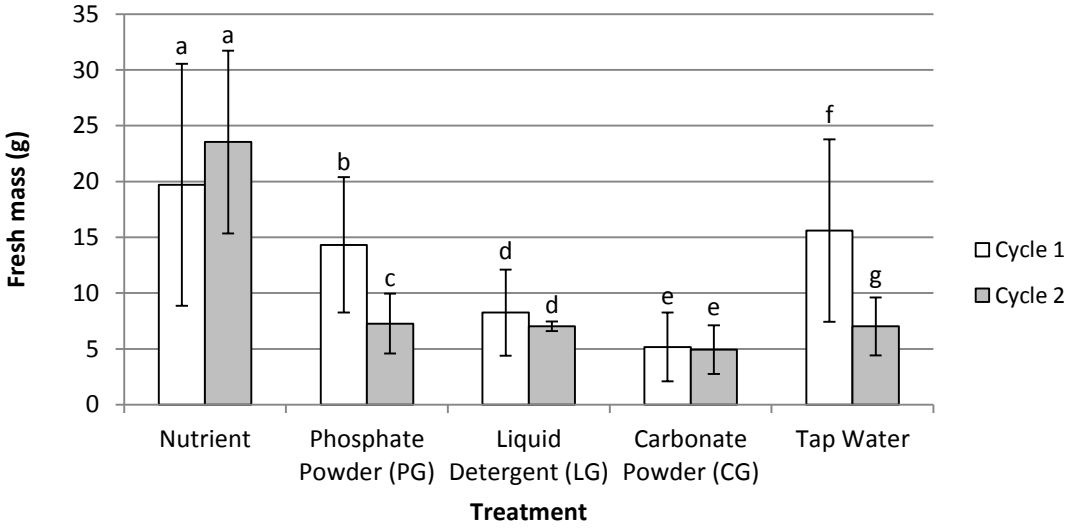

Fig. A4. Mean fresh mass of *C. annuum* stems and branches from the five irrigation treatments following 135 days of irrigation (Cycle 1: G3 = 97 days) for each growth cycle. Vertical bars represent standard deviations. Letters represent mean separation between cycles by Scheffe's multiple range test (P <0.05; n = 9 per treatment).

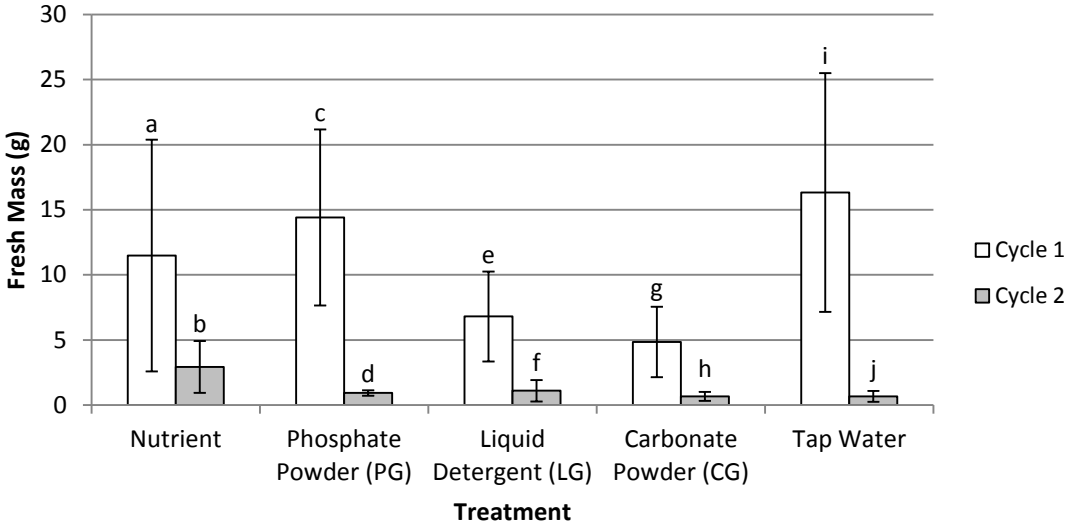

Fig. A5. Mean fresh mass of *C. annuum* roots from the five irrigation treatments following 135 days of irrigation (Cycle 1: G3 = 97 days) for each growth cycle. Vertical bars represent standard deviations. Letters represent mean separation between cycles by Scheffe's multiple range test (P <0.05; n = 9 per treatment).

6.2. APPENDIX B: UNIVERSITY EXPERIMENT SITE

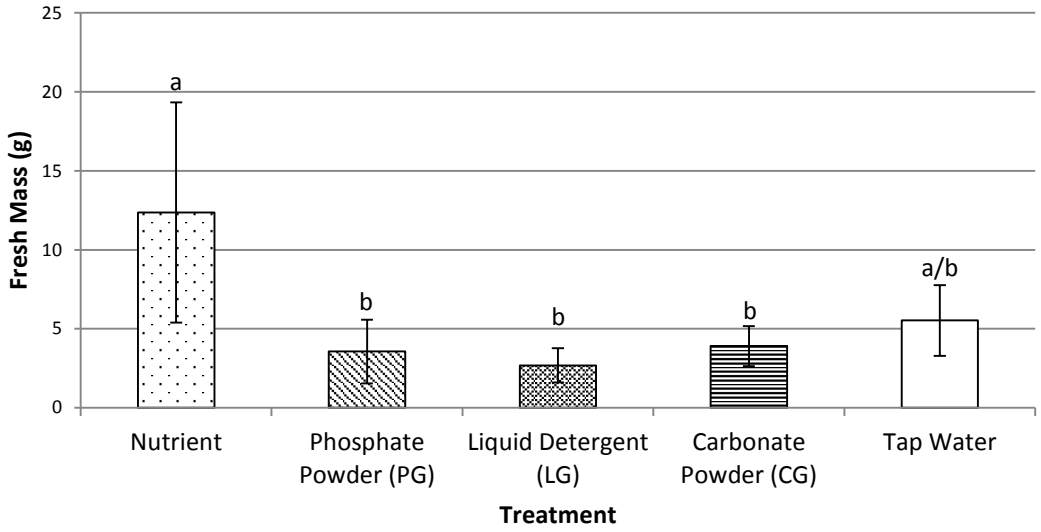

Fig. B1. Mean fresh mass of roots of *C. annuum* individuals from the five irrigation treatments following 96 days of irrigation at the University experimental site. Vertical bars represent standard deviations. Letters represent mean separation among treatments by Scheffe's multiple range test ($P< 0.05$; n = 9 per treatment).

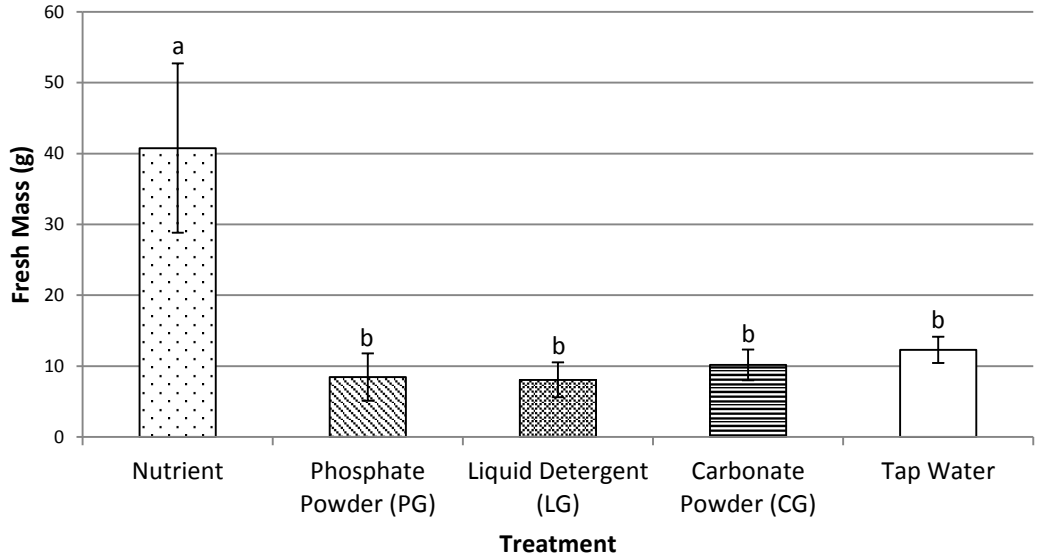

Fig. B2. Mean fresh mass of stems and branches of *C. annuum* individuals from the five irrigation treatments following 96 days of irrigation at the University experimental site. Vertical bars represent standard deviations. Letters represent mean separation among treatments by Scheffe's multiple range test ($P< 0.05$; n = 9 per treatment).

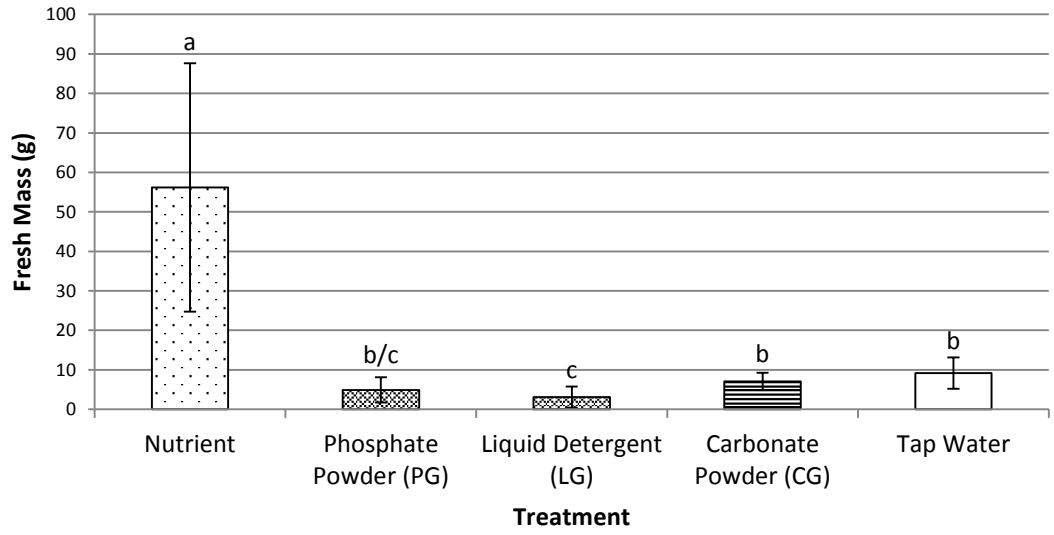

Fig. B3. Mean fresh mass of leaves of *C. annuum* individuals from the five irrigation treatments following 96 days of irrigation at the University experimental site. Vertical bars represent standard deviations. Letters represent mean separation among treatments by Scheffe's multiple range test ($P< 0.05$; n = 9 plants per treatment).

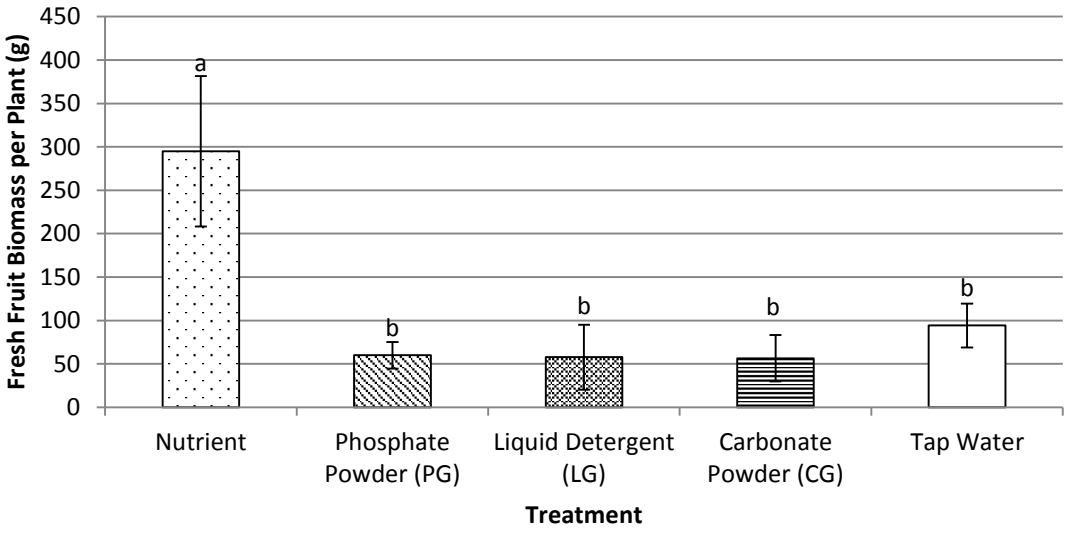

Fig. B4. Mean fresh fruit biomass of *C. annuum* individuals from the five irrigation treatments following 130 days of irrigation. Vertical bars represent standard deviations. Letters represent mean separation among treatments by Scheffe's multiple range test ($P< 0.05$; n = 9 plants per treatment).

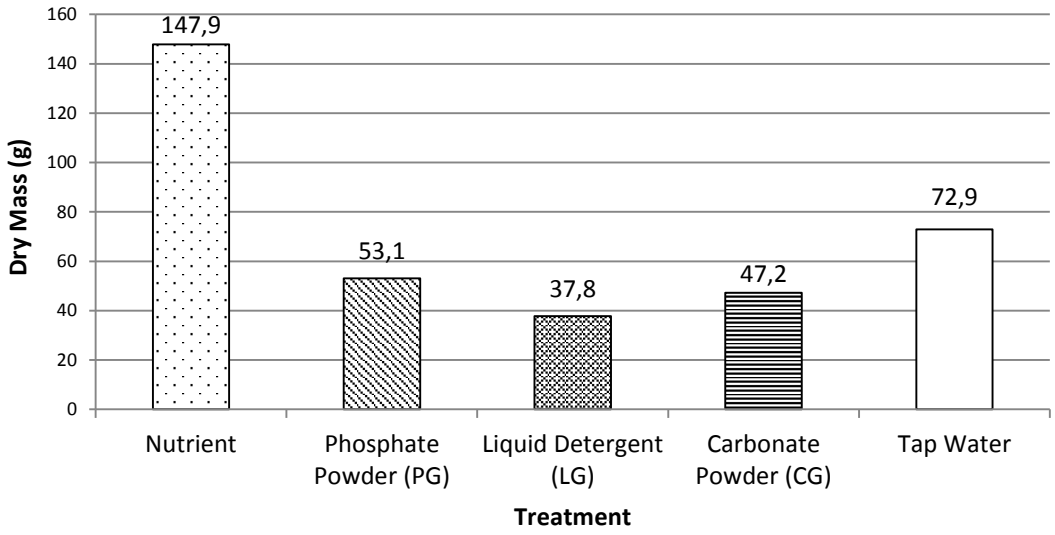

Fig. B5. Total dry fruit biomass yield of *C. annuum* fruit from the five irrigation treatments recorded post-harvest. Vertical bars represent standard deviations (n = 9 plants per treatment).

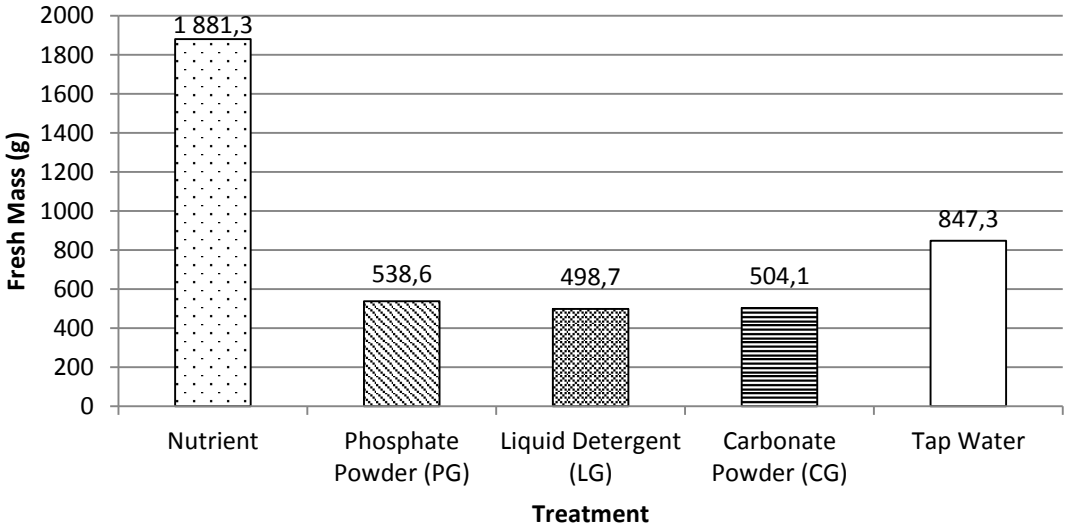

Fig. B6. Total fresh mass yield of *C. annuum* fruit at the University experimental site from the five irrigation treatments post-harvest. Vertical bars represent standard deviations. Letters represent mean separation among treatments by Scheffe's multiple range test ($P < 0.05$; n = 9 plants per treatment).

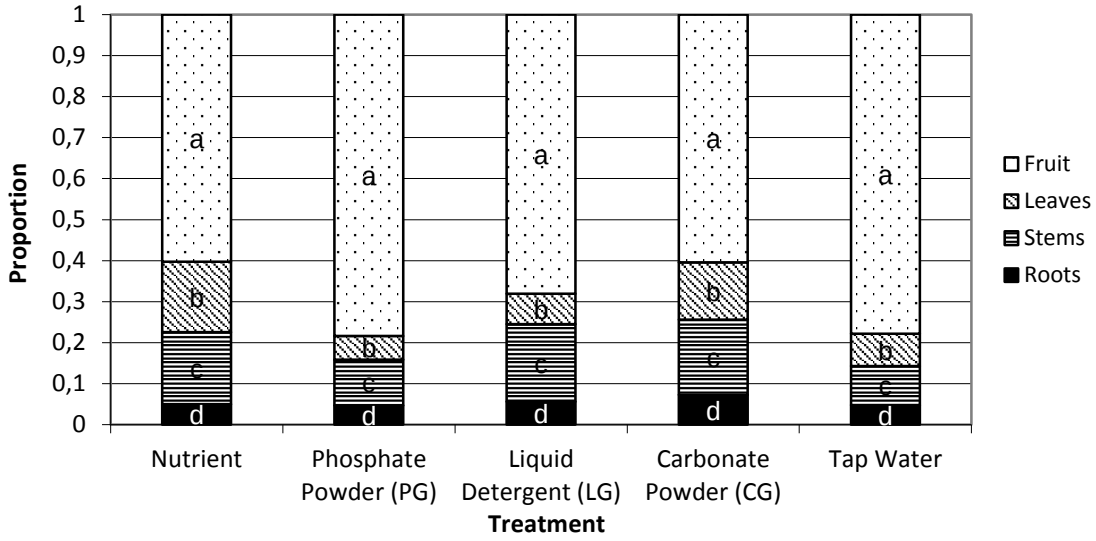

Fig. B7. Mean proportional fresh biomass allocation to various anatomical components of *C. annuum* among the five irrigation treatments. Vertical bars represent standard deviations. Letters represent mean separation among treatments by Scheffe's multiple range test (P <0.05; n = 9 plants per treatment).