

**Effects of organic and inorganic fertilisers on the growth of
Pseuderanthemum atropurpureum, soil fertility and leachate composition**

By

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DECLARATION


I, Duane Constance, declare that

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As the candidate's supervisors we have approved this thesis for submission.

Signed..........

Professor Isa Bertling (Supervisor)

Date.....1.11.2018.....

Signed.....

Dr A. (). Odindo (Co-Supervisor)

Date

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Acts 4:24 “And when they heard that, they lifted up their voice to God with one accord, and said, Lord, thou art God, which hast made heaven, and earth, and the sea, and all that in them is:”

All glory and honour to thee our Lord God, Father Almighty, for creatures great and small even for those that the naked eye cannot behold. O Lord, I thank you for this work, and all who have been a part of it coming together.

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ABSTRACT

The use of fertilisers in agricultural production systems, particularly nitrogen and phosphorous, has been shown to be one of the causes of eutrophication as a result of the excessive enrichment of freshwater systems through surface runoff and soil infiltration. The contamination of freshwater bodies from horticultural production systems in South Africa has, however, been rarely studied, although influx from such systems are considered highly polluting elsewhere. Eutrophication is particularly considered a major problem in areas with limited water resources. Phosphate is especially limiting in contributing to eutrophication in South African rivers and dams. The development of harmful algal blooms, particularly from cyanobacteria, has been a concern for a long time due to toxins introduced into freshwater systems from these algae.

This study investigated whether the use of organic fertilisers compared with inorganic fertilisers was potentially less detrimental to freshwater systems as a result of leachate nutrient and algal microorganism composition; further it was examined, if organic fertiliser was more beneficial to plant growth of *Pseuderanthemum atropurpureum*. Liquid and soluble granular organic and inorganic N equilibrated fertiliser treatments were applied at low, medium and high concentrations based on recommended label rates. Plant growth parameters were determined from mean height, number of leaves, size of leaves, number of nodes, internode length and number of branches. The species was grown over a period of three months and the experiment was repeated three times. Leaf tissue was analysed for mineral nutrient content and chlorophyll *a*, *b* and total chlorophyll. Leachate was analysed for mineral nutrient content including total phosphate, orthophosphate and chlorophyll *a*. Growth media was analysed for total nitrogen, ammonium and nitrate. A phase contrast light microscope was used to identify larger algal microorganisms and a scanning electron

microscope (SEM) to identify smaller algal microorganisms from growth media extracted leachate.

One specimen of green algae and some diatoms were identified, including two which may be found in eutrophic waters, but would not pose a threat similar to some species of cyanobacteria, if leached into freshwater systems over a period of time. Further, results showed that total phosphate and orthophosphate concentrations were significantly higher in leachate extracts from bark-based growth media across all fertiliser treatments and at all rates of treatment compared with soil-based growth media. This may have been due to a lack of binding sites in soilless media such as bark. Nitrate concentrations from organic soluble granular treatments were higher in both growth media types, whilst other treatments were similar. Ammonium and leachate nitrogen concentrations were found to be also similar. This may explain why plant growth traits assessed together were similar across all parameters tested. No single fertiliser compared with any other, produced plants that were superior in all growth characteristics measured. It is, therefore, suggested that the fertiliser treatments used in this study be applied at the half rate and plants be rather grown in randles growth medium than gromor for the production of *Pseuderanthemum atropurpureum*.

TABLE OF CONTENTS

DECLARATION.....	i
ACKNOWLEDGEMENTS.....	ii
ABSTRACT	iii
TABLE OF CONTENTS	v
LIST OF FIGURES	xi
LIST OF TABLES.....	xiii
CHAPTER 1	1
1.0 GENERAL INTRODUCTION.....	1
1.1 Background.....	1
1.2 Problem Statement.....	3
1.3 Research questions.....	4
1.4 Hypothesis.....	4
1.5 Objectives of this study.....	5
1.6 Reason for the study.....	6
1.7 Motivation for the study.....	6
1.8 Expected outcomes	6
1.9 Literature cited.....	7
CHAPTER 2 - Literature Review.....	10
2.0 Introduction.....	10
2.1 Eutrophication of fresh water systems.....	11
2.1.1 Causes of the eutrophication of fresh water systems.....	12
2.1.2 Pathways of total N and total P to fresh water systems.....	13
2.1.2.1 Total nitrogen (TN)	15
2.1.2.2 Total phosphorous	18
2.1.2.3 Silicon.....	20

2.1.3 Total N and total P as indicators of the eutrophication of fresh water systems	21
2.1.5 Freshwater microalgae dynamics	23
2.1.6 Soil microalgae	25
2.1.7 Current water situation in South Africa.....	25
2.1.8 Eutrophication of freshwater in South Africa: Current view	26
2.2 Greenhouse and nursery ornamental plant industry and production	28
2.2.1 Growth media	29
2.2.1.1 Properties of growth media.....	30
2.2.1.2 Effects of EC and pH on growth media characteristics.....	31
2.2.1.3 Composted pine bark and soil-based growth media mixes	35
2.2.1.4 Effects of growth medium texture on growth medium water leaching	38
2.2.2 Fertiliser use in pot plant production.....	39
2.2.2.1 Plant nutrition	42
2.2.3 Ornamental woody shrubs	47
2.2.4 Basic plant growth parameters	47
2.2.5 Effects of temperature on plant growth under cultivation.....	48
2.2.6 Greenhouse and nursery industry in South Africa.....	49
2.2.7 Conclusion	50
2.3 Literature cited	52
CHAPTER 3	69
Abstract.....	69
3.0 Introduction.....	71
3.1 Materials and methods	73
3.1.1 Study site and production environment	73
3.1.3 Growth media preparation and analysis	74
3.1.4 Experimental design	75

3.1.5 Fertiliser treatments	75
3.1.6 Plant analysis	75
3.1.6.1 Leaf analysis	78
3.1.6.2 Leaf chlorophyll determination	79
3.1.6.3 Plant growth parameter analyses	80
3.1.7 Cultural practices	81
3.1.8 Leachate collection and analysis	81
3.1.8.1 EC and pH analysis.....	81
3.1.8.2 Mineral element, total phosphate, orthophosphate and boron analysis	82
3.1.9 Temperature-logging	82
3.1.10 Statistical analysis.....	82
3.2 Results.....	83
3.2.1 Analyses of irrigation water	83
3.2.2 Analyses of growth media before planting.....	84
3.2.3. Leachate EC and pH.....	85
3.2.3.1 Leachate pH 60 days after planting	85
3.2.3.2 Leachate EC 60 days after planting.....	85
3.2.3.3 Leachate pH 90 days after planting	86
3.2.3.4 Leachate EC 90 days after planting.....	86
3.2.4 Analysis of growth medium inorganic nitrogen	89
3.2.4.1 Growth medium nitrate (NO ₃ -N).....	89
3.2.4.2 Growth medium ammonium (NH ₄ -N).....	89
3.2.5 Growth medium total nitrogen (TN)	90
3.2.6 Phosphate-P and TP in growth medium extracted leachate	90
3.2.6.1 Phosphate-P in extracted leachate	90
3.2.6.2 Total phosphates in extracted leachate	90

3.2.7	Macronutrients in growth medium extracted leachate	93
3.2.7.1	Nitrogen (N) in extracted leachate.....	93
3.2.7.2	Phosphorous (P) in extracted leachate.....	93
3.2.7.3	Potassium (K) in extracted leachate	93
3.2.7.4	Calcium (Ca) in extracted leachate.....	94
3.2.7.5	Magnesium (Mg) in extracted leachate	94
3.2.8	Micronutrients in growth medium extracted leachate	97
3.2.8.1	Iron (Fe) in extracted leachate	97
3.2.8.2	Boron (B) in extracted leachate	97
3.2.8.3	Manganese (Mn) in extracted leachate.....	97
3.2.8.4	Zinc (Zn) in extracted leachate.....	98
3.2.8.5	Copper (Cu) in extracted leachate	98
3.2.9	Nutrient composition of leaf tissue.....	100
3.2.9.1	Macronutrients in leaf tissue.....	100
3.2.9.2	Micronutrients in leaf tissue	103
3.2.10	Leaf chlorophyll <i>a</i> , chlorophyll <i>b</i> and total chlorophyll concentrations	106
3.2.11	Plant growth parameters	108
3.3	Discussion.....	115
3.3.1	Analyses of irrigation water and growth media.....	115
3.3.2	Effects of fertiliser treatments on growth medium EC and pH.....	117
3.3.3	Effects of fertiliser treatments and growth media on medium fertility	119
3.3.4	Effects of growth media on <i>Pseuderanthemum atropurpureum</i> growth.....	124
3.3.5	Effects of treatments on <i>Pseuderanthemum atropurpureum</i> growth	124
3.3.6	Effects of organic and inorganic fertiliser treatments on plant growth	126
3.4	Conclusion	128
3.5	Literature cited.....	129

CHAPTER 4.....	137
Abstract.....	137
4.0 Introduction.....	139
4.1 Materials and methods	142
4.1.1 Study site	142
4.1.2 Growth medium preparation and analysis	142
4.1.3 Plant preparation.....	143
4.1.4 Production environment	143
4.1.5 Experimental design	144
4.1.6 Plant treatment.....	145
4.1.7 Leachate collection and analysis	148
4.1.8 Algal chlorophyll <i>a</i> determination.....	148
4.1.9 Algal microorganism identification.....	150
4.1.10 SEM EDX qualitative analysis of growth media.....	150
4.1.11 Cultural practices.....	151
4.1.12 Statistical analysis.....	152
4.2 Results.....	153
4.2.1 Analyses of irrigation water	153
4.2.2 Analyses of growth media before planting.....	153
4.2.3 Leachate mineral composition (N, P, Ca, Mg, Fe, Al)	154
4.2.3.1 Nitrogen (N) in leachate	154
4.2.3.2 Phosphorus (P) in leachate	154
4.2.3.3 Calcium (Ca) in leachate	155
4.2.3.4 Magnesium (Mg) in leachate.....	155
4.2.3.5 Iron (Fe) in leachate.....	156
4.2.3.6 Aluminium (Al) in leachate	156

4.2.4 Phosphate (PO ₄ -P) and total phosphate in leachates	158
4.2.4.1 Phosphate (PO ₄ -P) in leachate	158
4.2.4.2 Total phosphate in leachate	158
4.2.5 Analysis of nitrate and total nitrogen in growth media	159
4.2.5.1 Growth medium nitrate (NO ₃ -N).....	159
4.2.5.2 Growth medium total nitrogen (TN)	159
4.2.6 Leachate composition	161
4.2.6.1 Algal chlorophyll concentration in leachate.....	161
4.2.6.2 Phase contrast light microscope algae identification.....	161
4.2.6.3 Scanning electron microscope (SEM) algae identification	161
4.2.7 SEM EDX qualitative analysis of mineral elements in growth media.....	162
4.3 Discussion.....	164
4.3.1 Analyses of irrigation water	164
4.3.2 Analyses of growth media before planting.....	164
4.3.3 Effects of treatments on leachate Al, Ca, Fe, Mg and forms of P concentration ..	165
4.3.4 Effects of treatments on growth media N.....	167
4.3.5 Algal presence in leachate	169
4.3.6 SEM EDX qualitative analysis of growth media.....	173
4.4 Conclusion	174
4.5 Literature cited.....	176
General conclusion and recommendations	190

LIST OF FIGURES

CHAPTER 3

- Figure 3.1: Effects of two organic (Nitrosol[®] and Organic Vitaboost) and two inorganic fertiliser treatment types (Polyfeed[®] and Lawn and Leaf) applied at three concentrations, on mean *Pseuderanthemum atropurpureum* height response, grown in two different growth media and compared with the two controls after a period of 90 days..... 108
- Figure 3.2: Effects of two organic (Nitrosol[®] and Organic Vitaboost) and two inorganic fertiliser treatment types (Polyfeed[®] and Lawn and Leaf) applied at three concentrations, on mean *Pseuderanthemum atropurpureum* leaf number response, grown in two different growth media and compared with the two controls after a period of 90 days..... 109
- Figure 3.3: Effects of two organic (Nitrosol[®] and Organic Vitaboost) and two inorganic fertiliser treatment types (Polyfeed[®] and Lawn and Leaf) applied at three concentrations, on mean *Pseuderanthemum atropurpureum* leaf size response, grown in two different growth media and compared with the two controls after a period of 90 days..... 110
- Figure 3.4: Effects of two organic (Nitrosol[®] and Organic Vitaboost) and two inorganic fertiliser treatment types (Polyfeed[®] and Lawn and Leaf) applied at three concentrations, on mean *Pseuderanthemum atropurpureum* node number response, grown in two different growth media and compared with the two controls after a period of 90 days..... 111
- Figure 3.5: Effects of two organic (Nitrosol[®] and Organic Vitaboost) and two inorganic fertiliser treatment types (Polyfeed[®] and Lawn and Leaf) applied at three concentrations, on mean *Pseuderanthemum atropurpureum* internode length response, grown in two different growth media and compared with the two controls after a period of 90 days 112
- Figure 3.6: Effects of two organic (Nitrosol[®] and Organic Vitaboost) and two inorganic fertiliser treatment types (Polyfeed[®] and Lawn and Leaf) applied at three concentrations, on mean *Pseuderanthemum atropurpureum* branch number, grown in two different growth media and compared with the two controls after a period of 90 days 113
- Figure 3.7: Google earth image of Randles Nursery where this study took place 136

CHAPTER 4

- Figure 4.1: Phase contrast microscope images: (A) Image of *Klebsormidium* sp. and (B) Brightfield image of *Klebsormidium* sp. 183
- Figure 4.2: SEM micrograph of (1) *Achnanthydium straubianum* (Lange-Bertalot) Lange-Bertalot and (2) *Planothydium engelbrechtii*. (Cholnoky) Round & Bukhityarova 183

Figure 4.3: SEM micrograph of (3) <i>Nitzschia amphibia</i> Grunow and (4) <i>Amphora montana</i> Krasske	184
Figure 4.4: SEM micrograph of (5) <i>Navicula veneta</i> Kützing and (6) <i>Navicula veneta</i> Kützing	184
Figure 4.5: SEM micrograph of (7) <i>Microcostatus</i> sp. and (8) <i>Planothidium</i> sp.....	185
Figure 4.6: SEM micrograph of (9) <i>Amphora montana</i> Krasske and (10) <i>Tryblionella levidensis</i> W Smith	185
Figure 4.7: SEM micrograph of an unidentified microorganism	186
Figure 4.8: Runoff from production areas at an ornamental plant nursery. Sharma <i>et al.</i> , 2008	186
Figure 4.9: Randles growth media control (CR) mineral element analysis from Energy Dispersive X-ray (EDX) spectroscopy with the corresponding image and EDX spectrum..	187
Figure 4.10: Gromor growth media control (CG) mineral element analysis from Energy Dispersive X-ray (EDX) spectroscopy with the corresponding image and EDX spectrum..	188
Figure 4.11: <i>Pseuderanthemum atropurpureum</i> grown at Randles Nursery at termination of study	188

LIST OF TABLES

CHAPTER 2

Table 2.1 Ranges of essential element concentrations in nutrient solutions and plant tissues (Silber and Bar-Tal, 2008).....	47
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CHAPTER 3

Table 3.1 Source and amount (by weight) of macronutrients and micronutrients in Nitrosol, Lawn and Leaf, Polyfeed and Organic Vitaboost fertilisers	76
Table 3.2 Dosage rate of N equilibrated fertiliser nutrients incorporated (g) into each 3 L plant bag or liquid fed·L ⁻¹ concentration as nutrient solution	78
Table 3.3 Chemical properties of irrigation water.....	83
Table 3.4 Physicochemical properties of randles and gromor start growth media.....	84

Table 3.5 Effects of two organic (Nitrosol® and Organic Vitaboost) and two inorganic fertiliser treatment types (Polyfeed® and Lawn and Leaf) applied at three concentrations to two different growth media on mean Pour thru extracted leachate EC ($\text{mS}\cdot\text{cm}^{-1}$) and pH compared to two controls after a period of 60 DAP and 90 DAP	88
Table 3.6 Effects of two organic (Nitrosol® and Organic Vitaboost) and two inorganic fertiliser treatment types (Polyfeed® and Lawn and Leaf) applied at three concentrations to two different growth media on mean growth media $\text{NO}_3\text{-N}$, $\text{NH}_4\text{-N}$ and TN and leachate $\text{PO}_4\text{-P}$ and TP concentrations compared to two controls after a period of 90 DAP	92
Table 3.7 Effects of two organic (Nitrosol® and Organic Vitaboost) and two inorganic fertiliser treatment types (Polyfeed® and Lawn and Leaf) applied at three concentrations to two different growth media on mean growth media Pour thru extracted nutrient solution N, P, K, Ca and Mg concentrations compared to two controls after a period of 90 DAP.....	96
Table 3.8 Effects of two organic (Nitrosol® and Organic Vitaboost) and two inorganic fertiliser treatment types (Polyfeed® and Lawn and Leaf) applied at three concentrations to two different growth media on mean growth media Pour thru extracted nutrient solution Fe, B, Mn, Zn and Cu concentrations compared to two controls after a period of 90 DAP ...	99
Table 3.9 Effects of two organic (Nitrosol® and Organic Vitaboost) and two inorganic fertiliser treatment types (Polyfeed® and Lawn and Leaf) applied at three concentrations to two different growth media on mean leaf tissue N, P, K, Ca and Mg concentrations compared to two controls after a period of 90 DAP	101
Table 3.10 Effects of two organic (Nitrosol® and Organic Vitaboost) and two inorganic fertiliser treatment types (Polyfeed® and Lawn and Leaf) applied at three concentrations to two different growth media on mean leaf tissue Fe, Mn, Zn and Cu concentrations compared to two controls after a period of 90 DAP	105
Table 3.11 Effects of two organic (Nitrosol® and Organic Vitaboost) and two inorganic fertiliser treatment types (Polyfeed® and Lawn and Leaf) applied at three concentrations to two different growth media on mean leaf tissue Chl <i>a</i> , Chl <i>b</i> and <i>Tchl</i> concentrations compared to two controls at 90 DAP.....	107
Table 3.12 Effects of two organic (Nitrosol® and Organic Vitaboost) and two inorganic fertiliser treatment types (Polyfeed® and Lawn and Leaf) applied at three concentrations to two different growth media on <i>Pseuderanthemum atropurpureum</i> growth parameters compared to two controls at 90 DAP.....	114

CHAPTER 4

Table 4.1 Source and amount (by weight) of macronutrients and micronutrients in Nitrosol, Lawn and Leaf, Polyfeed and Organic Vitaboost fertilisers	146
---	-----

Table 4.2 Dosage rate of N equilibrated fertiliser nutrients incorporated (g) into each 3 L plant bag or liquid fed·L ⁻¹ concentration as nutrient solution	147
Table 4.3 Chemical properties of irrigation water.....	153
Table 4.4 Physicochemical properties of randles (CR) and gromor start growth medium ...	154
Table 4.5 Effects of two organic (Nitrosol® and Organic Vitaboost) and two inorganic fertiliser treatment types (Polyfeed® and Lawn and Leaf) applied at three concentrations to two different growth media on mean growth media Pour thru extracted nutrient solution N, P, Ca, Mg, Fe and Al concentrations and compared with the two controls after a period of 90 days.....	157
Table 4.6 Effects of two organic (Nitrosol® and Organic Vitaboost) and two inorganic fertiliser treatment types (Polyfeed® and Lawn and Leaf) applied at three concentrations to two different growth media on mean growth media NO ₃ -N, and TN and leachate PO ₄ -P, TP and PChl <i>a</i> concentrations compared with the two controls after a period of 90 days...	160
Table 4.7 Effects of two organic (Nitrosol® and Organic Vitaboost) and two inorganic fertiliser treatment types (Polyfeed® and Lawn and Leaf) applied at three concentrations to two different growth media on SEM growth media qualitative analysis of Al, Si, P, Ca, Fe and Mg concentrations and compared with the two controls after a period of 90 days	163

This dissertation is a compilation of manuscripts where individual chapters are independent articles/manuscripts introduced disjointedly. Hence, some repetition between individual chapters has been inevitable. Chapters 3 and 4 are formatted to the requirements of Elsevier BV Publishers of *Scientia Horticulturae*. This dissertation consists of four chapters. Chapter 1 presents background information, reasons and justification for this study as well as objectives. Chapter 2 is a broad review of relevant literature pertaining to eutrophication, effects of ornamental plant production systems as one of its causes and its effects on South African freshwater resources. Chapter 3 was written in paper format and draws on information presented in the literature review in investigating the comparative effects of organic and inorganic fertilisers and soil and bark-based growth media on soil fertility and *Pseuderanthemum atropurpureum* growth. Chapter 4 was also written in paper format with a view to publishing, and also drawing on information presented in the literature review in investigating the comparative effects of organic and inorganic fertilisers and soil and bark-based growth media on leachate which may potentially pollute freshwater systems and result in eutrophication. General conclusions and recommendations for future study follows.

CHAPTER 1

1.0 GENERAL INTRODUCTION

1.1 Background

Agriculture has become the dominant land use over the past 300 years, mostly at the expense of forests, savannahs and grasslands. It is estimated that the global area of cropland in just less than three centuries increased from 265 Mha (mega hectares - 10^6) in 1700 to 1471 Mha in 1990 and pastureland from 524 Mha to 3451 Mha during the same period (Goldewijk, 2001). There is mounting pressure on the global agricultural system to provide sufficient food to an ever-increasing world population, which is expected to reach nine billion by 2050 (Wegner and Zwart, 2011). As developing countries adopt a more westernised diet (Tai *et al.*, 2014), the transition to livestock-based diets have mostly been embraced in developed countries (Alexandratos and Bruinsma, 2012). Undernourishment rates in developing countries are projected to decline substantially through a combination of economic growth and agricultural advancements (Tai *et al.*, 2014). Today only one third of all landscapes can be considered non-agricultural (Ostberg *et al.*, 2015).

Food provision is critical for human well-being, but its production has had adverse effects on the environment (Bennett *et al.*, 2014). Global threats from climate change, soil erosion, diminishing water resources as well as agricultural and horticultural practices present a formidable challenge to food security and plant production for commercial purposes. Agricultural and horticultural plant production are highly dependent on adequate amounts of water, but both do impact on its quality and availability as a resource necessary for its sustainability (Serediak, 2014; Bonacin *et al.*, 2015). Commercial greenhouse and nursery production systems are highly intensive and also require sufficient amounts of nutrients to maintain plant growth, thus ensuring crops of high value and quality (Smith *et al.*, 1999;

Taylor *et al.*, 2006; Andiru, 2010; Dennis *et al.*, 2010). Fertiliser usage per unit area is higher under protected cultivation, particularly in the greenhouse industry, than in any other agricultural system (van Iersel, 1999). This is partly due to the high nutrient supply to ornamental species, such as tropical foliage plants, produced in nursery environments; yet, little is known about the leaching of nutrients from containers to surrounding areas (Broschat, 1995). Literature on specialist crops, like potted chrysanthemums, poinsettias and geraniums exist (e.g. Tayama and Carver, 1992), but no information seems available on optimal nutrient supply to woody ornamentals (Ristvey, 2004). Nutritional requirements for container-grown plants are also known to vary widely among species and even between cultivars (Agro and Zheng, 2014). A newly transplanted seedling, for example, requires much less nitrogen than a rapidly growing forty day old plant (Evans, 2007). Several factors, however, complicate the management and economic considerations of fertiliser use.

Nitrogen (N) cannot be fully utilised in any production system (Schröder *et al.*, 2004), and along with phosphate (P) under moderate to excessive irrigation, leads to the risk of nutrient leaching, contamination of groundwater and eutrophication of receiving surface waters from runoff. (Goh *et al.*, 1979; van Iersel, 1999; Juntunen *et al.*, 2003; Merhaut *et al.*, 2006; Alem *et al.*, 2015). Eutrophication causes an increase in phytoplankton primary production and has been aligned with a number of environmental problems. These include depletion of dissolved oxygen, ultimately causing fish death, water turbidity and harmful algal blooms (HABs). Eutrophication also affects water usage for agriculture, human and animal consumption and recreation as well as for commercial enterprises, such as thermal power, pulp and paper and beverage plants (Carpenter *et al.*, 1998; Chislock *et al.*, 2013; Lemley *et al.*, 2015).

In some countries legislation dictates how open water resources should be used, maintained and sustained (Lemley *et al.*, 2015). Of particular concern is the use of fertilisers, the type,

mode of action and methods of application, as this determines the amount of discharge over a certain period of time. Controlled-release, liquid and water-soluble fertilisers have been the subject of many studies. Broschat (1995) investigated fertiliser types that could have low environmental impact, whilst at the same time producing plants of high quality and value. The Broschat study (Broschat, 1995) revealed that all controlled-release, liquid and water-soluble fertilisers leached N and P, with controlled-release fertilisers leaching the least. Comparative investigations have also been carried out between organic and inorganic fertilisers relating to plant growth (Bi *et al.*, 2010) and the leaching of nutrients.

1.2 Problem Statement

Eutrophication presents a major problem to South African agriculture, as the country has one of the most nutrient-enriched surface waters in the world (Frost and Sullivan, 2010). These water resources are under threat from HAB's (Chetty *et al.*, 2013) which are damaging to ecosystems (Ramkilowan *et al.*, 2013). Harmful algal blooms (especially cyanobacteria and dinoflagellates) appear to be increasing worldwide in frequency and the extent of the blooms (Van Ginkel, 2008). A broad definition of HAB's describes these planktonic algae as being potentially toxic species that can cause hypoxia and anoxia as a consequence of an extraordinary increase in biomass which may result in mortalities of aquatic life in a water system irrespective of whether toxins are produced or not (Heisler *et al.*, 2008). Agriculture is the largest consumer of surface water in South Africa, with large amounts used for irrigation purposes (Blignaut *et al.*, 2009). Ncube (2015) identified the agricultural industry as being the leading contributor of pollution to South African fresh water systems. It is, however, unclear to what extent the horticultural, floricultural and forestry seedling industry contribute to this problem. The use of organic fertilisers, particularly the fact that many of these are slow-release fertilisers (Carpio *et al.*, 2005) could allow South African nurseries to achieve high

plant quality for commercial plant production purposes rather than inorganic fertilisers that may discharge more N and P in nursery runoff.

1.3 Research questions

A range of fertilisers, both organic and inorganic, with different application methods are used in commercial nurseries. Plants (seedlings and cuttings) are mostly grown-on in plastic plant bags, pots and trays. Research has been carried out to identify best management practices (BMP's) in agricultural and horticultural enterprises worldwide with regard to fertiliser and irrigation usage and practices. There, however, appears to be little research on groundwater contamination and run-off from commercial ornamental plants, floriculture and forestry nurseries to natural and man-made water bodies in South Africa.

To identify the potential impact of organic fertiliser use in the ornamental/ nursery industry, the following research questions are addressed in this project:

- Are organic fertilisers more beneficial than inorganic fertilisers for plant growth and soil fertility?
- Are organic fertilisers less detrimental to freshwater systems in terms of its potential total nitrogen and total phosphorous leachate output, than inorganic fertilisers?
- What are the possible effects of nutrient leaching from any given ornamental plant nursery using the four types of fertiliser utilised in this study?

1.4 Hypothesis

The hypothesis of this study is that organic fertilisers are potentially less detrimental to freshwater systems, more beneficial for plant growth and soil fertility. This is based on reports that controlled poultry litter use from two formulations of organic poultry litter treatments at low to intermediate rates resulted in container grown plants with the highest dry

weights and similar quality compared to plants that received the highest rates of an inorganic treatment (Burnett *et al.*, 2016) and that organic fertilisers compared with inorganic fertilisers are often considered less damaging to the environment whilst improving soil quality (Bi *et al.*, 2010).

1.5 Objectives of this study

The main objective of this study was to establish potential beneficial effects of organic fertilisers on plant growth, while, at the same time, being less damaging to the environment than inorganic fertilisers due to less N and P runoff from a soil based growth medium and a bark based growth medium. The following set of specific objectives was carried out:

- comparison of plant growth traits and nutrient uptake of *Pseuderanthemum atropurpureum* under the different fertiliser regimes,
- determination of chlorophyll concentrations in leaf tissue,
- determination of mineral nutrients in leachates,
- determination of total nitrogen (TN), and inorganic nitrogen (NO₃-N plus NO₄-N) in the two types of growing medium,
- determination of total phosphate and Orthophosphate (PO₄-P) concentrations in leachates,
- comparison of growing media with respect to response of soil fertility, plant growth and fertiliser type,
- assessment of chlorophyll *a* concentrations in leachate from plant bags (water column phytoplankton biomass) since phytoplankton contain chlorophyll, and
- detect mineral nutrient content in growing media qualitatively using scanning electron microscope (SEM).

1.6 Reason for the study

The lack of information on runoff from tunnels and nurseries in South Africa makes it impossible to gauge its potential impact on eutrophication of surface waters. There also seems to be no specific legislation restricting the indiscriminate use of fertilisers in the horticultural and agricultural runoff of discharge water.

1.7 Motivation for the study

South Africa will have to provide food for an ever increasing population, similar to the rest of the world; in addition a fast-growing wealthier African population will put pressure on the available water sources. There is a rapidly increasing demand for water in a country that is classified as water scarce (Blignaut and Van Heerden, 2009) due to a relatively low annual rainfall. Water demand is expected to exceed availability by 2025 (Musvoto *et al.*, 2015). In turn, low water availability could result in increasing food insecurity. It has become important to regulate water consumption, prevent contamination and reduce pollution of water sources (Rouwenhorst, 2007). Agricultural impacts on eutrophication have been studied, but little emphasis has been placed on run-off from nursery and tunnel operations.

1.8 Expected outcomes

It is anticipated that organic fertilisers give rise to, lower amounts of N, P and K in the run-off, as well as greater plant growth compared to inorganic fertilisers.

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CHAPTER 2 - Literature Review

A literature review of the factors and elements affecting leachate composition, consequences and plant growth in soil and soilless growth media as a result of type and method of fertilisation under South African conditions

2.0 Introduction

The factors that affect the production of a field grown crop differ from those of a greenhouse or nursery container crop in some aspects. Fertigation, for instance, is not practical for large scale field crop production and may only be used if economically viable. It is widely used in greenhouses and nurseries, but nutrients applied in a dissolved form are likely to leach with the irrigation water from containers. Application of slow-release and controlled-release fertilisers are also used in both field and nursery production systems. Literature, however, has shown that these too leach nutrients into production areas. Nutrients are often applied in excess of plant requirements (Newman and Hayden-Smith, 2014). The extent of nutrients leached, notwithstanding irrigation quantities, is largely dependent on the type of growth medium and fertiliser used. This has been extensively investigated in container plant production. Organic and inorganic fertiliser use in field and nursery crop production has also been researched and there is support for its use in both systems of production. Consumers are willing to pay 10-15% more for organically grown crops Burnett *et al.* (2016) and organic fertiliser use has often been considered environmentally friendly Bi *et al.* (2010).

Runoff from production areas of greenhouses and agricultural lands carrying nutrient loads from fertiliser inputs can lead to excessive enrichment of surface waters which results in eutrophication. Symptomatic changes may include a considerable increase of algal biomass and aquatic macrophytes in freshwater systems, deteriorating the water quality and other

undesirable conditions affecting water usage (Van Ginkel, 2007). This chapter reviews literature on factors influencing the production of container grown plants, leachate composition and eutrophication of freshwater systems. The importance of this is that they are interrelated and a problem in many parts of the world.

2.1 Eutrophication of fresh water systems

The word ‘eutrophication’ comes from the Greek word ‘eutrophos’ meaning well-nourished and has also been described as well-fed (Barnard, 2009; Gilau, 2015). Eutrophication can be considered as natural, occurring as leaching of nutrients from a source over lengthy periods of time, or cultural, also called anthropogenic, which refers to human input and disturbance. The latter can result in an undesirable increase in algae biomass also called an algal bloom due to an often rapid, but generally excessive increase in nutrient content in surface water (Carpenter *et al.*, 1998; Khan and Ansari, 2005; Serediak, 2014). Khan and Ansari (2005), Yang *et al.* (2008) and Roy *et al.* (2013) defined eutrophication as ‘the sum of the effects of the excessive growth of phytoplankton leading to imbalanced primary and secondary productivity and a faster rate of succession from an existing ecosystem to a higher seral stage as caused by nutrient enrichment through runoffs that carry down excessive nutrient loads from agro-ecosystems and/or discharged human waste from settlements.

The American Heritage Science Dictionary, (2005) describes phytoplankton as plankton consisting of free-floating algae, protists, and cyanobacteria which forms the beginning of the food chain for aquatic animals and fixes large amounts of carbon, which would otherwise be released as carbon dioxide. The rate of carbon fixation by photosynthesis is referred to as primary production and the primary driving force for this is therefore light (Sathyendranath and Platt, 2001). Eutrophication forms part of a natural ageing process of lakes where the waterbody, through the accumulation of organic matter, becomes a wetland

and later a part of the terrestrial system. This takes a long time naturally, but is quickened through human input (Van Ginkel, 2007). There are a number of causes of eutrophication as a result of human influence and activity.

2.1.1 Causes of the eutrophication of fresh water systems

Available global sources of freshwater are groundwater (>98%) and surface water (<2%). Surface or freshwater systems include inland rivers, streams and lakes (Bouwer, 2000; Ndlovu, 2013). Rivers are streams by definition, just larger with more flow volume. Sources of groundwater are boreholes and springs (Ndlovu, 2013). Carpenter *et al.* (1998) and Yates (2008) have differentiated the causes of eutrophication in aquatic systems into point and non-point sources. Contaminants and mineral elements are transferred to fresh water systems and groundwater by precipitation, runoff and leaching (Yates, 2008). Runoff and soil infiltration from agricultural production systems are regarded as non-point or diffuse sources which lead to eutrophication, including the return flow of source irrigation water from irrigated agricultural lands (Carpenter *et al.*, 1998). Other non-point sources include runoff from pasture, unsewered areas, small construction sites, abandoned mines and atmospheric deposition over water surfaces. Point sources include wastewater discharge from municipalities and effluent from industry (Carpenter *et al.*, 1998).

Runoff from agricultural lands and nurseries contains varying, but significant, amounts of N and P (Carpenter *et al.*, 1998; Smith *et al.*, 1999; Taylor *et al.*, 2006; Evans, 2007). There are different schools of thought on which of these is the main cause of eutrophication in freshwater systems. Some studies have suggested that N is the limiting nutrient in marine systems and P the limiting nutrient in fresh water bodies, especially in temperate zones, but exceptions exist (Howarth and Marino, 2006). Elser *et al.* (2007) reported that either N or P can increase the primary production of a fresh water system, principally through

phytoplankton photosynthesis, whilst an increase in both simultaneously leads to a rather significant increase in phytoplankton biomass. Co-limitation of nutrients, more specifically N and P, has been suggested by Bracken *et al.* (2015) as a reason for an increase in phytoplankton biomass based on a study involving the collation, meta-analysis and review of data from long-term experiments. Additionally, silicon (Si) is needed by diatoms in their body structure and as such is limited by Si availability (Rabalais, 2002). The need for Si is greater than for N and P, to such an extent that a diatom assemblage in a column of water will die off (Tallberg, 2000).

Primary productivity of phytoplankton, is also affected by other factors besides nutrient inputs that may lead to a eutrophic event and these include light, temperature, salinity, thermal stratification and water pH, dissolved O₂ and CO₂, as well as turbidity (Khan and Ansari, 2005; Yang *et al.*, 2008; Serediak, 2014), river flow, water residence time and grazing (Mortazavi *et al.*, 2000). The interplay between these factors will determine how a freshwater ecosystem will respond to nutrient additions and over-enrichment (National Research Council, 2000). The nutrient loading of N and P to fresh waters is the most common cause of eutrophication. This is brought on principally by rain and hydrological factors in the catchment landscape and watershed, which ultimately determine the flow path to freshwater ecosystems (Ojwando, 2014). Literature on the mechanisms by which N and P may be transported from a plant nursery site to a freshwater body is limited.

2.1.2 Pathways of total N and total P to fresh water systems

Terrestrial runoff and atmospheric input are the primary sources of new nutrient loading to fresh water systems (Guildford and Hecky, 2000). N and P are critical mineral elements for the growth of algae and aquatic plants as well as terrestrial plants (Smith *et al.*, 1999; Elser *et al.* 2007; Li, 2016). Large quantities of water and nutrients, especially N and P, are used in

commercial container plant greenhouses and nurseries for the production of high value and high quality plants (Polomski *et al.*, 2007; Matysiak, 2015). A large amount of this nutrient-rich irrigation water, estimated at 78% by Furuta (1978), ends up as runoff. Samant (2010) suggested that between 60 to 80 % of irrigation water from a forestry container nursery is lost as runoff. This therefore implies that, in similar production systems, a large volume of irrigation water with dissolved nutrients has been - and still is - lost to runoff, given the time periods between these reports. Drechsel *et al.* (2015) reported that irrigation management in developing countries and fertilisation practices in developed countries have improved agricultural systems over the last thirty years. Van Iersel *et al.* (2013), Bayer (2014) and Million and Yeager (2015) have carried out investigations into the use of automated irrigation systems. The authors suggested that such technology can be used for more precise irrigation amounts and frequencies, thereby conserving irrigation water; however, this does not necessarily mitigate the effects of nutrients on runoff or groundwater contamination. Alem *et al.* (2015) have reported that up to 65% N or P from fertiliser applications can be lost in the production of potted plants using timer-controlled systems. Even lesser quantities than these lost amounts to wastage.

Nutrients applied to plants in container production are generally incorporated into or placed on the surface of the growing medium or delivered through fertigation systems. During irrigation or fertigation, the growing medium pores are filled, making dissolved nutrients and water available to the roots. The remaining pore water and dissolved nutrients is displaced during the next irrigation or fertigation event and discharged into the production area as runoff (Hoskins *et al.*, 2014). Total N and Total P forms in the irrigation water respond differently when entering the production environment and soil zone when either progressively becoming part of surface runoff or being absorbed through infiltration.

2.1.2.1 Total nitrogen (TN)

Nitrogen is often measured as TN in water samples and samples collected from runoff (Brosch, 2015). In leachate from production areas of container nurseries TN concentrations may exceed $500 \text{ mg}\cdot\text{L}^{-1}$ (Cregg *et al.*, 2004). One, however, has to take into account the “fate” of N in leachate as it becomes part of the surface water and groundwater watersheds. In leachate, TN is composed of four fractions: Inorganic N, including nitrate ($\text{NO}_3\text{-N}$), nitrite ($\text{NO}_2\text{-N}$) and ammonium ($\text{NH}_4\text{-N}$) and complex forms of organic N, including refractory nitrogen, amino sugars, amino acids, or proteins (Janßen and Koopmann, 2003; Bergese, 2013; Bhatt and Sapra, 2015). Refractory nitrogen is the nitrogen that cannot be biologically altered and is no longer available for biological productivity (Knicker and Hatcher, 1997).

Nitrate is more water-soluble than the other N fractions (Janßen and Koopmann, 2003; Follett, 2008; Serediak, 2014; Matysiak, 2015) and has the highest potential to leach from mineral soils and pot growing media. This is because of low anion-holding-capacity of most growth media and especially if composed of pinebark, peat, vermiculite and sand (Broschat, 1995; Follett and Delgado, 2002; Janßen and Koopmann, 2003; Matysiak, 2015; Bhatt and Sapra, 2015). McAvoy (1994) and Dunn *et al.* (2015) reported excessively high concentrations of $\text{NO}_3\text{-N}$ in the soil under greenhouses. The rate of $\text{NO}_3\text{-N}$ discharge in runoff or soil infiltration found in potted plant nurseries is dependent on factors such as the amount of rain or irrigation received, type and rate of fertilisers used, soil or growing media texture and structure, soil or growing media organic matter content and crop nutrient uptake (Howarth *et al.*, 1996; Letey and Vaughan, 2013). Dissolved $\text{NO}_3\text{-N}$ leaches readily into the upper soil profile, through an intermediate unsaturated area called the vadose zone and then into the ground water or saturated zone (Hermanson *et al.*, 2000; Follett and Delgado, 2002; Evans, 2007). The actual rate of movement through the soil profile may be slow (Follett *et al.*,

2010) taking several months in sandy soils with coarse-textured vadose zones (Ferguson and De Groot, 2000). Nitrate leaching will not occur unless the rate of water infiltration and deep percolation, through precipitation or irrigation, exceeds the rate of evapotranspiration (Smith and Cassel, 1991; Rubin, 2013).

High levels of nitrate in ground water destined for human consumption poses a health risk, potentially resulting in methaemoglobinaemia. Esterhuizen *et al.* (2015) found levels of nitrate on some farms in South Africa to be seven times more than the South African health limit of $11 \text{ mg}\cdot\text{L}^{-1}$ and reported increasing nitrate groundwater levels in rural areas of South Africa which are hazardous to bottle-fed infants (blue baby syndrome) and livestock. According to Ndlovu (2013), about 15% of South African water consumption emanates from groundwater sources.

Nitrate contaminated groundwater can discharge into surface water by base flow (Brosch, 2015). Ground water, including the contaminants in it, is able to move to rivers, lakes and dams from all landscapes such as agricultural and forestry (Lasagna *et al.*, 2016; Wang *et al.*, 2015). This interaction is also possible in the opposite direction. Surface water, including the contaminants in it, is able to move to adjacent ground water systems (Lasagna *et al.*, 2016). Brunner *et al.* (2011) refer to a 'gaining or losing fresh water regime flow', in the interaction between surface and groundwater.

A further pathway for $\text{NO}_3\text{-N}$ to enter fresh water systems is through surface or overland runoff. Given a preceding period of dry conditions, runoff is initiated through heavy rainfall or through irrigation due to infiltration excess whereby the intensity of rainfall or irrigation exceeds the soil infiltration rate (Qiu *et al.*, 2007; Nyawade, 2015). Runoff can also occur through saturation excess, where soils are nearly or fully saturated, and unable to accommodate more water. This scenario is considered the main mechanism of runoff in well-

vegetated areas with a humid climate and permeable soils (Steenhuis *et al.*, 1995; Nyawade, 2015); $\text{NO}_3\text{-N}$ concentrations in surface runoff from nursery and greenhouse operations have been reported to range between 1.6 and 55 $\text{mg}\cdot\text{L}^{-1}$ (Taylor *et al.*, 2006). The South African Environmental Protection Act (2002) stipulates that concentrations of $\text{NO}_3\text{-N}$ in discharged effluent onto land, into a watercourse or a freshwater body may not exceed 10 $\text{mg}\cdot\text{L}$. Actual data from soil analysis for $\text{NO}_3\text{-N}$ concentrations in catchment areas and riparian zones linking adjoining freshwater systems and agricultural lands is limited.

Nitrite ($\text{NO}_2\text{-N}$), like $\text{NO}_3\text{-N}$, does not readily adsorb to soil fractions because of its anionic nature and is able to move freely with water (Ghaly and Ramakrishnan, 2015). Nitrite occurs as an intermediate form when, during the process of mineralisation, ammonium ($\text{NH}_4\text{-N}$) is converted to $\text{NO}_3\text{-N}$. Nitrite is also the intermediate form in the process of nitrification, when, within aerobic conditions, $\text{NH}_4\text{-N}$ is converted to $\text{NO}_3\text{-N}$ (Follett, 2008; Ghaly and Ramakrishnan, 2015). This is a rapid process that may be completed within two days (Follett, 2008; Yates, 2008).

Ammonium adsorbs to soil fractions because of its cationic nature and will not readily leach into the soil profile nor be part of runoff (Follett, 2008; Grahmann *et al.*, 2013; Nyawade, 2015). Wind or surface flow transports $\text{NH}_4\text{-N}$ with eroded soil aggregates that are detached from the soil surface by rainfall. Ammonium is deposited either en route to freshwater bodies in the watershed or loaded directly into receiving surface waters (Follett, 2008; Nyawade, 2015).

In recent times, organic N has become an important and significant N fraction of fresh water systems, so that its transport dynamics and origin warrant more studies, including that of agricultural land use watersheds (Vogt *et al.*, 2011; Vogt *et al.*, 2015). The export of terrestrial N occurs in the form of dissolved and particulate organic nitrogen (Lee *et al.*,

2016). Organic N occurs in dissolved forms of N (DON) which include wastes excreted by organisms or from the degradation of particulate organic-N (Berman and Bronk, 2003) and particulate forms of N (PON) including small organisms, such as algae, bacteria and protozoa, both living and dead, and fragments of organisms (Johnson and Gerald, 2006). The amount of N leached or in surface runoff, more specifically organic N, is dependent on N mobility and results from factors of the environment including vegetation, hydrological processes, biological processes existent in the watershed and the primary source of N (Johnson and Gerald, 2006; Vogt *et al.*, 2011).

In greenhouse and field crop production, the N input refers primarily to use of fertilisers used. Organic N fractions predominate in magnitude depending on the land use. In most soils, organic N comprises more than 90% of total N (TN) and this could have a significant impact on DON in receiving waters from surface runoff (Wang *et al.*, 2015b). Similar to $\text{NH}_4\text{-N}$, PON is adsorbed to soil fractions and is also subject to rapid mineralisation (Veras *et al.*, 2016). The mode of transport to receiving surface waters is the same as for $\text{NH}_4\text{-N}$ (Durand *et al.*, 2011).

2.1.2.2 Total phosphorous

Total phosphorous exists in both organic and inorganic forms in soils (Evans, 2005; Davis, 2006; Jarosch, 2012; Nthejane *et al.*, 2012; Ojwando, 2014). These forms may also be classified as orthophosphate, polyphosphate or organic phosphate (Evans, 2005) and may either be dissolved or particulate and bound to soil fractions or the structure of some soil minerals (Evans, 2005; Davis, 2006; Ojwando, 2014). Davis (2006) reported that organic phosphate accounts for more than 50% of TP, while Nthejane *et al.* (2012) reported values of 15-80% of TP, while Johnson and Gerald (2006) reported organic phosphates to make up 25-65% of TP. These phosphate forms consist mainly of inositol phosphates, phospholipids and

nucleic acids (Nthejane *et al.*, 2012; Lyngsie, 2013). Microorganisms break down otherwise unavailable organic P to inorganic forms of plant available orthophosphate (Davis, 2006; Johnson and Gerald, 2006; Nthejane *et al.*, 2012). Orthophosphate (PO_4^{3-}) is also referred to by its hydrated forms (HPO_4^{2-} , H_2PO_4^- or H_3PO_4) of the ion (Pettersen, 2014). Temperature, soil water and organic matter in the soil influence the conversion rate and magnitude of organic to soluble phosphate (Nthejane *et al.*, 2012).

Dissolved phosphate, also referred to as soluble, reactive phosphate, is defined as the fraction that can pass through a 0.45 μm filter (Brothers, 2014; Shumaker and Paul, 2008). Particulate phosphate is smaller than 0.45 μm and is readily adsorbed to soil fractions or some soil minerals (Shumaker and Paul, 2008). Phosphate is the form of P that is readily taken up by both, terrestrial and aquatic plants, including phytoplankton, and is available for immediate uptake (Lee *et al.*, 1980).

Phosphate, like $\text{NO}_3\text{-N}$, can move from watersheds to receiving surface waters and groundwater through surface runoff, erosion and infiltration (Nthejane *et al.*, 2012; Shumaker and Paul, 2008). Phosphorous surface runoff occurs mainly in the form of particulate phosphate with eroded soil fractions (Miguntanna, 2009; Ojwando, 2014) although dissolved phosphate runoff also occurs (Shumaker and Paul, 2008); it can infiltrate the soil profile to groundwater as dissolved P (Davis, 2006) or particulate P (Lyngsie, 2013). Wind erosion of P is also a factor in its transport to fresh water bodies (Davis, 2006).

Studies of nutrients applied in container production have reported that up to 65% of P is lost through leaching (Alem *et al.*, 2015), with 0.01 to 20 $\text{mg}\cdot\text{L}^{-1}$ P lost through surface runoff from nurseries (Pomolski *et al.*, 2008). The phosphate ($\text{PO}_4\text{-P}$) concentrations in samples taken from nursery drainage areas ranged from 0.60 $\text{mg}\cdot\text{L}^{-1}$ during winter to 144 $\text{mg}\cdot\text{L}^{-1}$ during the growing season (Sharma *et al.*, 2008). The South African Environmental

Protection Act (2002) stipulates that concentrations of PO₄-P in discharged effluent onto land may not exceed 10 mg·L and may not exceed 1 mg·L when discharged into a watercourse or a freshwater body. Actual data from soil analyses for PO₄-P concentrations in catchment areas and riparian zones linking adjoining freshwater systems and agricultural lands is limited and therefore the possibility of predicting an algal bloom event before a storm is remote. This may be particularly important in a country like South Africa where water is scarce.

Most freshwater pollution studies focus on N and P as limiting nutrients, with silicon commonly being overlooked (Pearson *et al.*, 2016).

2.1.2.3 Silicon

Diatoms, which form a major part of phytoplankton communities (Choudhury and Bhadury, 2015), require silicon (Si), unlike other phytoplankton (Javaheri *et al.*, 2015). Silicate, a primary constituent of diatoms, is specifically used for the growth of these organisms which utilize this compound to produce their external shell called the frustule (Choudhury and Bhadury, 2015; Javaheri *et al.*, 2015). Studies have determined that growth is influenced by the N:Si ratio, when either N or Si are limiting (Gilpin *et al.*, 2004; Choudhury and Bhadury, 2015). Pearson *et al.* (2016) suggested that phytoplankton, more limited by N than Si, will outcompete diatoms for dominance, except for periods of lower temperatures, lower light levels and increased Si enrichment and that this limitation may result in eutrophication. With global warming, the length of these diatom algal bloom periods as a time factor are decreasing and toxic cyanobacterial blooms may potentially be more prevalent for longer periods of time (Pearson *et al.*, 2016).

Another major concern, is that diatom species are biological indicators of the health of freshwater ecosystems (De la Rey, 2008). Testing for every possible pollutant chemical is costly and difficult. Some of the reasons for using biological indicators include cost

effectiveness as the difficulty in testing for every possible pollutant is reduced; assemblages of organisms such as certain diatom species by their presence or disappearance and past history indicate the condition of a water body, techniques are quick, accurate and have been widely used to assess fresh water quality. Identifications and counts can be done by nonspecialists with a biological background if they are provided with illustrated guides (Taylor, 2006; De la Rey, 2008).

Soils containing, or amended with, Si have a good adsorption capacity and reduced P leaching (Matichenkov *et al.*, 2001). When plants were grown in soilless growing media, Si in plant tissue was found to be significantly lower in comparison with plants grown in soil-type media due to limited Si availability in soilless growth media (Voogt and Sonneveld, 2001). This implies that lower levels of Si in soilless growth media result in a limited capacity of the medium to prevent P leaching. Tubaña and Heckman (2015), citing findings from previous studies, stated that dissolved silicon in soil solution (H_4SiO_4) displaced P from slightly soluble phosphates of Al, Ca, Fe and Mg releasing it into the soil solution, making P more plant available.

2.1.3 Total N and total P as indicators of the eutrophication of fresh water systems

Dissolved inorganic mineral elements in aquatic ecosystems are assimilated by phytoplankton via metabolic processes (van Ginkel, 2011). Phytoplankton is critically dependent on N and P for growth (Kohn, 2016). Over-enrichment of water sources with these primary nutrients leads to increased phytoplankton biomass (Kitsios, 2004; Smith *et al.*, 2016) which is often too large for the phytoplankton consumer populations, such as zooplankton, to control (Kitsios, 2004).

In nutrient-phytoplankton relationships attention is often focused on TN and TP concentrations as well as the TN to TP ratio (Guildford and Hecky, 2000). Measurements of

the TN to TP ratio in freshwater samples are considered a true reflection of all N and P fractions in the system. Analysis for only NO₃-N, NH₄-N and PO₄-P fractions and not TP and TN as well, will only show what is immediately available for phytoplankton uptake but not for possible future use (Van Ginkel, 2007). The TN to TP ratio determines growth, abundance and taxonomic composition of a phytoplankton community but is particularly important when this ratio favours cyanobacteria dominance (Filstrup *et al.*, 2016). The community structure is determined by competition between the individual species for nutrients (Brauer, 2015) and also by physical factors such as light, temperature, turbidity and alkalinity (Reynolds *et al.*, 2002).

Cyanobacteria are strong competitors for N and weak competitors for P (Brauer, 2015), but will become the predominant phytoplankton species (toxic algal bloom) of a phytoplankton community at a low TN:TP ratio (Mihajlov, 2005; Brauer, 2015; Palus, 2015). *Microcystis* is the most dominant cyanobacteria in South Africa followed by *Anabaena* (Van Ginkel, 2004). *Microcystis* produces the toxin microcystin. *Anabaena* produces anatoxin and microcystin amongst others (Sivonen and Jones, 1999). This may impact human and livestock health adversely because of the toxicity in water if consumed (Van Ginkel, 2007). Blooms of this nature have been an increasing problem in South Africa over the last thirty years (Van Ginkel, 2011; Oberholster *et al.*, 2012).

The determination of TP and TN are frequently used to describe the ‘trophic’ status of freshwater bodies and are ideal parameters as the total nutrient content of the phytoplankton biomass are measured as well as those nutrients that are available to it (Dodds, 2003). All species of phytoplankton contain chlorophyll and the determination of this is a reliable and internationally used proxy for total phytoplankton biomass (Gregor and Maršálek, 2004) and an excellent trophic state indicator (Boyer *et al.*, 2009).

2.1.4 Algal chlorophyll *a* (PChl *a*)

There are many different parameters, which may differ from study to study, used to assess eutrophication and trophic status of which TN and TP are the basic ones (Yang *et al.*, 2008). Others include mean annual chlorophyll *a* (PChl *a*) and % of time PChl *a* > 30 g·L⁻¹ as used by Van Ginkel *et al.* (2001) or dissolved inorganic nitrogen, dissolved inorganic phosphate, dissolved oxygen and benthic diatom index as used by Lemley *et al.* (2015). It has been established that there is a relationship between TN, TP and Chl *a* that indicates the status of freshwater ecosystems (Magumba *et al.*, 2014). Chlorophyll *a* concentration is typically proportional to the concentration of a phytoplankton assemblage or community within a water column (Galvez-Cloutier and Sanchez, 2007).

There are four broad categories used in the classification of South Africa's aquatic ecosystems: oligotrophic, mesotrophic, eutrophic and hyper-eutrophic (De Villiers, 2007) which were categorized as 'Good', 'Fair', 'Poor' or 'Very Poor', respectively in the Lemley *et al.* (2015) study. Mean annual PChl *a* concentrations > 30 µg·L⁻¹ would indicate hyper-eutrophic conditions according to the classification system used by the Department of Water Affairs for sampling sites and should be considered a serious problem (Van Ginkel, 2011). Variables to determine the biological properties of freshwater ecosystems typically include Chl *a* as well as algal species composition (Van Ginkel and Hohls, 2001). Aquatic algal species composition has been determined by the use of scanning electron microscopes (SEM), transmission electron microscopes and optical phase contrast microscopy.

2.1.5 Freshwater microalgae dynamics

Aquatic microalgae may be classified as phytoplankton which are free-floating or periphyton which are attached to rocks, sediment or other aquatic organisms (Sigeo, 2005). Phytoplankton and periphyton are photosynthetic and autotrophic organisms that form as

primary producers, the base of the food web in both fresh and marine water systems (Striebel, 2008; Simmons, 2012; Fayiss, 2015) driving and sustaining the ecological functioning of these ecosystems (Lemley *et al.*, 2016). Some of the more common freshwater microalgae phyla occurring in South African dams and rivers are Chlorophyta (Green Algae), Cyanophyta (Blue-Green Algae), Chrysophyta (Golden-Brown Algae), Bacillariophyta (Diatoms), Euglenophyta (Euglenoids), Cryptophyta (Cryptomonads) and Dinophyta (Dinoflagellates) (Van Vuuren *et al.*, 2006). Each algal family has specific biotic and abiotic requirements for its survival (Lai, 2013). Light, temperature and nutrients are factors that regulate biomass, distribution, and structure of phytoplankton communities in the water column and freshwater body (Perez *et al.*, 2007; Striebel, 2008).

Tobin (2011) reported that populations of diatoms, green algae and cyanobacteria in lakes have often peaked sequentially, beginning with diatoms when temperatures are cooler in late winter and early spring followed by green algae at warmer temperatures and then cyanobacteria during hotter periods. A defined seasonal succession of phytoplankton species in temperate lakes, as reported by Wehr (2011), is of significance in the more temperate regions of South Africa. Toxic cyanobacterial blooms arising from sewer and industrial effluent, particularly in the summer months, have been a regular eutrophic occurrence in many, if not most, of the rivers and impoundments in South Africa (Oberholster and Ashton, 2008). Recorded cyanobacterial and dinoflagellate bloom events appear to have increased in frequency in South Africa and worldwide. The N:P ratio has been considered a potential management option in understanding the dynamics of phytoplankton proliferation which is influenced by dissolved, particulate and sediment-bound nutrients within a freshwater ecosystem and the control this ratio could have in reducing eutrophication (Van Ginkel, 2007).

2.1.6 Soil microalgae

Soil algae, also contain chlorophyll. Green algae are more abundant at the soil surface than below the surface, due to light accessibility (Messyasz, 2006). Terrestrial diatoms are also mostly found at the soil surface (Antonelli *et al.*, 2017), but may migrate deeper into soil due to possible desiccation at higher temperatures (Souffreau *et al.*, 2013). Top soil and river sand, used as growth media components in potting mixes, may contain soil algae. These may include the cyanobacteria – *Anabaena* and *Oscillatoria*, as identified in soil by Zancan *et al.* (2006) and in freshwater (Van Vuuren *et al.*, 2006), and the green algae – *Scenedesmus*, in soil (Zancan *et al.*, 2006) and in freshwater (Van Vuuren *et al.*, 2006). Some diatom species have also been described in freshwater systems, such as *Achnanthes minutissima* and *Nitzschia palea* (Kutzing) (Taylor *et al.*, 2007) and in soil (Zancan *et al.*, 2006). Living algal microorganisms in potting media may be able to enter freshwater systems in much the same way as N and P enter freshwater systems.

2.1.7 Current water situation in South Africa

South Africa is a semi-arid country (Snyman 1998; Turpie and Visser, 2013; Dalu, 2014). The demand for water in a country that has a relatively low annual rainfall of 497 mm per year (the global rainfall average is 860mm) is, however rapidly increasing (Kapuku, 2015). Rainfall distribution is also uneven with 21% of the country receiving less than 200 mm annually (Lai, 2013). South Africa's freshwater resources are under strain due to increased use and demand from a growing population, agricultural growth, socio-economic development and industrialisation (Oberholster and Ashton, 2008; Mwangi, 2014; Kapuku, 2015). Approximately 98% of these water resources, which include rivers, dams and groundwater, have been fully allocated (Hedden and Cilliers, 2014) since 2005 (Oberholster and Ashton, 2008). There have already been water restrictions across the whole country

(du Plessis *et al.*, 2015) as a result of critically low dam levels (Kapuku, 2015). The three main consumers of water are agriculture, industrial and domestic use (Rouwenhorst, 2007). These three are also the main causes of the deterioration of freshwater quality through salinisation, eutrophication, acidification, and microbial pathogens (Mwangi, 2014). Effluent from manufacturing, mining and power generation industries, sewage discharge as well as nutrient loads in agricultural, urban and forestry runoff and groundwater infiltration lead to a decline below the standards of water (Oberholster and Ashton, 2008; Jordaan and Bezuidenhout, 2013).

Agricultural irrigation accounts for about 62% of freshwater usage in South Africa (Rouwenhorst, 2007, Kapuku, 2015). The South African National Water Act (Act No. 36 of 1998) recognises that water is scarce, that government is responsible for water as a national asset and that there is a need for water quality and quantity protection, as well as its sustainable use for the people of this country into the near future (Africa, 1998). Water security remains a concern as South Africa's freshwater resources will be unable to sustain the current patterns of water use and discharge, as a result of anthropogenic activities (Oberholster and Ashton, 2008; du Plessis *et al.*, 2015).

2.1.8 Eutrophication of freshwater in South Africa: Current view

Agriculture is considered a major underlying cause of eutrophication in many catchments around the world (Withers *et al.*, 2014; Esterhuizen *et al.*, 2015). Intensive agricultural practices continue to result in the eutrophication of aquatic ecosystems due to high nutrient fluxes from agricultural landscapes (Heathcote, 2013; Jarvie *et al.*, 2013; Pettersen, 2014; Botha, 2015; Sakadevan and Nguyen, 2015).

Agricultural production systems in South Africa include intensive crop and mixed farming in winter rainfall and high summer rainfall areas with both, direct and indirect effects on surface

water quality (Shabalala *et al.*, 2013). The concerns surrounding eutrophication in South Africa are described by Thornton *et al.* (2013) as a “wicked” problem. Turton (2012) suggested that around one third of all South Africa’s water that is stored is eutrophic. The impairment of surface waters presents a major threat to potable and irrigation water (Oberholser *et al.*, 2009), which mostly comes from man-made dams to ensure supply (Pindihama *et al.*, 2011; Botha, 2015). Rural communities depend on water from sources that are frequently of poor quality, unreliable and inefficient; this part of the population is also often faced with polluted river water as an alternative source for domestic consumption (Oberholster *et al.*, 2009; Pindihama *et al.*, 2011).

Recently, studies have revealed toxins inside produce from crops irrigated with eutrophic waters (Turton, 2012). The National Eutrophication Monitoring Programme (NEMP) has been in place since 1985 but the problem of eutrophication remains a threat (Mwangi, 2014). Ally (2013) suggested that a solution to the eutrophication problem requires a multidisciplinary, multi-sector and multi-focal approach to arrest the impairment of the country’s freshwater systems. If the reservoirs are to fulfil the basic and multiple functions of resource provision, waste assimilation and recreation, then intense attention needs to be devoted to the concept of ‘integrated water resource management’ to prevent eutrophication (Botha, 2015).

The consequences of the over enrichment of freshwater as a result of nutrients in runoff and groundwater infiltration from agricultural lands and nurseries have been examined. Agricultural activity is considered one of the leading causes of the eutrophication problem in South Africa (Ncube, 2015). Literature relating to greenhouse and nursery practices, on the effects of fertiliser usage and subsequent surface runoff and infiltration, seems non-existent for South African conditions. There is practically no information on the types of growth

media and fertilisers used in ornamental commercial plant production in South Africa or on the cultivation of *Pseuderanthemum*.

2.2 Greenhouse and nursery ornamental plant industry and production

Greenhouse and nursery-cultivated ornamental plants include trees, shrubs, palms, groundcovers, climbers, ornamental grasses and bedding plants (Dobres, 2008; Chandler and Sanchez, 2012; Witcher, 2013), with many species and cultivars of each being propagated (Chandler, 2013; Zhu *et al.*, 2015) for the commercial market. The ornamental plant industry has grown tremendously worldwide as the search for new marketable plant species has increased (Drew, 2010). There are more ornamental plant species and cultivars propagated today than all other agricultural and horticultural crops put together (Middleton, 2012). Ornamental plants are sought after and grown for their aesthetic, amenity and cultural value they afford to indoor and outdoor open spaces integral to daily living (Afrin, 2009; Middleton, 2012; Chandler, 2013).

Common propagation systems for ornamentals include seed, cutting, grafting, and micro-propagation methods (Wu, 2013; Swelih, 2015). The type and species of a plant determines the propagation method and the optimal propagation environment. Cold-hardy ornamentals may be staged and grown on in open compounds after establishment, whereas tender plants must be propagated and grown on in a protected environment, such as a greenhouse, during the colder months until it has reached saleable age (Witcher, 2013). In some geographical locations, cuttings of certain ornamentals do not require sophisticated greenhouse conditions and controls. In most parts of South Africa, a plastic clad tunnel with a misting facility suffices. Often plants are produced more rapidly by vegetative propagation methods than from seed (Wu, 2013), avoiding the challenges aligned with seed dormancy (Ruchala, 2002) or with recalcitrant seeds (Pammenter *et al.*, 2007; Varghese *et al.*, 2011). Vegetative

propagation also has the distinct advantage of producing plant material that is true to type (Swelih, 2015), as vegetative propagation is cloning, where duplicates of the mother plant are produced, thus ensuring desirable characteristics are passed on (Ruchala, 2002)

Mass production of ornamental plants for commercial markets occurs mainly through propagation by stem cuttings (Jones *et al.*, 2010), commonly achieved in a relatively small area (Araya, 2005). Most ornamental plants are produced in plastic containers, which may be pots or bags, and are grown in growth medium (Witcher, 2013).

2.2.1 Growth media

A growth medium for plants, such as ornamentals, in containers commonly consists of a variety of components in order to offer plants adequate support, oxygen, water and nutrients for plant growth (Ingram *et al.*, 1993; Jackson, 2008). Knowledge of plant nutrition and soil fertility is essential in greenhouse and nursery plant production to ensure adequate, sustained and available forms of plant nutrients for plant uptake within the root zone (Jones and Jacobsen, 2005). Soil fertility may be defined as the capability of a soil to supply essential nutrients that will enhance plant growth, while soil productivity refers to a soil's ability to produce a crop (Follett *et al.*, 1987). Traditionally, soils were used to produce container ornamental plants (Adriaanse, 2013). The use of field soil for ornamental propagation is still common practice in some parts of the world today, such as Romania (Popescu and Popescu, 2015) and Nigeria, although there are concerns of soil-borne pathogens (Adriaanse, 2013).

Most growth media mixes used today in greenhouse and nursery production do not contain mineral soils (Richard, 2006), and are referred to as soilless or artificial media (Agro, 2014). Primary components of these media may include peat, pine bark, perlite, vermiculite, compost and coarse sand (Dewayne *et al.*, 2003). Peat and bark are both acidic with little buffering capacity (Bilderback *et al.*, 2013). Some media components, like peat, bark, and vermiculite,

possess a high cation exchange capacity CEC (Ingram *et al.*, 1993). These and other components either singly or blended in a growth media mix give rise to different biological, physical and chemical properties (Richard, 2006,).

2.2.1.1 Properties of growth media

Biological properties of growth media: Biological properties of soils are influenced by the presence and activities of soil macro-fauna (earthworms etc.) and micro-fauna (nematodes etc.) as well as soil micro-flora (bacteria, fungi, actinomycetes, algae etc.) which are involved in biochemical reactions and biological processes within the soil (Brussaard, 1997). Soil algae includes green algae (Messyasz, 2006), diatoms (Vacht *et al.*, 2014) and cyanobacteria. It has been reported that soilless media are preferable over soil as they are relatively free from soil-borne pathogens (Raviv *et al.*, 2008), affording more accurate control of the root zone, improved plant quality and increased plant production (Van Winden, 1987; Vallance *et al.*, 2010). Peat and coir possess low biological activity but might harbour root pathogens (Avilés *et al.*, 2011). Soilless cultures may develop root pathogenic problems soon after the introduction of the plant material and irrigation, even in inorganic growth media mixes which barely contain any microorganisms (Vallance *et al.*, 2010). Jackson (2008) reported that composts may suppress soil-borne diseases, although not all composts suppress all root pathogens (Avilés *et al.*, 2011). Schwarz and Krienitz (2005) reported that certain algae can prevent anaerobiosis around the root system and may even promote plant growth, while being potentially problematic in greenhouse production systems. Due to fertigation in hydroponic systems, algae are prone to cover all surfaces containing sufficient light, nutrients and moisture (Chase and Conover, 1993; Schwarz and Krienitz, 2005).

Physical properties of growth media: Physical properties of growth media include soil texture (Westervelt, 2003). A growth medium has a textural and a structural component, both

affecting porosity and water-holding capacity (Nimmo, 2004). Soil structure is the arrangement of the soil particles into aggregates of various sizes and shapes (Yoon, 2009), while texture refers to the size and distribution of the particles in a medium (Westervelt, 2003; Nimmo, 2004). The texture of a soilless growth medium has a similar impact on the growth medium's physical properties as it has on soil physical properties. A soilless growth medium with high porosity cannot hold nutrients easily, causing low nutrient uptake efficiency and a high run-off rate (Zhu *et al.*, 2015). It is critical that a growth medium provides a balance between aeration and water holding capacity to ensure a quality crop of containerised ornamental plants (Ingram *et al.*, 1993; Kukul *et al.*, 2012).

Chemical properties of growth media: Chemical properties of soils and growth media include pH, electrical conductivity (EC) and cation exchange capacity (CEC) (Ingram *et al.*, 1993; Richard, 2006; Witcher, 2013). Growth media chemical properties have a major influence on plant quality as they affect the solubility of nutrients, nutrient retention and plant nutrient uptake (Richard, 2006). The term CEC refers to a growth medium's ability to hold plant nutrients of positive charge, reducing nutrient leaching after rain or an irrigation event (Ingram *et al.*, 1993; Witcher, 2013). Chemical properties such as EC and pH can be determined from leachate extracted from growth media by a number of methods, including the pour-through extraction procedure (Jackson, 2008) and the saturated media extract method (Warncke, 1988).

2.2.1.2 Effects of EC and pH on growth media characteristics

The main distinguishing factor between the fertilisation management of soil-grown plants and that of plants grown in soilless media is the limited volume of growth media in the latter. This implies lower buffer capacity for solution composition and limited supply of nutrients due to inherent capacity (Silber and Bar-Tal, 2008). The pour-through extraction procedure

(Wright, 1986,) is a procedure for monitoring pot soluble salts, measured as EC, individual nutrient concentrations and pH.

The effectiveness of this procedure in monitoring the nutrient status of growth media for shrubs and foliage plants has been demonstrated (Wright *et al.*, 1990), and has been verified to test the soil solution available to plant roots (Richard, 2006). Suggestions of the ideal pH range of a growth medium for greenhouse crops are variable. Suggestions range from 5.0 to 6.5 (Ingram *et al.*, 1993), 5.4 to 6.4 (Bailey *et al.*, 2005), 5.4 to 6.5 (Jackson *et al.*, 2009), 5.5 to 6.5 (Witcher, 2013) and 5.4 to 6.0 for soilless media, and, with media having more than 20% incorporated soil, to lie between 6.2 and 6.8 (Westervelt, 2003). Plants such as azaleas, blue hydrangeas, rhododendrons and camellias require a more acidic growth medium, while Easter lilies prefer a higher pH (Bailey *et al.*, 2005) and, thus, optimal pH ranges are species-dependent (Miller and Jones, 1995).

Soilless growth media offer less pH buffering capacity than soil and soil-based growth media (Fisher and Argo, 2003). A growth medium's pH can change in response to fertiliser application, irrigation water alkalinity (Westervelt, 2003) and can even be altered by the plant itself (Westervelt, 2003; Raymond, 2004). The pH of a growth medium is important because it affects the solubility and availability of plant nutrients (Adriaanse, 2013), especially micronutrients (van Iersel, 1999). The medium pH can therefore also cause toxicity (Ingram *et al.*, 1993; Soti *et al.*, 2015). In general, micronutrients become more available as the pH decreases and less so as the pH rises (Jarvel, 1996). Ingram *et al.* (1993) reported that a pH above 7.5 usually results in the chemical binding of micronutrients, with the possibility of iron chlorosis (Landis, 1990), and a pH below 4.0 could result in toxic concentrations of ions such as aluminium (Al^{3+}), zinc (Zn^{2+}), copper (Cu^{2+}), iron (Fe^{3+}) and manganese (Mn^{2+}) (Ingram *et al.*, 1993; Soti *et al.*, 2015). Organic container medium components, such as bark

and peat moss, typically have lower pH values than mineral soils and can sometimes cause problems with Fe, Mn and Zn toxicity (Wada, 2005). Additionally, in alkaline soil media, Calcium (Ca), Magnesium (Mg), Potassium (K) and Orthophosphate (PO_4), exist in forms unavailable to plants, causing nutrient deficiency (Soti *et al.*, 2015).

The uptake and availability of phosphorus (P) in growth media is influenced by pH changes in a number of ways. The plant availability of several nutrients is affected by medium-pH, but one of nutrients most likely to leach is P (Fisher and Argo, 2003); P availability decreases in increasingly acidic soil media, due to P precipitation forming Al^{3+} and Fe^{3+} as well as Ca^{2+} and Mg^{2+} compounds under alkaline conditions (Landis, 1990; Raymond, 2004). Soilless growth media have little ability to hold P due to low phosphate sorption capacities of such substrates (Scagel, 2003; Oh *et al.*, 2016), especially with organic growth media components such as peat and bark lacking the binding sites that soils have to fix Al^{3+} and Fe^{3+} complexes (Williams and Nelson, 1996). As much as 79% applied P may be lost from soilless growth media (Yuan-Ling *et al.*, 1996).

Arguedas Rodriguez (2009) suggested an optimal soilless medium pH of 5.5 for P uptake, as P solubility decreases above this pH value. This is supported by Argo (1998). In soils, and on sites where P is bound, desorption may also occur, influenced by pH. As the pH is raised, bicarbonate (HCO_3^-) is able to exchange with adsorbed P and releases it into soil solution (Ojwando, 2014). Subsequently, this P is leached into the nursery production environment. The rate of desorption is higher in soils with a higher phosphate buffer capacity and soils are better able to buffer the phosphate concentration of the soil solution during the growing season (De Villiers, 2007), possibly due to prevailing higher temperatures.

Nitrogen can be absorbed by plants as either the ammonium (NH_4^+) cation or the nitrate (NO_3^-) anion (Nye, 1981; Silber and Bar-Tal, 2008) and in these forms has a significant

influence on rhizosphere pH and uptake (Marschner, 2011). When $\text{NH}_4\text{-N}$ is taken up by a plant, H^+ is secreted by the roots, inducing a decrease in soil solution pH (Marschner, 2011). Conversely, when $\text{NO}_3\text{-N}$ is assimilated by the plant, OH^- and HCO_3^- are secreted by the roots and the soil pH rises (Argo and Biernbaum, 1997; Silber and Bar-Tal, 2008). Bacterial nitrification of $\text{NH}_4\text{-N}$ into nitrite ($\text{NO}_2\text{-N}$) and nitrate ($\text{NO}_3\text{-N}$) may be inhibited by high levels of $\text{NH}_4\text{-N}$ and high solute concentrations (Silber and Bar-Tal, 2008). The critical pH for the inhibition of nitrification in soilless media lies in a pH range from 5.4 to 5.7. An increase in the concentration of $\text{NH}_4\text{-N}$ was found in media below this pH range with minimal concentrations of $\text{NH}_4\text{-N}$ in media above this range (Argo, 1998; Arguedas-Rodriguez, 2009). High concentrations of $\text{NH}_4\text{-N}$ are toxic to most plants, especially at high temperature and high salinity levels.

Electrical conductivity of growth medium solution is a measure of the ability of the solution to conduct electricity and indicates total soluble salts present but not individual nutrient species concentrations (Hanlon, 2012). As the level of soluble salts increases beyond recommended levels, the effect is of a decrease in plant growth; therefore, soluble salt determination has considerable significance (Jones Jr, 2001). High EC levels inhibit the root growth of some established crops (Whipker, 1999).

Electrical conductivity measurements give rapid results for growers, thereby allowing for timeous adjustments, if necessary. Levels of EC increase exponentially with increasing fertiliser input. Bilderback (2002) suggested minimal levels for liquid feed to range from 0.5 to 1 $\text{mS}\cdot\text{cm}^{-1}$, while Mathers *et al.* (2007) regarded an EC of 0.75 to 1.50 $\text{mS}\cdot\text{cm}^{-1}$ for pot-grown nursery plants in soilless media as optimal, when treated with a liquid feed at pH 5.2-6.2. Agro (2014) reported that an ideal growth medium will have an EC between 2.7 to 4.6 $\text{mS}\cdot\text{cm}^{-1}$ for most crops, whereas Raymond (2004) recommended that the maximum EC level

for ornamentals grown in containers to not exceed $1.5 \text{ mS}\cdot\text{cm}^{-1}$. An ideal growth medium for the production of containerised ornamental plants may have just one component or several components which may vary in proportions, depending on the type of plant being grown.

2.2.1.3 Composted pine bark and soil-based growth media mixes

Bark is a by-product of the timber and paper mill industries (Jackson, 2008). Composted bark is more preferable to shavings due to potential associated phytotoxicity problems (Carlile *et al.*, 2015). Physiochemical properties of bark-based soilless growth media can be quite different from soils (Hoskins *et al.*, 2014). Composted bark is stable over time and drains well, whilst maintaining adequate media moisture for plant growth (Schnelle and Henderson, 2003). It is acidic with little buffering capacity (Bilderback *et al.*, 2013) and lightweight, making it ideal for handling and transport of plants (Schnelle and Henderson, 2003). Miller and Jones (1995) suggested that as an amendment, composted bark is useful in increasing air porosity and water-holding capacity should this be required, although its water-holding capacity is not as high as peat. The size of the particles in the medium is important and the required size is achieved through hammer milling and screening (Landis, 1990). This is particularly important for smaller container use (Witcher, 2013) especially for seedling trays.

Pinebark is preferred over hardwood bark as it contains fewer leachable organic acids and can be composted relatively quickly, yielding the final product within five to seven weeks (Robbins and Evans, 2011). Composted pinebark also has anti-pathogenic effects (Bertrand, 2014). The similarity of composted pinebark to peat and its local availability have made this medium a common component of growth media in South Africa, despite it not having sufficient nutrients for plant needs (Mupondi *et al.*, 2006). Miller and Jones (1995) have reported significant, but low quantities of macro- and micronutrients in composted pinebark.

Initial pH ranges of composted pinebark have been reported to range between pH 4.0 to 4.3 (Mupondi *et al.*, 2006), 4.0 to 4.5 (Jackson *et al.*, 2009), pH 4.0 and 5.0 (Ingram *et al.*, 1993) and 3.5 to 6.0 (Witcher, 2013). Adding agricultural lime (CaCO_3), dolomitic limestone (MgCO_3) or a combination of calcium or magnesium hydroxides (CaOH_2), and (MgOH_2) will raise the pH of acidic media to required levels, but rates incorporated of any of these will depend on media characteristics, the lime particle size, surface area and irrigation water quality (Raymond, 2004).

Soil-based growth media should be amended with other components that will improve aeration and drainage, whilst maintaining water-holding capacity and should not contain more than thirty percent soil (Landis, 1990). Literature on media blends of topsoil, river sand and compost is limited. De Silva and Senarath (2013) reported a topsoil, river sand and compost blend (1:1:1, v:v:v) to be the best potting mix compared with coir dust substituting each of these components respectively (coir, river sand, compost; topsoil, coir, compost; topsoil, river sand, coir) , in a study on acclimatisation of tissue-cultured plants. Coconut coir dust is described as brown, spongy particle of low weight which falls out when the fiber is shredded from the husk.

Pine seedlings used to be raised only in topsoil for the South African forestry industry, but because of poor quality and insufficient topsoil supply, soilless growth media was investigated as alternatives (Hodgson, 1981). A lack of available peat in South Africa resulted in a comprehensive research programme to develop pine bark as a suitable alternate growth medium for the extensive containerised seedling industry in South Africa (Smith, 1992). The addition of topsoil, though, introduces desirable microorganisms into the growth medium and also adds weight for plant stability, but does contain weed seeds (Jacobs *et al.*, 2009). Sand is the coarse fraction of soil minerals (Tan, 2010). It is a commonly used component of growth

media mixes (Papadopoulos *et al.*, 2008). Sand is typically added to a growth media mix to increase its porosity (Bertrand, 2014), with coarse sand being most preferable to prevent aeration problems (Papadopoulos *et al.*, 2008). When combined with pine bark, sand reduces air space and total porosity (Bertrand, 2014). Growers generally use medium to coarse sands; 0.25 to 2 mm (Robbins and Evans, 2011). Sand is a chemically inactive medium and nutritionally valueless, but serves as diluent of more reactive components in growth media. Sand is very durable because it is neither chemically nor biologically altered during the course of its use as a growing medium component (Papadopoulos *et al.*, 2008).

The ornamental nursery industry worldwide has used growing media based on peat for many decades. Peat is obtained from wetlands and regarded as a non-renewable resource. Its rapid depletion, due to use as a growing medium, is causing rising environmental concerns that have led to many individual countries limiting the extent of peat mining, which has resulted in price increases (Ostos *et al.*, 2008). Increased commercial interest has been directed towards developing complete or partial alternatives for peat utilised in traditional growth media within nursery production (Mugnai *et al.*, 2007). Composts can improve the physical, chemical and biological properties of a growth media (Raviv, 2005; Jacobs *et al.*, 2009). Properly composted pine bark has been shown to possess most of the necessary chemical properties to produce a containerised pine seedling of desired quality (Jarvel, 1996). Compost is an ideal peat substitute, as it tends to have porosity and aeration properties comparable to peat (Ostos *et al.*, 2008) and enhances water retention and fertility (Jacobs *et al.*, 2009). Leaching from potting mixes containing composts is variable. Researchers have reported that some growth media leach equal or less N as $\text{NO}_3\text{-N}$ and $\text{NH}_4\text{-N}$ than a comparable peat-based substrate (Shober *et al.*, 2011). Frost (1997) reported that more P leached from a peat: vermiculite 1:1 blend than from sand: soil: peat 1:1:1 by volume. Compost is a valuable source of nutrients

(Mugnai *et al.*, 2007) and has been found to suppress seed-borne and soil-borne pathogens (Jacobs *et al.*, 2009). Gallo and Roberts (2010) reported that the benefits of compost amendments in growth media also include improved biological activity by bacteria, microalgae, mycorrhizal fungi, nematodes, protozoa and micro-arthropod organisms, as well as improved nutrient cycling, infiltration rate and growth medium texture.

2.2.1.4 Effects of growth medium texture on growth medium water leaching

A greenhouse or nursery crop growing in fine-textured media will be affected by poor aeration and a crop growing in a coarse-textured medium easily suffers from lack of water (Spomer *et al.*, 1997). Texture has, thus, a profound effect on the properties of a growth medium and is considered the most important physical property (McCauley *et al.*, 2005)

Soil texture is altered by the combination of sand, silt and clay, components determining the particle size distribution of a soil sample (Gee and Or, 2002; McCauley *et al.*, 2005). Porosity is affected by soil texture and this directly influences water and air movement through the growth medium and, subsequently, nutrient retention, nutrient uptake and plant growth (McCauley *et al.*, 2005). Fine-textured soils contain more clay particles and have smaller pore spaces than fine-textured components, resulting in media that is saturated after irrigation with little available oxygen. A coarse-textured medium is one comprised primarily of coarse to very coarse size particles resulting in large pores (Spomer *et al.*, 1997), like sandy soils; such media drain relatively quickly (Magdoff and Van Es, 2000). Rapidly draining media also leach mineral nutrients readily (Magdoff and Van Es, 2000; Fogg *et al.*, 2004). All soils are a combination of sand, silt, and clay-sized particles but vary in the proportion of these. Loamy soils have better water- and nutrient-holding capacities than sandy soils (Oyinlola and Jinadu, 2012) and, as with clay soils, benefit from the addition of compost. The ideal media mix, soil or soilless, should have a balance between medium and coarse particles with a minimum of

fine particles (Kukal *et al.*, 2012). This ‘perfect medium’ can be achieved by soil amendment or component mixing (Spomer *et al.*, 1997), and allows for optimal nutrient uptake by the cultivated plant.

2.2.2 Fertiliser use in pot plant production

Soil fertility is allowing adequate plant nutrition ensuring sustained provision and available forms of plant nutrients for uptake within the root zone (Foth and Ellis, 1997) Ornamental plant production in greenhouses and nurseries are highly intensive and require sufficient amounts of nutrients to enhance plant growth, thus, ensuring production of high value and quality crops (Taylor *et al.*, 2006; Dennis *et al.*, 2010;). On the other hand, fertiliser use can also create serious environmental hazards from leachate (Zhu *et al.*, 2015). Follett *et al.* (1987) defined fertiliser as any organic or inorganic material of natural or synthetic origin, which is added to a soil to supply certain elements essential to the growth of plants. Organic and inorganic fertilisers are used in greenhouse and nursery production systems as well as in field production systems in the form of dry-soluble, wet-soluble, slow-release or controlled-release fertilisers (Benson, 1997). Inorganic fertilisers are most commonly used in potted plant production, because nutrients are immediately available for plant uptake (Richard, 2006).

There is no legal definition of ‘organic fertilisers’ or ‘composts’. Regulatory bodies authorise its use for specific organic systems. Manufactured organic fertiliser products may include liquid fertilisers from hydrolysed animal waste (Cantrell *et al.*, 2008), solid forms, such as vermicompost, blood meal, hoof meal, horn meal and bone meal Bateman and Kelly, (2007) as well as animal manures in pellet form (Augustinus, 2007). Organic fertilisers, like compost, are considered to have a slow rate of nutrient release Ostrom, (2011) and may be less detrimental to receiving fresh waters than inorganic fertilisers (Reiter, 2008). Organic N

in organic fertilisers must undergo a mineralisation and nitrification process to inorganic N before being available for plant uptake (Bi *et al.*, 2010; Treadwell *et al.*, 2011). The release of nutrients from slow-release fertilisers depends on growth medium moisture, temperature and microorganism activity. Although variable and inconsistent in respect of demand, slow-release fertilisers allow nutrients to be available over an extended period of time (Bi *et al.*, 2010; Treadwell *et al.*, 2011).

Several studies have reported on the beneficial effects of organic fertilisers on the growth and yield of plants in production compared with inorganic fertilisers. Reiter (2008) stated that poultry litter, barring more total suspended solids, generally produced lower inorganic N, total Kjeldahl N, dissolved reactive P and TP in surface run-off concentrations than inorganic fertiliser sources applied at comparable rates.

In 2010, Guihong *et al.* conducted two experiments in a greenhouse study to evaluate the effects of two organic chicken litter fertilisers (4-2-2 and 3-3-3) and an inorganic controlled-release fertiliser (14-14-14) on the growth and flowering of potted French marigold plants. Plants fertilised with 4-2-2 and 3-3-3 produced the highest plant growth index, shoot dry weight, number of flowers per plant, total flower dry weight, and root rating, at low to intermediate rates and had higher tissue nutrient concentrations of N, P, K, Fe, Mn, Zn, and Cu than plants receiving 14-14-14. These results suggest that chicken litter as an organic fertiliser, may be used in commercial greenhouse crop production. Broschat (2008) suggested that pasteurized poultry litter was a suitable substitute for controlled-release fertilisers in greenhouse plant production, but also expressed concerns due to the initial rapid P leaching from a pine bark : Canadian peat : sand (5:4:1, by volume) blend during plant establishment.

Soluble, granular fertilisers are known to dissolve quickly in soil, but can result in plant injury coupled with leaching risks. Greenhouse operations typically apply a constant rate of

nutrients to plants through fertigation to meet the crop nutrient requirements, rarely changing the amount supplied during the production cycle (Majsztrik 2011). Fertigation is an efficient and uniform application of inorganic and organic sources of liquid fertilisers from dry or wet soluble forms (Saha *et al.*, 2005), when monitored correctly (Wells, 2013). Water-soluble fertiliser (WSF) provides considerable control over the fertility regime. Nutrients such as nitrate, however, present in liquid fertiliser formulations, are more likely to enrich drainage water as it is already dissolved (Wilson and Albano, 2011).

Ostrom (2011) conducted a study to evaluate the effect of four different fertilisers on plant growth, nutrition and nutrient leaching in New Guinea Impatiens (*Impatiens hawkeri* Bull) “Paradise New Red”. The author compared water soluble fertiliser (WSF) (20-4.4-16.6) at a rate of $75 \text{ mg}\cdot\text{L}^{-1}$ N, organic soybean-based fertiliser (SBF) (10-1.8-2.5) at a rate of $150 \text{ mg}\cdot\text{L}^{-1}$ N, controlled-release fertiliser (CRF) 15-4-10 at a rate of $7.11 \text{ kg}\cdot\text{m}^{-3}$ and granular slow-release turf fertiliser (AGT) (15-4-10), at a rate of $2.14 \text{ kg}\cdot\text{m}^{-3}$ with no fertiliser as control. The growth medium for this experiment consisted of a peat - perlite (7:3, v:v) mix. Rooted plugs were transplanted into 11.4 cm diameter plastic pots followed by an evenly distributed surface top-dressing of AGT and CRF. Results indicated increased EC in all growth media at 36 days after planting and were unacceptably high. Treatment with distilled water followed and EC came within acceptable limits ($<1.5 \text{ dS}\cdot\text{m}^{-1}$) by 66 days after planting.

Results of the study indicated CRF leached significantly more N than SBF and WSF, which were similar, in spite of SBF application rate being twice that of WSF. SBF and WSF P concentrations in leachate were similar and significantly higher than AGT, CRF and the control in decreasing concentration values respectively. Soybean-based fertiliser leached similar amounts of total N ($\text{N} = \text{NH}_4 + \text{NO}_3$) as WSF, however, SBF leachates may have had higher total N concentrations due to unmeasured urea in leachate. Ostrom (2011) suggested

that a three-month release CRF similar to that used in this experiment may not be ideal for New Guinea Impatiens production due to high EC and N leaching early in the production period, even though production of these plants occurred over a shorter period of time than three months. Use of WSF and SBF resulted in larger plants and less waste of nutrients through leaching and suggested that further investigation on the impact of SBF and CRF of varying longevities on N leaching and growth of bedding plants should be carried out. Perhaps reducing the concentration of SBF and WSF will reduce the amount of P leached, whilst still producing similar growth rates.

2.2.2.1 Plant nutrition

Plant nutrition and soil fertility are closely related in that the availability of nutrients through the substrate and the ability of the plant to take up the nutrients have to be coordinated. Soil available macro-nutrients including N, P and K, can arise out of mineralisation and available components of fertiliser contribute greatly to soil fertility (Dong *et al.*, 2012). Factors such as sufficient light, suitable temperature, and substances such as water, CO₂, oxygen, and mineral elements impact on plant growth and development (Roy *et al.*, 2006), affecting growth media nutrient availability, uptake as well as accumulation within the plant. Optimization of plant nutrition without due regard to the other primary factors may result in limited growth and yield (Osvalde, 2011).

More than 50 elements have been found in plants, though not all are considered to be essential (Silber and Bar-Tal, 2008). An element is considered to be essential, if a plant cannot complete its lifecycle without it or this element can be substituted by another one (Brown *et al.*, 1987). Some micronutrients, which may be considered beneficial, are essential for some taxa of plants (DalCorso *et al.*, 2014). An example with a special requirement is silicon (Si) for diatoms (Pearson *et al.*, 2016). Mineral elements are grouped in two categories

based on the amount required by a plant. Macronutrients are required in large quantities, whilst micronutrients are typically required only in smaller quantities or trace amounts (Marschner, 2011). carbon (C), hydrogen (H), oxygen (O), nitrogen (N), phosphorus (P), calcium (Ca), sulphur (S), potassium (K) and magnesium (Mg) are the macronutrients essential to the growth of most plants. Iron (Fe), manganese (Mn), boron (B), zinc, copper (Cu), molybdenum (Mo), sodium (Na), chlorine (Cl), and selenium (Se) are also essential but required in small amounts.

Furthermore, macronutrients can be considered as primary nutrients (N, P and K) or secondary nutrients (Mg, S and Ca) according to amounts needed by plants respectively (Tucker, 1999). To some extent and under most agricultural and horticultural conditions, only N, P and K are depleted to a point that growth and development are interrupted. Nitrogen deficiency is often characterised by stunted growth. Liebig's 'Law of the Minimum' is an important concept explaining that nutrients do not work in isolation. The nutrient that is in shortest supply regulates the development of the plant and only, when all minerals are available at certain ratios and concentrations, optimal growth can result (van der Ploeg *et al*, 1999). Concentrations of nutrients in media solutions above or below the optimal requirements can inhibit plant growth and reduce crop yields (Macnicol and Beckett, 1985) Deficiency or toxicity occurs when nutrients in solution are outside of a plants sufficiency range. When the concentration of nutrients is low, so is the plant growth rate. As the concentration of nutrients is increased so does plant growth rate increase up till the plants critical level and any further increases in concentration progressively impairs plant growth rate (Mengel and Kirkby, 1978). Knowledge of the functions and properties of the individual mineral elements is considered beneficial for its management and efficiency of use and essential for crops nutritional requirements which are guided by a sufficiency range. The

width of this range is dependent on plant species and the particular mineral element (van Maarschalkerweerd and Husted, 2015).

Ranges of the essential element concentrations in nutrient solutions and plant tissues are given in Table 2.1.

Macronutrients: Nitrogen – Nitrogen (N) is the element required in the largest quantity by plants and has many functions including the formation of amino acids, the building blocks of protein, in shoots or roots after uptake as $\text{NO}_3\text{-N}$ or $\text{NH}_4\text{-N}$ (Marschner, 2011). It is essential for plant cell division as a constituent of phytohormones and thus growth and photosynthesis as a major structural component of chlorophyll (Marschner, 2011).

Phosphorous – Phosphorous (P) exerts regulatory functions in energy transfer and storage and is a structural element in nucleic acids. It is necessary for photosynthesis, cell division and cell enlargement as a component of phospholipids. Phosphorous serves to increase the acquisition of nutrients and is involved in nutrient translocation (Marschner, 2011). Levels of between 0.01 to 20 $\text{mg}\cdot\text{L}^{-1}$ P in nursery runoff have been reported, which has led to eutrophication of freshwater ecosystems (Taylor *et al.*, 2006).

Potassium – The main function of potassium (K) in plants is osmotic regulation which is important in cell extension and opening and closing of the stomata and therefore plant respiration (Marschner, 2011). Potassium influences enzyme reactions, regulates photosynthesis and increases diseases resistance. (Barker and Pilbeam, 2015). This element is also involved in the synthesis of proteins from amino acid building blocks (Marschner, 2011). Potassium is a base cation and as such more weakly bound to the soil, which makes it prone to leaching at low pH (McCauley *et al.*, 2009b).

Calcium – Calcium (Ca) is a part of cell walls and membranes, and is used to stabilise and strengthen cell walls and plant tissue (Marschner, 2011). Calcium is a base cation and as such more weakly bound to the soil, which makes it prone to leaching at low pH (McCauley *et al.*, 2009b).

Magnesium – Magnesium (Mg) is a part of the chlorophyll molecule and as such a key element of photosynthesis and is also required for protein synthesis (Marschner, 2011). It stimulates P utilisation and its transport. Iron utilisation in plants is increased due to Mg (Negrea *et al.*, 2012). Magnesium is a base cation and as such more weakly bound to the soil, which makes it prone to leaching at low pH (McCauley *et al.*, 2009a).

Sulphur – Sulphur (S) is essential for the synthesis of amino acids, the building blocks of protein. It is also necessary for chlorophyll production and is a constituent of vitamins and some plant hormones. Sulphur is susceptible to leaching (Manjula, 2009).

Micronutrients: Most micronutrients are predominantly constituents of enzyme molecules and are thus essential in small amounts at the whole plant level (Marschner, 2011). The required range is quite narrow for several micronutrients (Jones Jr, 2016). Deficiency or toxicity occurs when the homeostatic mechanisms in plants breakdown and are unable to maintain the optimal range of supply of a particular micronutrient (Alloway, 2013). Deficiency symptoms detected early enough may be corrected during the current season for field crops or propagation cycle of container ornamentals (Tisdale and Nelson, 1958) but correction is more difficult for toxicity (Jones Jr, 2016). Trace metal (including Fe, Cu, Mn, and Zn) contamination of fresh water systems emanates from a variety of sources including agricultural activities. These essential plant micronutrients transported in runoff and eroded soil can have potentially toxic effects when they accumulate in sediment, aquatic organisms and fish of an aquatic ecosystem (Crafford and Avenant-Oldewage, 2011). Except for Fe, the

following micronutrients are only investigated for their potential effects on plant growth in the growth media used in this study.

Boron - Boron (B) is essential for seed and cell wall formation, activation of enzyme systems and transport of sugars in the plant. Promotes root growth, fertilisation and has been associated with lignin synthesis (Uchida, 2000).

Iron – Iron (Fe) functions for plants include involvement in the production of chlorophyll, as a constituent of some enzymes and proteins, in respiration, plant metabolism, energy transfer and nitrogen reduction and fixation. It has been suggested that low K availability can result in increased Fe uptake (Negrea *et al.*, 2012). South African legislation stipulates that concentrations of Fe in effluent may not exceed $2 \text{ mg}\cdot\text{L}^{-1}$ (Environmental Protection Act, 2002).

Manganese – Manganese (Mn) aids in chlorophyll synthesis, and assimilation of carbon dioxide during photosynthesis, activates enzyme systems including nitrate assimilation and fat forming enzymes. Mn increases the availability of P (Negrea *et al.*, 2012).

Zinc – Zinc is necessary for chlorophyll production, as enzyme activator and to form, auxin a growth hormone (Negrea *et al.*, 2012). It aids in seed formation and is necessary for carbohydrate and starch formation, as well as nitrogen metabolism (Prasad, 2007). Uptake of zinc is adversely affected by high levels of available phosphorus and iron in substrate (Marschner, 2011) .

Copper – Copper (Cu) is involved in the activation of enzymes and in chlorophyll formation (Tucker, 1999). It has a major function in photosynthesis and reproductive stages (Prasad, 2007).

Molybdenum – Molybdenum (Mo) is involved in several enzyme systems including the formation of nitrate reductase, forming ammonium out of nitrates. It is also involved in the formation of legume nodules, N fixation by legumes and protein synthesis (Roy *et al.*, 2006).

Table 2.1 Ranges of essential element concentrations in nutrient solutions and plant tissues (Silber and Bar-Tal, 2008).

Element	Chemical Symbol	Forms available to plants	Nutrient solution	Plant tissues
			mg·L ⁻¹	g·kg ⁻¹
Macronutrients				
Nitrogen	N	NO ₃ ⁻ , NH ₄ ⁺	50-200	10-56
Phosphorous	P	HPO ₄ ⁻² , H ₂ PO ₄ ⁺	5-50	1.2-5.0
Potassium	K	K ⁺	50-200	14-64
Calcium	Ca	Ca ⁺²	40-200	2.0-9.4
Magnesium	Mg	Mg ⁺²	10-50	1.0-2.1
Sulphur	S	SO ₄ ⁻²	5-50	2.8-9.3
			mg·L ⁻¹	µg·g ⁻¹
Micronutrients				
Boron	B	H ₃ BO ₃ , HBO ₃ ⁻	0.1-0.3	1.0-35
Iron	Fe	Fe ⁺³ , Fe ⁺²	0.5-3.0	53-550
Manganese	Mn	Mn ⁺²	0.1-1.0	50-250
Zinc	Zn	Zn ⁺²	0.01-0.1	10-100
Copper	Cu	Cu ⁺ , Cu ⁺²	0.001-0.01	2.3-7.0
Molybdenum	Mo	MoO ₄ ⁻²	0.01-0.1	1.0-2.0

2.2.3 Ornamental woody shrubs

Woody plants have a special place within the greenery elements of gardens, commercial sites and cities. They grow bigger than other plants with their biomass filling large areas of overhead space. It is vital to know their form and structure, growth requirements and ecological qualities for efficient utilisation. The functionality of the landscape area is increased through proper woody plant selection for specific conditions and the establishment of effective vegetation elements (Paganová and Jureková, 2012).

2.2.4 Basic plant growth parameters

Protected cultivation is based on the reduction of environmental stress, leading to fast growth and higher yields (Wittwer and Castilla, 1995). In order to assess plant growth, measurements

such as overall height, number of leaves, leaf area, number of branches, number of nodes and internode length can be made non-destructively (Evans, 1972). A specialised leaf-area meter or a portable leaf area meter for field measurements may be used to determine leaf area as the other parameters are relatively simple to quantify. Leaf area is an important variable for most physiological and agronomic studies involving plant growth, light interception, photosynthetic efficiency, evapotranspiration and response to fertilisers and irrigation (Blanco and Folegatti, 2005). Plants require adequate, but not excessive amounts of nitrogen. N deficiency due to low levels of available soil N or a decline in N uptake may result in plants that are stunted with narrow leaves (Marschner, 1995). Lynch *et al.* (1991) found that P deficiency primarily reduced leaf area by diminishing the number of leaves of bean plants through effects on the number of nodes, branching, and relative leaf appearance rate. Leaf area was also found to be significantly reduced in K deficient bean plants (Marschner, 1995). The over-application of fertiliser can also lead to nutrients being in excess and others deficient. High applications of P can induce Zn deficiency (Fageria *et al.*, 2002). Symptoms of Zn deficiency include stunted growth and inhibition of internode elongation (Marschner, 1995). The assessments of species-specific plant growth parameters are important tools in the choice of fertiliser for optimum growth of commercial crops and to avoid excess nutrients in leachate and runoff.

2.2.5 Effects of temperature on plant growth under cultivation

The importance of temperature in influencing the growth and development of plants has long been recognised (Haferkamp, 1988), due to its impact on photosynthesis, respiration, nutrient uptake and phytohormones (Marschner, 1995). These vital processes are restricted by a temperature range (Haferkamp, 1988), for each plant species, as represented by a minimum, optimum and maximum temperature range (Hatfield and Prueger, 2015). Increasing the

temperature in the environment in which the plant is grown in, results in an increase in vegetative growth up to the optimum temperature level for this species. The rate of leaf development is strongly regulated by temperature (Marschner, 1995). Protected cultivation of plants allows for greater control of environmental conditions including temperature. Tunnels covered with shade cloth are a less expensive option though, which serves to protect cultivated plants from excess heat and frequent drying out.

2.2.6 Greenhouse and nursery industry in South Africa

Protected horticultural cultivation is a relatively small part of the horticultural industry in South Africa, with plants produced under protected cultivation throughout the country, but predominantly close to Gauteng, Cape Town and Durban. Vegetables and cut flowers are mostly cultivated using greenhouse production systems (de Visser and Dijkxhoorn, 2011). The largest market for ornamental plants is in the Gauteng province, which includes the Highveld area, which has cold winters (Middleton, 2012). It is unclear how much of these plants are grown under protected cultivation.

South Africa's ornamental plant industry is characterised by its great diversity, especially in its indigenous plants (Middleton, 2012). Several of these indigenous species are well-known internationally in the floriculture and pot plant industry. These have been a source of genetic material for plant breeding and hybridisation (Reinten *et al.*, 2011). The South African nursery industry has been a professionally run industry with numerous member organisations representing the various sectors, and all falling under the South African Nursery Association (SANA). These organisations include the Allied, Bulb and Seed Trade Association, Bedding Plant Association, Garden Centre Association and Growers Association, representing allied trade to the nursery industry, seedling growers, retail nurseries and wholesale growers, respectively.

There has been very little investigation into the South African nursery industry or the volume of plants that are traded annually (Pollard, 2005). SANA-affiliated growers generally expect that maximum production and crop turnover time to last no longer than eight weeks for annuals, twelve weeks for herbaceous perennials and more than sixteen weeks for other horticultural groups of plants (Middleton, 2012). Another seedling organisation, the South African Seedling Growers Association, has members that produce about 381 million seedlings annually (Pollard, 2005). de Visser and Dijkxhoorn (2011) reported that on average seedling growers produce 40 million trays per annum but a few large growers produce in the region of 100 to 150 million trays per year. Most of these are vegetables and are for local growers. The forestry industry plants in the region of 84 million seedlings per annum (Pollard, 2005).

2.2.7 Conclusion

Current and older horticultural practices, especially the containerised production of ornamental plants in soilless growth media, have led to the eutrophication of freshwater systems as outlined. South Africa's water resources are currently under threat and have been for some time due to drought and environmental pollution. Literature has shown that eutrophication of South African freshwater systems is a serious problem, especially when harmful algal blooms occur. Agricultural practices in South Africa have been identified as a significant contributor of nutrients, especially P, which is limiting in South African freshwater systems. It is not clear to what extent the greenhouse and nursery industry in South Africa is a contributor to the over enrichment of freshwater but leachate composition studies elsewhere and its potential consequences, have confirmed this. Leachate composition and plant growth has been shown to differ due to fertiliser type and components of growth media used, as well as factors such as pH, EC and temperature. Pathways of N and P from agricultural lands to

freshwater systems, has been reviewed. There is, however, practically no literature on leachate chlorophyll analysis and algal content. This study compares the effects of organic and inorganic on the growth of *Pseuderanthemum atropurpureum* and leachate composition under South African conditions.

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CHAPTER 3

Effects of organic and inorganic fertilisers on growth of *Pseuderanthemum atropurpureum* and soil fertility

Abstract

Nutrients, especially N and P, are often applied in excess of plant requirements and leach with irrigation water in run-off to surface water systems; this may result in eutrophication. Components of growth media, fertiliser types and application methods differ in nutrient application, resulting in varying concentrations of plant growth responses, growth media fertility and nutrients leached. Organic (Nitrosol[®] and Wonder[™] Organic Vita Boost) and inorganic fertilisers (Wonder[™] Lawn and leaf and Polyfeed[®]) were, therefore, compared at different dosages when applied to two potting mixes (soil-based and bark-based) for their effects on *Pseuderanthemum atropurpureum* growth, growth media ability to sustain growth and leachate composition. The pot experiment was laid out in a completely randomised design in a 4x3x2 factorial design (fertiliser type x fertiliser concentration x growth media) with a control for each of the two growth media used. The experiment was repeated three times and there were seven N-equilibrated treatments per trial. Leachate extracts were obtained using the 'pour-through' method and used to determine electrical conductivity and pH and also used in laboratory determination of P, total phosphorous (TP), orthophosphate, B and other macro- and micronutrients. Growth media samples were taken for laboratory determination of total nitrogen (TN), nitrate and ammonium. Leaf samples were taken for laboratory determination of macro- and micronutrients and leaf chlorophyll concentration. Organic Vitaboost treatments to both growth media, especially at higher concentrations, resulted in significantly higher EC, lower pH, significantly higher NO₃-N growth media

concentrations and significantly higher leaf tissue N concentrations. Results from the other treatments for these parameters were similar to each other including leaf chlorophyll concentrations. Organic Vitaboost treatments also resulted in significantly more leaves, significantly more branches and significantly more nodes ($p=0,009$, $p=0.006$ and $p=0.023$, respectively) of *P. atropurpureum* plants. Bark-based growth media produced significantly more branches on the plants than soil-based growth media which might have been due to higher $\text{NH}_4\text{-N}$ than $\text{NO}_3\text{-N}$ supply in the bark-based growth media. The significant differences of bark-based growth media compared with soil-based ones on leaf number appear to be a result of significant differences in P-uptake evidenced by significantly higher leaf tissue P in bark-based media. Bark-based growth media leached significantly higher concentrations of P than soil based growth media. The effects of organic and inorganic fertiliser treatments did not produce significant differences for any plant growth characteristics measured at all levels of treatment. This may have been as a result of organic and inorganic fertiliser N concentrations being equilibrated prior to commencement of treatments. It is, therefore, recommended that *Pseuderanthemum atropurpureum* be propagated in a soil-based growth media with fertiliser treatments at the lowest concentrations used in this study due to environmental concerns of P leaching.

Keywords: Fertiliser, growth media, soil fertility, plant growth, leachate,

3.0 Introduction

Greenhouse- and nursery-cultivated ornamental plants include trees, shrubs, palms, groundcovers, climbers, ornamental grasses and bedding plants (Dobres, 2008; Chandler and Sanchez, 2012; Witcher, 2013), with many species and cultivars of each being propagated (Chandler, 2013; Zhu *et al.*, 2015) for the commercial market. The ornamental plant industry has grown tremendously worldwide, as the search for new marketable plant species has increased (Drew, 2010). More ornamental plant species and cultivars are grown today than all other agricultural and horticultural crops combined (Middleton, 2012). Ornamental plants are sought after as they present an aesthetic, amenity and cultural value to indoor and outdoor open spaces and enhance daily living.

Most ornamental plants are produced in containers, such as plastic pots or bags, and grown in a variety of media (Witcher, 2013). Such container production is the most widely used practice for growing shrubs (Richard, 2006). As the root volume of these plants is limited, such a system relies on optimal irrigation and fertilisation using conventional, slow-release and soluble fertiliser (Lea-Cox *et al.*, 2001; Majsztrik *et al.*, 2011). For ornamental plant production, containers result in better plant quality and faster plant growth in smaller areas, thus maximising space, production time and profit (De Lucia *et al.*, 2013; Agro and Zheng, 2014).

Fertilisation on an intensive scale is a necessity in this production system. The large amount of fertiliser applied to enhance plant growth can also have downsides. Broschat (1995) stated that $\text{PO}_4\text{-P}$ is rather immobile in many soils, but is readily leached from container media composed of pine bark, sphagnum peat, vermiculite or sand; $\text{NO}_3\text{-N}$ is readily leached from most mineral soils and also from container media. Nutrients applied in excess of plant demand have the potential to stunt plant growth (Liu *et al.*, 2014).

The movement towards naturally-managed gardens, and the growing interest in managing the environmental impacts of agriculture, have led to the development of organic and natural fertilisers that may have a lesser impact on the environment, while being suitable for commercial use. The use of organic fertilisers has been reported to be frequently similar to and often superior to inorganic fertilisers, when comparing growth, yield or quality of plants (Russo, 2005; Treadwell *et al.*, 2007; Bi *et al.*, 2010; Rochefort *et al.*, 2011). There does not appear to be any study comparing plant growth under the application of an organic liquid fertiliser, an organic pelletised chicken litter fertiliser, an inorganic granular fertiliser and an inorganic liquid fertiliser to soilless and soil-based growth media.

Pseuderanthemum atropurpureum belongs to the Acanthaceae family (Acanths) of herbaceous plants and shrubs mostly grown in warm climates (Pienaar, 1987). This family is one of the most popular ornamental tropical families (Meyer and Lavergne, 2004) with about 60 species commercially produced in the genus *Pseuderanthemum* (Pienaar, 1987). Acanths are widely used in horticulture for their numerous flowers or bracts of showy colours and for their variegated or bicolored foliage (Meyer and Lavergne, 2004). *Pseuderanthemum atropurpureum*, endemic to the tropical Pacific Islands, has leaves which are obovate, leathery, plum-coloured with rose, grey, green and cream markings in no set pattern. Rosy purple flowers with red markings appear in midsummer, borne in terminal spikes. The species grows to about 1 m in height (Sheat and Schofield, 1995). There has, however, been little research on the species, especially regarding its nutrient requirements.

The aim of this study was to compare the effects of organic and inorganic fertilisers on the growth of *Pseuderanthemum atropurpureum* in a soil-based and a bark-based growth medium and to determine whether these growth alterations are related to the general medium fertility.

3.1 Materials and methods

3.1.1 Study site and production environment

This research project was carried out at Randles Nursery (latitude 29°49'22"S, longitude 30°58'47"E), Durban, South Africa (Fig. 3.7). This nursery is operated by the eThekweni Municipality and is the site where most of the trees, shrubs, palms and ornamental grasses used in the municipality's landscaping and beautification operations are grown. A quonset-shaped tunnel structure covered with 40% shade cloth was used in this study which was located on the north-facing side of the tunnel to make maximum use of sunlight. There was no climate control. Precipitation in Durban averages 129mm in winter and 349mm in summer with annual precipitation averaging 828mm (www.durban.climatemps.com). Winter temperatures average a high of 22°C and a low of 11.3°C. Summer temperatures average a high of 27°C and a low of 22°C (www.durban.climatemps.com). The first and third experiments were carried out mostly in the summer and the second mostly in the winter, terminating in early spring.

3.1.2 Plant preparation

Plant material for cuttings was sourced from large, container-grown *Pseuderanthemum atropurpureum* plants at Randles nursery. Cuttings were inserted into washed river sand in large flat seedling trays (no cavities) and rooted in a greenhouse under manually operated mist, with no temperature control, at the Durban University of Technology nursery. Plants were potted, once cuttings had developed sufficient root mass. Two growth media were used for potting.

3.1.3 Growth media preparation and analysis

The soil-based medium used was a blended mix consisting of one part topsoil, one part compost and one part river sand (v/v/v); this mix is hereafter referred to as “randles”. The topsoil and river sand were acquired locally by Randles nursery and the compost obtained from Gromor (Cato Ridge, KwaZulu-Natal). These media components were thoroughly hand-mixed before use. The second growth medium was a commercial blend consisting of composted pinebark and 10% river sand, also purchased from Gromor. The supplier usually adds up to 2% Accelerator[®] (chicken litter fines) to bring the EC up to 2.4 (Jan van Vuuren, Gromor, personal communication, 2015). Samples of these growth media mixes, which served as controls, were sent to laboratories at the Soil Fertility and Analytical Service Section, KZN Department of Agriculture and Rural Development, Cedara for total nitrogen (TN), inorganic nitrogen (nitrate - $\text{NO}_3\text{-N}$ and ammonium - $\text{NH}_4\text{-N}$), mineral nutrient and soil texture analysis. Upon termination of the study (after a period of 90 days (DAP)) TN, $\text{NO}_3\text{-N}$ and $\text{NH}_4\text{-N}$ of growth media were again determined.

Total N was analysed by the automated Dumas dry combustion method using a LECO CNS 2000 (Leco Corporation, Michigan, USA; Matejovic, 1996). The $\text{NO}_3\text{-N}$ and $\text{NH}_4\text{-N}$ in filtered extracts were measured by segmented flow analysis with a Perstorp Flow Solution III analyser using the sodium salicylate - sodium nitroprusside-hypochlorite method for $\text{NH}_4^+ \text{- N}$ (Perstorp Analytical, 1993) and the sulphanilamide-naphthyl-ethylenediamine method for $\text{NO}_3^- \text{- N}$ after having reduced nitrate to nitrite with copperized cadmium wire (Willis & Gentry, 1987). For mineral element analysis, samples were dried at 105°C , and milled to pass through a 0.84 mm sieve. Subsamples were then dry-ashed at 450°C overnight and taken up in 1 M HCl. Concentrations of P, K, Ca, Fe, Mg, Cu, Mn and Zn in samples were determined using ICP-OES. Leachate N concentrations were determined on an Elementar Vario analyser

(Elementar Analysensysteme, Hanau, Germany). Soil texture analysis of samples involved suspended clay and fine silt content, determined after dispersion and sedimentation, and sand fractions by sieving. Once the particle size distributions of the soils was known, their textural class was determined from a textural triangle defining particle size limits of the various textural classes (Manson and Roberts, 2000).

3.1.4 Experimental design

Treatment was a 4 (four fertilisers used) x 3 (at three concentration levels) x 2 (in two growth media types) factorial design with a control for each of the two growth media used. A total of 208 cuttings were potted into either growth medium in 3 L black plastic plant bags. Plants were labelled and colour-coded according to each of the four fertiliser treatments and concentrations that they were going to receive, for ease of identification. This experiment was laid out in a completely randomised design to account for varying light exposure. Potted plants were randomly divided into two batches of 13 groups, arranged with eight plants per group (row) and each group representing an experimental unit. The two batches were laid out within adjacent areas measuring 3.5 m x 1.5 m on either side of a 1 m pathway. Each group had received either an N equilibrated organic or inorganic fertiliser treatment based on half label, label or double the recommended label rate except for the two controls. There were seven fertiliser treatments. The entire experiment was repeated three times. Experimental units in subsequent trials were not in the same position as previously sited.

3.1.5 Fertiliser treatments

The N content of the four fertilisers used in this study were not of equal concentration (Table 3.1). In order to equilibrate N and determine the dosage rate for treatments, the average N content of the four fertilisers used in this pot experiment was calculated and used to adjust the

recommended label dosage rates accordingly. This had the effect of either increasing or decreasing the recommended dosage rate, and, therefore, the recommended concentrations of the other mineral elements.

One of the organic fertilisers, Nitrosol[®] (NPK analysis 4:1:3 (16)), is a liquid, water-soluble treatment. This natural organic plant food is formulated from sterilised blood, bone and carcass meal and manufactured by Envirogreen (PTY) Ltd for Fleuron[®] (PTY) Ltd. Nitrosol[®] solution (200 ml·pot⁻¹) was applied every two weeks at concentrations of 2.61 ml·L⁻¹, 5.22 ml·L⁻¹ and 10.44 ml·L⁻¹ to plants in rows labelled as 1/2 label rate, label rate and 2 x label rate, respectively (Table 3.2). This liquid treatment also contained other macronutrients as well as micronutrients and gibberellic acid (Table 3.1).

Table 3.1 Source and amount (by mass) of macronutrients and micronutrients in Nitrosol[®], Lawn and Leaf, Polyfeed[®] and Organic Vitaboost fertilisers

Trade name	Nitrosol ¹	Lawn and Leaf	Polyfeed	Vitaboost
Formulation	4:1:3	7:1:3	6:1:3	6:1:3
N g/kg	80	95	266	60
P g/kg	20	14	44	13
K g/kg	58	41	134	27
Ca g/kg	6	-	-	25.3
Mg g/kg	7	-	4	6.4
S g/kg	4	-	5.3	-
Fe mg/kg	60	-	751	4452
Mn mg/kg	40	-	273	517
Zn mg/kg	1	-	699	319
Cu mg/kg	1	-	75	68
B mg/kg	23	-	1054	-
Mo mg/kg	15	-	63	-

1 – Nitrosol[®] contains Gibberellic acid at 0.003 g·kg⁻¹

Wonder[™] Organic Vitaboost (NPK analysis 6:1:3), a dry water soluble fertiliser, supplied by Efekto, (Bryanston, South Africa) was the second organic treatment used in this study and

consisted of chicken litter pellets. Organic Vita-Boost was applied every two weeks at rates of $2.77 \text{ g}\cdot\text{pot}^{-1}$, $5.54 \text{ g}\cdot\text{pot}^{-1}$ and $11.07 \text{ g}\cdot\text{pot}^{-1}$ to plants in rows labelled as 1/2 label rate, label rate and 2 x label rate, respectively (Table 3.2). This fertiliser, which was incorporated just below the surface of the growth media, also contained other macronutrients as well as micronutrients (Table 3.1).

Polyfeed[®] (NPK analysis 6:1:3 (44)), a highly concentrated dry, powdered, water-soluble fertiliser, was one of the two inorganic fertilisers treatments utilised in this study, and is manufactured by Nulandis (A Division of AECI Ltd, Kempton Park, South Africa). Polyfeed[®] solution ($200\text{ml}\cdot\text{pot}^{-1}$) was applied every two weeks at concentrations of $0.59 \text{ g}\cdot\text{L}^{-1}$, $1.18 \text{ g}\cdot\text{L}^{-1}$ and $2.36 \text{ g}\cdot\text{L}^{-1}$ to plants in rows labelled as 1/2 label rate, label rate and 2 x label rate respectively. This liquid treatment also contained other macronutrients as well as micronutrients (Table 3.1).

Wonder[™] Lawn and Leaf supplied by Efekto (NPK analysis 7:1:3 (15)) was the second inorganic fertiliser treatment used in this study. This fertiliser is a nitrogen, sustained-release fertiliser, with bio-carbon pellets which were removed. Lawn and Leaf was applied every two weeks at rates of $0.36 \text{ g}\cdot\text{pot}^{-1}$, $0.71 \text{ g}\cdot\text{pot}^{-1}$ and $1.42 \text{ g}\cdot\text{pot}^{-1}$ to plants in rows labelled as 1/2 label rate, label rate and 2 x label rate, respectively. The soil-based growth medium control and pinebark-based growth medium control received no fertiliser (Table 3.2).

Table 3.2 Dosage rate of N equilibrated fertiliser nutrients incorporated (g) into each 3 L plant bag or liquid fed·L⁻¹ concentration as nutrient solution

Formulation	Treatment	Source	Form	Application method	Dosage rate
Control	CR	-	-	-	-
Control	CG	-	-	-	-
4.1.3	NRA	organic	liquid	liquid feed	2.61 ml·L ⁻¹
4.1.3	NRB	organic	liquid	liquid feed	5.22 ml·L ⁻¹
4.1.3	NRC	organic	liquid	liquid feed	10.44 ml·L ⁻¹
4.1.3	NGA	organic	liquid	liquid feed	2.61 ml·L ⁻¹
4.1.3	NGB	organic	liquid	liquid feed	5.22 ml·L ⁻¹
4.1.3	NGC	organic	liquid	liquid feed	10.44 ml·L ⁻¹
6.1.3	PRA	inorganic	powder	liquid feed	0.59 g·L ⁻¹
6.1.3	PRB	inorganic	powder	liquid feed	1.18 g·L ⁻¹
6.1.3	PRC	inorganic	powder	liquid feed	2.36 g·L ⁻¹
6.1.3	PGA	inorganic	powder	liquid feed	0.59 g·L ⁻¹
6.1.3	PGB	inorganic	powder	liquid feed	1.18 g·L ⁻¹
6.1.3	PGC	inorganic	powder	liquid feed	2.36 g·L ⁻¹
7.1.3	LRA	inorganic	granule	incorporated	0.36 g·pot ⁻¹
7.1.3	LRB	inorganic	granule	incorporated	0.71 g·pot ⁻¹
7.1.3	LRC	inorganic	granule	incorporated	1.42 g·pot ⁻¹
7.1.3	LGA	inorganic	granule	incorporated	0.36 g·pot ⁻¹
7.1.3	LGB	inorganic	granule	incorporated	0.71 g·pot ⁻¹
7.1.3	LGC	inorganic	granule	incorporated	1.42 g·pot ⁻¹
6.1.3	ORA	organic	pellet	incorporated	2.77 g·pot ⁻¹
6.1.3	ORB	organic	pellet	incorporated	5.54 g·pot ⁻¹
6.1.3	ORC	organic	pellet	incorporated	11.07 g·pot ⁻¹
6.1.3	OGA	organic	pellet	incorporated	2.77 g·pot ⁻¹
6.1.3	OGB	organic	pellet	incorporated	5.54 g·pot ⁻¹
6.1.3	OGC	organic	pellet	incorporated	11.07 g·pot ⁻¹

* - CR – Control in Randles growth medium, CG – Control in Gromor growth medium, N – Nitrosol fertiliser, P – Polyfeed fertiliser, L – Lawn and Leaf fertiliser, O – Organic Vita boost fertiliser, R – Randles growth medium, G – Gromor growth medium, A – ½ label rate, B – label rate and C – 2 x label rate

3.1.6 Plant analysis

3.1.6.1 Leaf analysis

At the termination of the experiment, after a period of 90 days after planting (DAP), all leaves from all plants were harvested, counted, measured and chlorophyll a, b, and total chlorophyll of each replication determined. Leaf samples were separately packaged, labelled and sent to the Plant Laboratory at the Soil Fertility and Analytical Service Section, KZN Department of

Agriculture and Rural Development, Cedara, for tissue mineral element analysis. Samples were dried at 105°C, and milled to pass through a 0.84 mm sieve. Subsamples were then dry-ashed at 450°C overnight and taken up in 1 M HCl and P, K, Ca, Fe, Mg, Cu, Mn and Zn concentration of the leaf tissue determined using ICP-OES. Leaf N was determined by the Automated Dumas dry combustion method using a LECO CNS 2000.

3.1.6.2 Leaf chlorophyll determination

Three fully mature leaves were selected at random from three plants in each row (experimental unit) for chlorophyll a, chlorophyll b and total chlorophyll analyses. Leaf samples were separately packaged into paper bags, labelled and transported in a cooler box with ice bricks to the laboratory for same day analysis.

Determination of chlorophyll was carried out according to Lichtenthaler (1987) allowing for the simultaneous determination of chlorophylls (*a* and *b*) using exact absorbance readings of plant extracts in certain solvents. Fresh leaf tissue (1.0g) was placed into test tubes and kept on ice in a covered cooler box. Chlorophyll was extracted using 80% acetone. Samples were macerated using an Ultra Turrax[®] (IKA, Staufen, Germany) to achieve a homogeneous suspension. This suspension was then decanted into 10 ml centrifuge tubes and centrifuged for 5 minutes at 4500 rpm in a PLC Series table top centrifuge (Gemmy Industrial Corp., Taipei, Taiwan). The centrifuged supernatant was diluted by a factor of 10 and absorbance units read at 664 nm, 630 nm and 647 nm in a Hellma glass cuvette (1 cm light path, Type 100-T4) (Hellma Analytics, Müllheim, Germany) using a Shimadzu Spectrophotometer UV-1800 (Shimadzu Corp, Kyoto, Japan).

Chlorophyll content in extract ($\mu\text{g}\cdot\text{ml}^{-1}$) was calculated using formulas by Lichtenthaler (1987):

$$\text{Chl}_a = 12.25 A_{664} - 2.79 A_{647}$$

$$\text{Chl}_b = 21.50 A_{647} - 5.10 A_{664}$$

$$\text{Chl}_{a+b} = 7.15 A_{664} + 18.71 A_{647}$$

Chlorophyll content in leaves was calculated using the formula used by Mitić *et al.* (2013),

$$C = \frac{C_1 V r}{m} (\text{mg}\cdot\text{g}^{-1})$$

where, C_1 = concentration of extract chlorophyll pigment ($\text{mg}\cdot\text{L}^{-1}$), V = initial volume of extract (ml), r = dilution factor and m = fresh leaf mass (g)

3.1.6.3 Plant growth parameter analyses

Plant height, leaf number, leaf size, number of nodes, internode length and number of branches were determined 90 days after planting, at termination of the experiment. Data was recorded from three replications and means determined.

Height: Height was measured from the surface of the growth media (base of plants) to the tips of the new leaves using a stainless steel ruler and TESA 0.02mm dial callipers, model CCMA-M (TESA Technology, Switzerland), after all leaves were removed.

Number of leaves: Leaves were counted as they were removed from the plants. These were packaged separately, labelled and batched in preparation for individual leaf measurements and mineral analyses.

Leaf size: Leaf area was determined using a LI-COR Biosciences portable area meter, model LI-3000C, with transparent belt conveyer accessory, model LI-3050C (LI-COR, Lincoln, NE, USA) and expressed in $(\text{cm})^2$, as a unit of area.

Number of nodes: The number of all nodes, including those on all stems and branches, was recorded.

Internode length: Internode length was measured on the main stem only using callipers and expressed in centimetres (cm).

Number of branches: All side branches on the main stem were counted.

3.1.7 Cultural practices

Weeding was carried out regularly throughout the course of the experiment. Plants were irrigated by hosepipe using tap water, and this was limited to the well (volume between growth media surface and lip of each pot) of each pot being filled. Plants were watered on Mondays, Wednesdays and Fridays. Irrigation water was analysed by the Soil Salinity Laboratory at the Soil Fertility and Analytical Service Section, KZN Department of Agriculture and Rural Development, Cedara, for EC, pH, cations and anions.

3.1.8 Leachate collection and analysis

Leachate collection was carried out from each plant bag, using the pour-through extraction method (Wright, 1986). Plants were watered at least an hour before collection to ensure that pots were at container capacity. This method recommends pouring enough water to yield 50 ml leachate, as not to dilute the leachate too much, which may result in lower EC values. Approximately 200 ml deionised water was applied to each 3 L bag. Leachate was collected in foil trays, previously washed in 5% nitric acid and transferred into labelled sample bottles.

3.1.8.1 EC and pH analysis

Analysis of EC and pH of the leachate were carried out after potting, 60 DAP and at termination of the experiment (90 DAP). EC and pH were determined using a Hanna HI98130 combo tester meter (Hanna Instruments[®], Woonsocket, RI, USA) after being calibrated according to manufacturer's guidelines, before each set of analyses.

3.1.8.2 Mineral element, total phosphate, orthophosphate and boron analysis

Leachate samples collected at termination of the experiment (after a period of 90 days) were sent to the Plant Laboratory at the Soil Fertility and Analytical Service Section, KZN Department of Agriculture and Rural Development, Cedara, for mineral element analysis. Leachate N concentrations were measured using an elemental analyzer (Vario EL III; Elementar Analysensystem GmbH, Germany), while the other elements were determined by ICP-OES. Samples were also sent to Regen Waters laboratory (Witbank, Mpumalanga, South Africa) for total phosphate as well as orthophosphate analysis (Aquakem 600 Photometric Discrete Analyser, Thermo Scientific) and boron analysis (ICP-OES).

3.1.9 Temperature-logging

Two Brannan 'quick set push-button minimum maximum' thermometers (Brannan, Cumbria, England) were placed between the plants at ground level. These were moved randomly after temperatures were logged daily. Mean minimum and maximum temperatures for replications 1, 2 and 3 were $20.90^{\circ}\text{C}^{\text{min}}$ and $27.76^{\circ}\text{C}^{\text{max}}$, $12.76^{\circ}\text{C}^{\text{min}}$ and $25.14^{\circ}\text{C}^{\text{max}}$ and $20.31^{\circ}\text{C}^{\text{min}}$ and $29.75^{\circ}\text{C}^{\text{max}}$ with temperature ranges recorded for the same periods $18.13^{\circ}\text{C}^{\text{min}} - 31.5^{\circ}\text{C}^{\text{max}}$, $8.67^{\circ}\text{C}^{\text{min}} - 27.83^{\circ}\text{C}^{\text{max}}$ and $16.08^{\circ}\text{C}^{\text{min}} - 36^{\circ}\text{C}^{\text{max}}$, respectively. Minimum temperatures were significantly lower during replication two. The variance in temperature between the three experiments was accounted for by checking the blocks box and entering the column data containing the three experiments in the box alongside, as a blocking factor in the Genstat ANOVA analysis of data.

3.1.10 Statistical analysis

Statistical analyses were performed using GenStat® 12th Edition (VSN International, Hemel Hempstead, UK). Data collected were subjected to analysis of variance and means separated

using the Duncan's Multiple Range Test at 5% probability level. Treatment for all parameter means were compared with each control (randles (CR) and gromor (CG)) separately, by partitioning each comparison separately into polynomial contrasts to determine main effects and interactions at $p < 0.05$ and elucidate treatment responses for each parameter investigated. Fertiliser main effects were subjected to polynomial contrasts to determine, if there were any linear or quadratic components.

3.2 Results

3.2.1 Analyses of irrigation water

Electrical conductivity (EC) is usually used as an indicator of salinity and sodium adsorption ratio (SAR) as an indicator of sodicity when water quality is classified (Culverwell and Swinford, 1986). Interpretations of SAR and EC are based on criteria developed by the United States Department of Agriculture (Meyer and van Antwerpen, 1995). Electrical conductivity of the irrigation water was measured as $24.32 \text{ mS}\cdot\text{m}^{-1}$ and pH as 7.64 (Table 3.3).

Table 3.3 Chemical properties of irrigation water

Parameter	Elements	Value
EC ($\text{mS}\cdot\text{m}^{-1}$)		24.32
pH		7.64
Cations ($\text{me}\cdot\text{L}^{-1}$)	Na	0.93
	Ca	0.95
	Mg	0.23
	K	0.05
Anions ($\text{me}\cdot\text{L}^{-1}$)	TA	1.1
	Cl	1.1
SAR		1.21
Class of water		C1-S1

TA – Total alkalinity

SAR – Sodium adsorption ratio

3.2.2 Analyses of growth media before planting

Chemical properties (EC, pH, % Fe, % Mn and % Cu) of the two growth media used in this study showed some significant differences between the soil-based potting mix (randles) and the bark-based potting mix ‘gromor’ whereas soil texture was similar. The two controls, control randles and control gromor (CR and CG), were not treated.

Table 3.4 Physicochemical properties of randles (CR) and gromor (CG) growth media (elements as %DM)

Parameter		CR	CG
EC (mS·cm ⁻¹)	Leachate	2.58	3.05
pH	Leachate	6.28	5.84
C/N ratio		20.91:1	20.40:1
Moisture (%)		6.24	29.68
Elements			
C (%)		2.17	17.27
N (%)		0.64	1.38
P (%)		0.18	0.22
K (%)		0.28	0.83
Ca (%)		1.04	1.22
Mg (%)		0.14	0.22
S (%)		0.26	0.32
Fe (mg/kg)		24028.81	17374.56
Mn (mg/kg)		237.79	302.17
Zn (mg/kg)		83.43	94.97
Cu (mg/kg)		157.69	106.62
Soil Texture			
Sand (%)	(0.02 - 2 mm)	80.33	73.80
Silt (%)	(0.02 - 0.002 mm)	6.17	7.80
Clay (%)	(<0.002 mm)	13.83	18.50
Soil texture classification*		Loamy sand	Sandy loam

* According to: - Soil Classification, A Taxonomic System for South Africa - Soil Classification Working Group and Macvicar 1991

3.2.3. Leachate EC and pH

Results indicated were determined from extracted leachate.

3.2.3.1 Leachate pH 60 days after planting

All mean pH values increased after a period of 60 DAP from the start (initial) values, except for Organic Vitaboost in randles growth media at label rate (ORB), Organic Vitaboost in randles growth media at twice label rate (ORC) and Organic Vitaboost in gromor growth media at twice label rate (OGC) (Table 3.5).

Mean growth medium pH at 60 DAP (Table 3.5) was significantly ($p < 0.001$) affected by the main effects of fertiliser type and growth media type. Soil-based growth media maintained higher pH than bark-based growth media. Inorganic Lawn and Leaf fertiliser at the lower dosages added to randles soil-based growth media (LRA and LRB) resulted in a significantly higher ($p = 0.004$) pH compared with Organic Vitaboost at the highest level of treatment in randles growth medium (ORC), Organic Vitaboost, at all levels of treatment, in gromor bark-based media (OGA, OGB, and OGC), organic Nitrosol at the highest level of treatment in gromor growth medium and inorganic Polyfeed at the lowest and highest levels of treatment in gromor growth medium (PGA and PGC).

The pH for all treatments decreased linearly with increasing fertiliser rates except for PRC, PGB and OGB.

3.2.3.2 Leachate EC 60 days after planting

Overall, all mean EC values ($\text{mS}\cdot\text{cm}^{-1}$) had decreased after a period of 60 DAP, from the start values except for Nitrosol (NGC) and Polyfeed (PRC), Lawn and Leaf (LRC, LGB and LGC) and all Organic Vitaboost treatments (ORA, ORB, ORC, OGA, OGB and OGC) (Table 3.5).

At 60 DAP, EC values of all leachates had increased with increasing fertiliser rates, except for NGB. Mean EC values were significantly affected by the main effects of fertiliser type and levels of treatment (concentration) at $p < 0.001$ and $p = 0.002$, for both effects and comparisons (Table 3.5). Differences between treatments were significant at $p < 0.001$.

3.2.3.3 Leachate pH 90 days after planting

Leachate pH values had decreased from 60 DAP to 90 DAP, except for several of the gromor treatments (NGA, NGC, PGA and PGB) (Table 3.5). The pH of Lawn and Leaf treatments in randles growth medium were the only treatments that became more acidic with increasing fertiliser rates.

Statistically, pH was not significantly affected by the differences between treatments ($p = 0.080$), but PGB was significantly different from some treatments and main effects were significantly affected by fertiliser type at $p < 0.001$ (Table 3.5).

3.2.3.4 Leachate EC 90 days after planting

All mean leachate EC values decreased from 60 to 90 DAP (Table 3.5), while the EC of all treatments increased with increasing fertiliser rates, except for three gromor (PGC, LGC and OGB) and one randles treatment (PRB).

The EC values for the Organic Vitaboost treatments (OR and OG) were in general higher than those of all other groups with the EC of high addition (ORC and OGC) to both media being significantly different from all other treatments at $p < 0.001$. The EC results of the other organic fertiliser, Nitrosol (NR and NG), were similar to the other inorganic treatments. Controls (CR and CG) EC mean values were not the lowest, as might have been expected, despite not receiving any additional solutes by fertiliser treatment.

Fertiliser type and level of treatment (concentration) significantly affected the EC values at $p < 0.001$ and $p = 0.001$, respectively. There were also significant interactions for both comparisons to the controls between the fertiliser used and its concentration on EC values (CR: $p = 0.008$; CG: $p = 0.007$).

Table 3.5 Effects of two organic (Nitrosol[®] and Organic Vitaboost) and two inorganic fertiliser treatment types (Polyfeed[®] and Lawn and Leaf) applied at three concentrations to two different growth media, on mean pour-through extracted leachate EC (mS·cm⁻¹) and pH compared to two controls after a period of 60 DAP and 90 DAP

Type	Treatment	EC start	pH start	EC 60 DAP	pH 60 DAP	EC end	pH end
Control	CR ⁶	2.58	6.28	1.95 ± 0.63ab ¹	6.77 ± 0.02fg	0.93 ± 0.31 ab	6.26 ± 0.35bcd
Control	CG	3.05	5.84	1.55 ± 0.33a	6.48 ± 0.39c-g	1.13 ± 0.12abc	6.18 ± 0.21a-d
4.1.3	NRA	2.58	6.28	1.86 ± 0.45ab	6.58 ± 0.11c-g	0.88 ± 0.22ab	6.09 ± 0.33a-d
4.1.3	NRB	2.58	6.28	2.15 ± 0.34ab	6.40 ± 0.18a-g	1.08 ± 0.34ab	6.12 ± 0.42a-d
4.1.3	NRC	2.58	6.28	2.52 ± 0.48ab	6.39 ± 0.18a-g	1.12 ± 0.36abc	6.25 ± 0.33bcd
4.1.3	NGA	3.05	5.84	2.18 ± 0.58ab	6.28 ± 0.37a-g	0.75 ± 0.16a	6.32 ± 0.22bcd
4.1.3	NGB	3.05	5.84	2.10 ± 0.61ab	6.21 ± 0.23a-g	0.88 ± 0.16ab	6.11 ± 0.16a-d
4.1.3	NGC	3.05	5.84	3.29 ± 0.92ab	6.10 ± 0.26a-f	1.29 ± 0.41a-e	6.38 ± 0.30cd
6.1.3	PRA	2.58	6.28	1.73 ± 0.42ab	6.78 ± 0.05fg	0.83 ± 0.19a	6.11 ± 0.33a-d
6.1.3	PRB	2.58	6.28	1.80 ± 0.32ab	6.38 ± 0.34a-g	0.78 ± 0.12a	6.19 ± 0.26a-d
6.1.3	PRC	2.58	6.28	2.64 ± 0.27ab	6.61 ± 0.11d-g	1.22 ± 0.16a-d	6.12 ± 0.35a-d
6.1.3	PGA	3.05	5.84	1.74 ± 0.77ab	6.10 ± 0.40a-f	0.87 ± 0.27ab	6.13 ± 0.06a-d
6.1.3	PGB	3.05	5.84	2.51 ± 1.20ab	6.39 ± 0.34a-g	1.35 ± 0.44a-e	6.44 ± 0.170d
6.1.3	PGC	3.05	5.84	2.84 ± 1.03ab	6.06 ± 0.20a-e	0.96 ± 0.04ab	5.94 ± 0.21a-d
7.1.3	LRA	2.58	6.28	1.90 ± 0.48ab	6.82 ± 0.06g	1.30 ± 0.44a-e	5.99 ± 0.45a-d
7.1.3	LRB	2.58	6.28	2.25 ± 0.72ab	6.79 ± 0.04g	1.37 ± 0.35a-e	5.96 ± 0.45a-d
7.1.3	LRC	2.58	6.28	3.79 ± 0.51ab	6.71 ± 0.13efg	2.13 ± 0.63a-e	5.85 ± 0.44abc
7.1.3	LGA	3.05	5.84	2.62 ± 1.62ab	6.51 ± 0.32c-g	0.95 ± 0.19ab	6.14 ± 0.29ab
7.1.3	LGB	3.05	5.84	4.00 ± 2.22ab	6.48 ± 0.33c-g	1.81 ± 0.30a-e	5.80 ± 0.21ab
7.1.3	LGC	3.05	5.84	4.44 ± 1.51abc	6.47 ± 0.16b-g	1.72 ± 0.44a-e	5.93 ± 0.36a-d
6.1.3	ORA	2.58	6.28	2.88 ± 0.49ab	6.40 ± 0.15a-g	2.55 ± 0.76de	5.75 ± 0.41ab
6.1.3	ORB	2.58	6.28	4.38 ± 0.62abc	6.27 ± 0.13a-g	2.63 ± 0.59e	5.85 ± 0.21abc
6.1.3	ORC	2.58	6.28	6.87 ± 0.91c	5.80 ± 0.32ab	4.78 ± 0.57f	5.77 ± 0.14ab
6.1.3	OGA	3.05	5.84	4.09 ± 0.67ab	5.92 ± 0.21abc	2.50 ± 0.50cde	5.80 ± 0.13ab
6.1.3	OGB	3.05	5.84	4.69 ± 0.95bc	5.97 ± 0.37a-d	2.26 ± 0.78b-e	5.90 ± 0.20a-d
6.1.3	OGC	3.05	5.84	6.81 ± 1.37c	5.76 ± 0.30a	4.22 ± 0.84f	5.67 ± 0.28a
Sig p<0.05				<0.001	0.004	<0.001	0.080
LSD ²				2.43	0.56	1.17	0.46
CV % ³				48.50	5.30	43.80	4.70
Fertiliser (F) ⁴				<0.001	<0.001	<0.001	<0.001
CR	Medium (G)			ns	<0.001	ns	ns
vs	Levels (C)			0.002	ns	0.001	ns
Treatments	F x G			ns	ns	ns	ns
	F x C			ns	ns	0.008	ns
	G x C			ns	ns	ns	ns
Sig. ⁵				Q**	L***Q***	Q***	ns
Fertiliser (F) ⁴				<0.001	<0.001	<0.001	<0.001
CG	Medium (G)			ns	<0.001	ns	ns
vs	Levels (C)			0.002	ns	0.001	ns
Treatments	F x G			ns	ns	ns	ns
	F x C			ns	ns	0.007	ns
	G x C			ns	ns	ns	ns
Sig. ⁵				Q**	L**Q*	Q***	ns

1. Means (±SE) within columns followed by the same letter do not differ significantly according to Duncan's Multiple Range Test at p < 0.05. Non-significant (ns) or significant at p < 0.05 (*), 0.01 (**) or 0.001 (***)

2. LSD – Least significant difference at p < 0.05

3. CV % - Percentage coefficient of variance

4. Main effects and interactions at both control levels. Non-significant (ns) or significant at P < 0.05 (*), 0.01 (**) or 0.001 (***)

5. Significant linear (L) or quadratic contrast at p < 0.05 (*), 0.01 (**) or 0.001 (***) across fertiliser types and growth media

6. CR – Control in Randles growth medium, CG – Control in Gromor growth medium, N – Nitrosol fertiliser, P – Polyfeed fertiliser, L – Lawn and Leaf fertiliser, O – Organic vita boost fertiliser, R – Randles growth medium, G – Gromor growth medium, A – ½ label rate, B – label rate and C – 2 x label rate, Table adapted from (Bi *et al.*, 2010)

3.2.4 Analysis of growth medium inorganic nitrogen

3.2.4.1 Growth medium nitrate (NO₃-N)

Concentrations of NO₃-N in the media increased for all treatments with increasing fertiliser rates, except for the gromor treatments NGB and PGC (Table 3.6). Nitrate-N concentrations for the Organic Vitaboost addition to the randles (OR) and gromor (OG) treatments were higher than the other fertiliser treatments.

Nitrate-N level main effects were significantly influenced by fertiliser type ($p < 0.001$). The statistical analysis for NO₃-N was not significant overall ($p = 0.087$), but the high randles Organic Vitaboost fertiliser (ORC) produced significantly higher NO₃-N growth media concentrations compared with most treatments.

3.2.4.2 Growth medium ammonium (NH₄-N)

The NH₄-N concentrations in the media only increased with increasing fertiliser rates (at all concentrations A to C) from Nitrosol (NR, NG), the gromor Lawn and Leaf (LG) and the gromor Organic Vitaboost (OG) fertiliser treatments (Table 3.6). The Vitaboost fertiliser applied to gromor at twice the recommended rate (OGC) resulted in a significantly higher NH₄-N concentration.

Mean growth media NH₄-N concentrations were significantly ($p = 0.044$) affected by the main effects of growth medium when treatments were compared with each control (CR and CG). Ammonium-N concentrations in the randles medium were significantly lower than those in the gromor medium.

3.2.5 Growth medium total nitrogen (TN)

The TN concentrations in the growth media were significantly affected by the main effects of growth medium type ($p < 0.001$) for all treatments compared with each control (Table 3.6). There were significantly lower TN concentrations in the soil-based randles media mixes than those containing the bark-based mixes. The Nitrosol and Polyfeed (NGA and PGA) applications had significantly higher ($p = 0.018$) %TN concentrations than all soil-based treatments.

3.2.6 Phosphate-P and TP in growth medium extracted leachate

Leachate was collected from growth medium for orthophosphate ($\text{PO}_4\text{-P}$) and total phosphate analyses using the pour through extraction method (Wright, 1986). This method tests the amount of phosphate available to the plant roots.

3.2.6.1 Phosphate-P in extracted leachate

The orthophosphate ($\text{PO}_4\text{-P}$) concentrations in leachate were, in general, lower for fertiliser groups containing randles media than those containing the bark-based media (Table 3.6). The high Organic Vitaboost-supplied gromor medium (OGC) had a tendency towards the highest phosphates in the leachate ($p < 0.001$).

The main effects of the factors fertiliser, growth medium and levels of fertiliser used in treatments, were statistically significantly different at $p = 0.035$, $p < 0.001$ and $p = 0.050$, respectively, for randles medium control comparison, while only the factors growth medium and fertiliser level were significant at $p < 0.001$ and $p = 0.050$, respectively for the gromor medium control.

3.2.6.2 Total phosphates in extracted leachate

The TP concentrations in leachate were in general lower for fertiliser groups containing randles medium mixes than those containing the bark-based medium. The overall effect of growth medium type (randles or gromor) differed significantly ($p < 0.001$) due to fertiliser treatments when compared with either of the controls (CR or CG). Gromor-based media showed a tendency to leach significantly higher concentrations of TP compared with randles-based ones. The higher organic Vitaboost and higher Polyfeed applications to the gromor medium (OGC and PGC) resulted in statistically higher phosphate leachates than most treatments at $p < 0.001$ (Table 3.6).

Table 3.6 Effects of two organic (Nitrosol[®] and Organic Vitaboost) and two inorganic fertiliser treatment types (Polyfeed[®] and Lawn and Leaf) applied at three concentrations to two different growth media, on mean growth media nitrogen (nitrate, ammonium and total N) and phosphorus (phosphate and total phosphorus concentrations) after a period of 90DAP

Type	Treatment	NO ₃ -N mg L ⁻¹	NH ₄ -N mg L ⁻¹	TN %	PO ₄ -P mg L ⁻¹	TP mg L ⁻¹
Control	CR ⁶	2.63 ± 1.11a ¹	5.15 ± 1.24a	0.17 ± 0.06a	0.46 ± 0.21ab	0.69 ± 0.36
Control	CG	3.19 ± 1.76a	5.52 ± 0.66a	0.39 ± 0.12a-d	3.80 ± 0.33a-e	4.16 ± 0.35a-d
4.1.3	NRA	8.50 ± 4.13ab	5.44 ± 0.48a	0.17 ± 0.04a	0.50 ± 0.17ab	1.17 ± 0.33ab
4.1.3	NRB	16.70 ± 11.33abc	5.84 ± 0.39a	0.18 ± 0.08a	0.51 ± 0.17ab	0.93 ± 0.32ab
4.1.3	NRC	33.46 ± 24.64abc	6.36 ± 0.73a	0.22 ± 0.03ab	0.99 ± 0.47abc	1.48 ± 0.48ab
4.1.3	NGA	40.43 ± 40.21abc	7.18 ± 1.11a	1.09 ± 0.68d	5.91 ± 2.38a-e	7.17 ± 2.69a-e
4.1.3	NGB	15.47 ± 9.83abc	7.46 ± 1.55a	0.78 ± 0.23a-d	7.34 ± 2.54a-f	8.23 ± 2.88a-e
4.1.3	NGC	38.77 ± 19.36abc	7.60 ± 1.35a	0.65 ± 0.18a-d	8.01 ± 2.41b-f	10.40 ± 3.56b-e
6.1.3	PRA	10.26 ± 6.26ab	6.09 ± 1.07a	0.18 ± 0.07a	0.62 ± 0.32ab	0.99 ± 0.29ab
6.1.3	PRB	14.95 ± 8.36abc	5.06 ± 0.44a	0.21 ± 0.03ab	0.31 ± 0.01ab	0.41 ± 0.04a
6.1.3	PRC	35.95 ± 35.26abc	5.74 ± 1.05a	0.31 ± 0.05abc	0.26 ± 0.10ab	0.34 ± 0.10a
6.1.3	PGA	16.64 ± 15.10abc	6.76 ± 0.68a	1.09 ± 0.40d	6.83 ± 2.58a-e	7.50 ± 2.64a-e
6.1.3	PGB	26.58 ± 26.09abc	6.43 ± 1.04a	0.78 ± 0.24a-d	5.74 ± 4.33a-e	6.77 ± 4.78a-e
6.1.3	PGC	12.34 ± 9.84abc	7.78 ± 0.17a	0.79 ± 0.28a-d	10.36 ± 5.19ef	14.20 ± 8.35e
7.1.3	LRA	21.94 ± 16.22abc	5.76 ± 0.58a	0.20 ± 0.03ab	0.21 ± 0.05a	0.52 ± 0.21a
7.1.3	LRB	25.23 ± 20.40abc	5.57 ± 0.70a	0.16 ± 0.09a	0.17 ± 0.02a	0.34 ± 0.05a
7.1.3	LRC	64.74 ± 57.94a-d	6.10 ± 0.87a	0.18 ± 0.03a	0.53 ± 0.09ab	0.98 ± 0.28ab
7.1.3	LGA	6.03 ± 5.80ab	6.97 ± 0.63a	1.04 ± 0.60cd	6.04 ± 2.43a-e	8.23 ± 3.93a-e
7.1.3	LGB	15.64 ± 15.27abc	7.46 ± 0.75a	0.93 ± 0.44bcd	6.79 ± 2.48a-e	10.93 ± 5.76cde
7.1.3	LGC	22.76 ± 13.69abc	8.32 ± 1.28a	0.67 ± 0.19a-d	9.14 ± 3.54ef	11.55 ± 4.90de
6.1.3	ORA	61.11 ± 45.04a-d	6.15 ± 0.62a	0.24 ± 0.02ab	1.07 ± 0.49a-d	1.61 ± 0.63abc
6.1.3	ORB	109.96 ± 94.05bcd	5.77 ± 0.98a	0.22 ± 0.02ab	1.46 ± 0.62a-d	3.06 ± 1.45a-d
6.1.3	ORC	147.32 ± 88.45d	5.76 ± 0.87a	0.23 ± 0.07ab	8.65 ± 6.45def	10.37 ± 6.59b-e
6.1.3	OGA	40.53 ± 20.63abc	6.95 ± 1.19a	0.77 ± 0.35a-d	8.43 ± 3.07c-f	9.47 ± 3.47a-e
6.1.3	OGB	85.86 ± 24.79a-d	8.61 ± 1.67a	0.81 ± 0.37a-d	7.42 ± 3.94a-f	9.10 ± 4.83a-e
6.1.3	OGC	115.89 ± 10.98cd	52.09 ± 31.54b	0.56 ± 0.12a-d	14.51 ± 4.58f	16.05 ± 4.95e
Sig p<0.05		0.087 (ns)	0.012	0.018	<.001	<.001
LSD ²		85.79	17.60	0.63	6.38	7.86
CV (%) ³		137	130.40	61.60	87.10	85.00
Fertiliser (F) ⁴		<0.001	ns	ns	0.035	ns
CR	Medium (G)	ns	0.044	<0.001	<0.001	<0.001
vs	Levels (C)	ns	ns	ns	0.050	ns
Treatments	F x G	ns	ns	ns	ns	ns
	F x C	ns	ns	ns	ns	ns
	G x C	ns	ns	ns	ns	ns
Sig. ⁵		Q*	ns	ns	Q*	ns
Fertiliser (F) ³		<0.001	ns	ns	ns	ns
CG	Medium (G)	ns	0.044	<0.001	<0.001	<0.001
vs	Levels (C)	ns	ns	ns	0.050	ns
Treatments	F x G	ns	ns	ns	ns	ns
	F x C	ns	ns	ns	ns	ns
	G x C	ns	ns	ns	ns	ns
Sig. ⁵		Q*	ns	ns	ns	ns

1. Means (±SE) within columns followed by the same letter do not differ significantly according to Duncan's Multiple Range Test at p < 0.05. Non-significant (ns) or significant at p < 0.05 (*), 0.01 (**), or 0.001 (***)

2. LSD –Least significant difference at p < 0.05

3. CV (%) - Percentage coefficient of variance

4. Main effects and interactions at both control levels. Non-significant (ns) or significant at P < 0.05 (*), 0.01 (**), or 0.001 (***)

5. Significant linear (L) or quadratic contrast at p < 0.05 (*), 0.01 (**), or 0.001 (***) across fertiliser types and growth media

6. CR – Control in Randles growth medium, CG – Control in Gromor growth medium, N – Nitrosol fertiliser, P – Polyfeed fertiliser, L – Lawn and Leaf fertiliser, O – Organic vita boost fertiliser, R – Randles growth medium, G – Gromor growth medium, A – ½ label rate, B – label rate and C – 2 x label rate, Table adapted from (Bi *et al.*, 2010)

3.2.7 Macronutrients in growth medium extracted leachate

Leachate was collected from growth medium for macronutrient analyses using the pour-through extraction method (Wright, 1986). This method tests the nutrients available in the growth medium solution for plant roots (Richard, 2006).

3.2.7.1 Nitrogen (N) in extracted leachate

There were no significant differences between any of the treatments, including comparison with the controls, at $p=0.919$, nor were there any significant main effects or interactions for mean N concentrations in the leachates (Table 3.7).

3.2.7.2 Phosphorous (P) in extracted leachate

Phosphorous concentrations in extracted leachate were significantly lower from soil-based growth media (randles) than from bark-based media (gromor). The gromor medium leachate containing the highest Organic Vitaboost supply (OGC) had a significantly higher P concentration than most treatments (Table 3.7).

The main effects of the factor fertiliser type were statistically significantly different when treatments were compared with the randles control and also gromor control ($p=0.026$ and $p<0.001$, respectively) Growth medium main effects were also significantly different when treatments were compared with the randles and gromor controls ($p=0.011$ and $p<0.001$, respectively) (Table 3.7).

3.2.7.3 Potassium (K) in extracted leachate

The leachate K concentrations were higher in the randles Organic Vitaboost treatments, whilst the other fertiliser treatments were similar, irrespective of growth medium effects. The organic fertiliser supplied to the randles medium at double the recommended rate (ORC) was

significantly different from most treatments ($p=0.001$), except the high Lawn and Leaf (LRC) and the lower Organic Vitaboost application to the randles medium (ORA and ORB) (Table 3.7).

The main effects of fertiliser type, growth medium type and level of fertiliser were statistically significantly different ($p<0.001$, $p<0.001$ and $p=0.038$, respectively) for the randles medium (CR) comparison and ($p<0.001$, $p<0.001$ and $p=0.040$, respectively) for the gromor comparison.

3.2.7.4 Calcium (Ca) in extracted leachate

The Ca concentrations in the leachate increased with increasing fertiliser application, except for the PG fertiliser group (Table 3.7). The Ca concentrations in the growth medium were higher when it contained randles mix than the bark-based mix. Similar to the potassium figures, the high Organic Vitaboost application to the randles medium (ORC) was significantly different from all treatments ($p<0.001$), except for the high Lawn and Leaf and the standard Organic Vitaboost to the randles medium (LRC and ORB).

The main effects of the factors fertiliser type and growth medium type and levels of fertiliser used in treatments were statistically significantly different (<0.001 , $p<0.001$ and $p=0.046$, respectively) for the CR comparison and ($p<0.001$, $p<0.001$ and $p=0.050$, respectively) for the CG comparison (Table 3.7).

3.2.7.5 Magnesium (Mg) in extracted leachate

The Mg concentrations increased with increasing fertiliser rate, except for the Polyfeed applied to the randles mix (PR) group (Table 3.7). Leachate Mg concentrations were, in general, higher for fertiliser groups containing the randles medium than those containing the bark-based medium.

The main effects of fertiliser, growth medium and levels of fertiliser used were statistically significantly different ($p < 0.001$, $p = 0.001$ and $p = 0.009$, respectively) when treatments were compared with the randles medium control (CR) and similarly when compared with the gromor control ($p < 0.001$, $p = 0.002$ and $p = 0.010$, respectively).

Table 3.7 Effects of two organic (Nitrosol[®] and Organic Vitaboost) and two inorganic fertiliser treatment types (Polyfeed[®] and Lawn and leaf) applied at three concentrations to two different growth media, on mean growth media pour-through extracted nutrient solution N, P, K, Ca and Mg concentrations compared to two controls after a period of 90 DAP

Type	Treatment	N mg·L ⁻¹ (x1000)	P mg·L ⁻¹	K (mg·L ⁻¹)	Ca mg·L ⁻¹	Mg mg·L ⁻¹
Control	CR ⁶	2.57 ± 1.11a ¹	2.34 ± 1.75a	121.20 ± 54.89a	58.00 ± 28.65ab	13.41 ± 6.31ab
Control	CG	2.30 ± 1.57a	5.26 ± 1.47abc	91.90 ± 38.25a	39.00 ± 10.67a	10.83 ± 0.86ab
4.1.3	NRA	3.43 ± 1.70a	4.34 ± 3.74ab	166.00 ± 76.88ab	136.90 ± 100.88a-d	23.51 ± 16.33a-d
4.1.3	NRB	3.68 ± 1.93a	4.48 ± 3.89ab	198.70 ± 92.89ab	154.30 ± 99.15a-e	24.60 ± 13.92a-d
4.1.3	NRC	3.27 ± 1.82a	7.52 ± 6.52a-d	258.20 ± 124.85abc	179.70 ± 94.55a-e	35.14 ± 18.30a-e
4.1.3	NGA	3.61 ± 2.17a	13.04 ± 5.65a-f	130.60 ± 47.35a	29.80 ± 8.51a	9.89 ± 2.49ab
4.1.3	NGB	3.86 ± 1.91a	15.42 ± 5.17a-g	150.30 ± 37.74ab	54.30 ± 4.04ab	18.25 ± 2.10abc
4.1.3	NGC	2.89 ± 1.50a	22.36 ± 8.46fg	208.80 ± 67.17ab	61.80 ± 17.32ab	24.89 ± 8.62a-d
6.1.3	PRA	3.91 ± 2.39a	6.66 ± 6.26a-d	142.40 ± 61.63ab	115.20 ± 77.38abc	20.70 ± 13.23a-d
6.1.3	PRB	3.33 ± 1.63a	8.10 ± 7.70a-e	157.60 ± 80.31ab	130.20 ± 79.77a-d	20.59 ± 10.80a-d
6.1.3	PRC	2.61 ± 1.25a	7.32 ± 6.93a-d	238.00 ± 103.97abc	173.90 ± 110.03a-e	31.91 ± 19.20a-d
6.1.3	PGA	2.71 ± 1.88a	15.22 ± 6.83a-g	94.60 ± 31.67a	32.00 ± 9.68a	8.78 ± 2.33a
6.1.3	PGB	3.67 ± 2.73a	13.98 ± 5.36a-f	142.70 ± 12.01ab	44.60 ± 8.23a	14.51 ± 1.45ab
6.1.3	PGC	4.03 ± 2.01a	17.96 ± 7.85b-g	155.50 ± 61.19ab	38.40 ± 8.05a	15.24 ± 4.74ab
7.1.3	LRA	4.01 ± 2.33a	2.96 ± 2.56a	242.30 ± 114.06abc	152.70 ± 95.93a-e	26.36 ± 14.75a-d
7.1.3	LRB	2.85 ± 1.38a	6.24 ± 5.84abc	289.10 ± 129.65abc	183.60 ± 103.29a-e	37.63 ± 21.31a-e
7.1.3	LRC	3.04 ± 1.38a	4.75 ± 4.15ab	478.50 ± 217.36cd	297.50 ± 160.56def	64.69 ± 36.17def
7.1.3	LGA	2.40 ± 1.72a	12.76 ± 5.63a-f	104.20 ± 19.31a	40.10 ± 16.59a	11.40 ± 3.40ab
7.1.3	LGB	2.88 ± 1.57a	16.12 ± 8.67a-g	195.10 ± 67.72ab	67.50 ± 18.80ab	23.90 ± 6.52a-d
7.1.3	LGC	4.26 ± 3.27a	20.36 ± 10.33d-g	209.40 ± 84.89ab	88.00 ± 36.81abc	30.98 ± 11.69a-d
6.1.3	ORA	3.15 ± 1.90a	5.82 ± 5.03abc	390.70 ± 191.62bcd	246.90 ± 110.22cde	62.05 ± 28.03c-f
6.1.3	ORB	5.50 ± 3.41a	9.2 ± 8.21a-f	480.20 ± 253.72cd	311.70 ± 121.46ef	89.70 ± 38.83fg
6.1.3	ORC	4.60 ± 2.94a	14.68 ± 12.13a-f	560.80 ± 256.25d	408.10 ± 87.90f	119.13 ± 38.69g
6.1.3	OGA	2.92 ± 1.76a	21.72 ± 13.06efg	242.10 ± 98.69abc	132.10 ± 39.88a-d	48.62 ± 17.33a-f
6.1.3	OGB	4.12 ± 2.79a	18.96 ± 9.30c-g	226.10 ± 111.08ab	141.50 ± 9.97a-d	54.87 ± 18.31b-f
6.1.3	OGC	3.83 ± 1.77a	28.52 ± 14.88g	293.00 ± 133.81abc	220.00 ± 35.81b-e	76.34 ± 21.85ef
Sig p<0.05		0.919 (ns)	<0.001	0.001	<.001	<.001
LSD ²		2.75	11.47	110.40	142.85	37.14
CV (%) ³		48.80	59.20	56.40	64.00	63.50
Fertiliser (F) ⁴		ns	0.011	<.001	<.001	<.001
CR	Medium (G)	ns	<.001	<.001	<.001	0.001
vs	Levels (C)	ns	ns	0.038	0.046	0.009
Treatments	F x G	ns	ns	ns	ns	ns
	F x C	ns	ns	ns	ns	ns
	G x C	ns	ns	ns	ns	ns
Sig. ⁵		ns	L*Q*	Q**	Q**	Q***
Fertiliser (F) ⁴		ns	0.026	<.001	<.001	<.001
CG	Medium (G)	ns	<.001	<.001	<.001	0.002
vs	Levels (C)	ns	ns	0.040	0.050	0.010
Treatments	F x G	ns	ns	ns	ns	ns
	F x C	ns	ns	ns	ns	ns
	G x C	ns	ns	ns	ns	ns
Sig. ⁵		ns	Q*	Q**	Q**	Q***

1. Means (±SE) within columns followed by the same letter do not differ significantly according to Duncan's Multiple Range Test at p < 0.05. Non-significant (ns) or significant at p < 0.05 (*), 0.01 (**), or 0.001 (***)

2. LSD –Least significant difference at p < 0.05

3. CV (%) - Percentage coefficient of variance

4. Main effects and interactions at both control levels. Non-significant (ns) or significant at P < 0.05 (*), 0.01 (**), or 0.001 (***)

5. Significant linear (L) or quadratic contrast at p < 0.05 (*), 0.01 (**), or 0.001 (***) across fertiliser types and growth media

6. CR – Control in Randles growth medium, CG – Control in Gromor growth medium, N – Nitrosol fertiliser, P – Polyfeed fertiliser, L – Lawn and Leaf fertiliser, O – Organic vita boost fertiliser, R – Randles growth medium, G – Gromor growth medium, A – ½ label rate, B – label rate and C – 2 x label rate, Table adapted from (Bi *et al.*, 2010)

3.2.8 Micronutrients in growth medium extracted leachate

3.2.8.1 Iron (Fe) in extracted leachate

The Fe concentrations were higher in leachate extracted from the randles mix than in leachates from the bark-based mix, except for OR and OG (Table 3.8). There were no significant differences between treatments overall ($p=0.227$). Treatments compared with each control showed that the main effect of growth medium type was statistically significantly different ($p=0.001$) for both comparisons.

3.2.8.2 Boron (B) in extracted leachate

The B concentrations increased with increasing fertiliser rate. The high Organic Vitaboost application to the randles medium (ORC) resulted in significantly higher amounts of B leached than from most treatments at $p=0.03$ (Table 3.8).

The main effects of the factors fertiliser type and levels of fertiliser, were statistically significantly different ($p=0.028$ and $p=0.006$) for the randles (CR) comparison, respectively, and ($p=0.033$ and $p=0.006$) respectively for the gromor (CG) comparison. There was also a statistically significant interaction between the effects of fertiliser and levels of fertiliser on boron concentrations for both media.

3.2.8.3 Manganese (Mn) in extracted leachate

The Mn leachate concentrations increased in all fertiliser groups with increasing fertiliser rates, except for the Polyfeed supplemented randles medium (PR) (Table 3.8). Again, ORC Mn concentrations were significantly higher ($p=0.016$) than all other treatments. The Mn concentrations had a tendency to be lower in substrates containing the soil-based growth medium (randles) than those containing the bark-based medium (gromor) mix. The main

effects of fertiliser type used in treatments were significantly different ($p < 0.001$) for both media.

3.2.8.4 Zinc (Zn) in extracted leachate

Overall, the Zn concentration in the leachates was not significantly different ($p = 0.091$), although ORC was significantly higher than several treatments (Table 3.8). The Zn concentrations had a tendency to be lower in the fertiliser groups containing the soil-based growth medium than the bark-based medium. The fertiliser type used in treatments significantly affected the Zn concentration in both media ($p < 0.001$).

3.2.8.5 Copper (Cu) in extracted leachate

The leachate Cu concentrations were statistically significantly higher for leachate extracted from the randles media than those containing the gromor medium (Table 3.8). The randles medium supplied with high concentrations of Organic Vitaboost (ORC) or Polyfeed (PRC) were significantly higher than most other treatments ($p = 0.033$). The main effects of growth medium type were statistically significantly different ($p < 0.001$).

Table 3.8 Effects of two organic (Nitrosol[®] and Organic Vitabooost) and two inorganic fertiliser treatment types (Polyfeed[®] and Lawn and Leaf) applied at three concentrations to two different growth media, on mean growth media pour-through extracted nutrient solution Fe, B, Mn, Zn and Cu concentrations compared to two controls after a period of 90 DAP

Type	Treatment	Fe mg·L ⁻¹ (x 1000)	B mg·L ⁻¹	Mn mg·L ⁻¹ (x 1000)	Zn mg·L ⁻¹	Cu mg·L ⁻¹
Control	CR ⁶	5.17 ± 2.61ab ¹	0.12 ± 0.03abc	0.32 ± 0.24a	321.80 ± 243.80a	286.40 ± 177.10a-d
Control	CG	3.82 ± 2.88a	0.13 ± 0.01a-d	0.56 ± 0.35a	358.10 ± 234.70a	49.30 ± 24.90a
4.1.3	NRA	7.72 ± 4.95abc	0.11 ± 0.03ab	0.37 ± 0.23a	208.10 ± 115.80a	409.50 ± 130.50a-e
4.1.3	NRB	13.28 ± 6.66abc	0.14 ± 0.04a-d	0.50 ± 0.36a	325.00 ± 242.80a	464.60 ± 177.90a-e
4.1.3	NRC	17.12 ± 8.63bc	0.22 ± 0.07a-e	0.68 ± 0.48a	351.10 ± 236.10a	763.80 ± 391.30de
4.1.3	NGA	5.06 ± 3.67ab	0.16 ± 0.03a-d	0.52 ± 0.14a	207.10 ± 115.70a	97.60 ± 51.30abc
4.1.3	NGB	5.45 ± 5.25abc	0.22 ± 0.04a-e	1.25 ± 0.99a	493.20 ± 362.50a	150.40 ± 108.10a-d
4.1.3	NGC	5.43 ± 4.26abc	0.29 ± 0.07cde	4.00 ± 3.60a	744.10 ± 430.70abc	129.00 ± 97.40abc
6.1.3	PRA	17.21 ± 8.30bc	0.10 ± 0.03a	0.71 ± 0.47a	359.80 ± 234.40a	711.50 ± 324.00 b-e
6.1.3	PRB	12.42 ± 9.46abc	0.12 ± 0.03abc	0.58 ± 0.35a	352.70 ± 235.70a	460.40 ± 91.40a-e
6.1.3	PRC	18.22 ± 9.89c	0.18 ± 0.05a-d	1.07 ± 0.67a	334.70 ± 240a	990.70 ± 667.90e
6.1.3	PGA	8.94 ± 7.15abc	0.16 ± 0.02a-d	0.70 ± 0.25a	638.90 ± 161.10ab	87.30 ± 17.10ab
6.1.3	PGB	5.36 ± 3.79abc	0.25 ± 0.06a-e	1.06 ± 0.67a	731.10 ± 438.50abc	97.90 ± 51.20abc
6.1.3	PGC	7.58 ± 5.21abc	0.30 ± 0.13de	1.88 ± 1.67a	621.10 ± 495.30ab	58.30 ± 11.70a
7.1.3	LRA	11.34 ± 6.08abc	0.10 ± 0.02ab	0.49 ± 0.36a	177.40 ± 117.70a	490.20 ± 199.10a-e
7.1.3	LRB	9.65 ± 5.83abc	0.13 ± 0.03a-d	0.67 ± 0.48a	203.00 ± 115.50a	466.90 ± 162.10a-e
7.1.3	LRC	9.17 ± 5.09abc	0.15 ± 0.05a-d	0.87 ± 0.77a	569.70 ± 326.50ab	728.90 ± 351.10cde
7.1.3	LGA	5.99 ± 4.10abc	0.14 ± 0.03a-d	0.97 ± 0.52a	772.50 ± 255.10abc	218.90 ± 99.60a-d
7.1.3	LGB	5.01 ± 5.01ab	0.15 ± 0.03a-d	1.38 ± 1.12a	762.10 ± 420.20abc	115.20 ± 62.80abc
7.1.3	LGC	6.52 ± 6.32abc	0.16 ± 0.04a-d	2.89 ± 2.56a	777.00 ± 412.00abc	119.60 ± 23.50abc
6.1.3	ORA	5.45 ± 5.06abc	0.19 ± 0.06a-d	3.04 ± 2.88a	469.30 ± 370.30a	487.00 ± 206.40a-e
6.1.3	ORB	6.70 ± 5.73abc	0.28 ± 0.13b-e	9.19 ± 9.01a	1147.40 ± 1028.70abc	600.00 ± 400.00a-e
6.1.3	ORC	5.02 ± 4.63ab	0.36 ± 0.18e	31.95 ± 17.51b	2337.3 ± 1335.20c	1038.70 ± 629.50e
6.1.3	OGA	5.44 ± 5.44abc	0.17 ± 0.03a-d	7.27 ± 6.77a	1594.70 ± 1407.00abc	62.50 ± 11.80a
6.1.3	OGB	4.40 ± 4.40ab	0.18 ± 0.05a-d	12.32 ± 12.04a	2168.10 ± 1716.20bc	97.40 ± 32.30abc
6.1.3	OGC	5.32 ± 5.12abc	0.22 ± 0.04a-e	13.75 ± 13.33a	1872.60 ± 1463.70abc	61.45 ± 11.60a
Sig p<0.05		0.227 (ns)	0.038	0.016	0.091(ns)	0.033
LSD ²		10.64	0.143	13.63	1383.20	633.00
CV (%) ³		78.20	48.40	118.30	116.00	108.60
Fertiliser (F) ⁴		ns	0.028	<0.001	<0.001	ns
CR Medium (G)		0.001	ns	ns	ns	<0.001
vs Levels (C)		ns	0.006	ns	ns	ns
Treatments F x G		ns	0.017	ns	ns	ns
F x C		ns	ns	ns	ns	ns
G x C		ns	ns	ns	ns	ns
Sig. ⁵		ns	L*Q*	Q*	ns	ns
Fertiliser (F) ⁴		ns	0.033	<0.001	<0.001	ns
CG Medium (G)		0.001	ns	ns	ns	<0.001
vs Levels (C)		ns	0.006	ns	ns	ns
Treatments F x G		ns	0.016	ns	ns	ns
F x C		ns	ns	ns	ns	ns
G x C		ns	ns	ns	ns	ns
Sig. ⁵		ns	L*	Q*	ns	ns

1. Means (±SE) within columns followed by the same letter do not differ significantly according to Duncan's Multiple Range Test at p < 0.05. Non-significant (ns) or significant at p < 0.05 (*), 0.01 (**), or 0.001 (***)

2. LSD –Least significant difference at p < 0.05

3. CV (%) – Percentage coefficient of variance

4. Main effects and interactions at both control levels. Non-significant (ns) or significant at P < 0.05 (*), 0.01 (**) or 0.001 (***)

5. Significant linear (L) or quadratic contrast at p < 0.05 (*), 0.01 (**) or 0.001 (***) across fertiliser types and growth media

6. CR – Control in Randles growth medium, CG – Control in Gromor growth medium, N – Nitrosol fertiliser, P – Polyfeed fertiliser, L – Lawn and Leaf fertiliser, O – Organic Vita boost fertiliser, R – Randles growth medium, G – Gromor growth medium, A – ½ label rate, B – label rate and C – 2 x label rate, Table adapted from (Bi *et al.*, 2010)

3.2.9 Nutrient composition of leaf tissue

As the mineral concentration in leachates of the various treatments was affected by the medium and the fertiliser applied, nutrients in leaf tissues could also be affected by growth medium and fertiliser application.

3.2.9.1 Macronutrients in leaf tissue

Nitrogen (N) The N concentration in leaf tissue (Table 3.9) were significantly affected by the main effects of fertiliser type and applied concentration ($p < 0.001$ and $p = 0.013$, respectively) for treatments compared with the randles control (CR) and the gromor control ($p < 0.001$ and $p = 0.016$, respectively). The N concentrations of leaf tissue from media containing Organic Vitaboost (OR and OG) were the highest amongst all treatments. Leaf N concentrations increased as fertiliser rates increased. High Organic Vitaboost applications (OGC) produced significantly higher N leaf tissue concentrations than most treatments ($p < 0.001$).

Phosphorous (P) – Leaf tissue P concentrations were significantly affected by growth medium type ($p < 0.001$) compared with the controls (Table 3.9). The P concentrations in leaf tissue were significantly lower for randles-containing treatments than gromor-containing treatments. Leaf phosphorous concentrations of plants in randles media mixes decreased linearly as applied concentrations increased. Leaf P concentrations in both controls were even higher than those of randles soil-based treatments.

Potassium (K) – There were no significant differences in leaf tissue K concentrations amongst treatments and between treatments and the controls ($p = 0.811$) (Table 3.9).

Calcium (Ca) – Mean leaf tissue Ca concentrations were significantly affected by the main effect of growth medium type ($p < 0.001$) when treatments were compared with the controls (Table 3.9). Leaf Ca concentrations were significantly higher for fertiliser groups containing

the soil- than the bark-based medium. There were no significant differences overall between treatments, including the controls ($p=0.081$) in leaf tissue Ca concentrations.

Magnesium (Mg) – Leaf Mg concentrations were significantly affected by fertiliser type ($p=0.002$ and $p=0.012$), when treatments were compared with the controls (CR and CG, respectively). Significantly more Mg was taken up by the randles control plants than most Organic Vitaboost treatments (Table 3.9). Treatments were not significantly different overall ($p=0.325$). Leaf Mg concentrations decreased or had a tendency to decrease as fertiliser rates increased.

Table 3.9 Effects of two organic (Nitrosol[®] and Organic Vitaboost) and two inorganic fertiliser treatment types (Polyfeed[®] and Lawn and Leaf) applied at three concentrations to two different growth media, on mean leaf tissue N, P, K, Ca and Mg concentrations compared to two controls after a period of 90 DAP

Type	Treatment	N %	P %	K %	Ca %	Mg %
Control	CR ⁶	3.04 ± 0.63abc ¹	0.89 ± 0.11cde	4.64 ± 0.70a	2.80 ± 0.59a-d	1.67 ± 0.34b
Control	CG	2.63 ± 0.58a	0.93 ± 0.35cde	5.68 ± 0.45a	2.38 ± 0.30abc	1.47 ± 0.21ab
4.1.3	NRA	2.89 ± 0.39ab	0.73 ± 0.18a-e	4.78 ± 0.95a	3.06 ± 0.25a-d	1.53 ± 0.23ab
4.1.3	NRB	3.34 ± 0.65a-d	0.60 ± 0.16a-d	4.93 ± 0.96a	3.31 ± 0.22cd	1.51 ± 0.27ab
4.1.3	NRC	3.43 ± 0.76a-d	0.53 ± 0.13abc	5.01 ± 1.06a	3.37 ± 0.47cd	1.39 ± 0.22ab
4.1.3	NGA	2.95 ± 0.39ab	1.01 ± 0.41de	5.30 ± 0.46a	2.44 ± 0.17abc	1.42 ± 0.22ab
4.1.3	NGB	3.14 ± 0.56abc	1.06 ± 0.26e	5.05 ± 0.58a	2.44 ± 0.28abc	1.44 ± 0.21ab
4.1.3	NGC	3.55 ± 0.69a-e	1.07 ± 0.21e	4.92 ± 0.67a	2.46 ± 0.29a-d	1.40 ± 0.16ab
6.1.3	PRA	3.04 ± 0.70abc	0.84 ± 0.19b-e	4.61 ± 0.96a	3.00 ± 0.37a-d	1.56 ± 0.30ab
6.1.3	PRB	3.16 ± 0.72abc	0.66 ± 0.16a-e	4.55 ± 1.08a	3.49 ± 0.76d	1.51 ± 0.30ab
6.1.3	PRC	3.49 ± 0.68a-d	0.56 ± 0.10abc	4.99 ± 1.11a	3.27 ± 0.52bcd	1.38 ± 0.22ab
6.1.3	PGA	3.15 ± 0.24abc	1.07 ± 0.28e	5.02 ± 0.35a	2.55 ± 0.34a-d	1.55 ± 0.24ab
6.1.3	PGB	3.43 ± 0.43a-d	1.03 ± 0.24e	5.05 ± 0.63a	2.46 ± 0.32a-d	1.48 ± 0.16ab
6.1.3	PGC	3.41 ± 0.65a-d	0.96 ± 0.16cde	4.94 ± 0.65a	2.15 ± 0.21a	1.38 ± 0.17ab
7.1.3	LRA	3.06 ± 0.72abc	0.78 ± 0.07a-e	4.95 ± 1.11a	2.78 ± 0.29a-d	1.55 ± 0.27ab
7.1.3	LRB	3.14 ± 0.78abc	0.55 ± 0.12abc	5.21 ± 1.11a	3.03 ± 0.61a-d	1.47 ± 0.31ab
7.1.3	LRC	3.35 ± 0.63a-d	0.43 ± 0.13ab	5.83 ± 0.98a	3.06 ± 0.65a-d	1.42 ± 0.30ab
7.1.3	LGA	3.05 ± 0.24abc	0.95 ± 0.27cde	5.32 ± 0.31a	2.23 ± 0.25ab	1.49 ± 0.25ab
7.1.3	LGB	3.39 ± 0.46a-d	1.04 ± 0.32e	5.60 ± 0.42a	2.37 ± 0.30abc	1.55 ± 0.28ab
7.1.3	LGC	3.65 ± 0.59a-e	0.87 ± 0.20cde	5.40 ± 0.32a	2.24 ± 0.24ab	1.38 ± 0.18ab
6.1.3	ORA	3.96 ± 0.84b-f	0.56 ± 0.20abc	5.64 ± 0.98a	2.57 ± 0.30a-d	1.25 ± 0.14a
6.1.3	ORB	4.16 ± 0.56c-f	0.44 ± 0.15ab	5.91 ± 1.08a	2.83 ± 0.45a-d	1.32 ± 0.20a
6.1.3	ORC	4.61 ± 0.33ef	0.40 ± 0.12a	6.19 ± 1.16a	2.76 ± 0.51a-d	1.31 ± 0.22a
6.1.3	OGA	4.39 ± 0.58def	0.88 ± 0.12cde	5.18 ± 0.22a	2.43 ± 0.53abc	1.36 ± 0.21ab
6.1.3	OGB	4.98 ± 0.62fg	0.92 ± 0.15cde	4.87 ± 0.27a	2.48 ± 0.65a-d	1.32 ± 0.24a
6.1.3	OGC	5.61 ± 0.62g	0.95 ± 0.13cde	5.31 ± 0.19a	2.50 ± 0.79a-d	1.27 ± 0.29a
Sig p<0.05		<0.001	<0.001	0.811 (ns)	0.081 (ns)	0.325 (ns)
LSD ²		0.96	0.36	1.45	0.86	0.27
CV (%) ³		16.40	27.50	17.30	19.40	11.40
Fertiliser (F) ⁴		<0.001	ns	ns	ns	0.002
CR	Medium (G)	ns	<0.001	ns	<0.001	ns
vs	Levels (C)	0.013	ns	ns	ns	ns
Treatments	F x G	ns	ns	ns	ns	ns
	F x C	ns	ns	ns	ns	ns
	G x C	ns	ns	ns	ns	ns
Sig. ⁵		L**Q***	ns	ns	ns	Q**
Fertiliser (F) ⁴		<0.001	ns	ns	ns	0.012
CG	Medium (G)	ns	<0.001	ns	<0.001	ns
vs	Levels (C)	0.016	ns	ns	ns	ns
Treatments	F x G	ns	ns	ns	ns	ns
	F x C	ns	ns	ns	ns	ns
	G x C	ns	ns	ns	ns	ns
Sig. ⁵		L**Q***	ns	ns	ns	Q*

1. Means (±SE) within columns followed by the same letter do not differ significantly according to Duncan's Multiple Range Test at p < 0.05. Non-significant (ns) or significant at p < 0.05 (*), 0.01 (**), or 0.001 (***)

2. LSD –Least significant difference at p < 0.05

3. CV (%) – Percentage coefficient of variance

4. Main effects and interactions at both control levels. Non-significant (ns) or significant at P < 0.05 (*), 0.01 (**), or 0.001 (***)

5. Significant linear (L) or quadratic contrast at p < 0.05 (*), 0.01 (**), or 0.001 (***) across fertiliser types and growth media

6. CR – Control in Randles growth medium, CG – Control in Gromor growth medium, N – Nitrosol fertiliser, P – Polyfeed fertiliser, L – Lawn and Leaf fertiliser, O – Organic Vita boost fertiliser, R – Randles growth medium, G – Gromor growth medium, A – ½ label rate, B – label rate and C – 2 x label rate, Table adapted from (Bi *et al.*, 2010)

3.2.9.2 Micronutrients in leaf tissue

Irons (Fe) – Mean Fe concentrations in leaf tissue from treatments were similar. Concentrations were, however, significantly affected by the fertiliser type ($p=0.004$ and $p=0.002$, for CR and CG comparisons, respectively), when treatments were compared with the controls (Table 3.10). The high concentration of Organic VitabooSt fertiliser applied to the bark-based media (OGC) resulted in significantly more leaf tissue Fe than all other treatments, except the lower VitabooSt amendment OGB ($p=0.036$).

Copper (Cu) – Mean leaf Cu tissue concentrations were significantly affected by the fertiliser type ($p=0.033$) for CG comparison only, and were significantly affected by the main effects of growth medium ($p<0.001$) for CG and CR when treatments were compared with the controls (Table 3.10). The leaf Cu concentrations were significantly higher for fertiliser groups containing the randles media mix than those containing the bark-based medium. There were no statistically significant differences overall ($p=0.095$), although ORC had a significantly higher Cu concentration than several treatments.

Manganese (Mn) – Mean leaf tissue Mn concentrations were significantly affected by the main effects fertiliser type and growth medium ($p<0.001$ and $p=0.002$, respectively for CR comparison, and $p<0.001$ and $p=0.002$, respectively for CG comparison) when treatments were compared with the controls (Table 3.10). Leaf tissue Mn concentrations were significantly lower for fertiliser groups containing the randles mix than those containing the bark-based mix. Leaf tissue concentrations of OGC plants had significantly higher Mn concentrations than most other fertiliser treatments ($p=0.018$).

Zinc (Zn) - Leaf tissue Zn concentrations were similar for most treatments, including the controls. Concentrations were significantly affected by fertiliser type and growth medium ($p<0.001$ for both factors) when treatments were compared to the both controls (Table 3.10).

The Zn concentrations in leaf tissue of plants grown in randles as the basic medium were in general lower than those grown in the bark-based medium.

Table 3.10 Effects of two organic (Nitrosol[®] and Organic Vitaboost) and two inorganic fertiliser treatment types (Polyfeed[®] and Lawn and Leaf) applied at three concentrations to two different growth media, on mean leaf tissue Fe, Mn, Zn and Cu concentrations compared to two controls after a period of 90 DAP

Type	Treatment	Fe mg·kg ⁻¹	Cu mg·kg ⁻¹	Mn mg·kg ⁻¹	Zn mg·kg ⁻¹
Control	CR ⁶	375.00 ± 60.89ab ¹	8.75 ± 1.28bcd	123.20 ± 31.99abc	56.81 ± 2.58abc
Control	CG	287.30 ± 29.77a	6.26 ± 0.40ab	84.30 ± 21.90ab	55.32 ± 8.85abc
4.1.3	NRA	308.80 ± 32.09a	8.30 ± 0.60a-d	77.00 ± 127.03a	47.18 ± 5.38abc
4.1.3	NRB	320.00 ± 29.95ab	9.43 ± 1.02cd	69.40 ± 16.99a	47.49 ± 8.56abc
4.1.3	NRC	327.90 ± 54.30ab	8.39 ± 0.84a-d	64.50 ± 64.50a	41.23 ± 7.12abc
4.1.3	NGA	275.60 ± 75.71a	6.95 ± 1.08abc	115.70 ± 43.23abc	57.10 ± 6.90abc
4.1.3	NGB	325.00 ± 84.52ab	6.50 ± 0.41abc	163.20 ± 58.99a-d	54.39 ± 7.02abc
4.1.3	NGC	343.20 ± 83.03ab	6.66 ± 0.52abc	209.70 ± 81.41a-d	57.41 ± 3.45abc
6.1.3	PRA	399.90 ± 104.56ab	8.08 ± 0.63a-d	110.00 ± 7.10abc	48.93 ± 2.91abc
6.1.3	PRB	339.40 ± 75.26ab	8.47 ± 1.03bcd	80.90 ± 4.89ab	44.75 ± 4.88abc
6.1.3	PRC	296.00 ± 54.07ab	8.63 ± 1.27bcd	72.10 ± 3.94a	49.23 ± 5.22abc
6.1.3	PGA	348.10 ± 94.10ab	7.47 ± 1.17a-d	137.40 ± 46.10abc	56.64 ± 1.43abc
6.1.3	PGB	374.00 ± 84.63ab	7.26 ± 0.31a-d	144.60 ± 49.54abc	56.51 ± 46.963abc
6.1.3	PGC	296.00 ± 54.07a	5.48 ± 0.55a	138.80 ± 50.37abc	45.48 ± 1.15abc
7.1.3	LRA	301.30 ± 47.47a	7.21 ± 0.53a	112.10 ± 17.38abc	42.83 ± 2.24abc
7.1.3	LRB	405.00 ± 111.67ab	8.39 ± 1.25a-d	75.10 ± 2.99a	39.79 ± 5.18a
7.1.3	LRC	371.50 ± 18.67ab	8.22 ± 0.81a-d	69.00 ± 3.13a	40.45 ± 4.63ab
7.1.3	LGA	318.70 ± 56.14ab	7.16 ± 0.66abc	143.40 ± 53.12abc	54.65 ± 4.42abc
7.1.3	LGB	417.90 ± 99.07ab	7.80 ± 0.49a-d	189.70 ± 75.00a-d	68.43 ± 3.98b-e
7.1.3	LGC	369.60 ± 58.72ab	6.88 ± 0.25abc	205.10 ± 80.74a-d	58.78 ± 6.11abc
6.1.3	ORA	341.00 ± 70.75ab	9.33 ± 2.16cd	244.60 ± 107.45a-d	68.16 ± 23.17b-e
6.1.3	ORB	364.60 ± 68.55ab	9.14 ± 1.66bcd	184.40 ± 19.22a-d	60.70 ± 16.97abc
6.1.3	ORC	391.60 ± 85.86ab	9.94 ± 1.21d	260.40 ± 98.58bcd	63.44 ± 5.22a-d
6.1.3	OGA	410.40 ± 122.68ab	8.19 ± 0.67a-d	221.30 ± 79.21a-d	69.07 ± 10.64cde
6.1.3	OGB	464.80 ± 152.95bc	8.05 ± 0.92a-d	286.60 ± 109.98cd	91.29 ± 25.34e
6.1.3	OGC	549.60 ± 144.16c	7.88 ± 1.81a-d	327.40 ± 132.42d	87.17 ± 13.89de
Sig p<0.05		0.036*	0.095 (ns)	0.018	0.003
LSD ²		124.80	2.42	148.60	23.15
CV (%) ³		21.10	18.80	60.30	25.10
Fertiliser (F) ⁴		0.004	ns	<0.001	<0.001
CR	Medium (G)	ns	<0.001	0.002	<0.001
vs	Levels (C)	ns	ns	ns	ns
Treatments	F x G	ns	ns	ns	ns
	F x C	ns	ns	ns	ns
	G x C	ns	ns	ns	ns
Sig. ⁵		ns	ns	Q*	Q*
Fertiliser (F) ⁴		0.002	0.033	<0.001	<0.001
CG	Medium (G)	ns	<0.001	0.002	<0.001
vs	Levels (C)	ns	ns	ns	ns
Treatments	F x G	ns	ns	ns	ns
	F x C	ns	ns	ns	ns
	G x C	ns	ns	ns	ns
Sig. ⁵		ns	Q*	Q**	Q*

1. Means (±SE) within columns followed by the same letter do not differ significantly according to Duncan's Multiple Range Test at p < 0.05. Non-significant (ns) or significant at p < 0.05 (*), 0.01 (**) or 0.001 (***)

2. LSD –Least significant difference at p < 0.05

3. CV (%) – Percentage coefficient of variance

4. Main effects and interactions at both control levels. Non-significant (ns) or significant at P < 0.05 (*), 0.01 (**) or 0.001 (***)

5. Significant linear (L) or quadratic contrast at p < 0.05 (*), 0.01 (**) or 0.001 (***) across fertiliser types and growth media

6. CR – Control in Randles growth medium, CG – Control in Gromor growth medium, N – Nitrosol fertiliser, P – Polyfeed fertiliser, L – Lawn and Leaf fertiliser, O – Organic Vita boost fertiliser, R – Randles growth medium, G – Gromor growth medium, A – ½ label rate, B – label rate and C – 2 x label rate, Table adapted from (Bi *et al.*, 2010)

3.2.10 Leaf chlorophyll *a*, chlorophyll *b* and total chlorophyll concentrations

Chlorophyll *a* (Chl *a*) – Mean leaf Chl *a* concentrations were statistically similar between treatments ($p=0.452$) (Table 3.11). Leaf Chl *a* concentrations were significantly affected by fertiliser type for the CG comparison only ($p=0.024$). Concentrations ranged from 39.54 to 80.26 $\mu\text{g}\cdot\mu\text{g}\cdot\text{Chl } a\text{ g}^{-1}$ (OGB).

Chlorophyll *b* (Chl *b*) – Mean leaf Chl *b* concentrations were significantly affected by fertiliser type ($p=0.001$, $p=0.004$ for CR and CG comparisons to treatments, respectively). The high Organic Vitaboost treatment (ORC) resulted in significantly higher leaf tissue Chl *b* concentrations than CR, CG and the lower Nitrosol (NRA, NRB) and lower Polyfeed (PRA) treatments. There were no significant differences overall between treatments ($p=0.064$) (Table 3.11). Concentrations ranged from 24.52 $\mu\text{g}\cdot\text{g}^{-1}$ (PRA) to 48.11 $\mu\text{g}\cdot\text{g}^{-1}$ (ORC).

Total chlorophyll (TChl) – Leaf total chlorophyll concentrations (TChl) were significantly affected by the fertiliser type compared with the gromor control only ($p=0.013$). There were no significant differences overall between treatments ($p=0.262$) (Table 3.11). Concentrations ranged from 71.00 $\mu\text{g}\cdot\text{g}^{-1}$ to 119.90 $\mu\text{g}\cdot\text{g}^{-1}$ (OGB).

Table 3.11 Effects of two organic (Nitrosol[®] and Organic Vitaboost) and two inorganic fertiliser treatment types (Polyfeed[®] and Lawn and leaf) applied at three concentrations to two different growth media, on mean leaf tissue Chl *a*, Chl *b* and Tchl concentrations compared to two controls after a period of 90 DAP

Type	Treatment	Chl <i>a</i> µg g ⁻¹	Chl <i>b</i> µg g ⁻¹	Total Chl µg g ⁻¹
Control	CR ⁶	51.56 ± 2.15ab ¹	30.48 ± 3.53abc	82.00 ± 2.92ab
Control	CG	39.54 ± 4.44a	31.41 ± 5.29a-d	71.00 ± 9.65a
4.1.3	NRA	62.68 ± 5.80ab	29.28 ± 5.96ab	92.00 ± 4.74abc
4.1.3	NRB	60.14 ± 1.79ab	31.39 ± 7.13a-d	91.50 ± 8.92abc
4.1.3	NRC	78.95 ± 10.80b	37.45 ± 4.11a-e	116.40 ± 10.31bc
4.1.3	NGA	64.79 ± 18.06ab	33.06 ± 5.70a-e	97.80 ± 20.86abc
4.1.3	NGB	79.22 ± 12.53b	46.56 ± 8.75de	125.80 ± 16.76c
4.1.3	NGC	70.28 ± 10.14b	35.23 ± 8.87a-e	105.50 ± 16.22abc
6.1.3	PRA	70.83 ± 10.31b	24.52 ± 10.10a	95.30 ± 5.65abc
6.1.3	PRB	70.97 ± 10.21b	35.26 ± 7.88a-e	106.20 ± 16.01abc
6.1.3	PRC	68.95 ± 10.34ab	37.19 ± 4.01a-e	106.10 ± 12.55abc
6.1.3	PGA	59.91 ± 10.91ab	39.75 ± 7.82a-e	99.70 ± 18.47abc
6.1.3	PGB	71.95 ± 9.09b	38.66 ± 7.42a-e	110.60 ± 14.26abc
6.1.3	PGC	65.32 ± 2.65ab	35.57 ± 8.12a-e	100.90 ± 8.06abc
7.1.3	LRA	64.88 ± 4.52ab	33.85 ± 5.52a-e	98.70 ± 3.58abc
7.1.3	LRB	68.28 ± 4.41ab	36.05 ± 5.79a-e	104.30 ± 4.13abc
7.1.3	LRC	72.87 ± 2.81b	37.77 ± 8.37a-e	110.60 ± 10.24abc
7.1.3	LGA	75.46 ± 7.77b	38.23 ± 9.16a-e	113.70 ± 16.13bc
7.1.3	LGB	72.18 ± 7.12b	37.76 ± 10.05a-e	109.90 ± 16.51abc
7.1.3	LGC	69.84 ± 8.86b	38.30 ± 11.17a-e	108.10 ± 19.47abc
6.1.3	ORA	68.85 ± 9.00ab	44.38 ± 6.92b-e	113.00 ± 15.84bc
6.1.3	ORB	74.57 ± 9.36b	45.91 ± 6.83cde	120.50 ± 16.06bc
6.1.3	ORC	76.83 ± 10.97b	48.11 ± 6.60e	124.90 ± 17.06c
6.1.3	OGA	74.62 ± 12.11b	45.31 ± 9.33cde	119.90 ± 21.35bc
6.1.3	OGB	80.26 ± 8.00b	42.74 ± 10.27b-e	123.00 ± 18.28bc
6.1.3	OGC	62.83 ± 6.13ab	37.72 ± 7.77a-e	100.50 ± 13.86abc
Sig p<0.05		0.452 (ns)	0.064 (ns)	0.262 (ns)
LSD ²		24.97	12.61	33.44
CV (%) ³		22.30	20.60	19.30
Treatments				
	Fertiliser (F) ⁴	ns	0.001	ns
CR	Medium (G)	ns	ns	ns
vs	Levels (C)	ns	ns	ns
	F x G	ns	ns	ns
	F x C	ns	ns	ns
	G x C	ns	ns	ns
Sig. ⁵		ns	Q*	Q*
Treatments				
	Fertiliser (F) ⁴	0.024	0.004	0.013
CG	Medium (G)	ns	ns	ns
vs	Levels (C)	ns	ns	ns
	F x G	ns	ns	ns
	F x C	ns	ns	ns
	G x C	ns	ns	ns
Sig. ⁵		Q*	Q*	Q**

1. Means (±SE) within columns followed by the same letter do not differ significantly according to Duncan's Multiple Range Test at p < 0.05. Non-significant (ns) or significant at p < 0.05 (*), 0.01 (**), or 0.001 (***)

2. LSD –Least significant difference at p < 0.05

3. CV (%) – Percentage coefficient of variance

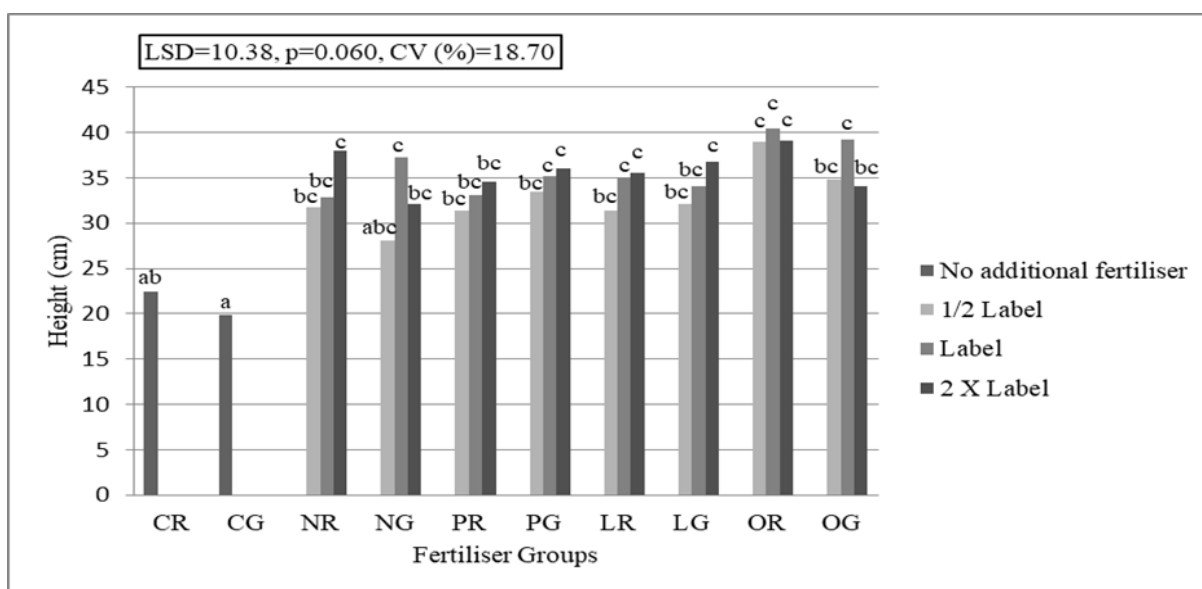
4. Main effects and interactions at both control levels. Non-significant (ns) or significant at P < 0.05 (*), 0.01 (**), or 0.001 (***)

5. Significant linear (L) or quadratic contrast at p < 0.05 (*), 0.01 (**), or 0.001 (***) across fertiliser types and growth media

6. CR – Control in Randles growth medium, CG – Control in Gromor growth medium, N – Nitrosol fertiliser, P – Polyfeed fertiliser, L – Lawn and Leaf fertiliser, O – Organic Vita boost fertiliser, R – Randles growth medium, G – Gromor growth medium, A – ½ label rate, B – label rate and C – 2 x label rate, Table adapted from (Bi *et al.*, 2010)

3.2.11 Plant growth parameters

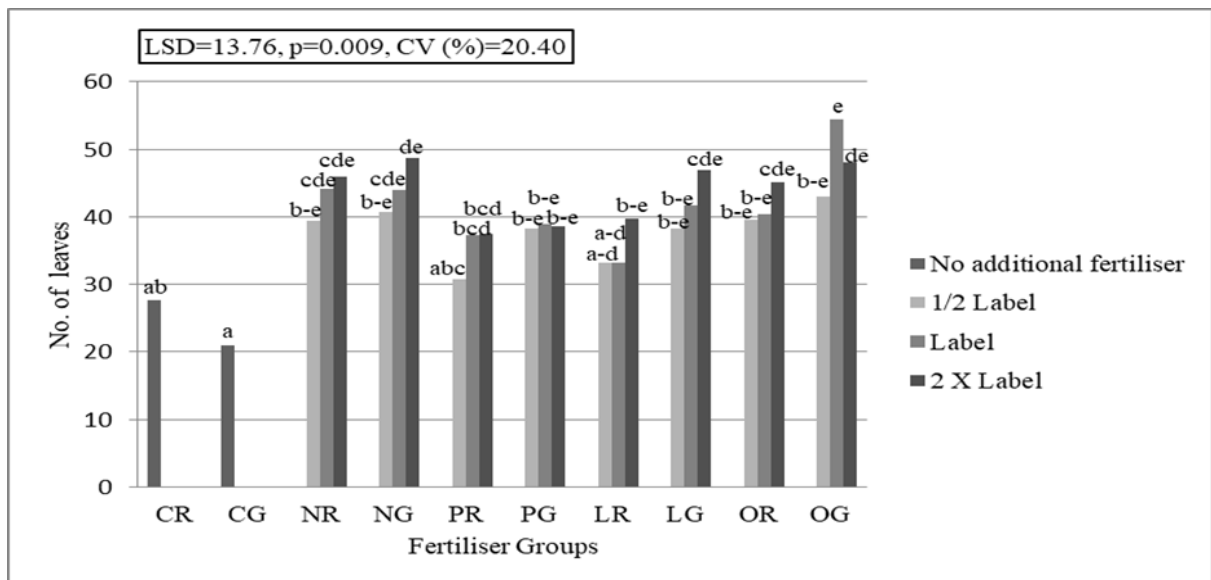
Height – Plant height was significantly affected by fertiliser type ($p < 0.001$) for both media, when treatments were compared with both controls (Table 3.12). Height increased linearly or quadratically with increasing fertiliser rate. Several treatments (NRC, NGB, PGB, PGC, LRB, LRC, LGC, ORA, ORB, ORC and OGB) produced significantly taller plants than both controls. (Fig.3.1). Plant height ranged from 19.84 cm to 40.39 cm.



1. Means followed by the same letter do not differ significantly according to Duncans Multiple Range Test at $p < 0.05$.
2. LSD – Least significant difference
3. CV (%) – Percentage coefficient of variance
4. CR – Control in Randles growth medium, CG – Control in Gromor growth medium, N – Nitrosol fertiliser, P – Polyfeed fertiliser, L – Lawn and Leaf fertiliser, O – Organic Vita boost fertiliser, R – Randles growth medium, G – Gromor growth medium

Figure 3.1: Effects of two organic (Nitrosol[®] and Organic Vitaboost) and two inorganic fertiliser treatment types (Polyfeed[®] and Lawn and Leaf) applied at three concentrations, on mean height of *Pseuderanthemum atropurpureum*, grown in two growth media and compared with the two controls after a period of 90 days (DAP)

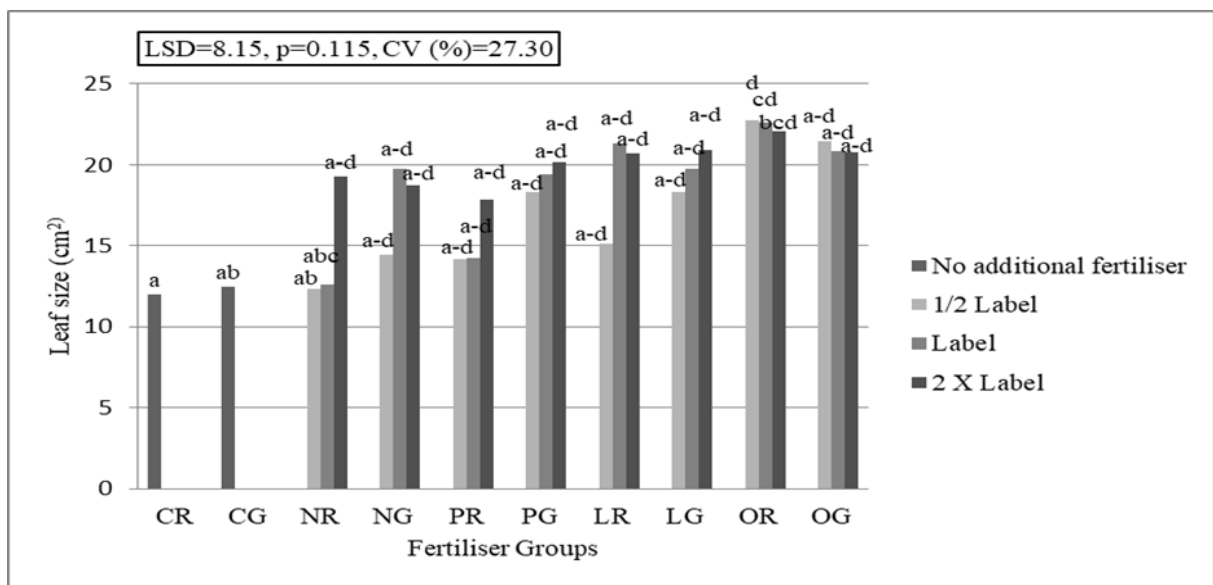
Number of leaves – Leaf number was significantly affected by fertiliser type ($p < 0.001$) and growth medium ($p = 0.014$ and $p = 0.022$, for CR and CG, respectively; when treatments were compared with both controls Table 3.12). Organic Vitaboost (OGB) applied to the gromor media produced significantly more leaves than Polyfeed at all treatment levels in the randles growth medium (PRA, PRB and PRC) and significantly more leaves than Lawn and Leaf in randles growth medium at the lower treatment levels (LRA and LRB). Mean plant leaf number ranged from 21 in the gromor control to 54 in gromor amended with the highest Organic Vitaboost rate (OGC). The mean number of leaves increased with increasing fertiliser rate for most treatments (Fig. 3.2).



1. Means followed by the same letter do not differ significantly according to Duncan's Multiple Range Test at $p < 0.05$.
2. LSD – Least significant difference
3. CV (%) – Percentage coefficient of variance
4. CR – Control in Randles growth medium, CG – Control in Gromor growth medium, N – Nitrosol fertiliser, P – Polyfeed fertiliser, L – Lawn and Leaf fertiliser, O – Organic Vita boost fertiliser, R – Randles growth medium, G – Gromor growth medium

Figure 3.2: Effects of two organic (Nitrosol[®] and Organic Vitaboost) and two inorganic fertiliser treatment types (Polyfeed[®] and Lawn and Leaf) applied at three concentrations, on mean leaf number of *Pseuderanthemum atropurpureum*, grown in two growth media and compared with the two controls after a period of 90 days (DAP)

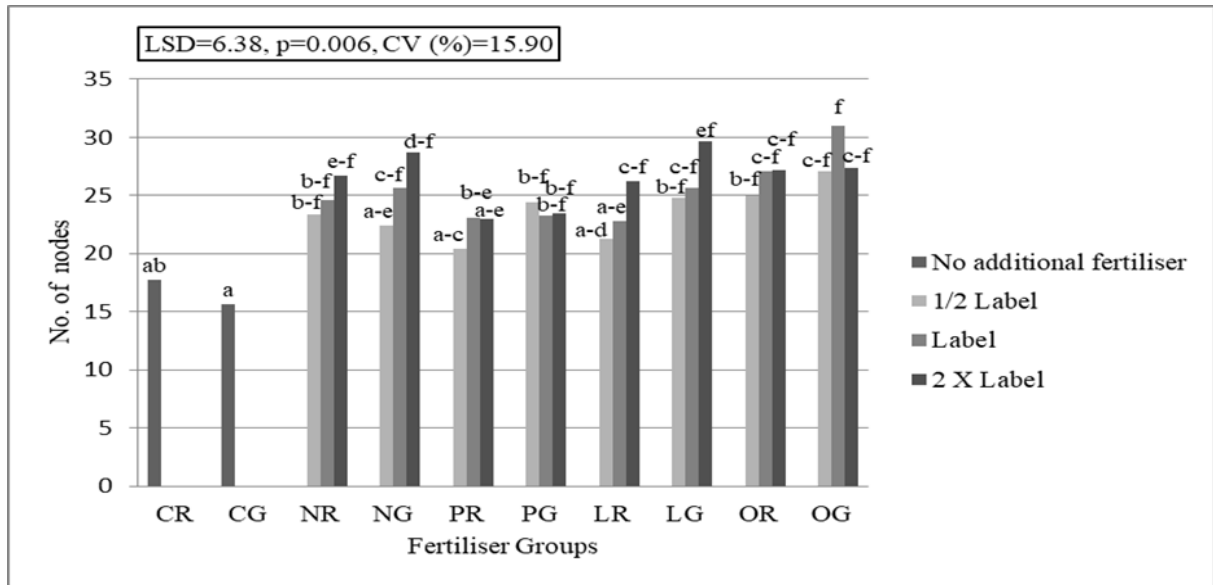
Leaf size - Leaf size was significantly affected by the fertiliser type ($p=0.003$ and $p=0.004$, for CR and CG comparisons, respectively (Table 3.12). Statistical analysis showed no significant effect of treatments on leaf size overall ($p=0.115$). Low organic Vitaboost amendments (ORA) produced significantly larger leaves on plants grown in randles growth medium than when lower Nitrosol levels (NRA and NRB) were used in the same medium (Fig. 3.3). Mean leaf area ranged from a low 11.99 cm^2 for the randles control (CR) to 22.77 cm^2 in the randles Organic Vitaboost low treatment (ORA).



1. Means followed by the same letter do not differ significantly according to Duncans Multiple Range Test at $p < 0.05$.
2. LSD – Least significant difference
3. CV (%) – Percentage coefficient of variance
4. CR – Control in Randles growth medium, CG – Control in Gromor growth medium, N – Nitrosol fertiliser, P – Polyfeed fertiliser, L – Lawn and Leaf fertiliser, O – Organic Vita boost fertiliser, R – Randles growth medium, G – Gromor growth medium

Figure 3.3: Effects of two organic (Nitrosol[®] and Organic Vitaboost) and two inorganic fertiliser treatment types (Polyfeed[®] and Lawn and Leaf) applied at three concentrations, on mean leaf size of *Pseuderanthemum atropurpureum*, grown in two growth media and compared with the two controls after a period of 90 days (DAP)

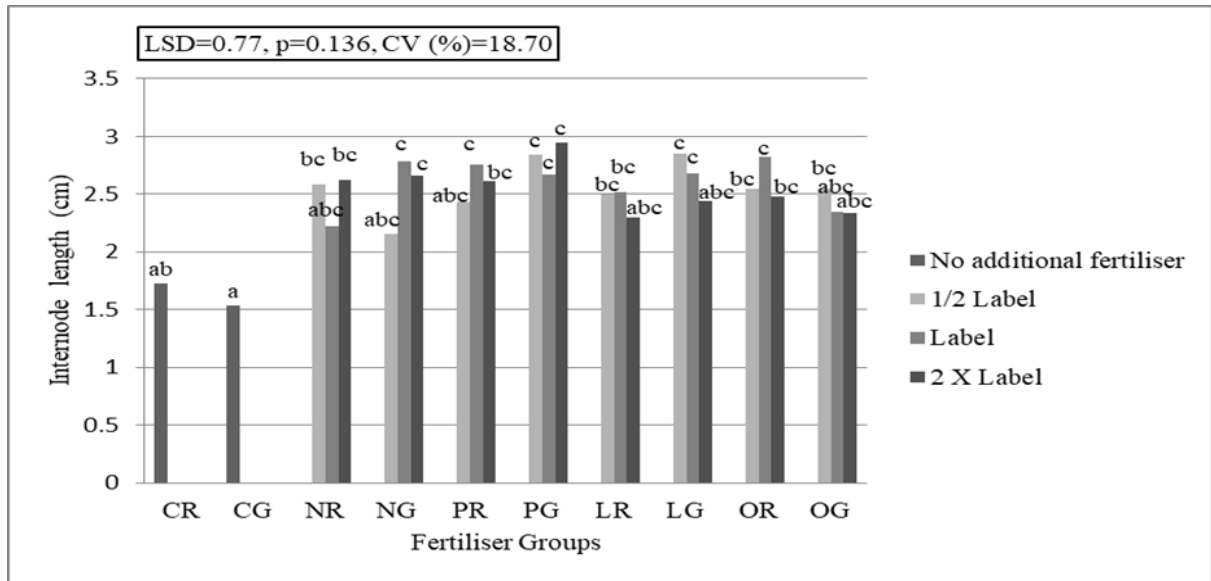
Number of nodes - Mean plant node number was significantly affected by the fertiliser type ($p < 0.001$) for both media as well as by the fertiliser level ($p = 0.017$ and $p = 0.042$ for CR and CG comparisons, respectively; Table 3.12). Overall statistical analysis showed significant differences ($p = 0.006$). The plant node number ranged from 15.69 to 31.00 (OGB). In most treatments, the number of nodes increased with increasing fertiliser rate (Fig. 3.4).



1. Means followed by the same letter do not differ significantly according to Duncan's Multiple Range Test at $p < 0.05$.
2. LSD - Least significant difference
3. CV (%) - Percentage coefficient of variance
4. CR - Control in Randles growth medium, CG - Control in Gromor growth medium, N - Nitrosol fertiliser, P - Polyfeed fertiliser, L - Lawn and Leaf fertiliser, O - Organic Vita boost fertiliser, R - Randles growth medium, G - Gromor growth medium

Figure 3.4: Effects of two organic (Nitrosol[®] and Organic Vitaboost) and two inorganic fertiliser treatment types (Polyfeed[®] and Lawn and Leaf) applied at three concentrations, on mean node number of *Pseuderanthemum atropurpureum*, grown in two growth media and compared with the two controls after a period of 90 days (DAP)

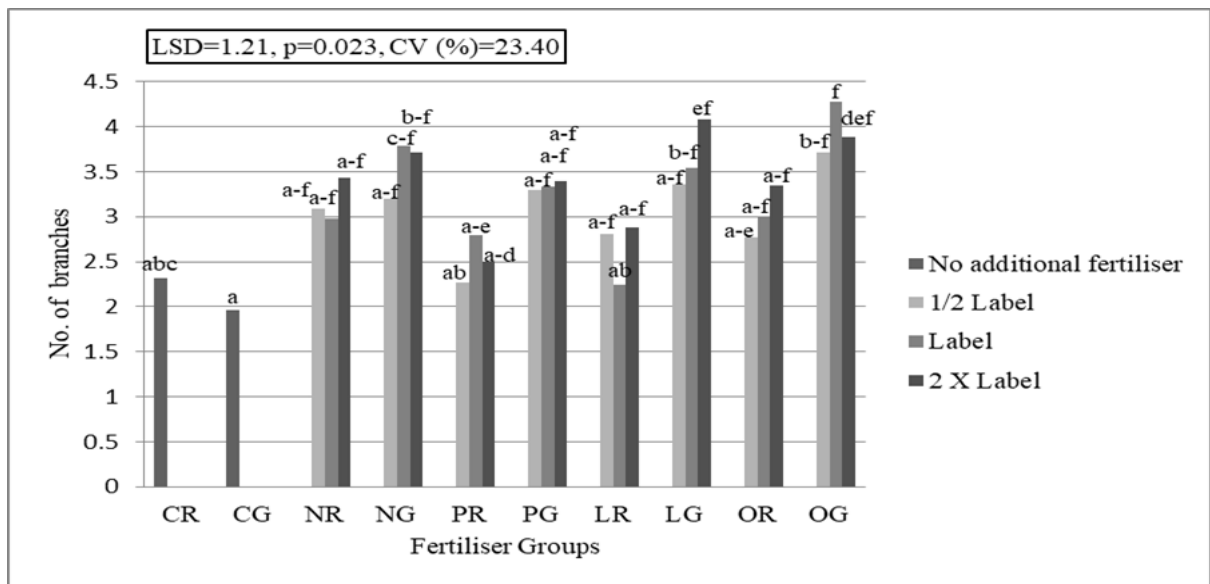
Internode length - Mean internode length was significantly affected by the main effect of fertiliser type ($p=0.011$ and $p=0.005$) for CR and CG comparisons, respectively (Table 3.12). Statistical analysis showed no significant differences in internode length overall ($p=0.136$), although there were some treatments that were significantly different from the controls (Fig. 3.5). Mean internode length ranged from 1.54 cm (CR) to 2.95 cm (PGC).



1. Means followed by the same letter do not differ significantly according to Duncans Multiple Range Test at $p < 0.05$.
2. LSD – Least significant difference
3. CV (%) – Percentage coefficient of variance
4. CR – Control in Randles growth medium, CG – Control in Gromor growth medium, N – Nitrosol fertiliser, P – Polyfeed fertiliser, L – Lawn and Leaf fertiliser, O – Organic Vita boost fertiliser, R – Randles growth medium, G – Gromor growth medium

Figure 3.5: Effects of two organic (Nitrosol[®] and Organic Vitaboost) and two inorganic fertiliser treatment types (Polyfeed[®] and Lawn and Leaf) applied at three concentrations, on mean internode length of *Pseuderanthemum atropurpureum*, grown in two growth media and compared with the two controls after a period of 90 days (DAP)

Number of branches - Branch number per plant was significantly affected by fertiliser type ($p=0.033$ and $p=0.009$) for comparisons to CR and CG, respectively and growth medium at ($p<0.001$) for both comparisons (Table 3.12). The overall statistical analysis showed significant differences at $p=0.023$. The number of branches of plants in grown in the randles-containing media mixes was significantly lower in bark-based mixes (Fig. 3.6). The mean plant branch number ranged from 1.96 to 4.28 (OGB).



1. Means followed by the same letter do not differ significantly according to Duncans Multiple Range Test at $p < 0.05$.
2. LSD – Least significant difference
3. CV (%) – Percentage coefficient of variance
4. CR – Control in Randles growth medium, CG – Control in Gromor growth medium, N – Nitrosol fertiliser, P – Polyfeed fertiliser, L – Lawn and Leaf fertiliser, O – Organic Vitaboost fertiliser, R – Randles growth medium, G – Gromor growth medium

Figure 3.6: Effects of two organic (Nitrosol[®] and Organic Vitaboost) and two inorganic fertiliser treatment types (Polyfeed[®] and Lawn and Leaf) applied at three concentrations, on mean branch number of *Pseuderanthemum atropurpureum*, grown in two growth media and compared with the two controls 90 days after planting (DAP)

Table 3.12 Effects of two organic (Nitrosol[®] and Organic Vitaboost) and two inorganic fertiliser treatment types (Polyfeed[®] and Lawn and Leaf) applied at three concentrations to two different growth media, on *Pseuderanthemum atropurpureum* growth parameters compared to two controls at 90 DAP

Type	Treatment	Height (cm)	No of Leaves	Leaf Size (cm ²)	No of nodes	Internode Length (cm)	No of Branches
Control	CR ⁶	22.46 ± 6.91ab ¹	27.67 ± 8.25ab	11.99 ± 3.45a	17.77 ± 2.46ab	1.73 ± 0.45ab	2.32 ± 1.00abc
Control	CG	19.84 ± 9.22a	21 ± 11.53a	12.47 ± 4.46ab	15.69 ± 4.99a	1.54 ± 0.44a	1.96 ± 0.58a
4.1.3	NRA	31.73 ± 9.51bc	39.40 ± 8.03b-e	12.33 ± 1.88ab	23.37 ± 2.90b-f	2.58 ± 0.89bc	3.09 ± 0.71a-f
4.1.3	NRB	32.84 ± 9.45bc	44.14 ± 11.57cde	12.64 ± 2.19abc	24.64 ± 5.08b-f	2.22 ± 0.73abc	2.98 ± 1.02a-f
4.1.3	NRC	37.95 ± 11.40c	45.99 ± 11.97cde	19.28 ± 5.52a-d	26.69 ± 6.42e-f	2.62 ± 0.91bc	3.43 ± 0.47a-f
4.1.3	NGA	28.02 ± 7.96abc	40.78 ± 8.24b-e	14.46 ± 4.36a-d	22.43 ± 3.93a-e	2.16 ± 0.53abc	3.20 ± 0.33a-f
4.1.3	NGB	37.28 ± 11.22c	44.00 ± 3.06cde	19.72 ± 7.00a-d	25.62 ± 2.84c-f	2.78 ± 0.97c	3.79 ± 0.91c-f
4.1.3	NGC	32.1 ± 7.06bc	48.68 ± 2.87de	18.74 ± 6.53a-d	28.69 ± 3.98d-f	2.66 ± 0.70c	3.72 ± 0.68b-f
6.1.3	PRA	31.42 ± 8.54bc	30.67 ± 5.81abc	14.19 ± 2.86a-d	20.43 ± 4.51a-c	2.43 ± 0.86abc	2.27 ± 0.68ab
6.1.3	PRB	33.05 ± 10.74bc	37.32 ± 7.94bcd	14.23 ± 3.38a-d	23.09 ± 4.40b-e	2.76 ± 0.95c	2.79 ± 0.61a-e
6.1.3	PRC	34.58 ± 13.03bc	37.43 ± 12.51bcd	17.83 ± 5.46a-d	22.95 ± 5.65a-e	2.61 ± 0.68bc	2.50 ± 0.92a-d
6.1.3	PGA	33.45 ± 8.31bc	38.33 ± 3.53b-e	18.30 ± 5.64a-d	24.38 ± 1.94b-f	2.84 ± 0.89c	3.30 ± 0.53a-f
6.1.3	PGB	35.15 ± 10.15c	38.83 ± 2.07b-e	19.43 ± 6.56a-d	23.25 ± 2.96b-f	2.67 ± 0.89c	3.34 ± 0.13a-f
6.1.3	PGC	36.03 ± 11.88c	38.60 ± 4.07b-e	20.13 ± 7.08a-d	23.42 ± 3.44b-f	2.95 ± 0.99c	3.39 ± 0.45a-f
7.1.3	LRA	31.42 ± 10.72bc	33.16 ± 6.91a-d	15.14 ± 4.84a-d	21.25 ± 3.76a-d	2.50 ± 1.08bc	2.81 ± 0.61a-f
7.1.3	LRB	35.12 ± 12.39c	33.33 ± 7.75a-d	21.32 ± 8.02a-d	22.81 ± 4.99a-e	2.52 ± 0.93bc	2.25 ± 0.45ab
7.1.3	LRC	35.61 ± 12.04c	39.67 ± 8.88b-e	20.73 ± 7.44a-d	26.21 ± 6.02c-f	2.30 ± 0.75abc	2.88 ± 0.65a-f
7.1.3	LGA	32.16 ± 8.58c	38.18 ± 3.16b-e	18.34 ± 6.61a-d	24.75 ± 2.46b-f	2.85 ± 1.08c	3.36 ± 0.26a-f
7.1.3	LGB	34.12 ± 11.97bc	41.67 ± 2.19b-e	19.78 ± 7.57a-d	25.62 ± 2.56c-f	2.68 ± 0.82c	3.54 ± 2.29b-f
7.1.3	LGC	36.82 ± 12.11c	46.89 ± 5.28cde	20.94 ± 7.87a-d	29.62 ± 5.04ef	2.44 ± 0.84abc	4.08 ± 0.58ef
6.1.3	ORA	38.97 ± 13.27c	39.49 ± 9.42b-e	22.77 ± 8.15d	25.01 ± 4.19b-f	2.55 ± 0.87bc	2.77 ± 0.62a-e
6.1.3	ORB	40.39 ± 15.50c	40.42 ± 12.21b-e	22.60 ± 8.36cd	27.07 ± 7.80c-f	2.82 ± 1.01c	3.01 ± 0.91a-f
6.1.3	ORC	39.15 ± 14.71c	45.06 ± 12.49cde	22.06 ± 8.10bcd	27.21 ± 6.47c-f	2.48 ± 0.84bc	3.35 ± 0.71a-f
6.1.3	OGA	34.76 ± 12.23bc	42.96 ± 4.28b-e	21.43 ± 8.22a-d	27.04 ± 2.96c-f	2.55 ± 0.81bc	3.71 ± 0.38b-f
6.1.3	OGB	39.21 ± 12.31c	54.38 ± 5.33e	20.82 ± 7.49a-d	31.00 ± 5.92f	2.35 ± 0.65abc	4.28 ± 0.15f
6.1.3	OGC	34.09 ± 12.10bc	48.01 ± 9.31de	20.77 ± 7.73a-d	27.39 ± 3.81c-f	2.34 ± 0.79abc	3.89 ± 0.11def
Sig p<0.05		0.060 (ns)	0.009**	0.115 (ns)	0.006**	0.136 (ns)	0.023*
LSD ²		10.38	13.76	8.15	6.38	0.77	1.21
CV (%) ³		18.70	20.40	27.30	15.90	18.70	23.40
Fertiliser (F) ⁴		< 0.001***	<0.001***	0.003**	<0.001***	0.011*	0.033*
Treatments	CR vs CG	ns	0.014*	ns	ns	ns	<0.001***
	Medium (G) vs Levels (C)	ns	ns	ns	0.017*	ns	ns
	F x G	ns	ns	ns	ns	ns	ns
	F x C	ns	ns	ns	ns	ns	ns
	G x C	ns	ns	ns	ns	ns	ns
Sig. ⁵		L*Q**	Q***	ns	Q***	L*	Q**
Fertiliser (F) ⁴		<0.001	<0.001***	0.004**	<0.001***	0.005**	0.009**
Treatments	CG vs LRA	ns	0.022*	ns	ns	ns	<0.001***
	Medium (G) vs Levels (C)	ns	ns	ns	0.042*	ns	ns
	F x G	ns	ns	ns	ns	ns	ns
	F x C	ns	ns	ns	ns	ns	ns
	G x C	ns	ns	ns	ns	ns	ns
Sig. ⁵		L*Q**	Q***	ns	Q***	L*	Q***

1. Means (±SE) within columns followed by the same letter do not differ significantly according to Duncan's Multiple Range Test at p < 0.05. Non-significant (ns) or significant at p < 0.05 (*), 0.01 (**), or 0.001 (***)

2. LSD –Least significant difference at p < 0.05

3. CV (%) – Percentage coefficient of variance

4. Main effects and interactions at both control levels. Non-significant (ns) or significant at P < 0.05 (*), 0.01 (**), or 0.001 (***)

5. Significant linear (L) or quadratic contrast at p < 0.05 (*), 0.01 (**), or 0.001 (***) across fertiliser types and growth media

6. CR – Control in Randles growth medium, CG – Control in Gromor growth medium, N – Nitrosol fertiliser, P – Polyfeed fertiliser, L – Lawn and Leaf fertiliser, O – Organic Vita boost fertiliser, R – Randles growth medium, G – Gromor growth medium, A – ½ label rate, B – label rate and C – 2 x label rate, Table adapted from (Bi *et al.*, 2010)

3.3 Discussion

3.3.1 Analyses of irrigation water and growth media

Irrigation water can be placed into various categories according to salinity and sodicity levels (Richard, 1954). The salinity level (EC) of irrigation water ($24.32 \text{ mS}\cdot\text{cm}^{-1}$) used in this study fell into the lowest category (C1 < $250 \text{ mS}\cdot\text{cm}^{-1}$). The other categories (C2, $250 - 750 \text{ mS}\cdot\text{cm}^{-1}$; C3, $750-2250 \text{ mS}\cdot\text{cm}^{-1}$ and C4, $2250 - 5000 \text{ mS}\cdot\text{cm}^{-1}$) are of decreasing fitness for use as salinity increases. The sodicity level (SAR) of irrigation water used in this study (1.21) also fell within the 'low' category (S1, 1-10). A concern of high sodicity levels is, that as concentrations of sodium in relation to calcium and magnesium in irrigation water increases, soil permeability decreases (Menezes *et al.*, 2014). The other categories (S2, 10-18; S3, 18-26 and S4 > 26) are therefore of decreasing suitability for use as irrigation water (Richard, 1954). The water used was, thus, very acceptable from a salinity as well as a sodicity perspective (Table 3.3); pH was, however, higher than the acceptable range of 5.4 to 6.8 for container production (Bailey *et al.*, 2005). No remedial action was taken as plants grown at Randles nursery were of good quality. Sodium (Na), calcium (Ca), magnesium (Mg), potassium (K), total alkalinity (TA) and chlorine (Cl) concentrations ($\text{me}\cdot\text{L}^{-1}$) of the irrigation water were all below the recommended upper limits (Bailey *et al.*, 1999) of 3, 6, 2, 0.26, 2 and $2 \text{ me}\cdot\text{L}^{-1}$, respectively.

Results of EC (CR – 2.58, CG – $3.05 \text{ mS}\cdot\text{cm}^{-1}$) and pH (CR – 6.28, CG – 5.84) (Table 3.4) were within recommended ranges of $0.75 - 3.49 \text{ mS}\cdot\text{cm}^{-1}$ and pH 5.2 – 6.3, respectively (Abad *et al.*, 2001), for growth media. The bark-based control had a higher EC and a lower pH than the soil-based growth medium control. Most mineral nutrients were present in higher concentration in the bark-based growth media (Table 3.4). The only exceptions were Fe and Cu, which were higher in the soil-based growth medium. The Carbon to Nitrogen (C:N) ratio

was similar between the growth media, with no likelihood of immobilization (at a C:N ratio >30:1) with no demand for immediate N treatment to growth media (Tripepi, 2014).

Chemical properties, particularly N (0.43%), P (0.15%), Ca (1.03%) and Fe (25800 mg·kg⁻¹), of the commercial potting medium Metro-Mix 360 (Scotts, Marysville, Ohio) used by Atiyeh *et al.* (2001) were comparable to soil-based growth medium N, P, Ca, Fe concentrations in this study. The bark-based growth medium in this study had higher concentrations of N, P and Ca than soil-based growth media and Metro-Mix 360, except for Fe. In a later study, Atiyeh *et al.* (2002) reported lower Cu concentration and higher Mn and Zn concentrations in Metro-Mix 360 than either of the growth media used in this study. Metro-Mix 360 contains a starter nutrient fertiliser and is formulated from soilless components (Canadian sphagnum peat moss, bark ash and sand). Approximate concentrations of nutrients required for healthy plant growth are N (1.4%), P (0.2%), K (1%), Ca (0.5%), Mg (0.2%), Fe (100 mg·kg⁻¹), Mn (50 mg·kg⁻¹), Zn (20 mg·kg⁻¹) and 6 mg·kg⁻¹ for Cu (Jones Jr, 2012); Fe, Mn, Zn and Cu concentrations (Table 3.4) were significantly higher than those recommended by Jones Jr (2012) in both growth media used in this study.

Nutrients in both growth media used in this study were at acceptable levels when compared with commercial Metro-Mix 360 and those recommended by Jones Jr (2012), including Fe, Mn and Zn when compared with Metro-Mix 360, but not Cu in soil-based medium. Bunt (2012) suggested that Cu concentrations in potting mixes below 150 mg·kg⁻¹ were not likely to result in toxicity. The soil-based growth medium Cu concentration was marginally over this limit (157.69 mg·kg⁻¹), but was not a major concern given that the Cu concentration would decrease due to leaching into irrigation water, prior to first treatments.

3.3.2 Effects of fertiliser treatments on growth medium EC and pH

Organic treatments improved growth and development of *Pseuderanthemum atropurpureum* to a similar and sometimes greater extent as the other conventional fertilisers considered to produce saleable plants. Growth media EC values of solutions obtained by the pour-through method showed similar trends at 60 DAP and at termination of the study (90 DAP), indicating that the amounts supplied had been used for plant growth. Statistically, EC results showed higher values following Organic Vitaboost treatments for both growth media, with the highest treatment concentrations significantly different ($p < 0.001$), whilst the other treatments were similar at both sampling times. There were also significant interactions between the factors of fertiliser treatment and levels of treatment at termination (90 DAP). Electrical conductivity variations which occurred for Organic Vitaboost treatments in both growth media, were similar, but above the recommended range at the highest level of application.

Bi *et al.* (2010) reported on two poultry-based organic fertilisers and an inorganic controlled-release fertiliser for the production of marigolds in a growth medium that contained peatmoss, vermiculite and perlite (7.5:1:1.5 ratio by volume). The presented results (Table 3.5) indicate that incorporated organic treatments may lead to high EC and that high EC may adversely affect plant growth. The same authors reported that poultry litter EC levels were significantly higher than those from the inorganic treatment and considerably higher at the highest concentration used. Poultry litter-based Organic Vitaboost applied at the highest concentration in this study resulted in EC levels that were also significantly higher than the inorganic Lawn and Leaf and Polyfeed treatments as well as Nitrosol organic treatment at all levels of treatment. The EC of Lawn and Leaf treatment was similar to that of liquid feeds (Nitrosol and Polyfeed). Bi *et al.* (2010) found, as in this study with leachate, that EC levels of growth media decreased over time..

Ruter (1992), investigating the growth response of 'Burfordii' and 'Nellie R. Stevens' holly in pine bark and sand (4: 1 by volume) to multicoated controlled-release fertilisers, found a strong linear relationship between media $\text{NO}_3\text{-N}$ and EC values. . There were also similarities between $\text{NO}_3\text{-N}$ concentrations in both growth media and EC in this study. This was especially seen at the upper application rates of Organic Vitaboost, where nitrate seemed to affect EC and resulted in an interaction, evident when treatments were compared statistically to both controls (Table 3.5). Similar to Organic Vitaboost EC values, Organic Vitaboost treated growth media had $\text{NO}_3\text{-N}$ concentrations that were virtually higher than all other growth media $\text{NO}_3\text{-N}$ concentrations. This is also evident in leaf N concentrations, with Organic Vitaboost treatments generally increasing leaf N, even significantly so at upper treatment levels.

The pelleted organic (Organic Vitaboost) and granular (Lawn and Leaf) inorganic treatments caused a greater decrease in pH from 60 DAP to termination (90 DAP), compared with the liquid feeds. This is in line with findings by Fisher and Argo (2003) that soilless growth media offer less pH-buffering capacity than soil and soil-based growth media. Goh (1979) found that the presence of soil in peat-sand-soil growth media buffered drastic changes in pH. This was not as evident in pH results from fertiliser treatments at 60 DAP as at 90 DAP, comparing soil-based and bark-based (soilless) growth media used in this study. Changes were more gradual in the soil-based (90 DAP), more drastic in the same media at 60 DAP and in bark-based media 90 DAP (Table 3.5). The pH of a growth medium affects solubility and availability of plant nutrients and thereby impacts nutrient uptake (Fisher and Argo, 2003; Adriaanse, 2013). These drastic pH changes did, however, not affect leaf N concentrations, which increased linearly with increasing treatment levels (Table 3.9).

Organic Vitaboost applied to gromor at twice amended rate (OGC) resulted in a significantly higher $\text{NH}_4\text{-N}$ concentration at termination of this study. Leachate displayed the lowest pH at both, 60 DAP and 90 DAP. The lowered pH may have been due to $\text{NH}_4\text{-N}$ uptake (Argo and Biernbaum, 1997; Silber and Bar-Tal, 2008). The critical pH range for the inhibition of nitrification, whereby $\text{NH}_4\text{-N}$ is oxidised to $\text{NO}_3\text{-N}$, is 5.4 – 5.7 and may explain the significant difference between the pH for OGC at 5.67, while the other treatments were characterised by significantly higher pH.

At 60 DAP bark-based growth media displayed pH values significantly lower than those of soil-based growth media. The reasons why are not clear as analysis for growth media $\text{NO}_3\text{-N}$ and $\text{NH}_4\text{-N}$ did not take place at 60 DAP, but may be related to lower pH values typical of bark and peat moss compared to mineral soil (Wada, 2005) and this was the case in start pH unit values. Bark based growth media initial pH values were lower than soil based growth media. Results for pH at 60 DAP in this study, which had decreased in general, were comparable to results obtained before termination of the study by Bi *et al.* (2010) and Ostrom (2011) and pour-through extraction results from Wright *et al.* (1990), but not final pH values in this study which were quite variable at termination of the study.

The N in leachate solutions from growth media, as determined by pour-through extraction, was similar among treatments. The variability in pH between treatments may have been solely due to variations in $\text{NO}_3\text{-N}$ uptake, as the effects of growth media on $\text{NH}_4\text{-N}$ concentrations were relatively similar at final sampling, albeit significantly different between the growth media types (Table 3.6).

3.3.3 Effects of fertiliser treatments and growth media on medium fertility

Effects from fertiliser treatments used in this study on growth medium fertility were significantly affected by growth media type. Soil fertility, or availability of nutrients in the

growing medium for plants, is typically expressed as the concentration of dissolved N or P in soil or total N (TN) or total P in soil (Fujita *et al.*, 2013). There were similar, significant main effects for the factor growth medium type relating to TN and TP. Concentrations of both were significantly higher in the bark-based growth media (gromor) than in the soil-based growth media (randles). Like TP, PO₄-P, exhibited significant main effects for the factor growth medium and was also significantly different for the main effect factor, fertiliser type.

Additionally, significant main effect growth medium differences were found in NH₄-N concentrations of growth media tested (Table 3.6). There were significantly higher NH₄-N concentrations in pine bark-based media than in soil-based growth media. These significant differences may be explained by similar growth media adsorption capacity and pH. Knowles *et al.* (1993), Jarvel (1996) and Yu and Zinati (2006) have reported on pine bark capacity to adsorb NH₄-N. Foster *et al.* (1983) showed that NH₄-N adsorption to pine bark increased with an increase in pH of growth media. Although NH₄-N concentrations were not strongly correlated with pH (not statistically tested), the degree of significant difference between the two media with respect to NH₄-N was quite high at $p < 0.001$.

In a study evaluating the release characteristics of a range of slow- and rapid-release nitrogen (N) fertilisers in potting mixes contained in PVC (polyvinyl chloride) columns, greater levels of NH₄-N compared with NO₃-N in leachate were found in a peat-sand-sawdust medium and very low levels of NH₄-N in a peat-sand-soil medium (Goh, 1979). The significant growth media main effect differences in TN concentrations appeared to be more related to those of NH₄-N, as NO₃-N was not significantly different in growth media (Table 3.6).

There appeared to be a correlation (not statistically tested) between TP, PO₄-P and P (Table 3.6), all determined by leachate extraction and any differences may be a result of experimental error. Merhaut *et al.* (2006), in a simulated plant production cycle, found that

leachate TP concentrations extracted from a peatmoss, pinebark and sand (5:4:1 ratio) growth media mix prepared separately with four controlled release fertilisers at equal rates of N, averaged below 10 mg TP·L⁻¹ in the last 27 weeks of a 47 week study. Results in this study showed bark based media solution TP concentrations range from 6.77 mg·L⁻¹-16.05 mg·L⁻¹ at termination with an average of 9.97 mg·L⁻¹ (Table 3.6); TP concentrations in soil-based media were much lower in general. Zinati *et al.* (2011) investigated leachate PO₄-P concentrations, extracted from a pinebark, Canadian sphagnum peatmoss, and builders sand (8:1:1 ratio) media and fertilized at recommended and half-label rates with Polyon controlled release fertiliser. The authors found leachate PO₄-P, whilst growing *Leucothoe axillaris* and *Pieris japonica*, showed mean values of 8.35 mg·L⁻¹ and 3.91 mg·L⁻¹ and 8.64 mg·L⁻¹ and 3.32 mg·L⁻¹, respectively in such media. These label rate concentrations were comparable to the gromor bark-based treatment results and the half label concentrations to the gromor control results in this study, except for OGC (Table 3.6). P concentrations in bark-based media leachates were about twice as high as those of soil-based media, in agreement with Raymond (2004) who evaluated soilless and soil-based media. The significant differences in leachate TP, P and PO₄-P may also be explained by growth media adsorption capacity. Williams and Nelson (2000), Scagel (2003) and Oh *et al.* (2016) reported that soilless growth media, such as bark, have little ability to hold P due to low sorption capacities.

Adsorption–precipitation and desorption–dissolution reactions regulate the removal of nutrients from, or release into, the soil solution (Comerford, 2005). Concentrations of K, Ca, Mg, Fe and Cu were significantly higher in leachate extracted from randles growth media than from gromor-based media leachate (Tables 3.8 and 3.9). Concentrations of Mn (0.1-1 mg·L⁻¹ suggested by Silber and Bar-Tal (2008) and 0.02-3 mg·L⁻¹ suggested by LeBude and Bilderback (2009) and Zn (0.01-0.1 mg·L⁻¹ suggested by Silber and Bar-Tal (2008) as well as

0.3-3 mg·L⁻¹ suggested by LeBude and Bilderback (2009) far exceeded the recommended range in all extracted leachate from all fertiliser treatments (Table 3.8).

Plants in low-pH growth media may experience toxicities in Fe, Mn, Zn and Cu along with deficiencies in Ca or Mg (Mathers *et al.*, 2007). The pH values were within recommended limits (5.2 – 6.3) proposed by Abad *et al* (2001) at 90 DAP or mostly higher at 60 DAP (Table 3.5). Calcium was however, below the recommended range of 40-200 mg·L⁻¹ suggested by Silber and Bar-Tal (2008) and LeBude and Bilderback (2009) in the bark-based media CG, NGA, PGA, PGC. Magnesium was below the recommended range of 10-50 mg·L⁻¹ as suggested by Silber and Bar-Tal (2008) and LeBude and Bilderback (2009) in NGA and PGA (Table 3.7). Magnesium is a part of the chlorophyll molecule and as such a key element of photosynthesis and is also required for protein synthesis whilst Ca is used to stabilise and strengthen cell walls and plant tissue; a severe lack of these elements will impact plant growth adversely (Marschner, 2011). This did not appear to be the case on plant growth parameters tested.

Fe and Cu leachate concentrations also exceeded the recommended range. The Fe concentrations in all solution analysed were much higher than 0.3-3 mg·L⁻¹ suggested by LeBude and Bilderback (2009) and the 0.5-3 mg·L⁻¹ suggested by (Silber and Bar-Tal, 2008). Mean Cu concentrations, including the controls, were above the sufficiency range (0.001-0.01 mg·L⁻¹) suggested by Silber and Bar-Tal (2008) and the sufficiency range (0.01-0.5 mg·L⁻¹) suggested by LeBude and Bilderback (2009). Smith *et al.* (2004) stated that Fe and Mn are mutually antagonistic and excess soluble Fe or Mn can suppress the uptake of the less soluble nutrient. There did not appear to be any suppression to deficient levels in this study, as suggested by Smith *et al.* (2004).

It would, however, appear that these growth media effects are more due to cation competition for sites within growth media than effects of leaching carrying nutrients with it and that Mn and Zn were significantly desorbed from exchange sites in the bark-based media (Table 3.8). Dominy (2010) reported competition for Zn^{2+} uptake by Cu^{2+} , Fe^{2+} and Mn^{2+} , as these cations are of similar ionic size, and, therefore, compete for Zn^{2+} exchange sites. It has been stated that Zn availability is significantly reduced at higher pH levels (pH > 6.5). The same research stated that Mn^{2+} exhibits similar characteristics to other divalent cations and competition for uptake with Mg^{2+} and Ca^{2+} is common, whilst Mn^{2+} availability can be limited at higher soil solution pH levels (pH levels > 6.5). In this study Ca and Cu leaf tissue concentrations were significantly higher from soil based treatments, Mn and Zn leaf tissue concentrations significantly higher from bark based treatments whilst Mg and Fe leaf tissue concentrations were similar between the two growth media after a period of 90 days (Tables 3.10 and 3.11). Since all pH unit values at 90 DAP were < 6.5, it would appear that Mn and Zn were more strongly bound to exchange sites in soil-based growth media.

Significantly higher concentrations of P were also found in leaf tissue of plants grown in gromor growth media compared with those in randles growth media (Table 3.9). Reasons why significant differences between growth media did not extend to leaf tissue concentrations for K, Mg and Fe may be explained by cation competition. Uptake processes of Mg, K, and Ca are strongly antagonistic, Mg being the nutrient of the lowest uptake preference (Farhat *et al.*, 2015; Farhat *et al.*, 2016). Significant and excessive Ca leaf tissue of plants grown in the studied media would suggest that Ca influenced the uptake of K and Mg, but not reducing these elements to deficient levels, as the concentrations for these elements in leaf tissue were similar between treatments and within the sufficiency range (Table 3.9). The same

principle may explain why Mn leaf tissue concentrations were significantly different as affected by growth media, while Fe leaf tissue concentrations were not.

3.3.4 Effects of growth media on *Pseuderanthemum atropurpureum* growth

There were significant main effects of growth media on leaf number and branch number (Table 3.12). Plants grown in media containing gromor potting mix produced more leaves and more branches than those grown in the randles potting mix. The significantly higher number of branches of plants grown in the gromor media may have been related to the significant main effects of NH₄-N in gromor media compared with the randles media. Handreck and Black (2002) suggested increasing the NH₄-N proportion of N in the fertiliser treatment, if more shoot growth is desired. Raymond (2004) stated that growers focus on NH₄-N nutrition in the early stages of plant growth in order to achieve lush green growth, including greater leaf expansion and stem internode length. Leaf size and height differences, however, were not significantly affected as a result of growth media.

Insufficient P impacts, to a relatively greater extent, on leaf number by reducing the time to new leaf appearance (Atwell, 1999). Significantly higher concentrations of P may have resulted in significantly more leaves and leaf tissue concentrations of plants grown in gromor media. The treatments producing the highest number of leaves (NGC, OGB and OGC) were, however, only significantly different from the two controls and PRA.

3.3.5 Effects of treatments on *Pseuderanthemum atropurpureum* growth

Mineral elements in foliage were generally within the sufficiency range (Table 2.1) (Silber and Bar-Tal, 2008). Plant nutrient (especially NO₃-N) uptake resulted in higher levels of leaf N concentrations from Organic Vitaboost treatments than from the other treatments.

The strong relationship between leaf chlorophyll content and leaf N content is well documented and chlorophyll is one of the indices in photosynthesis (Bojović and Stojanović, 2005; Yang *et al.*, 2009). Incorporation of higher Organic Vitaboost leaf N did, however, not result in significantly higher leaf chlorophyll *a* or *b* concentrations (Table 3.11). Leaf chlorophyll *a* concentrations (Table 3.11.) were similar between treatments, but leaf chlorophyll *b* appeared more correlated with leaf N concentrations.

Organic Vitaboost (OGB) produced significantly more leaves, significantly more branches and significantly more nodes ($p=0,009$, $p=0.006$ and $p=0.023$, respectively). Results for leaf size in the Organic Vitaboost (OR and OG) treatment groups and for internode length in the commercial fertiliser (LG treatment group) decreased linearly (Fig, 3.3, 3.5). The decrease in certain plant growth parameters before increasing again may have been due to a reduction in available $\text{NH}_4\text{-N}$. The decrease in internode length from the medium Polyfeed application rate (PGB) compared to lower and higher rates (PGA and PGC), corresponds to the highest pH result in this study at termination (Table 3.5) and reduced $\text{NH}_4\text{-N}$ concentration in growth media. Ammonium nutrition increases internode length (Raymond, 2004).

The second experiment conducted during the winter period (July, August and September) had lower temperatures which affected growth rate and resulted in larger standard errors. Randles combined with Nitrosol increased plant growth parameters in all treatments with increasing fertiliser rate, except for the number of branches produced and internode length and warrants a repeat investigation to determine if results are similar. Combining 'gromor' with Lawn and Leaf treatment increased plant growth parameters in all treatments with increasing fertiliser rate, except for internode length and warrants further investigation as it does not contain all essential plant nutrients. These would have been supplied by the growth medium initially. It appeared that randles supplied with Organic Vitaboost will only produce more leaves and

more branches at higher concentrations than those used in this study. Similarly, Polyfeed will only increase plant height, leaf size and the number of nodes at higher concentrations than those used in this study in either of the growth media used. Increasing the concentration of the fertilisers used in this study would require careful consideration. This could be of concern due to possible eutrophication of freshwater systems as a result of surface runoff or soil infiltration. South African standards for effluent discharge restricts $\text{NO}_3\text{-N}$ to $<10 \text{ mg}\cdot\text{L}^{-1}$ and $\text{PO}_4\text{-P}$ to $<10 \text{ mg}\cdot\text{L}^{-1}$ for land discharges whilst $\text{PO}_4\text{-P}$ discharges to watercourses is restricted to $1 \text{ mg}\cdot\text{L}^{-1}$. Growth media $\text{NO}_3\text{-N}$ concentrations could potentially exceed $10 \text{ mg}\cdot\text{L}^{-1}$ in leachate due to its anionic nature as several treatments contained more than this. Phosphorous species are however the limiting nutrient in freshwater systems in South Africa. Bark medium Polyfeed (PGC - $12.34 \text{ mg}\cdot\text{L}^{-1} \text{ PO}_4\text{-P}$) and Organic Vitaboost (OGC - $14.51 \text{ mg}\cdot\text{L}^{-1} \text{ PO}_4\text{-P}$) exceeded the $10 \text{ mg}\cdot\text{L}^{-1}$.

3.3.6 Effects of organic and inorganic fertiliser treatments on plant growth

There were no significant differences between organic and inorganic fertiliser effects on the determined plant growth parameters of height, leaf size and internode length. Pelleted Organic vitaboost (OGB) added to the gromor medium produced significantly more leaves than addition of the liquid, inorganic treatment Polyfeed, at all levels of treatment in randles growth medium (PRA, PRB and PRC) and significantly more leaves than the granular inorganic treatment, Lawn and Leaf, in randles growth media at the lower levels of treatment (LRA and LRB). Organic vitaboost (OGB) added to the gromor medium produced significantly more branches than the inorganic treatment, Polyfeed, at all levels of treatment when added to the randles medium (PRA, PRB and PRC).

Similarly, OGB resulted in significantly more branches than the randles inorganic treatment, Lawn and Leaf (LRA). Organic vitaboost (OGB) added to the gromor medium produced

significantly more nodes than the inorganic treatment, Polyfeed, at all levels of treatment in randles growth medium (PRA, PRB and PRC) and significantly more nodes than randles inorganic Lawn and Leaf (LRA and LRB). Results in this study were mostly consistent with a decrease in height, number of leaves and leaf size when the level of Organic vitaboost exceeded the intermediate rate, whilst Lawn and Leaf fertiliser mostly increased these growth parameters further (Table 3.12).

Mbangcolo (2008), evaluating the effects of organic fertiliser and cutting position on *Cyclopia* species grown in fine pinebark and river sand (1:1 ratio by volume), found that Nitrosol applied at two rates ($1.67 \text{ ml}\cdot\text{L}^{-1}$ and $3.33 \text{ ml}\cdot\text{L}^{-1}$), significantly affected plant height and the number of shoots produced among other parameters tested when compared with a control in that study. In this study, results were similar for Nitrosol treatment when comparing height and number of branches in that NGB and NGC produced significantly taller plants and significantly more branches than the gromor control.

Bi *et al.* (2010) found that only the inorganic treatment increased plant growth characteristics with increasing fertiliser rate and that the organic treatments (poultry litter) responded linearly or quadratically. Results were similar in this study (Table 3.12), although the growth media used differed. Bi *et al.* (2010) reported that growth of French marigolds (*Tagetes patula* L. 'Janie Deep Orange') varied and suggested that such variation may be the result of differences in nutrient availability and mineralisation rates of various nutrient fractions under the environmental and horticultural conditions of a particular study. Treatments effects in this study increased plant growth parameters or showed luxury consumption at higher treatment concentrations as also reported by Bi *et al.*, (2010).

Statistical analysis of variance of fertiliser treatments only, based on the average of means of all plant growth parameters determined, showed the following overall effect in order on plant

growth: Organic Vitabooost (23.18^b) > Nitrosol (20.78^a) > Lawn & leaf (20.52^a) > Polyfeed (19.47^a) with means separated by Duncan's multiple range test at $p < 0.05$. This suggests that Organic Vitabooost was superior to the other treatments in effects on plant growth. Results however, by parameters assessed, indicate that the effects of treatment were more similar. This may have been as a result of N being equilibrated. Bojović and Stojanović (2005) stated that, amongst all nutrients, N has the greatest influence on plant growth and leaf development, with P, and to a lesser extent by K. The results of this study may be of importance to a Grower of *Pseuderanthemum* or plants from the same Family, who is seeking more shoots and more leaves from his crop.

3.4 Conclusions

Organic and inorganic fertilisers used in this study enhanced plant growth, especially when compared to the soil based growth medium control (randles) and the bark based growth medium control (gromor). Plants were at a marketable size after a period of 90 DAP and of acceptable quality visually at the termination of this experiment.

The objective of this study was to determine which of the organic or inorganic fertilisers used in study was more desirable for its effects on plant growth. Some treatments showed significance for some parameters tested. Results from this study would suggest that *Pseuderanthemum atropurpureum* be grown at the lowest fertiliser concentrations used in this study in either of the growth media, as there was no single treatment which showed significant differences for all plant growth characteristics measured at all levels of treatment.

3.5. Literature cited

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Figure 3.7: Google earth image of Randles Nursery where this study took place

CHAPTER 4

Effects of organic and inorganic fertilisers on growth media leachate composition under *Pseuderanthemum atropurpureum* production

Abstract

In South Africa, agriculture is considered to be a major contributor to eutrophication, but little is known of the contribution by the greenhouse and nursery industry. Nutrients, especially N and P, are readily leached in container production systems which may potentially result in nutrient over-enrichment of freshwater ecosystems, followed by algal blooms or harmful algal blooms. Leachates from container production systems are mostly analysed for nutrient content and pollutants due to recognised impacts on the environment, but not for chlorophyll and algal composition. A tunnel pot experiment was, therefore, conducted to evaluate the effects of organic and inorganic fertilisers and the rate of application and soil- as well as bark-based growth media on leachate composition. Nitrogen equilibrated treatments were administered to the two growth media in a 4 x 3 x 2 (fertiliser type x fertiliser concentration x growth media) factorial design in a completely randomised layout with a control for each growth media. Leachate from container grown *Pseuderanthemum atropurpureum* was extracted using the pour-through method at termination of the study (90 DAP) for laboratory determination of TP, PO₄-P, Al, Ca, Fe, N, Mg and P concentrations. Extracted leachate was also filtered and used to determine algal chlorophyll *a* (PChl *a*) concentrations and to identify the prevalence of algal organisms. Growth media samples were used for laboratory determination of TN and NO₃-N and SEM-EDX qualitative analysis of Al, Ca, Fe, Mg, P, and Si. Soil-based growth media leached significantly more Al, Ca, Fe and Mg than bark-based growth media which leached significantly more TP, PO₄-P and P than soil-based growth

media. Concentrations of leached $\text{PO}_4\text{-P}$ from all bark-based treatments and soil-based Organic Vitaboost treatments were above the permissible limit of $1 \text{ mg}\cdot\text{L}^{-1}$ for direct discharge into a freshwater ecosystem or surface water courses in South Africa. Leached N was similar between treatments. Growth media $\text{NO}_3\text{-N}$ concentrations were significantly higher under the higher application rates of Organic Vitaboost to both growth media and represent a potential for increased $\text{NO}_3\text{-N}$ leaching. The low presence of microalgae was most likely influenced by irrigation, followed by leaching during the course of the study, as PChl *a* concentrations at the end of study had decreased from initial concentrations. Diatoms identified, except for *Microcostatus*, represented those found in a variety of conditions in freshwater systems, including *Nitzschia amphibia* Grunow and *Navicula veneta* Kützing, both organisms associated with eutrophic waters. The implications from this study are that leachate $\text{NO}_3\text{-N}$ and forms of P as well as algal content from nurseries may potentially result in over-enrichment and increased algal biomass of surface water.

Keywords: Fertiliser, growth media, leaching, eutrophication, algae

4.0 Introduction

Successful agricultural and horticultural plant production requires a continuous supply of uncontaminated water, but also impacts on quality and availability of freshwater (Bonacin *et al.*, 2015). Commercial production systems in the greenhouse and nursery industries are highly intensive and utilise large quantities of water and nutrients to enhance plant growth and ensure crop production of high value and quality (Taylor *et al.*, 2006; Dennis *et al.*, 2010).

Greenhouse and nursery produced plants are grown almost exclusively in soilless growth media (Margenot *et al.*, 2018). Nutrients are however readily leached from soilless growth media compared to soils due to its higher porosity and lower anionic and cationic properties (Majsztzik *et al.*, 2011). Nutrients that have leached, particularly Nitrogen (N) and Phosphorous (P), can result in the contamination of groundwater and eutrophication of receiving surface waters through runoff (Juntunen *et al.*, 2003; Alem *et al.*, 2015; Majsztzik *et al.*, 2017). Eutrophication causes an increase in phytoplankton biomass and has been aligned with a number of environmental problems (Kyewalyanga, 2015), including algal and harmful algal blooms (Van Ginkel, 2011).

Toxic cyanobacterial blooms dominate eutrophic freshwater systems in South Africa in the warmer seasons (Van Ginkel, 2004), brought on by nutrient enrichment. Phosphorus is usually the limiting factor in South African freshwater systems for eutrophication (Lai, 2013) and initiatives to curb the release of P into freshwater systems have been advocated. The Environmental Protection Act (No. 44 of 2003) limits land and underground reactive P (PO_4^{3-}) discharge to $10 \text{ mg}\cdot\text{L}^{-1}$. The current standard for surface water course and point source effluent discharge is $1 \text{ mg}\cdot\text{L}^{-1}$ P (Pillay, 1994; Van Ginkel, 2011). There does not appear to be any literature on specific nutrients and amounts leached from greenhouse and

nurseries operations or from agricultural production systems in South Africa. Studies have taken place in America which has reported on concentrations of nutrients that are leached into the environment. For instance, Nitrate-N concentrations in surface runoff from nursery and greenhouse operations have been reported to lie between 1.6 and 55mg L⁻¹ (Taylor *et al.*, 2006). Phosphate concentrations in samples taken from nursery drainage areas ranged from 0.60 mg·L⁻¹ during the winter to 144 mg·L⁻¹ during the growing season (Sharma *et al.*, 2008).

Broschat (1995) investigated environmental impact from controlled-release, liquid and granular slow release fertiliser leachate and reported lower primary nutrient losses from controlled release fertilisers. Few studies have compared the effects of organic and inorganic fertilisers on leachate content (Altland *et al.*, 2000; Carpio *et al.*, 2005). Fertiliser type (liquid or soluble granular, organic or inorganic) may significantly affect the amount of N and P leached, as surface runoff, over the course of a study as a consequence of growth media type.

Rainfall (intensity and duration) and hydrological factors in the watershed principally influence the nutrient loading of N (Durand *et al.*, 2011) and P (Ojwando, 2014) to freshwater ecosystems. A key implication for freshwater management from the findings of Sun *et al.* (2018) suggests that all hydrological variables should be taken into consideration for bio-monitoring protocols. Chlorophyll *a* concentrations in surface runoff (Johnson and Gerald, 2006) have been little researched. Nutrients in leachate from container production have been widely investigated (Merhaut *et al.*, 2006; Alem *et al.*, 2015; Shreckhise *et al.*, 2018) but not the microalgae that are contained therein nor its chlorophyll *a* concentrations.

The addition of soil to growth media increases P retention in the medium (Logsdon, 2017). Soils containing, or amended with, Si have good adsorption capacity and reduced P leaching (Matichenkov *et al.*, 2001). Goh (1979), Raymond (2004) and Logsdon (2017) appear to be the only studies found in available literature, comparing the effects of soilless and soil-

containing growth media, on nutrient leaching. There does not appear to be any study comparing leachate concentrations under the application of organic liquid, organic pelletised chicken litter, inorganic granular and inorganic liquid fertilisers to soilless and soil based growth media.

It was therefore investigated if organic or inorganic fertilisers, at varying rates, affect the nutrient leachate with respect to N and more especially P, as P is mostly instrumental in freshwater eutrophication. Additionally, the leachate content from soil and soilless bark-based growth media used to grow potted *Pseuderanthemum atropurpureum* was determined to grasp, if fertiliser applications in nurseries and algal organisms in leachate could potentially contribute to eutrophication.

The objectives of this study were, therefore, to

- determine N and P forms, chlorophyll a (PChl *a*) concentrations as well as the concentration of the mineral elements Al, Ca, Fe and Mg in nursery container leachate,
- determine concentrations of total phosphate and orthophosphate (PO₄-P) in leachate and growth media TN and NO₃-N concentrations,
- detect Si, P, Al, Ca, Fe, and Mg in growing media using a scanning electron microscope (SEM) and identify soil algal organisms in leachate using SEM as well a phase contrast light microscope.

4.1 Materials and methods

4.1.1 Study site

This research project was carried out at Randles Nursery (latitude 29°49'22"S, longitude 30°58'47"E), Durban, South Africa).

4.1.2 Growth medium preparation and analysis

Two basic growth media were used. A soil-based growth medium, a blended mix consisting of topsoil, compost and river sand (1:1:1, v/v/v) (herein referred to as “randles”). The topsoil and river sand were acquired locally by Randles nursery and the compost was supplied by Gromor® (Cato Ridge, KwaZulu-Natal). The media components were thoroughly hand-mixed before potting up. The gromor medium was a commercial blend consisting of composted pinebark and 10% river sand, also purchased from Gromor®. The supplier usually adds up to two percent Accelerator® poultry litter fertiliser fines to bring the EC up to 2.4 (Jan van Vuuren, Gromor, personal communication, 2015). These two growth media served as the controls.

Samples of all growth media mixes were sent to laboratories at the Soil Fertility and Analytical Service Section, KZN Department of Agriculture and Rural Development, Cedara, for total nitrogen, inorganic nitrogen (nitrate - $\text{NO}_3\text{-N}$ and ammonium - $\text{NH}_4\text{-N}$) and mineral nutrient content as well as soil texture analysis. These growth media parameters (TN, $\text{NO}_3\text{-N}$ and $\text{NH}_4\text{-N}$) were again determined at termination of the study (90 days after planting (DAP)).

Total N was analysed by the automated Dumas dry combustion method using a LECO CNS 2000 analyser (Leco Corporation, St. Joseph, MI) which measures total carbon, nitrogen and sulphur in soils. Both, $\text{NO}_3\text{-N}$ and $\text{NH}_4\text{-N}$ were measured in filtered extracts by segmented

flow analysis with a Perstorp Flow Solution III analyser using the sodium salicylate - sodium nitroprusside-hypochlorite method for NH_4^+ - N (Perstorp Analytical, Warrington, UK) and the sulphanilamide-naphthyl-ethylenediamine method for NO_3^- - N after having reduced nitrate to nitrite with copperized cadmium wire (Willis and Gentry, 1987). For mineral element analysis, samples were dried at 105°C and milled to pass through a 0.84 mm sieve. Subsamples were then dry-ashed at 450°C overnight and taken up in 1 M HCl. Minerals (P, K, Ca, Fe, Mg, Cu, Mn and Zn) were determined by inductively coupled plasma optical emission spectrometry (ICP-OES). Soil texture analysis involved determination of the suspended clay and fine silt content after dispersion and sedimentation, while sand fractions were determined by sieving. Once the particle size distributions of the soils was known, their textural class was determined from a textural triangle defining particle size limits of the various textural classes (Manson and Roberts, 2000).

4.1.3 Plant preparation

Cuttings were prepared from plant material sourced from large container-grown *Pseuderanthemum atropurpureum* plants at Randles nursery. Cuttings were rooted in flat seedling trays (no cavities) filled with washed river sand and kept under manually operated mist in a greenhouse at the Durban University of Technology nursery with no temperature control. Cuttings were ready for potting in either of the growth media once sufficient root mass had developed.

4.1.4 Production environment

This project was carried out in a tunnel covered with 40% black shade cloth. Rooted cuttings were potted into 3 L black plastic growing bags and placed on the north-facing side of the tunnel on black plastic sheeting on top of a layer of gravel to prevent weed growth. Plants were labelled according to rows and separately colour coded according to each of the four

fertiliser treatments and concentrations for ease of identification. This tunnel had no climate control and temperatures in the structure varied over the experimental period. The experiment in this study was repeated three times with the second conducted mostly during winter (July, August and September). Minimum temperatures were significantly lower during this period in comparison to the other experiments. Mean minimum and maximum temperatures for replications 1, 2 and 3 were $20.90^{\circ}\text{C}^{\text{min}}$ and $27.76^{\circ}\text{C}^{\text{max}}$, $12.76^{\circ}\text{C}^{\text{min}}$ and $25.14^{\circ}\text{C}^{\text{max}}$ and $20.31^{\circ}\text{C}^{\text{min}}$ and $29.75^{\circ}\text{C}^{\text{max}}$ with temperature ranges recorded for the same periods $18.13^{\circ}\text{C}^{\text{min}} - 31.5^{\circ}\text{C}^{\text{max}}$, $8.67^{\circ}\text{C}^{\text{min}} - 27.83^{\circ}\text{C}^{\text{max}}$ and $16.08^{\circ}\text{C}^{\text{min}} - 36^{\circ}\text{C}^{\text{max}}$ respectively. The variance in temperature between the three experiments was accounted for by checking the blocks box and entering the column data containing the three experiments in the box alongside, as a blocking factor prior to running Genstat ANOVA analysis of data.

4.1.5 Experimental design

The experiment was laid out as randomized complete block design in a 4x3x2 factorial design (fertiliser type x fertiliser level x growth media) with a control for each of the two growth media used. In total, 208 *Pseuderanthemum atropurpureum* plants in either growth medium were randomly divided (blocking) into two batches of 13 groups including a control group for each growth medium, with eight plants per group (row) and each group representing an experimental unit. The batches (two) were staged within adjacent areas measuring 3.5 m x 1.5 m and laid out in rows on either side of a 1 m pathway. Each group received either an N equilibrated organic or inorganic fertiliser treatment based on half, recommended or double the recommended rate, except for the two controls. There were seven fertiliser treatments per trial (Table 4.2). The experiment was repeated three times (3 trials).

4.1.6 Plant treatment

The fertiliser treatments included two organic and two inorganic fertilisers. Fertiliser application rates were equilibrated according to N as the N content of the four fertilisers used in this study varied (Table 4.1). For the dosage rates of the treatments, the average nitrogen content of the four fertilisers used in the pot experiment was calculated, and fertiliser treatments were adjusted according to their N concentration. This had the effect of either increasing or decreasing the stipulated dosage rate and, therefore, also the concentrations of the other mineral elements. Comparisons were made between Nitrosol[®], Wonder[™] Organic Vita Boost, Wonder[™] Lawn and Leaf, Polyfeed[®] at three concentrations in two types of growth media.

Nitrosol[®] (NPK analysis 4:1:3 (16)), an organic liquid water-soluble fertiliser, is formulated using sterilised blood, bone and carcass meal. It is manufactured by Envirogreen (Pty) Ltd for Fleuron[®] (Pty) Ltd. Nitrosol[®] solution (200ml·pot⁻¹) was applied to each plant every two weeks at 2.61 ml·L⁻¹, 5.22 ml·L⁻¹ and 10.44 ml·L⁻¹ as 1/2 label rate, label rate and 2 x label rate, respectively (Table 4.2). This liquid treatment also contained other macronutrients, as well as micronutrients and gibberellic acid (Table 4.1).

Table 4.1. Source and amount (by weight) of macronutrients and micronutrients in Nitrosol[®], Lawn and Leaf, Polyfeed[®] and Organic Vitaboost fertilisers

Trade name	Nitrosol ¹	Lawn and Leaf	Polyfeed	Vitaboost
Formulation	4:1:3	7:1:3	6:1:3	6:1:3
N g/kg	80	95	266	60
P g/kg	20	14	44	13
K g/kg	58	41	134	27
Ca g/kg	6	-	-	25.3
Mg g/kg	7	-	4	6.4
S g/kg	4	-	5.3	-
Fe mg/kg	60	-	751	4452
Mn mg/kg	40	-	273	517
Zn mg/kg	1	-	699	319
Cu mg/kg	1	-	75	68
B mg/kg	23	-	1054	-
Mo mg/kg	15	-	63	-

1 – Nitrosol also contains Gibberellic acid at 0.003 g·kg⁻¹

Wonder[™] Organic Vita Boost (NPK analysis 6:1:3), a dry water-soluble fertiliser, supplied by Efekto was the second organic treatment used in this study and consisted of chicken litter pellets. Vita Boost was applied every two weeks at 2.77 g·pot⁻¹, 5.54 g·pot⁻¹ or 11.07 g·pot⁻¹ to each plant in rows as 1/2 label rate, label rate or 2 x label rate, respectively (Table 4.2). This fertiliser, which was incorporated just below the surface, also contained other macro and micronutrients.

Polyfeed[®] (NPK analysis 6:1:3 (44)), a highly concentrated dry, powdered, water-soluble inorganic fertiliser is manufactured by Nulandis (A Division of AECI Ltd). Polyfeed[®] (200ml·pot⁻¹) was applied as a liquid treatment every two weeks at concentrations of 0.59 g·L⁻¹, 1.18 g·L⁻¹ and 2.36 g·L⁻¹ as 1/2 label rate, label rate and 2 x label rate, respectively (Table 4.2). This liquid treatment also contained other macro and micronutrients.

Wonder[™] Lawn and Leaf (NPK analysis 7:1:3 (15)), supplied by Efekto, an inorganic fertiliser was used as the 4th fertiliser treatment in this study. This is a high N, sustained-

release fertiliser, with bio-carbon pellets, which were removed prior to application. Lawn and Leaf was applied every two weeks at rates of 0.36 g·pot⁻¹, 0.71 g·pot⁻¹ and 1.42 g·pot⁻¹ to each plant in rows as 1/2 label rate, label rate and 2 x label rate, respectively. The controls of the soil-based medium and the pinebark-based medium received no fertiliser (Table 4.2).

Table 4.2 Dosage rate of N equilibrated fertiliser nutrients incorporated (g) into each 3 L plant bag or liquid fed·L⁻¹ concentration as nutrient solution

Formulation	Treatment	Source	Form	Application method	Dosage rate
Control	CR*	-	-	-	-
Control	CG	-	-	-	-
4.1.3	NRA	organic	liquid	liquid feed	2.61 ml·L ⁻¹
4.1.3	NRB	organic	liquid	liquid feed	5.22 ml·L ⁻¹
4.1.3	NRC	organic	liquid	liquid feed	10.44 ml·L ⁻¹
4.1.3	NGA	organic	liquid	liquid feed	2.61 ml·L ⁻¹
4.1.3	NGB	organic	liquid	liquid feed	5.22 ml·L ⁻¹
4.1.3	NGC	organic	liquid	liquid feed	10.44 ml·L ⁻¹
6.1.3	PRA	inorganic	powder	liquid feed	0.59 g·L ⁻¹
6.1.3	PRB	inorganic	powder	liquid feed	1.18 g·L ⁻¹
6.1.3	PRC	inorganic	powder	liquid feed	2.36 g·L ⁻¹
6.1.3	PGA	inorganic	powder	liquid feed	0.59 g·L ⁻¹
6.1.3	PGB	inorganic	powder	liquid feed	1.18 g·L ⁻¹
6.1.3	PGC	inorganic	powder	liquid feed	2.36 g·L ⁻¹
7.1.3	LRA	inorganic	granule	incorporated	0.36 g·pot ⁻¹
7.1.3	LRB	inorganic	granule	incorporated	0.71 g·pot ⁻¹
7.1.3	LRC	inorganic	granule	incorporated	1.42 g·pot ⁻¹
7.1.3	LGA	inorganic	granule	incorporated	0.36 g·pot ⁻¹
7.1.3	LGB	inorganic	granule	incorporated	0.71 g·pot ⁻¹
7.1.3	LGC	inorganic	granule	incorporated	1.42 g·pot ⁻¹
6.1.3	ORA	organic	pellet	incorporated	2.77 g·pot ⁻¹
6.1.3	ORB	organic	pellet	incorporated	5.54 g·pot ⁻¹
6.1.3	ORC	organic	pellet	incorporated	11.07 g·pot ⁻¹
6.1.3	OGA	organic	pellet	incorporated	2.77 g·pot ⁻¹
6.1.3	OGB	organic	pellet	incorporated	5.54 g·pot ⁻¹
6.1.3	OGC	organic	pellet	incorporated	11.07 g·pot ⁻¹

* - CR – Control in Randles growth medium, CG – Control in Gromor growth medium, N – Nitrosol fertiliser, P – Polyfeed fertiliser, L – Lawn and Leaf fertiliser, O – Organic Vita boost fertiliser, R – Randles growth medium, G – Gromor growth medium, A – ½ label rate, B – label rate and C – 2 x label rate

4.1.7 Leachate collection and analysis

The ‘pour-through’ extraction method (Wright, 1986), was used to produce and collect leachate. The ‘pour-through’ extraction method recommends pouring sufficient water to yield 50 ml of leachate. It is also recommended that plants were watered at least an hour before collection, to ensure that the moisture content of pots was at container capacity. Approximately 200 ml deionised water was applied to each 3 L pot. Foil trays, washed with nitric acid except for those collecting algal samples, were used to collect the leachate. The leachate was transferred to labelled sample bottles. Samples were sent to the Plant Laboratory at the Soil Fertility and Analytical Service Section, KZN Department of Agriculture and Rural Development, Cedara, for mineral element analysis. The leachate N concentration was measured using an elemental analyzer (Vario EL III; Elementar Analysensysteme GmbH, Germany). Leachate P, K, Ca, Mg, Fe, Cu, Mn and Zn was determined using Inductively Coupled Plasma – Optical Emission Spectroscopy (ICP-OES). Samples were also sent to Regen Waters laboratory (Witbank, South Africa) for total phosphate and orthophosphate analysis (Aquakem 600 Photometric Discrete Analyser, Thermo-Scientific, South Africa). Samples were also sent to Talbot Laboratories for an initial analysis of leachate algal chlorophyll *a* (PChl *a*) concentrations from soil-based growth media (CR) and bark-based growth media (CG) controls before commencement of fertilization.

4.1.8 Algal chlorophyll *a* determination

The spectrophotometric algal chlorophyll *a* determination was based on the method suggested by Arar (1997), to measure the pigment concentrations of freshwater phytoplankton. Leachate was collected using the pour through extraction method (Wright, 1986). Plants were watered at least an hour before collection, to ensure that pot moisture content was at container capacity. Approximately 200 ml deionised water was applied to each 3 L plant bag. Leachate

was collected in foil trays, then the liquid was transferred to labelled sample bottles and placed on ice in cooler bags. Chlorophyll-containing algal microorganisms in the sample solution were filtered at low vacuum through Whatman 25 mm GF/F glass microfiber fibre filters under low light. This was achieved by using a sidearm filter flask connected to a vacuum pump. The leachate volume was recorded prior to filtration. Filters were folded, wrapped in foil, labelled and immediately frozen. On arrival in the laboratory, the entire filter was cut up and placed into a 30 mm test tube, adding 10 ml of 90% laboratory grade acetone. Samples were kept on ice in a closed cooler box under low light. An Ultra Turrax[®] T-25 (IKA, Germany) digital high speed homogeniser was used to extract pigments from algal organisms. Each sample was allowed to steep for at least two hours. The filter slurry was centrifuged at 1000G for 5 min to clarify the solution using a PLC Series tabletop centrifuge (Gemmy Industrial Corp, Taiwan). The absorbance of the centrifuged supernatant was read at 750 nm, 664 nm, 647 nm and 630 nm in a Hellma glass cuvette (1 cm light path, Type 100-T4) (Hellma Analytics, Müllheim, Germany) using a Shimadzu Spectrophotometer UV-1800 (Shimadzu Corp., Japan). The sample absorbance at 750 nm was subtracted from the values at 664, 647 and 630 nm to correct for turbidity. These samples were then filtered through nylon 25 mm Pall Acrodisc PSF syringe filters (0.45 µm). Acidification of samples before reading on the spectrophotometer was not carried out, as Stich and Brinker (2005) suggested this methodological procedure for photometric determination of chlorophyll concentrations from non-acidified extracts to be less time-consuming with a better accuracy of resulting data.

The amount of algal chlorophyll *a* (PChl*a*) in the extract (mg·L⁻¹) was calculated using the formula provided by Lichtenthaler (1987)) with amendments recommended by Arar (1997).

$$\text{PChl}a_{\text{extract}} = 11.85_{(\text{Abs } 664)} - 1.54_{(\text{Abs } 647)} - 0.08_{(\text{Abs } 630)}$$

PChl*a* concentration in the sample was calculated using the formula used by Arar (1997),

$$PChla = \frac{PChla_{\text{extract}} \times \text{extract volume (L)} \times DF}{\text{sample volume (L)} \times \text{cell length (cm)}} \text{ (mg} \cdot \text{L}^{-1}\text{)}$$

where, $PChla_{\text{extract}}$ = concentration ($\text{mg} \cdot \text{L}^{-1}$) of pigment in extract measured in the cuvette,

extract volume = volume (L) of extract (before any dilution),

DF = dilution factor

sample volume = volume (L) of whole water sample that was filtered, and

cell length = optical path length (cm) of cuvette used.

4.1.9 Algal microorganism identification

A phase contrast light microscope (Olympus BHS Phase Contrast Microscope, Tokyo, Japan) was used to identify larger algal microorganisms and a scanning electron microscope (Zeiss EVO LS15 SEM, Oberkochen, Germany) to identify smaller algal microorganisms. Wet mounts from leachate extraction, a droplet at a time, were placed between a microscope glass slide and a cover slip for observation at 20x and 40x objective lens magnitude for the possible presence of microalgae.

Scanning electron microscope (SEM) samples were filtered at low vacuum through Whatman 25 mm GF/F glass microfiber filters in a Buchner funnel into a sidearm filter flask connected to a hand-held vacuum pump. The filter paper was cut up and sections mounted onto aluminium stubs using double-sided carbon tape. Further sample preparation was carried out as described by Whitton (2012). Sections were sputter-coated with gold palladium using a Polaron E5100 ion sputter coater (Eiko IB-3, Japan) before microalgae observation.

4.1.10 SEM EDX qualitative analysis of growth media

A qualitative analysis of the mineral element composition of the growth media samples was carried out using a scanning electron microscope (Zeiss EVO LS15 SEM, Oberkochen,

Germany) fitted with an Oxford X-Max 80mm SSD (Silicon Drift Detector) using INCA Analysis Software (version 4) for the EDX (Energy Dispersive X-ray) analysis. The samples were evenly dispersed and mounted onto aluminium stubs with double-sided carbon tape for viewing, without coating for conductivity, as variable pressure was used in the SEM operation. Three spots were observed per sample. The qualitative results of SEM EDX analysis of growth media reflect all detectable mineral elements in the sample in relation to each other, and minerals are expressed as percentage of 100%. The Al, Ca, Fe, Mg, P and Si percentages of the three replicates per sample were read from the EDX report, averaged and means from the three experiments determined statistically. Means were then partitioned into polynomial contrasts to determine the main effects of Al, Ca, Fe, Mg, P and Si within growth media from treatments as a qualitative reference and to compare the two growth media with the leachates.

4.1.11 Cultural practices

Weeding and irrigation were carried out regularly throughout the course of each successive experiment. Plants were irrigated by hosepipe using tap water, with the irrigation amount limited to the well (volume between growth media surface and lip of each pot) of each pot. Plants were watered on Mondays, Wednesdays and Fridays, except in the event of sufficient rain.

Irrigation water was analysed by the Soil Salinity Laboratory at the Soil Fertility and Analytical Service Section, KZN Department of Agriculture and Rural Development, Cedara for EC, pH, cations and anions. Electrical conductivity (EC) of irrigation water is usually used as an indicator of salinity and sodium adsorption ratio (SAR) of sodicity when water quality is classified (Culverwell and Swinford, 1986). The determination of the Sodium Adsorption Ratio (SAR) describes the level of sodicity of irrigation water and the ratio of

sodium to the sum of calcium and magnesium ions. Higher levels of calcium and magnesium counteract the potential for degradation of soil structure which may result in infiltration problems in soils and growth media due to high sodium levels (Menezes *et al.*, 2014). EC and SAR interpretation are based on criteria developed by the United States Department of Agriculture (Meyer and van Antwerpen, 1995).

4.1.12 Statistical analysis

GenStat® 12th Edition (VSN International, Hemel Hempstead, UK) was the statistical package used in the analyses of data. The data that was collected were subjected to analysis of variance and treatment means separated using the Duncan Multiple Range Test at 5% probability level. Treatment for all parameter means were compared to each control, randles (CR) and gromor (CG) by partitioning each comparison separately into polynomial contrasts to determine possible main effects and interactions at ($p < 0.05$) and elucidate treatment responses for each parameter investigated.

4.2 Results

4.2.1 Analyses of irrigation water

Results from the analysis of irrigation water are contained in Table 4.3. All parameters were acceptable except for irrigation water pH.

Table 4.3 Chemical properties of irrigation water

Parameter	Elements	Value
EC ($\text{mS}\cdot\text{m}^{-1}$)		24.32
pH		7.64
Cations ($\text{me}\cdot\text{L}^{-1}$)	Na	0.93
	Ca	0.95
	Mg	0.23
	K	0.05
Anions ($\text{me}\cdot\text{L}^{-1}$)	TA	1.1
	Cl	1.1
SAR		1.21
Class of water		C1-S1

TA – Total alkalinity

SAR – Sodium adsorption ratio

4.2.2 Analyses of growth media before planting

Results from the analysis of growth media are contained in Table 4.4. EC and pH analyses were at acceptable levels for growth media. Bark-based control had a higher EC and lower pH than soil-based growth media. Most mineral nutrients were present in higher concentrations in the bark-based growth media. The only exceptions were Fe and Cu, which was higher in the soil-based growth media. Carbon:Nitrogen (C:N) ratio in growth media were similar.

Table 4.4 Physicochemical properties of randles (CR) and gromor start growth medium

Parameter		CR		CG	
EC (mS·cm ⁻¹)	Leachate	2.58	Leachate	3.05	
pH	Leachate	6.28	Leachate	5.84	
C/N ratio		20.91:1		20.40:1	
Moisture (%)		6.24		29.68	
Elements					
C (%)		13.38		28.27	
N (%)		0.64		1.38	
P (%)		0.18		0.22	
K (%)		0.28		0.83	
Ca (%)		1.04		1.22	
Mg (%)		0.14		0.22	
S (%)		0.26		0.32	
Fe (mg/kg)		24028		17374	
Mn (mg/kg)		237.79		302.17	
Zn (mg/kg)		83.43		94.97	
Cu (mg/kg)		157.69		106.62	
Soil Texture					
Sand (%)	(0.02 - 2 mm)	80.33	(0.02 - 2 mm)	73.8	
Silt (%)	(0.02 - 0.002 mm)	6.17	(0.02 - 0.002 mm)	7.8	
Clay (%)	(<0.002 mm)	13.83	(<0.002 mm)	18.5	
Soil texture classification*		Loamy sand		Sandy loam	

* According to: - Soil Classification, A Taxonomic System for South Africa - Soil Classification Working Group and Macvicar, 1991

4.2.3 Leachate mineral composition (N, P, Ca, Mg, Fe, Al)

4.2.3.1 Nitrogen (N) in leachate

There were no significant differences between any of the treatments, including comparison with the controls, at $p < 0.05$ level of confidence ($p = 0.919$), nor were there any significant main effects or interactions for mean N concentrations in leachate (Table 4.5).

4.2.3.2 Phosphorus (P) in leachate

Mean leachate P concentrations were significantly lower in the fertiliser groups contained in the soil-based growth media than those of the groups contained in the bark-based media mix. The main effects of the factors, fertiliser type and growth medium type, were significantly different at $p = 0.011$ and $p < 0.001$, respectively, when treatments were statistically compared

to the randles control (CR), with $p=0.026$ and $p<0.001$, respectively when treatments were compared statistically to the gromor control (CG). The amendment of the gromor-containing media with the high concentration of Organic Vitaboost (OGC) resulted in significantly higher leachate P, different from most treatments at $p<0.001$ (Table 4.5). Phosphorous leachate concentrations ranged from $2.34 \text{ mg}\cdot\text{L}^{-1}$ to $21.72 \text{ mg}\cdot\text{L}^{-1}$.

4.2.3.3 Calcium (Ca) in leachate

Mean Ca concentrations increased with increasing fertiliser rate, except for the Polyfeed in gromor (PG) fertiliser group (Table 4.5). Leachate Ca concentrations were in general higher, from soil-based randles media than from bark-based gromor media. The highest application of Organic Vitaboost to the randles medium (ORC) was significantly different from all treatments at $p<0.001$, other than the high Lawn and Leaf (LRC) and the medium Organic Vitaboost addition to the randles medium. The main effects of the factors, fertiliser type, growth medium and levels of fertiliser, were statistically significantly different at $p<0.001$, $p<0.001$ and $p=0.046$, respectively when treatments were statistically compared to the randles control (CR) and at $p<0.001$, $p<0.001$ and $p=0.050$, respectively, when treatments were statistically compared to the gromor control (CG) (Table 4.5).

4.2.3.4 Magnesium (Mg) in leachate

Mean Mg concentrations increased with increasing fertiliser rates, except for the Polyfeed addition to the soil-based medium (PR) (Table 4.5). Leachate Mg concentrations were, in general, higher from soil-based randles media than from bark-based gromor media. The Organic Vitaboost treatment produced significantly higher Mg concentrations in randles growth medium (ORC) compared to all treatments ($p<0.001$), except the lower randles Organic Vitaboost treatment (ORA). The main effects of fertiliser type, growth medium and level of fertiliser were significantly different at $p<0.001$, $p=0.001$ and $p=0.009$, respectively,

when treatments were statistically compared to the randles control (CR) and at $p < 0.001$, $p = 0.002$ and $p = 0.010$, respectively, when treatments were statistically compared to the gromor control (CG) (Table 4.5).

4.2.3.5 Iron (Fe) in leachate

Mean leachate Fe concentrations followed, in general, a trend similar to Ca and Mg concentrations, with higher amounts of Fe leached from the soil-based randles media than from the bark-based gromor media, except for OR and OG (Table 4.5). The main effect of the factor growth medium type was significantly different at $p = 0.001$ for treatments statistically compared to both controls (CR and CG). Statistical analysis showed no significant differences between treatments overall ($p = 0.227$), although PRC leachate contained significantly higher Fe concentrations than the leachate from a few treatments (CR, CG, NGA, LGB, ORC and OGB).

4.2.3.6 Aluminium (Al) in leachate

Mean leachate Al concentrations were, in general, higher from soil-based randles media than from bark-based gromor media. Treatment comparisons showed that growth medium type was significantly different at $p = 0.001$ and $p < 0.001$ for CR and CG, respectively (Table 4.5). Statistical analysis showed no significant differences between treatments overall ($p = 0.250$), although PRA leachate contained significantly higher Al concentrations than the bark-based treatments CG, NGA, NGB, NGC, PGB, PGC, LGA and OGC.

Table 4.5 Effects of two organic (Nitrosol[®] and Organic Vitaboost) and two inorganic fertiliser treatment types (Polyfeed[®] and Lawn and Leaf) applied at three concentrations to two different growth media, on mean growth media pour-through extracted nutrient solution N, P, Ca, Mg, Fe and Al concentrations and compared with the two controls after a period of 90 days

Type	Treatment	N mg·l ⁻¹ (x1000)	P mg·l ⁻¹	Ca mg·l ⁻¹	Mg mg·l ⁻¹	Fe mg·l ⁻¹ (x 1000)	Al mg·l ⁻¹ (x1000)
Control	CR ⁵	2.57 ± 1.11a ¹	2.34 ± 1.75a	58.00 ± 28.65ab	13.41 ± 6.31ab	5.17 ± 2.61ab	19.37 ± 4.61ab
Control	CG	2.30 ± 1.57a	5.26 ± 1.47abc	39.00 ± 10.67a	10.83 ± 0.86ab	3.82 ± 2.88a	12.56 ± 6.43a
4.1.3	NRA	3.43 ± 1.70a	4.34 ± 3.74ab	136.90 ± 100.88a-d	23.51 ± 16.33a-d	7.72 ± 4.95abc	20.40 ± 5.89ab
4.1.3	NRB	3.68 ± 1.93a	4.48 ± 3.89ab	154.30 ± 99.15a-e	24.60 ± 13.92a-d	13.28 ± 6.66abc	30.54 ± 3.89ab
4.1.3	NRC	3.27 ± 1.82a	7.52 ± 6.52a-d	179.70 ± 94.55a-e	35.14 ± 18.30a-e	17.12 ± 8.63bc	34.77 ± 5.05ab
4.1.3	NGA	3.61 ± 2.17a	13.04 ± 5.65a-f	29.80 ± 8.51a	9.89 ± 2.49ab	5.06 ± 3.67ab	15.05 ± 7.54a
4.1.3	NGB	3.86 ± 1.91a	15.42 ± 5.17a-g	54.30 ± 4.04ab	18.25 ± 2.10abc	5.45 ± 5.25abc	13.83 ± 6.72a
4.1.3	NGC	2.89 ± 1.50a	22.36 ± 8.46fg	61.80 ± 17.32ab	24.89 ± 8.62a-d	5.43 ± 4.26abc	11.34 ± 4.47a
6.1.3	PRA	3.91 ± 2.39a	6.66 ± 6.26a-d	115.20 ± 77.38abc	20.70 ± 13.23a-d	17.21 ± 8.30bc	42.65 ± 9.25b
6.1.3	PRB	3.33 ± 1.63a	8.10 ± 7.70a-e	130.20 ± 79.77a-d	20.59 ± 10.80a-d	12.42 ± 9.46abc	26.90 ± 9.55ab
6.1.3	PRC	2.61 ± 1.25a	7.32 ± 6.93a-d	173.90 ± 110.03a-e	31.91 ± 19.20a-d	18.22 ± 9.89c	34.43 ± 9.19ab
6.1.3	PGA	2.71 ± 1.88a	15.22 ± 6.83a-g	32.00 ± 9.68a	8.78 ± 2.33a	8.94 ± 7.15abc	22.94 ± 12.45ab
6.1.3	PGB	3.67 ± 2.73a	13.98 ± 5.36a-f	44.60 ± 8.23a	14.51 ± 1.45ab	5.36 ± 3.79abc	13.64 ± 7.07a
6.1.3	PGC	4.03 ± 2.01a	17.96 ± 7.85b-g	38.40 ± 8.05a	15.24 ± 4.74ab	7.58 ± 5.21abc	11.58 ± 5.93a
7.1.3	LRA	4.01 ± 2.33a	2.96 ± 2.56a	152.70 ± 95.93a-e	26.36 ± 14.75a-d	11.34 ± 6.08abc	31.61 ± 8.80ab
7.1.3	LRB	2.85 ± 1.38a	6.24 ± 5.84abc	183.60 ± 103.29a-e	37.63 ± 21.31a-e	9.65 ± 5.83abc	20.97 ± 2.49ab
7.1.3	LRC	3.04 ± 1.38a	4.75 ± 4.15ab	297.50 ± 160.56def	64.69 ± 36.17def	9.17 ± 5.09abc	28.35 ± 8.93ab
7.1.3	LGA	2.40 ± 1.72a	12.76 ± 5.63a-f	40.10 ± 16.59a	11.40 ± 3.40ab	5.99 ± 4.10abc	15.59 ± 7.51a
7.1.3	LGB	2.88 ± 1.57a	16.12 ± 8.67a-g	67.50 ± 18.80ab	23.90 ± 6.52a-d	5.01 ± 5.01ab	30.39 ± 22.44ab
7.1.3	LGC	4.26 ± 3.27a	20.36 ± 10.33d-g	88.00 ± 36.81abc	30.98 ± 11.69a-d	6.52 ± 6.32abc	24.70 ± 15.11ab
6.1.3	ORA	3.15 ± 1.90a	5.82 ± 5.03abc	246.90 ± 110.22cde	62.05 ± 28.03c-f	5.45 ± 5.06abc	25.26 ± 16.32ab
6.1.3	ORB	5.50 ± 3.41a	9.2 ± 8.21a-f	311.70 ± 121.46ef	89.70 ± 38.83fg	6.70 ± 5.73abc	21.87 ± 12.36ab
6.1.3	ORC	4.60 ± 2.94a	14.68 ± 12.13a-f	408.10 ± 87.90f	119.13 ± 38.69g	5.02 ± 4.63ab	21.76 ± 13.75ab
6.1.3	OGA	2.92 ± 1.76a	21.72 ± 13.06efg	132.10 ± 39.88a-d	48.62 ± 17.33a-f	5.44 ± 5.44abc	20.96 ± 13.73ab
6.1.3	OGB	4.12 ± 2.79a	18.96 ± 9.30c-g	141.50 ± 9.97a-d	54.87 ± 18.31b-f	4.40 ± 4.40ab	18.27 ± 12.59ab
6.1.3	OGC	3.83 ± 1.77a	28.52 ± 14.88g	220.00 ± 35.81b-e	76.34 ± 21.85ef	5.32 ± 5.12abc	13.29 ± 7.13a
Sig p<0.05		0.919 (ns)	<0.001	<0.001	<0.001	0.227 (ns)	0.250 (ns)
LSD ²		2.75	11.47	142.85	37.14	10.64	20.97
CV % ³		48.80	59.20	64.00	63.50	78.20	57.00
Treatments	Fertiliser (F) ⁴	ns	0.011	<0.001	<0.001	ns	ns
	Medium (G)	ns	<0.001	<0.001	0.001	0.001	0.001
	vs Levels (C)	ns	ns	0.046	0.009	ns	ns
	F x G	ns	ns	ns	ns	ns	ns
	F x C	ns	ns	ns	ns	ns	ns
	G x C	ns	ns	ns	ns	ns	ns
Treatments	Fertiliser (F) ⁴	ns	0.026	<0.001	<0.001	ns	ns
	Medium (G)	ns	<0.001	<0.001	0.002	0.001	<0.001
	vs Levels (C)	ns	ns	0.050	0.010	ns	ns
	F x G	ns	ns	ns	ns	ns	ns
	F x C	ns	ns	ns	ns	ns	ns
	G x C	ns	ns	ns	ns	ns	ns

1. Means (±SE) within columns followed by the same letter do not differ significantly according to Duncan's Multiple Range Test at p < 0.05. Non-significant (ns) or significant at p < 0.05 (*), 0.01 (**) or 0.001 (***)

2. LSD –Least significant difference at p < 0.05

3. CV (%) – Percentage coefficient of variance

4. Main effects and interactions at both control levels. Non-significant (ns) or significant at P < 0.05 (*), 0.01 (**) or 0.001 (***)

5. CR – Control in Randles growth medium, CG – Control in Gromor growth medium, N – Nitrosol fertiliser, P – Polyfeed fertiliser, L – Lawn and Leaf fertiliser, O – Organic Vita boost fertiliser, R – Randles growth medium, G – Gromor growth medium, A – ½ label rate, B – label rate and C – 2 x label rate, Table adapted from (Bi *et al.*, 2010)

4.2.4 Phosphate (PO₄-P) and total phosphate in leachates

4.2.4.1 Phosphate (PO₄-P) in leachate

Mean leachate orthophosphate (PO₄-P) concentrations were in general lower from the soil-based randles media than from the bark-based gromor media (Table 4.6). The high Organic Vitaboost application to the gromor medium (OGC) resulted in a significantly higher phosphate concentration ($p < 0.001$) than most other treatments (Table 4.6).. The main effects of the factors fertiliser type, growth medium type and the level of fertiliser were significantly different at $p = 0.035$, $p < 0.001$ and $p = 0.050$, respectively, when treatments were statistically compared to the randles control (CR) but only the factors growth medium ($p < 0.001$) and levels of fertiliser ($p = 0.050$) were significantly different when treatments were statistically compared to the gromor control (CG). Phosphate-P concentrations in leachates ranged from 0.17 to 8.65 mg·L⁻¹ in the soil-based growth media and from 3.80 to 14.51 mg·L⁻¹ in the bark-based ones.

4.2.4.2 Total phosphate in leachate

Mean leachate TP concentrations were, like the orthophosphates, in general, lower from the soil-based growth media than from the bark-based ones. Leachates from the bark-based high Organic Vitaboost (OGC) and the high Polyfeed-containing (PGC) soil-based medium displayed significantly higher TP concentrations than most treatments ($p < 0.001$, Table 4.6). The main effects factor, growth medium type, showed significant differences as a result of the effects of the two growth media ($p < 0.001$), when treatments were statistically compared with both controls (CR and CG) Leachate TP concentrations ranged from 0.34 mg·L⁻¹ to 16.05mg·L⁻¹.

4.2.5 Analysis of nitrate and total nitrogen in growth media

4.2.5.1 Growth medium nitrate (NO₃-N)

Mean NO₃-N concentrations for all treatments increased with increasing fertiliser applications, except for the medium Nitrosol level (NGB) and the high Polyfeed amount added to the bark-based medium (PGC, Table 4.6). The NO₃-N concentrations of the organic fertiliser (OR and OG) were, in general, higher than the other treatments. The statistical analysis for this parameter was not significant overall ($p=0.087$), but the high organic fertiliser application to the soil-based medium (ORC) increased NO₃-N concentrations significantly over most treatments. Levels of NO₃-N were significantly affected by Organic Vitaboost fertiliser applied at the highest dosage ($p<0.001$).

4.2.5.2 Growth medium total nitrogen (TN)

There were no uniform effects on growth medium total nitrogen (TN) concentrations as a result of increasing fertiliser application (Table 4.6). Mean growth medium TN concentrations were significantly affected by both growth media ($p<0.001$). The TN concentrations for treatments containing randles medium were significantly lower than for those containing the gromor bark-based medium; TN in the low Nitrosol (NGA) and the low Polyfeed application to the bark medium (PGA) was significantly higher ($p=0.018$) than in all soil-based treatments, including the randles control.

Table 4.6 Effects of two organic (Nitrosol[®] and Organic Vitaboost) and two inorganic fertiliser treatment types (Polyfeed[®] and Lawn and Leaf) applied at three concentrations, to two different growth media, on mean growth media NO₃-N, and TN and leachate PO₄-P, TP and Pchl *a* concentrations compared with the two controls after a period of 90 days

Type	Treatment	NO ₃ mg L ⁻¹	TN %	PO ₄ mg L ⁻¹	TP mg L ⁻¹	PhChl <i>a</i> µg g ⁻¹
Control	CR ⁵	2.63 ± 1.11a ¹	0.17 ± 0.06a	0.46 ± 0.21ab	0.69 ± 0.36	1.92 ± 0.76ab
Control	CG	3.19 ± 1.76a	0.39 ± 0.12a-d	3.80 ± 0.33a-e	4.16 ± 0.35a-d	2.05 ± 0.93ab
4.1.3	NRA	8.50 ± 4.13ab	0.17 ± 0.04a	0.50 ± 0.17ab	1.17 ± 0.33ab	5.65 ± 2.82b-e
4.1.3	NRB	16.70 ± 11.33abc	0.18 ± 0.08a	0.51 ± 0.17ab	0.93 ± 0.32ab	5.62 ± 2.59b-e
4.1.3	NRC	33.46 ± 24.64abc	0.22 ± 0.03ab	0.99 ± 0.47abc	1.48 ± 0.48ab	4.53 ± 2.02a-e
4.1.3	NGA	40.43 ± 40.21abc	1.09 ± 0.68d	5.91 ± 2.38a-e	7.17 ± 2.69a-e	4.95 ± 2.43a-e
4.1.3	NGB	15.47 ± 9.83abc	0.78 ± 0.23a-d	7.34 ± 2.54a-f	8.23 ± 2.88a-e	8.34 ± 3.90e
4.1.3	NGC	38.77 ± 19.36abc	0.65 ± 0.18a-d	8.01 ± 2.41b-f	10.40 ± 3.56b-e	3.58 ± 1.79a-d
6.1.3	PRA	10.26 ± 6.26ab	0.18 ± 0.07a	0.62 ± 0.32ab	0.99 ± 0.29ab	3.22 ± 1.43a-d
6.1.3	PRB	14.95 ± 8.36abc	0.21 ± 0.03ab	0.31 ± 0.01ab	0.41 ± 0.04a	3.03 ± 1.10a-d
6.1.3	PRC	35.95 ± 35.26abc	0.31 ± 0.05abc	0.26 ± 0.10ab	0.34 ± 0.10a	1.20 ± 0.32a
6.1.3	PGA	16.64 ± 15.10abc	1.09 ± 0.40d	6.83 ± 2.58a-e	7.50 ± 2.64a-e	3.05 ± 1.54a-d
6.1.3	PGB	26.58 ± 26.09abc	0.78 ± 0.24a-d	5.74 ± 4.33a-e	6.77 ± 4.78a-e	6.40 ± 2.57cde
6.1.3	PGC	12.34 ± 9.84abc	0.79 ± 0.28a-d	10.36 ± 5.19ef	14.20 ± 8.35e	4.97 ± 1.95a-e
7.1.3	LRA	21.94 ± 16.22abc	0.20 ± 0.03ab	0.21 ± 0.05a	0.52 ± 0.21a	3.92 ± 1.65a-d
7.1.3	LRB	25.23 ± 20.40abc	0.16 ± 0.09a	0.17 ± 0.02a	0.34 ± 0.05a	1.80 ± 0.77ab
7.1.3	LRC	64.74 ± 57.94a-d	0.18 ± 0.03a	0.53 ± 0.09ab	0.98 ± 0.28ab	3.53 ± 1.45a-d
7.1.3	LGA	6.03 ± 5.80ab	1.04 ± 0.60cd	6.04 ± 2.43a-e	8.23 ± 3.93a-e	2.26 ± 0.98ab
7.1.3	LGB	15.64 ± 15.27abc	0.93 ± 0.44bcd	6.79 ± 2.48a-e	10.93 ± 5.76cde	4.09 ± 1.93a-d
7.1.3	LGC	22.76 ± 13.69abc	0.67 ± 0.19a-d	9.14 ± 3.54ef	11.55 ± 4.90de	4.39 ± 2.05a-d
6.1.3	ORA	61.11 ± 45.04a-d	0.24 ± 0.02ab	1.07 ± 0.49a-d	1.61 ± 0.63abc	2.91 ± 1.47a-d
6.1.3	ORB	109.96 ± 94.05bcd	0.22 ± 0.02ab	1.46 ± 0.62a-d	3.06 ± 1.45a-d	3.08 ± 1.57a-d
6.1.3	ORC	147.32 ± 88.45d	0.23 ± 0.07ab	8.65 ± 6.45def	10.37 ± 6.59b-e	1.18 ± 0.48a
6.1.3	OGA	40.53 ± 20.63abc	0.77 ± 0.35a-d	8.43 ± 3.07c-f	9.47 ± 3.47a-e	6.73 ± 3.57de
6.1.3	OGB	85.86 ± 24.79a-d	0.81 ± 0.37a-d	7.42 ± 3.94a-f	9.10 ± 4.83a-e	2.45 ± 0.67abc
6.1.3	OGC	115.89 ± 10.98cd	0.56 ± 0.12a-d	14.51 ± 4.58f	16.05 ± 4.95e	4.83 ± 2.68a-e
Sig p<0.05		0.087 (ns)	0.018	<.001	<.001	0.005
LSD ²		85.79	0.63	6.38	7.86	3.29
CV % ³		137.00	61.60	87.10	85.00	52.30
Fertiliser (F) ⁴		<0.001	ns	0.035	ns	0.008
CR	Medium (G)	ns	<0.001	<0.001	<0.001	0.006
vs	Levels (C)	ns	ns	0.050	ns	ns
Treatments	F x G	ns	ns	ns	ns	ns
	F x C	ns	ns	ns	ns	ns
	G x C	ns	ns	ns	ns	ns
Fertiliser (F) ⁴		<0.001	ns	ns	ns	0.009
CG	Medium (G)	ns	<0.001	<0.001	<0.001	0.006
vs	Levels (C)	ns	ns	0.050	ns	ns
Treatments	F x G	ns	ns	ns	ns	ns
	F x C	ns	ns	ns	ns	ns
	G x C	ns	ns	ns	ns	ns

1. Means (±SE) within columns followed by the same letter do not differ significantly according to Duncan's Multiple Range Test at p < 0.05. Non-significant (ns) or significant at p < 0.05 (*), 0.01 (**) or 0.001 (***)

2. LSD –Least significant difference at p < 0.05

3. CV (%) – Percentage coefficient of variance

4. Main effects and interactions at both control levels. Non-significant (ns) or significant at P < 0.05 (*), 0.01 (**) or 0.001 (***)

5. CR – Control in Randles growth medium, CG – Control in Gromor growth medium, N – Nitrosol fertiliser, P – Polyfeed fertiliser, L – Lawn and Leaf fertiliser, O – Organic Vita boost fertiliser, R – Randles growth medium, G – Gromor growth medium, A – ½ label rate, B – label rate and C – 2 x label rate, Table adapted from (Bi *et al.*, 2010)

4.2.6 Leachate composition

4.2.6.1 Algal chlorophyll concentration in leachate

Initial analysis of leachate from the soil-based (CR) and the bark-based (CG) growth media before commencement of fertilisation, revealed PChl *a* to be lower in the soil ($11 \mu\text{g}\cdot\text{L}^{-1}$) than in the bark-based medium ($52 \mu\text{g}\cdot\text{L}^{-1}$). Mean leachate PChl *a* concentrations ranged from $1.20 \mu\text{g}\cdot\text{L}^{-1}$ to $8.34 \mu\text{g}\cdot\text{L}^{-1}$, with NGB significantly higher ($p=0.005$, Table 4.6) than most treatments. The main effects of the factors, fertiliser type and growth medium type had affected leachate chlorophyll significantly, evidenced when treatments were statistically compared to the randles control ($p=0.008$ and $p=0.006$, respectively) and when treatments were statistically compared to the gromor control ($p=0.009$ and $p=0.006$, respectively).

4.2.6.2 Phase contrast light microscope algae identification

Only one algal microorganism was identified from all samples observed under the microscope using keys provided by John *et al.* (2002) and Bellinger and Sigeo (2015). These keys only allow identification up to the genus level. It was, therefore not certain whether *Klebsormidium flaccidum* or *K. nitens* was the observed specimen, as these species bear similarities in morphology and chloroplast characteristics (Fig. 4.1). Filamentous cells of *K. nitens* should be thinner than cells of *K. flaccidum* (Škaloud, 2006). Strains with a cell width of $6 \mu\text{m}$ and below should represent *K. nitens* (Škaloud, 2006). Although cells of *K. flaccidum* appeared to be thicker, the results were not conclusive. This species is, therefore, described as *Klebsormidium sp.*

4.2.6.3 Scanning electron microscope (SEM) algae identification

Only *Bacillariophyta* algal organisms were present in the growth medium leachates. Several pennate diatom cells were observed under the SEM. Various sources and literature, including

keys by Weber, (1971), Compère (2000) and Bellinger and Sigeo (2015) aided in the identification of the diatoms as well as Van Vuuren *et al.* (2006); Taylor *et al.* (2007); Spaulding *et al.* (2010) and Antonelli *et al.* (2017). Diatom taxa were identified to species level using valve face structure and morphological characteristics, such as shape and ornamentation. Five diatom species were identified, belonging to five genera: *Amphora montana* Krasske, *Navicula veneta* Kützing, *Nitzschia amphibia* Grunow, *Planothidium engelbrechtii* (Cholnoky) Round & Bukhityarova, and *Tryblionella levidensis* W Smith. A further two genera, *Microscostatus sp.* and *Planothidium sp.*, were identified, but not the species.

4.2.7 SEM EDX qualitative analysis of mineral elements in growth media

Energy Dispersive X-ray (EDX) spectroscopy was used to identify mineral elements in the growth media (Figure 4.9, 4.10). These qualitative results showed, as expected, a much higher Si content in the soil-based randles than the bark-based gromor medium (Table 4.7. Mean Al, Ca, Fe, Mg and P results showed significant differences between treatments ($p < 0.05$); the growth medium influenced the presence of all of these elements significantly. The trends obtained by statistical analysis show Al, Fe and Si concentrations to be significantly higher in the randles growth medium than in the gromor one and Ca, Mg and P concentrations significantly higher in gromor than in randles growth medium.

Table 4.7 Effects of two organic (Nitrosol[®] and Organic Vitaboost) and two inorganic fertiliser treatment types (Polyfeed[®] and Lawn and Leaf) applied at three concentrations to two different growth media, on SEM growth media qualitative analysis of Al, Si, P, Ca, Fe and Mg concentrations compared with the two controls after a period of 90 days

Type	Treatment	Al %	Si %	P %	Ca %	Fe %	Mg %
Control	CR ⁵	6.93 ± 2.60e ¹	18.89 ± 4.20d	0.08 ± 0.02a	0.46 ± 0.07a	2.94 ± 0.68ab	0.16 ± 0.00a-d
Control	CG	1.48 ± 0.55ab	9.30 ± 5.08abc	0.26 ± 0.11a	1.71 ± 0.13a	1.17 ± 0.32ab	0.27 ± 0.08bcd
4.1.3	NRA	4.73 ± 0.77de	17.92 ± 0.48d	0.13 ± 0.00a	0.59 ± 0.11a	3.42 ± 0.86ab	0.11 ± 0.02ab
4.1.3	NRB	4.94 ± 1.33de	16.42 ± 4.16cd	0.10 ± 0.02a	0.56 ± 0.08a	3.27 ± 1.38ab	0.14 ± 0.03abc
4.1.3	NRC	5.68 ± 2.70e	20.19 ± 3.09d	0.08 ± 0.06a	0.49 ± 0.13a	3.12 ± 1.51ab	0.17 ± 0.06a-d
4.1.3	NGA	1.28 ± 0.33ab	5.69 ± 1.56ab	0.36 ± 0.19a	2.36 ± 1.49a	0.95 ± 0.19a	0.32 ± 0.13d
4.1.3	NGB	1.46 ± 0.53ab	5.93 ± 3.50ab	0.33 ± 0.23a	2.21 ± 1.17a	1.27 ± 0.59ab	0.25 ± 0.08a-d
4.1.3	NGC	1.37 ± 0.32ab	13.45 ± 0.36bcd	0.30 ± 0.18a	1.27 ± 0.71a	1.25 ± 0.80ab	0.17 ± 0.06a-d
6.1.3	PRA	6.30 ± 1.52e	15.91 ± 3.23cd	0.14 ± 0.05a	0.58 ± 0.18a	5.74 ± 2.52c	0.11 ± 0.02ab
6.1.3	PRB	4.07 ± 1.10a-e	17.63 ± 0.19d	0.20 ± 0.18a	1.06 ± 0.79a	3.11 ± 0.17ab	0.14 ± 0.04abc
6.1.3	PRC	5.16 ± 0.61e	18.48 ± 2.89d	0.16 ± 0.07a	0.83 ± 0.40a	3.60 ± 0.38b	0.23 ± 0.04a-d
6.1.3	PGA	1.51 ± 0.33ab	8.00 ± 0.33ab	0.40 ± 0.32a	1.84 ± 1.22a	1.15 ± 0.43ab	0.25 ± 0.12a-d
6.1.3	PGB	1.36 ± 0.01ab	5.55 ± 0.60ab	0.37 ± 0.25a	2.44 ± 1.22a	1.46 ± 0.32ab	0.29 ± 0.11cd
6.1.3	PGC	2.15 ± 0.11a-d	6.79 ± 0.60ab	0.34 ± 0.18a	1.73 ± 0.81a	1.33 ± 0.10ab	0.24 ± 0.09a-d
7.1.3	LRA	4.90 ± 1.01de	19.80 ± 2.27d	0.13 ± 0.08a	0.51 ± 0.12a	3.06 ± 0.23ab	0.13 ± 0.02abc
7.1.3	LRB	6.05 ± 0.36e	18.04 ± 0.22d	0.16 ± 0.11a	0.73 ± 0.44a	2.92 ± 0.50ab	0.10 ± 0.00a
7.1.3	LRC	4.36 ± 1.20cde	17.88 ± 0.19d	0.16 ± 0.07a	0.57 ± 0.24a	3.00 ± 0.80ab	0.13 ± 0.02abc
7.1.3	LGA	1.40 ± 0.16ab	7.19 ± 2.41ab	0.29 ± 0.17a	1.64 ± 0.66a	1.03 ± 0.06a	0.22 ± 0.03a-d
7.1.3	LGB	1.33 ± 0.11ab	7.56 ± 3.31ab	0.29 ± 0.14a	2.08 ± 0.54a	1.37 ± 0.03ab	0.21 ± 0.05a-d
7.1.3	LGC	1.64 ± 0.08abc	5.01 ± 1.59a	0.47 ± 0.27a	2.52 ± 1.55a	1.21 ± 0.56ab	0.32 ± 0.12d
6.1.3	ORA	5.74 ± 0.91e	19.69 ± 0.63d	0.12 ± 0.04a	0.52 ± 0.20a	2.42 ± 0.16ab	0.11 ± 0.04ab
6.1.3	ORB	5.37 ± 0.21e	16.80 ± 1.05cd	0.16 ± 0.05a	0.54 ± 0.10a	3.14 ± 1.10ab	0.14 ± 0.01abc
6.1.3	ORC	4.20 ± 0.04b-e	17.45 ± 3.07d	0.18 ± 0.07a	0.61 ± 0.02a	2.98 ± 0.26ab	0.19 ± 0.04a-d
6.1.3	OGA	1.36 ± 0.01ab	6.50 ± 2.20ab	0.40 ± 0.24a	2.13 ± 0.97a	1.21 ± 0.11ab	0.29 ± 0.05cd
6.1.3	OGB	1.15 ± 0.02a	5.54 ± 1.28ab	0.41 ± 0.24a	2.22 ± 1.05a	1.10 ± 0.11ab	0.25 ± 0.05a-d
6.1.3	OGC	1.49 ± 0.04ab	5.78 ± 1.34ab	0.47 ± 0.29a	2.59 ± 1.23a	1.16 ± 0.13ab	0.25 ± 0.07a-d
Sig p<0.05		<0.001	<0.001	0.829	0.241	0.008	0.032
LSD ²		2.51	7.08	0.44	1.99	2.13	0.14
CV % ³		36.20	27.30	87.20	72.50	46.10	34.30
Fertiliser(F) ⁴		0.011	ns	ns	ns	ns	ns
CR	Medium (G)	<0.001	<0.001	0.002	<0.001	<0.001	<0.001
vs	Levels (C)	ns	ns	ns	ns	ns	ns
Treatments	F x G	ns	ns	ns	ns	ns	ns
	F x C	ns	ns	ns	ns	ns	ns
	G x C	ns	ns	ns	ns	ns	ns
Fertiliser(F) ⁴		ns	ns	ns	ns	ns	ns
CG	Medium (G)	<0.001	<0.001	0.001	<0.001	<0.001	<0.001
vs	Levels (C)	ns	ns	ns	ns	ns	ns
Treatments	F x G	ns	ns	ns	ns	ns	ns
	F x C	ns	ns	ns	ns	ns	ns
	G x C	ns	ns	ns	ns	ns	ns

1. Means (±SE) within columns followed by the same letter do not differ significantly according to Duncan's Multiple Range Test at p < 0.05. Non-significant (ns) or significant at p < 0.05 (*), 0.01 (**) or 0.001 (***)

2. LSD –Least significant difference at p < 0.05

3. CV (%) – Percentage coefficient of variance

4. Main effects and interactions at both control levels. Non-significant (ns) or significant at P < 0.05 (*), 0.01 (**) or 0.001 (***)

5. CR – Control in Randles growth medium, CG – Control in Gromor growth medium, N – Nitrosol fertiliser, P – Polyfeed fertiliser, L – Lawn and Leaf fertiliser, O – Organic Vita boost fertiliser, R – Randles growth medium, G – Gromor growth medium, A – ½ label rate, B – label rate and C – 2 x label rate, Table adapted from (Bi *et al.*, 2010)

4.3 Discussion

4.3.1 Analyses of irrigation water

Analysis of irrigation water demonstrated the lowest salinity level (C1) and lowest sodicity level (S1), according to the United States Department of Agriculture classification. The EC of irrigation water ($24.32 \text{ mS}\cdot\text{cm}^{-1}$) used in this study was acceptable (Table 4.3) as it fell into the most suitable category ($\text{C1} < 250 \text{ mS}\cdot\text{cm}^{-1}$). Concentration of sodium (Na) relative to calcium (Ca) plus magnesium (Mg), known as the sodium adsorption ratio (SAR) in analysed water, was considered to be also acceptable for irrigation use. The sodicity level (SAR) of irrigation water used in this study (1.21) fell within the 'low' category (S1, 1-10). The pH of 7.64 was higher than the acceptable range of 5.4 to 6.8 for container production according Bailey *et al.* (2005). This did not seem to have an effect on the various species grown at Randles nursery for landscape use within the Ethekeeni municipality. No remedial action was, therefore, taken. Sodium (Na), calcium (Ca), magnesium (Mg), potassium (K), total alkalinity (TA) and chlorine (Cl) concentrations ($\text{me}\cdot\text{L}^{-1}$) of irrigation water (Table 4.3) were all below the recommended upper limits (Bailey *et al.*, 1999) of 3, 6, 2, 0.26, 2 and 2, respectively.

4.3.2 Analyses of growth media before planting

Growth media EC and pH were also within recommended (Abad *et al.*, 2001) ranges ($0.75 - 3.49 \text{ mS}\cdot\text{cm}^{-1}$ and pH 5.2 – 6.3, respectively). Soil-based growth medium N, P, Ca, Fe concentrations in both used media (Table 4.4) were comparable to N (0.43%), P (0.15%), Ca (1.03%) and Fe ($25800 \text{ mg}\cdot\text{kg}^{-1}$) in the commercial potting medium Metro-Mix 360 (Scotts, Marysville, Ohio) used by Atiyeh *et al.* (2001) for tomato (*Solanum lycopersicum*) production. Concentrations of N, P and Ca in bark-based growth medium used in this study were higher

than in soil-based growth media and Metro-Mix 360, except for Fe, which was lower than in soil-based growth media and Metro-Mix 360. All nutrients analysed in growth media were, therefore, at acceptable levels for potting media use, except for Cu ($157.69 \text{ mg}\cdot\text{kg}^{-1}$) in soil-based growth medium, which was marginally over the limit ($150 \text{ mg}\cdot\text{kg}^{-1}$) recommended by Bunt (2012). This would have corrected through irrigation prior to treatments.

4.3.3 Effects of treatments on leachate Al, Ca, Fe, Mg and forms of P concentration

The leaching of fertilisers from nurseries is contributing to the pollution of freshwater systems due to nurseries applying fertiliser in excess of plant needs (Majsztrik *et al.*, 2011). In this particular study the effects of organic and inorganic fertilisers on soil- *versus* bark-based growth media leachate composition were investigated using two growth media.

Amongst the leached nutrients investigated, P, Al and Fe are the only elements listed in the South African Environmental Protection Act (No. 44 of 2003) for which effluent discharge limits to freshwater bodies have been set. Significantly more Fe and Al were leached by soil-based water soluble treatments compared with water-soluble bark-based treatments. Pelleted and granular fertiliser treatments leached similar concentrations of Al and Fe from both growth media. Concentrations exceeded the permissible limits (5 and $2 \text{ mg}\cdot\text{L}^{-1}$, respectively) by more than 1000-fold at the time of sampling (Table 4.5) from both growth media. Ingestion of high concentrations of Al and Fe in water is harmful to human and fish health and toxicity can cause irreversible tissue damage (Crafford and Avenant-Oldewage, 2011). Calcium and magnesium affect hardness of water and have significance in aquaculture (Wurts, 1993), but, along with K, are not an environmental threat. Calcium and magnesium compounds which may have formed insoluble phosphates under alkaline conditions were not favoured, as pH was slightly acidic for all treatments.

According to water quality criteria set by the US Environmental Protection Agency (USEPA, 1986) total P concentration should not exceed $0.025 \text{ mg}\cdot\text{L}^{-1}$ within lakes or reservoirs (Owen *et al.*, 2008). To achieve this in the US, the recommended best management practice advocated for P concentrations in growth media nutrient solutions is $10\text{-}15 \text{ mg}\cdot\text{L}^{-1}$ (Yeager *et al.*, 1997). Leachates from most bark-based treatments (Table 4.5) exceeded this level when extracted using Wright's (1986) pour-through method. The results of this study are in agreement with Scagel (2003) and Oh *et al.* (2016). These authors described P to leach more readily from peat-amended and peat-perlite growth media than from coir-amended and soil-amended peat-perlite media, respectively.

Treating the bark medium with high concentrations of Polyfeed (PGC) or high amounts of Vitaboost (OGC) resulted in the highest $\text{PO}_4\text{-P}$ leachates, exceeding the limit of $10 \text{ mg}\cdot\text{L}^{-1}$ $\text{PO}_4\text{-P}$ (South African Environmental Protection Act, 2002), for land or underground discharge (Table 4.6). All bark-based treatments and soil-based Organic Vitaboost treatments (ORA, ORB and ORC) exceeded the $1 \text{ mg}\cdot\text{L}^{-1}$ $\text{PO}_4\text{-P}$ discharge limit into water courses or water bodies. The TP leachate concentrations were significantly different between growth media, with randles producing less phosphate, leachate TP ($0.34 \text{ mg}\cdot\text{L}^{-1}$ - $10.37 \text{ mg}\cdot\text{L}^{-1}$, 0.000034% - 0.0010%) than the bark-based medium ($6.77 \text{ mg}\cdot\text{L}^{-1}$ - $16.05 \text{ mg}\cdot\text{L}^{-1}$, 0.00068% - 0.0016%). No significant differences between liquid, granular and pelletised fertiliser were observed with respect to P forms (P, $\text{PO}_4\text{-P}$ and TP) in the leachate and by extension organic and inorganic fertilisers.

It is unclear, what the $\text{PO}_4\text{-P}$ leaching losses from treatments amounted to over the course of this study, but $49.4 \text{ kg}\cdot\text{ha}^{-1}$ may be potentially lost annually, assuming a density of $80\,000 \text{ pots}\cdot\text{ha}^{-1}$, with foliage plants grown in Margate fine sand and fertilised with a soluble granular fertiliser at rate of $6.1 \text{ g}\cdot\text{pot}^{-1}$ (Broschat, 1995). If the same density of plants would have been

used in this study, a potential release of between $0.014 \text{ kg}\cdot\text{L}^{-1}$ at the lowest leached concentration $\text{PO}_4\text{-P}$ and $0.69 \text{ kg}\cdot\text{L}^{-1}$ at the highest leached concentration $\text{PO}_4\text{-P}$ from the randles mix would have resulted, using the lower and upper levels of leachate $\text{PO}_4\text{-P}$ (Table 4.6). For the bark-based medium between 0.304 and $1.16 \text{ kg PO}_4\text{-P}\cdot\text{L}^{-1}$ would have been leached in only one irrigation event. Leached $\text{PO}_4\text{-P}$ concentrations exceeded recommended levels for most treatments used in this study and represent the potential to cause eutrophication. Extrapolating leached $\text{PO}_4\text{-P}$ over the production cycle of a nursery crop, as presented here, would suggest the urgent need for strategies to mitigate the effects of fertiliser use, especially the use of soilless growth media. Runoff from production areas of some ornamental plant nurseries may discharge directly into fresh water bodies during irrigation, storm or heavy rainfall events (Sharma *et al.* 2008, Fig 4.8), which may enrich and pollute water quicker than from sites further away.

4.3.4 Effects of treatments on growth media N

Nitrate dissolves readily in water and leaches easily with water due to its anionic nature and the generally low anion-holding capacity of growth media (Matysiak, 2015). Although P is the primary limiting nutrient in South Africa (Lai, 2013), N may be equally or more important than P as a limiting nutrient for enrichment in low latitude warm-water and eutrophic systems in general (Hart and Harding, 2015). Samples for mean growth media nitrate ($\text{NO}_3\text{-N}$) and total nitrogen (TN) concentrations were taken at termination of the experiment to correlate concentrations at end of the production period, with plant growth and nutrient leaching potential.

The increase in $\text{NO}_3\text{-N}$ in all growth media with increasing fertiliser rates, except for NGB and PGC (Table 4.6) may imply that $\text{NO}_3\text{-N}$ leached or was taken up from growth media for these treatments. Both, NGB and PGC were among the eight treatments resulting in the tallest

plants with PGC achieving the longest internode (Table 3.12). It is not clear whether $\text{NO}_3\text{-N}$ leached at higher rates than the limit set by the South African Environmental Protection Act in discharge onto land or into a watercourse (higher than $10 \text{ mg}\cdot\text{L}^{-1}$, General notice No. 44 of 2003) as leachate was not tested for $\text{NO}_3\text{-N}$.

Broschat (1995) found soluble granular fertilisers to leach more $\text{NO}_3\text{-N}$ than liquid fertilisers when used in a pinebark, peat and sand potting mix (5:4:1 v/v/v). Application and reapplication of Organic Vitaboost fertiliser resulted in higher growth media $\text{NO}_3\text{-N}$ concentrations at the end of this study compared with the other treatments in both growth media. It may be reasonable to assume that higher concentrations of $\text{NO}_3\text{-N}$ leached from growth media treated with Organic Vitaboost given its anionic nature. In the Broschat (1995) study, a sandy soil growth medium leached 54% of applied N, whilst a 5 pine bark : 4 sedge peat : 1 sand medium (by volume) -potting mix leached 52% N of soluble granular fertiliser treatment.

Total N concentrations in randles medium were significantly lower (0.16% - 0.31%) than those of the groups containing the bark-based medium (0.56%-1.09%). Lack of P is, however of more concern, in South African freshwater systems with respect to growth media leachate runoff.

The TN:TP ratio is used to determine the limiting nutrient (N or P) in freshwater systems, with a TN:TP ratio greater than 10:1 signifying P limitation. This scenario is desirable from a management perspective, as P is easier to control and favours population growth of green algae which may be less problematic than that of cyanobacteria (van Ginkel, 2002). It appears highly probable that TN leachates, if having been measured from samples in this study, would yield a TN:TP ratio that is greater than 10:1. It, therefore, appears highly probable, that TN

and TP leachate in runoff to fresh water bodies over a period of time and assessed from a study like this, is desirable with respect to the high TN:TP ratio.

4.3.5 Algal presence in leachate

Chlorophyll *a* (PChl *a*) in water indicates the presence of algae. Results of initial leachate PChl *a* analysis were $11 \mu\text{g}\cdot\text{L}^{-1}$ and $52 \mu\text{g}\cdot\text{L}^{-1}$ for the two growth media controls, CR and CG, respectively. The PChl *a* concentration in the leachate from the soil-based growth medium (CR) was one fifth of the leachate from the bark-based growth medium. The addition of Nitrosol to the bark-based medium at medium rate (NGB) resulted in highest PChl *a* concentration, while the Nitrosol addition to the randles growth medium (NRA, NRB), Polyfeed to gromor at medium rate (PGB) and Organic Vitabooost to gromor at low label rate (OGA) resulted in PChl *a* concentrations that were significantly higher than all the other treatments ($p=0.005$).

The main effects of fertiliser and growth medium were statistically significantly different at $p=0.008$ and $p=0.006$, respectively for the CR comparison, with $p=0.009$ and $p=0.006$, respectively for the CG comparison. Mean PChl *a* concentrations had dropped from the initial analysis especially during the third repetition of this study, and ranged from $1.20 \mu\text{g}\cdot\text{L}^{-1}$ to $8.34 \mu\text{g}\cdot\text{L}^{-1}$ (Table 4.6). This implies that algal organisms had leached out. It also suggests that the rate of increase of these organisms in the growth media was lower than the rate at which they leached. Concentrations of PChl *a* were much lower than the $30 \mu\text{g}\cdot\text{L}^{-1}$, and more, in a freshwater system which indicate hyper-eutrophic status due to a large population of microalgae. This is only one of the parameters used to assess trophic status. The high PChl *a* concentration in the gromor control ($52 \mu\text{g}\cdot\text{L}^{-1}$) may not appear to be of major concern but does warrant further investigation into algal content when using these media as it represents the potential to increase biomass when loading to freshwater systems.

Of the algal organisms identified, the green algae *Klebsormidium flaccidum* or *Klebsormidium nitens* (Fig. 4.1) are terrestrial and no literature seems to indicate that either of them can be found in freshwater. *Klebsormidium sp.* are considered cosmopolitan and widespread in different soil types (Zancan *et al.*, 2006). Diatoms and one unidentified specimen (Fig. 4.7) were the only algal organisms observed under the scanning electron microscope (SEM).

Amphora montana Krasske (Fig. 4.3, 4.6) is a cosmopolitan species which rarely becomes dominant in the alkaline waters in which it is found (Taylor *et al.*, 2007). *Amphora montana* has been found in soil on sites that are characteristically wet and periodically dry (Stanek-Tarkowska and Noga, 2012). The genus *Microcostatus* most commonly develops on wet aerial surfaces, mosses and wet soils (Taylor *et al.*, 2010; Stanek-Tarkowska *et al.*, 2016) although these diatom cells have also been found in water (Stanek-Tarkowska *et al.*, 2016). Taylor *et al.* (2010) described three new species of *Microcostatus* in South Africa which survive in very dry and periodically wet sandy soils, that can be dry for several consecutive months. A new species of *Microcostatus*, found in brackish to saline waters, was recently described by Li *et al.* (2016). The habitat of the observed *Microcostatus sp.* (Fig. 4.2, 4.5) is unknown, as these diatom cells could only be identified up to the genera level. *Navicula veneta* Kützing (Fig. 4.4) is a high nutrient indicator (Potapova and Charles, 2007), often found in heavily eutrophic, electrolyte-rich to brackish water (Taylor *et al.*, 2007). These cosmopolitan species are very pollution-tolerant (Potapova *et al.*, 2005; Taylor *et al.*, 2007) and are often the dominant species in polluted water resulting from industrial effluent and runoff (Taylor *et al.*, 2007). Leachate containing heavy metals from plant production to surface runoff may therefore be potentially harmful. *Nitzschia amphibia* Grunow (Fig. 4.3) is

considered a eutrophic species (Faustino *et al.*, 2016) and is found in waters over a range from electrolyte-poor to electrolyte-rich waters (Taylor *et al.*, 2007).

Bate (2013) reported that *Planothidium engelbrechtii* (Fig. 4.2) is a marine, brackish water and freshwater species. These diatom cells are often associated with saline freshwater, but have also been found in permanent pans (Riato *et al.*, 2014). *Planothidium engelbrechtii* are found abundantly in saline inland waters with very high electrolyte content and are capable of tolerating critical to very heavy organic pollution (Taylor *et al.*, 2007). *Tryblionella levidensis* W Smith (Fig. 4.6) is a cosmopolitan species, especially common in brackish waters, and in waters ranging from moderate electrolyte content to electrolyte-rich waters. These diatom cells are tolerant of strongly polluted conditions (Taylor *et al.*, 2007). Some diatom cells observed had ruptured frustules. It is not clear, if the chloroplasts of those diatom cells shown here were intact.

The types of diatoms identified from leachate in this study are characterised by a variety of conditions found in freshwater systems. Two of the diatom species, *Nitzschia amphibia* Grunow and *Navicula veneta* Kützing, are found in eutrophic waters and have been found in riparian zones and wetlands. Little information is available on soil microalgae found in areas surrounding agricultural sites and commercial nurseries or the hillslopes of watersheds in South Africa. It is plausible that when water flows between such areas, microalgae are able to migrate to freshwater bodies. Plants are watered regularly in commercial production nurseries in order to maintain high plant quality. Runoff areas therefore also remain moist. Diatoms may be transported in water flow and between elements of the hydrological cycle due to their small size (Pfister *et al.*, 2009). *Hantzschia amphioxys* (Ehrenberg) Grunow, which favours periodically dry habitats including soils and rock crevices, lives also in freshwater systems. It

grows larger in comparison to *N. amphibia* Grunow and *N. veneta* Kützingand, and is said to be washed into freshwater bodies from soils (Taylor *et al.*, 2007).

In a study by Martínez-Carreras *et al.* (2015) in the Weierbach catchment, Luxembourg, aerial diatoms (*i.e.*, diatoms nearly exclusively occurring outside water bodies and in wet and moist or temporarily dry places) served as natural tracers to investigate connectivity between hillslopes, riparian zones and stream water during rainfall events. Results from that study showed diatom cell abundance in overland flow from hillslope to be low and that there was greater connectivity between the riparian zone and stream water because of aerial diatoms found in stream water that were discharged after rainfall events. Similar studies under South African conditions may yield different results due to different vegetation and climatic conditions. Pfister *et al.* (2017) confirmed that terrestrial diatoms may be flushed from their terrestrial habitats to freshwater bodies. Although not detected in this study, cyanobacteria and other green algal species do develop on container growth medium (Brissette *et al.*, 1991), and may also be transported by the similar hydrological effects (surface runoff and erosion) that transport P.

The effects of surface run-off and infiltration from greenhouses and nurseries, over months and years, pose questions. Literature investigating run-off and infiltration from ornamental container plant nurseries seems non-existing in South Africa. Ferreira (2008) found that agricultural land use and practices changed the water quality of the Harts and Vaal rivers in South Africa at the sites studied. The diatom community structure had changed as a result compared with those communities upstream. This was mostly due to mineralisation and salinisation, which appeared to mask the effects of nutrients on diatom communities. The influences of agricultural land use on fresh water systems is further highlighted in a study carried out by Walsh and Wepener (2009) at sites in South Africa on the Crocodile and

Magalies rivers. These authors reported that high intensity agriculture is characterised by motile diatom species of the genus *Nitzschia* and low intensity agriculture by motile diatom species of the genus *Navicula*. It is unclear from both studies if any plant nurseries were in the vicinity of these study sites and whether *Nitzschia amphibia* Grunow and *Navicula veneta* Kützing which are associated with eutrophic waters, could have washed into freshwater bodies and impacted trophic status.

4.3.6 SEM EDX qualitative analysis of growth media

Statistical trend analysis showed patterns in response to treatments. The Al, Fe and Si levels detected (SEM EDX) tended to be higher in the soil-based randles than in the bark-based gromor media (Table 4.7) as expected in soil versus bark. Levels of Ca, Mg and P detected (SEM EDX) tended to be lower in soil-based randles than in the bark-based gromor media. Leachate analysis showed that Ca, Mg, Fe and Al leachates were significantly higher in soil-based than from bark-based media and P concentrations significantly higher in the bark-based than the soil-based media.

It has been described that bark-based (soilless) growth media have little ability to hold P (Scagel, 2003; Oh *et al.*, 2016). The P sorption of growth media is most efficient in the presence of Fe or Al hydroxides or easily soluble Ca or Mg compounds (Klimeski *et al.*, 2012). Higher P retention can be achieved with a high Ca or Mg presence and a basic pH or high Fe or Al content at lower pH (Klimeski *et al.*, 2012).

The mean leachate pH determined from leachate was slightly acidic, ranging from 5.77 – 6.26 for randles and 5.67 – 6.44 for gromor media (Table 3.5). It seems likely that higher Ca and Mg levels at a lower pH range in the bark-based media could not retain P to the extent that soil-based media with higher Fe or Al levels at a lower pH range did. The Si concentrations

detected were significantly higher in randles than in gromor growth media (Table 4.7), but it is unclear, whether Si helped to displace P from Ca and Mg compounds in bark media sites or assisted in good adsorption capacity in soil-based media.

4.4 Conclusion

Elemental Al, Ca, Fe and Mg were only considered in this study because of their relationship with P, the major driver of eutrophication in freshwater bodies. The high concentrations of Al and Fe leached from both growth media are, however, a cause for concern due to possible toxicity to humans and aquatic life as a result of Al and Fe potentially leaching to freshwater systems.

Organic Vitaboost treatments resulted in higher growth media NO₃-N concentrations which potentially represents a higher concentration of applied N leached compared to the other treatments but P is of greater concern in South African freshwater systems. Intensive plant production using bark-based growth media and the fertilisers used in this study poses a potential threat to the eutrophication of freshwater systems. Pelleted Organic Vitaboost and water soluble Polyfeed applied at the highest concentration leached PO₄-P exceeding the legislated South African limit (10 mg·L⁻¹) for land or underground discharge. Application of Lawn and Leaf, Nitrosol and Polyfeed to soil-based growth media were the only treatments with leachates within the limit (PO₄-P < 1 mg·L⁻¹) for watercourse or surface water discharge. The use of soil based growth media is therefore key when applying organic and inorganic fertilisers to keep leachate PO₄-P concentrations within acceptable limits in South Africa.

Chlorophyll *a* concentrations extracted from leachate indicated the presence of microalgae and this was confirmed by the identification of a green algal species and several genera of diatoms. Concentrations of 52 µg·L⁻¹ PChl *a* in freshwater would indicate hyper-eutrophic conditions and a high abundance of microalgae. Chlorophyll *a* concentrations had dropped

from $11 \mu\text{g}\cdot\text{L}^{-1}$ and $52 \mu\text{g}\cdot\text{L}^{-1}$ for the two growth media controls, CR and CG respectively, to $1.20 \mu\text{g}\cdot\text{L}^{-1}$ to $8.34 \mu\text{g}\cdot\text{L}^{-1}$ by the end of the experimental period. This would have most likely been as a result of microalgae leaching. It is, therefore, plausible that microalgae, including cyanobacteria, are able to be flushed from watersheds or directly from close proximity nurseries to freshwater systems. This may result in a substantial increase in algal biomass during heavy rain events, and possibly eutrophication when accompanied by high $\text{PO}_4\text{-P}$ in the runoff.

4.5 Literature cited

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Figure 4.1: Phase contrast microscope images: (A) Image of *Klebsormidium* sp. and (B) Brightfield image of *Klebsormidium* sp.

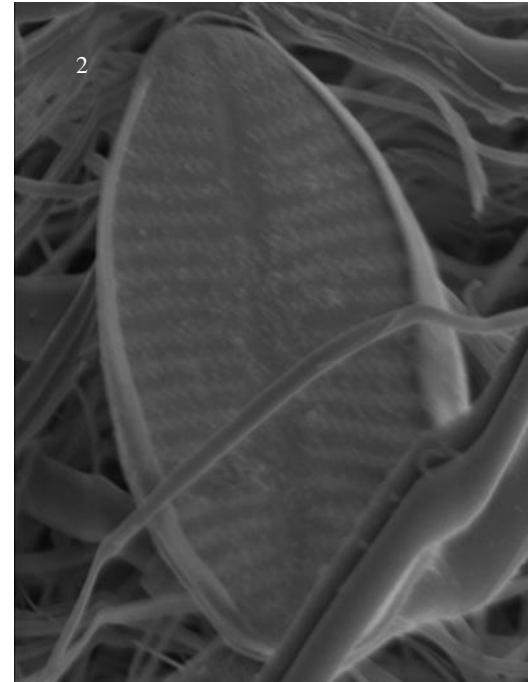
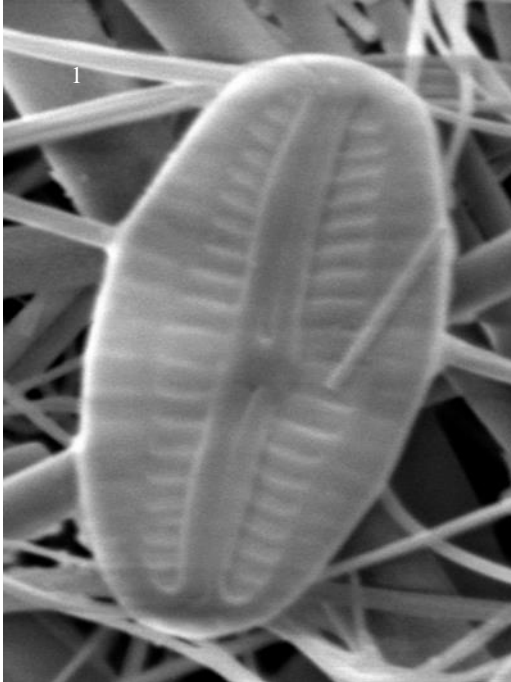


Figure 4.2: SEM micrograph of (1) *Microcostatus* sp. and (2) *Planothidium engelbrechtii*. (Cholnoky) Round & Bukhityarova

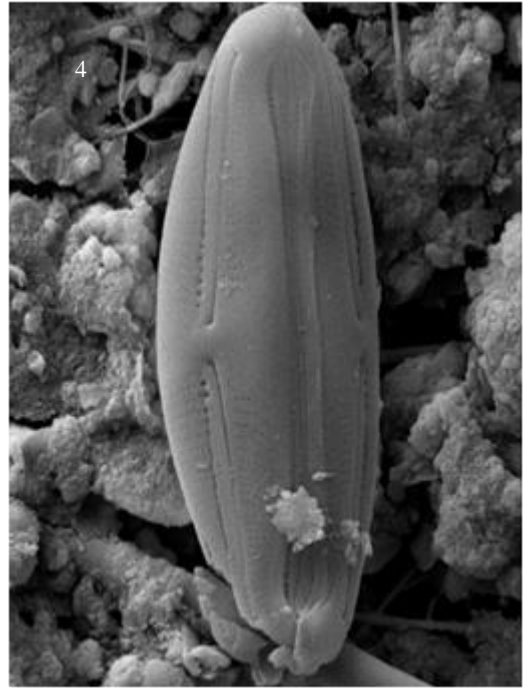
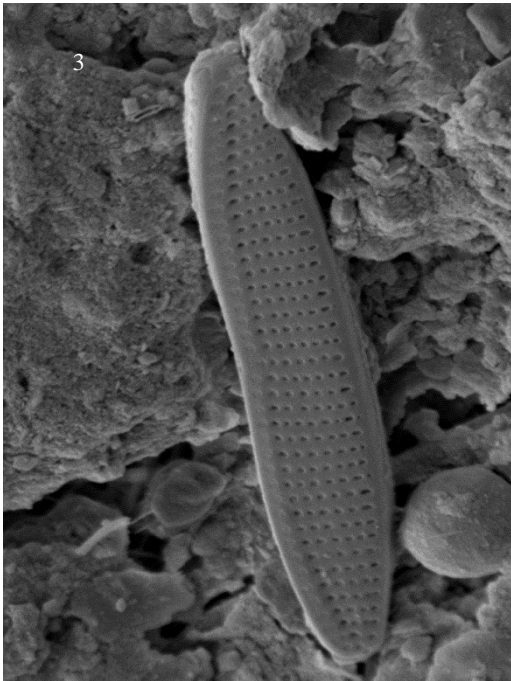


Figure 4.3: SEM micrograph of (3) *Nitzschia amphibia* Grunow and (4) *Amphora montana* Krasske

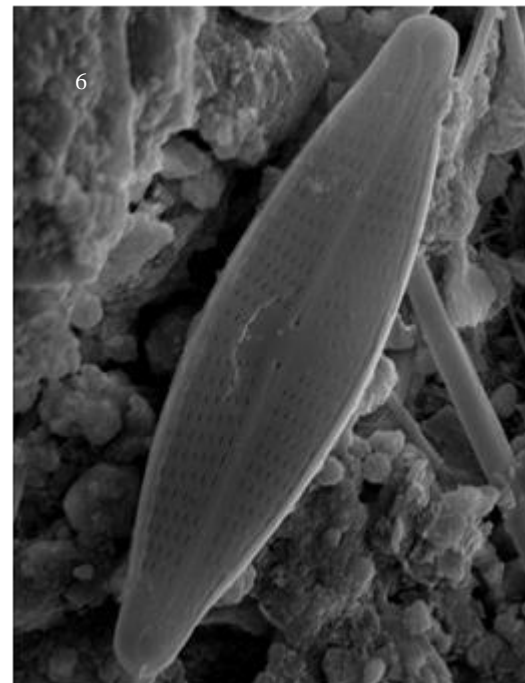


Figure 4.4: SEM micrograph of (5) *Navicula veneta* Kützing and (6) *Navicula veneta* Kützing

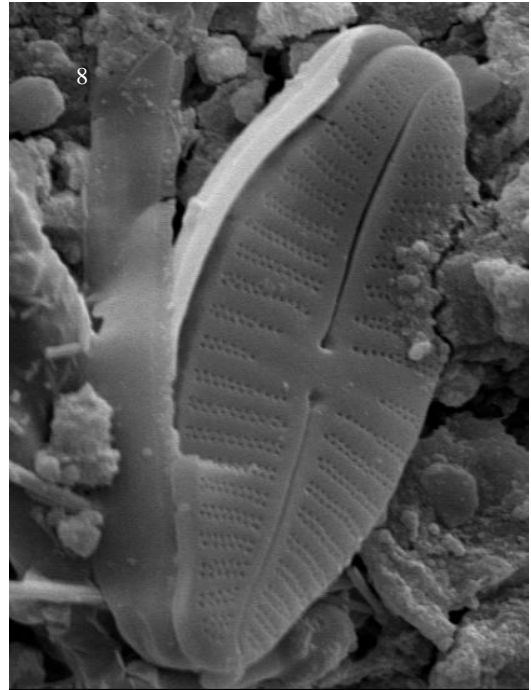
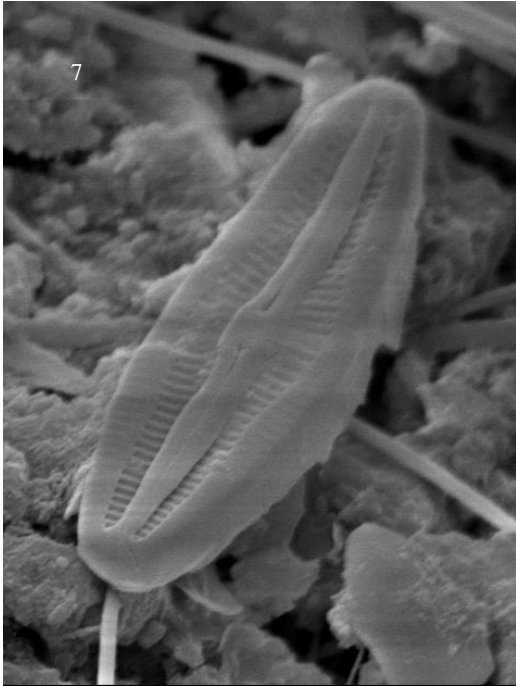


Figure 4.5: SEM micrograph of (7) *Microcostatus* sp. and (8) *Planothidium* sp.

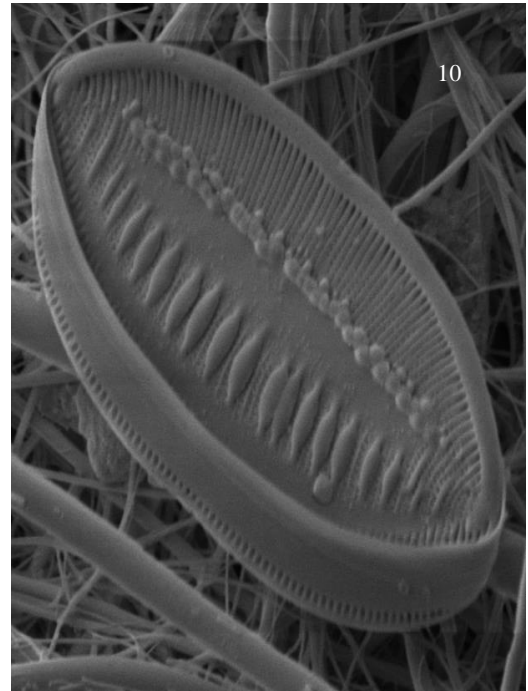
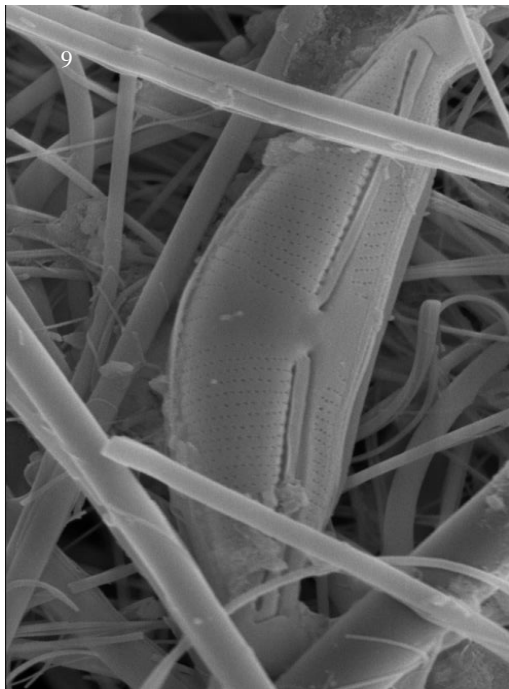


Figure 4.6: SEM micrograph of (9) *Amphora montana* Krasske and (10) *Tryblionella levidensis* W Smith

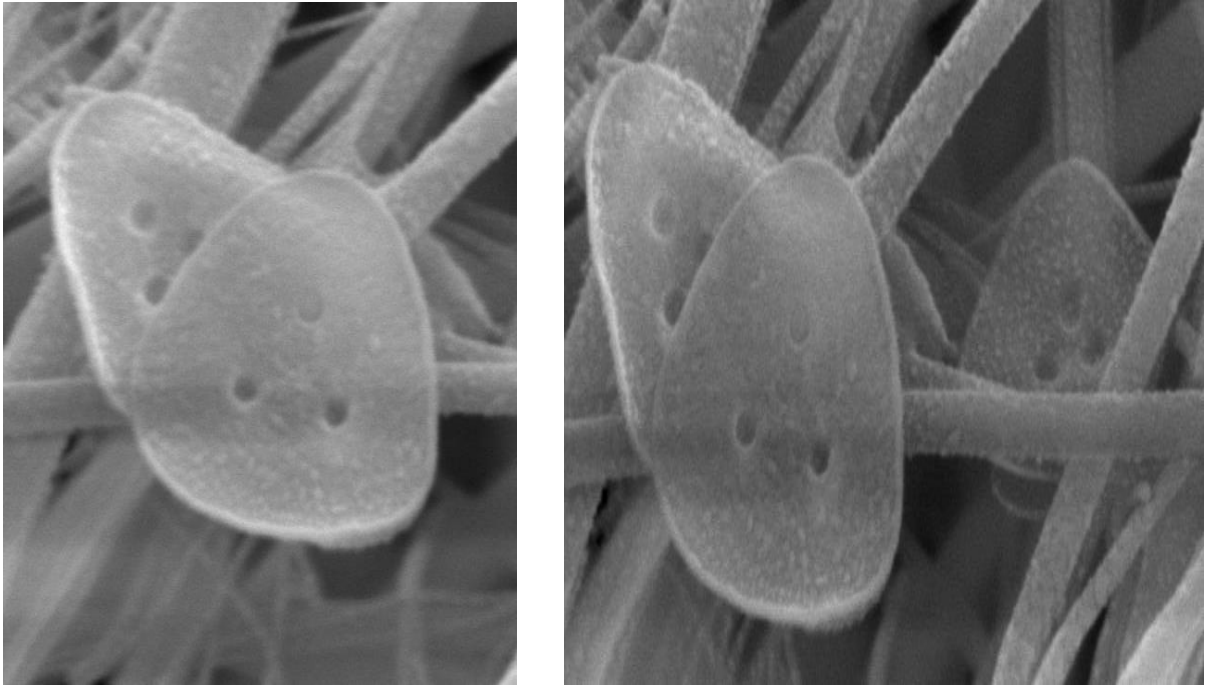


Figure 4.7: SEM micrograph of an unidentified microorganism



Figure 4.8: Runoff from production areas at an ornamental plant nursery. Sharma *et al.*, 2008

Element	Weight%	Atomic%
C K	15.59	22.84
O K	52.70	57.96
Al K	2.85	1.86
Si K	26.14	16.38
S K	0.19	0.10
K K	0.23	0.10
Ca K	0.28	0.12
Ti K	0.18	0.07
Fe K	1.61	0.51
Cu K	0.24	0.07
Totals	100.00	

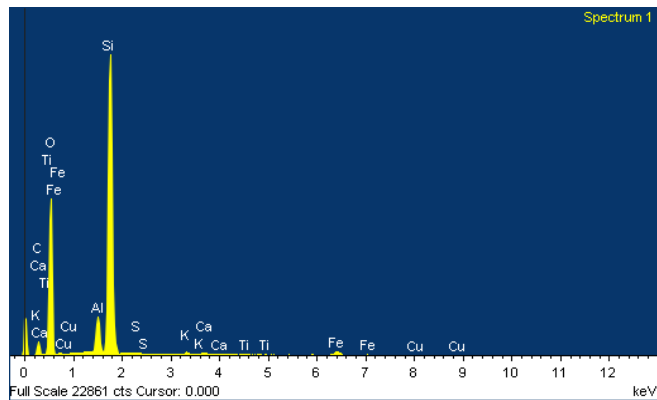
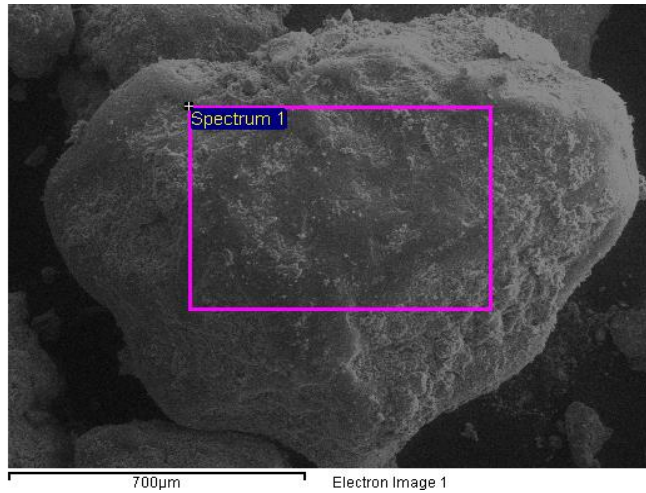


Figure 4.9: Randles growth media control (CR) mineral element analysis from Energy Dispersive X-ray (EDX) spectroscopy with the corresponding image and EDX spectrum.

Element	Weight%	Atomic%
C	56.49	65.43
O	35.74	31.08
Na K	0.32	0.19
Mg K	0.15	0.09
Al K	0.97	0.50
Si K	3.48	1.73
P K	0.19	0.08
S K	0.17	0.07
Cl K	0.45	0.18
K K	0.56	0.20
Ca K	0.79	0.28
Fe K	0.69	0.17
Totals	100.00	

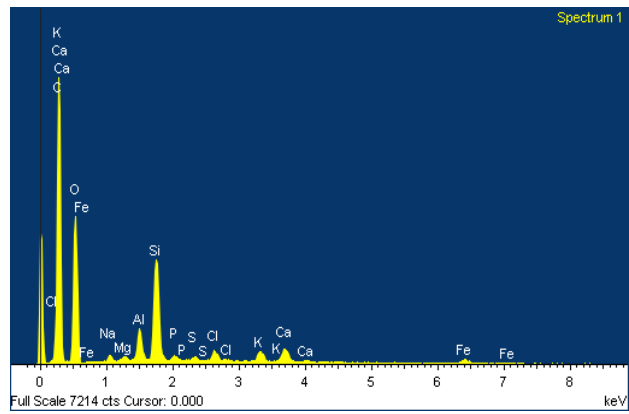
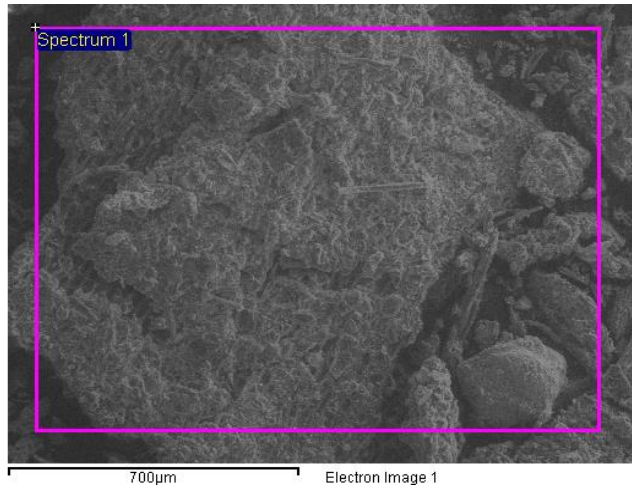


Figure 4.10: Gromor growth media control (CG) mineral element analysis from Energy Dispersive X-ray (EDX) spectroscopy with the corresponding image and EDX spectrum



Figure 4.11: *Pseuderanthemum atropurpureum* grown at Randles Nursery at termination of study

General conclusion and recommendations

Results of this study showed that there were no significant differences between the two types of growth media with respect to the treatments on plant growth parameters or elemental N in leachate. There were also no significant differences between treatments due to type of fertiliser (organic or inorganic) or application method (liquid feed or slow release). The reasons for this may have been due to N being equilibrated. Organic Vitaboost did produce more leaves, branches and nodes than some of the other treatments but growth media $\text{NO}_3\text{-N}$ concentrations were significantly higher than the other treatments at final sampling. This represents the potential to leach more $\text{NO}_3\text{-N}$ but also the potential to fare better as saleable product if not sold relatively quickly in a retail plant nursery. There were, however, significant differences between the two types of growth media with respect to nutrients leached.

The effect of equilibrating N increased the concentrations of Nitrosol, Lawn and Leaf and Organic Vitaboost nutrients applied, including N and P, and decreased the same of Polyfeed from supplier recommended rates. The increase in nutrient dosage was not substantial for Nitrosol and Lawn and Leaf, but more than doubled for Organic Vitaboost and was more than halved for Polyfeed. There may have been more or less $\text{PO}_4\text{-P}$ and TP leached at recommended rates than these results suggest - had no adjustments been made. Organic Vitaboost, therefore, may have been more suitable at recommended rates and Polyfeed only suitable at rates lower than recommended.

Two treatments leached more than $10 \text{ mg}\cdot\text{L}^{-1} \text{ PO}_4\text{-P}$, the South African standard for non-point source discharge onto land or underground. Discharge into surface water courses, however, is $1 \text{ mg}\cdot\text{L}^{-1}$ and only some of the soil-based growth media treatments (Lawn and Leaf, Nitrosol and Polyfeed) were below this limit at experiment termination. Testing for leachate $\text{NO}_3\text{-N}$

and PO₄-P during the course of a study will help better understand discharge rates from fertiliser treatments over the course of a study and will help to quantify the potential effects of these amendments. Literature on the effects of effluent discharge from greenhouses and nurseries in South Africa appears limited. Results from this study would suggest that fertiliser treatments applied at the lowest rate would suffice for the growth of *Pseuderanthemum atropurpureum*, when produced in a soil-based growth medium.

Composted pinebark is commonly used as a growth medium in South Africa and future research should include the amendment of this growth medium with soil. Controlled release fertilisers and nitrogen use efficiency (NUE) were not tested in this study and although these still leach nutrients, controlled release fertiliser nutrients may leach appreciably less from soilless growth media amended with soil. Some literature indicates lower N applications when plants are small with N rates increasing as plants grow, thereby reducing fertiliser usage without compromising plant quality. The same should hold true for P, but increasing rates within a production cycle may be a problem for controlled release fertilisers which are usually incorporated in the growth medium. Increasing rates through the use of water soluble fertilisers would afford this control. It is also recommended that algal identification from leachate and watersheds be carried out during the course of a study as growth medium algal chlorophyll *a* concentrations appear to decrease over time.