The Influence of Carcass Decomposition on Soil Nutrient Composition and its Effects on Growth of *Gazania rigens* (L.).

By

Erika Anne Kullander

Student number - 53109627

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Supervisor – Dr MS Deutschländer
Co-supervisor – Prof VM Ngole-Jeme

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DEDICATION

Dedicated in loving memory to my dearest late Father, Per Erik Kullander

(4 October 1944 – 10 April 2019)

Jeg savner deg veldig!

UNISA | college of agriculture and environmental sciences

DECLARATION

I, Erika Anne Kullander, hereby declare that this dissertation, titled: The Influence of Carcass Decomposition on Soil Nutrient Composition and its Effects on Growth of *Gazania rigens* (L.)., which I hereby submit for the degree of Master of Science in Ornamental Horticulture at the University of South Africa, is my own work and has not previously been submitted by me for a degree at this or any other institution.

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I declare that during my study, I adhered to the Research Ethics Policy of the University of South Africa, received ethics approval for the duration of my study prior to the commencement of data gathering, and have not acted outside the approval conditions.

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Date: February 2022

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LIST OF ABBREVIATIONS

Abbreviation	Description
AF	Arrest front
B	Boron
C	Carbon
C:N ratio	Carbon and nitrogen ratios
Ca	Calcium
CA	Caged
CAES	College of Agriculture and Environmental Sciences
CDI	Cadaver decomposition island
CEC	Cation exchange capacity
Cl	Chlorine
CRD	Completely randomised design
Cu	Copper
EC	Electrical conductivity
EDTA	Ethylenediaminetetraacetic acid
Fe	Iron
ICP-OES	Inductively coupled plasma - optical emission spectrometry
K	Potassium
KSb-tartrate	Antimony potassium tartrate
M	Molecule
Mg	Magnesium
Mn	Manganese
Mo	Molybdenum
N	Nitrogen
Na	Sodium
Ni	Nickel
Nm	Nanometre
NPK	Nitrogen, phosphorus, potassium
NRN	Ninhydrin-reactive nitrogen
OM	Organic matter
P	Phosphorus

PE	Pegged
pH	Potential hydrogen
PMI	Post-mortem interval
Rpm	Revolutions per minute
S	Sulphur
TC	Total carbon
TN	Total nitrogen
UNISA	University of South Africa
VOC	Volatile Organic Compounds
W1	Week 1: before placement of carcasses
W2	Week 2: 3 weeks after placement of carcasses: Active decay
W3	Week 3: 6 weeks after placement of carcasses: Advanced decay
W4	Week 4: 9 weeks after placement of carcasses: Dry/Remains
Zn	Zinc

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ABSTRACT

This research project forms part of the Department of Nature Conservation, Unisa's: Death in the long grass: The ecological implications of carcass decomposition in a southern African grassland project. Decomposing carcasses leach nutrients into soils, forming dense nutrient rich islands in grasslands, which contribute to the recycling of nutrients and energy through the ecosystem. Large decomposing herbivore species, such as blue wildebeest (*Connochaetes taurinus*), create much larger volumes of nutrient influx into soils of cadaver decomposition islands (CDI) compared to smaller decomposing animals, such as rodents (*Rattus norvegicus*). Plant growth is negatively affected immediately after the influx of carcass decomposing liquids in CDI soils, resulting in plant death within the CDI; however, field observations have indicated plant growth returns after several months.

The aim of the project was to develop an understanding of the ecological dynamics associated with the decomposition of wild animal carcasses in a grassland ecosystem as nutrient islands.

This project investigated the effect of liquid influx from decomposing blue wildebeest (*Connochaetes taurinus*) carcasses on soil nutrient content at various stages of decomposition, and the effect thereof on the growth of the indigenous plant *Gazania rigens* (L.). The findings of this research project could be applied positively in the development of organic growth media by making use of animal waste products incorporated with sustainable plant products, such as coir and bagasse, to enhance their performance, as the current source of organic growth media, peat moss, is seen as unsustainable.

Ten blue wildebeest carcasses, five caged and five pegged, were placed at suitable localities in grasslands at Telperion Nature Reserve, Mpumalanga, South Africa. Soil samples were collected at each site before the placement of carcasses and at a three-week interval after placement of carcasses. Soil samples collected from each carcass site were homogenised, air-dried, some frozen (for nitrogen analyses) and analysed at Unisa's CAES laboratories for soil texture, colour, electrical conductivity (EC), and potential hydrogen (pH), organic matter (OM), exchangeable

bases; sodium (Na), magnesium (Mg), calcium (Ca) and potassium (K) and cation exchange capacity (CEC), total nitrogen (TN), total carbon (TC) and C:N ratios, available phosphorus (P), and micronutrients; copper (Cu), iron (Fe) and zinc (Zn). To assess the influence of soil nutrient inputs on plant growth, 120 five-week-old *G. rigens* seedlings were planted in the soils collected from around each carcass before placement of carcasses, and at three-week intervals during the decomposition process. After 12 weeks of growth under controlled environmental conditions, plants were harvested and individual organs measured, counted, weighed and air-dried to obtain dried organ weights.

Soil properties showed significant temporal changes after placement of caged and pegged carcasses, especially EC, pH, OM, exchangeable bases; Na, Mg, Ca and K, nitrogen (N), C:N ratios, and Cu, compared to before placement of carcasses. Soil colours and textures remained unchanged throughout carcass decomposition periods compared to before the placement of carcasses.

Noticeable differences were observed between plants grown in soils collected from decomposing caged and pegged carcasses, compared to those grown in soils collected before placement of carcasses, especially in the number of leaves, leaf lengths, fresh and dry leaf weights and number of dead leaves, as well as fresh buds, flowers and seed heads.

Soil aspects reported to have the most significant temporal influence on *G. rigens* growth were EC, pH, OM, Mg, CEC, N, carbon (C), C:N ratios, P, Cu, Fe and Zn. Each of the growth parameters was affected by one or more of these soil aspects, especially plant leaves and flowering organs.

This study reported significant changes in soils and plant growth parameters; however, no significant changes were reported in the two treatments, caged carcasses compared to pegged carcasses. The implication of this study is that the use of soils from around decomposing carcasses as a medium for growth of ornamental crops is not detrimental to plant growth. Recommendations are to investigate methods to use carcass waste in composting techniques for growth medium in which to grow ornamental and edible plants.

Keywords: Carcass decomposition, soil analysis, soil nutrients, plant growth parameters, *Gazania rigens*, leaf lengths, pegged carcasses, caged carcasses, blue wildebeest, *Connochaetes taurinus.*

CHAPTER 1
INTRODUCTION

1.1 Background

All terrestrial organisms undergo six stages of decomposition when they die: fresh, bloated, active decay, advanced decay, dry, and remains (Carter & Tibbett, 2010). The time for decomposition is affected by various climatic conditions, such as temperature and moisture, as well as the size of the carcass (Benbow, Tarone & Tomberlin, 2016). Immediately after death during the fresh period, a rapid initial breakdown of the carcass occurs as internal chemical processes, such as autolysis (internal cellular enzyme breaks down organs and tissue), take place. During the bloated stage, putrefaction occurs where proteins in the remaining organs are liquefied by bacteria and fungi, releasing gases. Putrefaction liquids leach in soils through carcass orifices and skin tears resulting from the internal bloating pressure (Benbow *et al*., 2016). Insects and maggots start to feed on the carcass, leading to further stages of advanced decay, “dry” decomposition stages and “remains” stages of decomposition (Carter & Tibbett, 2010). Advanced decay has indicated increases in soil pH levels, and increases of carbon and nitrogen in soils (Carter & Tibbett, 2010), as well as fungi fruiting structures, formed post putrefaction (Carter, Yellowlees & Tibbett, 2007).

Carcass, also referred to as carrion matter, as with decomposing plant matter or animal faeces, recycles energy and nutrients back into soils (Carter & Tibbett, 2010). The amount of nutrient recycled varies with the size of the carcass (Carter & Tibbett, 2010). According to Benninger, Carter and Forbes (2008), increased soil nutrient content, including total nitrogen, soil-extractable phosphorus, and lipid-phosphorus, as well as soil pH, was observed as a result of the presence of pig (*Sus scrofa*) carcasses. The carcasses of large herbivore species, such as blue wildebeest (*Connochaetes taurinus*) and elephant (*Loxodonta africana*), create large cadaver decomposition islands (CDI) with large volumes of nutrient influx into soils when compared to other smaller animals such as rodents.

Carcasses vary in their chemical composition. A carcass of blue wildebeest (*C. taurinus*), for example, consists of 76.5-77.5% water, 20.5-21.6% protein, 1.8% intramuscular fat and between 1.01 and 1.07% ash (inorganic residue) (Van Heerden & Hoffman, 2018). According to Fitzhenry (2016), a carcass of Fallow deer (*Dama dama*) consists of 73.3-76.2% water, 20.4-23.1% protein, 2.2-3.2% intramuscular fat and between 1.1 and 1.5% ash. In a study conducted by Hoffman, Kidd, Laubscher and, Mostert (2009) on a carcass of kudu (*Tragelaphus strepsiceros*), the chemical composition was found to consist of 75.66-75.77% water, 22.25-22.75% protein, 1.48-1.49% intramuscular fat and between 1.14 and 1.19% ash.

Studies of large carcass decomposition, such as bison *(Bison bison)* in North America, have revealed that initial nutrients fluxed into soils were potassium and sodium, followed by nitrogen and sulphur, then phosphorus and magnesium, and lastly calcium (Barton, Cunningham, Lindenmayer & Manning, 2013). In Australia, Barton, Cunningham, Farrell, Macdonald, Manning and Tuomia, (2014) found that kangaroo (*Macropus giganteus*) carcasses were an increased source of soil nitrogen (N), adding an average of 4.4 kg m^{-2} into soils, as well as 40 mg/kg proteins and 25 mg/kg amino acids. Initial influx of carcass decomposition fluids, ammonia, organic acids, and nutrients such as nitrogen, phosphorus, and potassium into surrounding environments could have a negative effect on plants directly below and around carcasses (Carter & Tibbett, 2010). During putrefaction, leaching acids lower soil pH, a process enhanced during warmer seasons, specifically within the first week after death (Benbow *et al*., 2016). In addition, carcass decomposition releases methane, hydrogen sulphide, and volatile organic compounds (VOC) into soils, which may affect plant growth negatively (Ackermann, Amir, Bibat, Bucheli, Carter, Gebert, Gilbert, Haarmann, Humphrey, Hyde, Knight, Larsen, Lauber, Lax, Lynne, Metcalf, Nicholas, Petrosino, Reed, Sangwan, Song, Thompson, Van Treuren, Weiss & Xu, 2016; Benbow *et al*., 2016). Vertebrate scavengers' impact on the speed at which carcasses decompose, as carcasses create readily available organic matter for scavengers to feed on. The scavengers affect surrounding soils through deposition of faecal matter, feathers, nails and fur (Carter *et al*., 2007), as well as spreading parts of the carcass into surrounding areas (Benbow *et al*., 2016). This could also affect the properties of surrounding soils.

Not much research has been conducted concerning the effects of carcass decomposition on soils (Barton *et al*., 2013). Limited data exists regarding the effects of soil nutrient influx from carcass decomposition on ornamental plant growth. Good growth media is important for optimal plant growth as it could affect root, stem and leaf growth, as well as bud and flower formation (Growing Media Europe, 2016). In addition, plants require macro-nutrients such as nitrogen (N), phosphorus (P), potassium (K), calcium (C), sulphur (S) and magnesium (Mg), and micro-nutrients such as boron (B), chlorine (Cl), manganese (Mn), iron (Fe), zinc (Zn), copper (Cu), molybdenum (Mo), and nickel (Ni) (Brown & White, 2010). Other important factors for optimal plant growth include soil pH and Electrical Conductivity (EC). Soil pH determines how acidic or alkaline the substrate is, whereas soil EC indicates the amount of nutrients available for plants to take up (Bloodnick, 2018). These plant growth requirements are necessary, especially at the seedling stage to enable healthy growth and establishment. This project was conducted to determine the specific effects carcass decomposition has on soil physiochemical properties and nutrients, as well as the implication on an ornamental plant growth (*Gazania rigens* 'Strawberry Shortcake Mix') (Figure 1.1). Ornamental plants are grown for their aesthetic value, such as foliar colour or texture, shapes, sizes, and fragrance (McMahon, 2022).

Figure 1.1: *Photograph of the experimental plant G. rigens that was used in the execution of the experiment*

Source: newPlantsandFlowers, 2019

This research forms part of the larger project titled Death in the long grass: The ecological implications of carcass decomposition in a southern African grassland project, a research initiative of the Department of Environmental Sciences, Nature Conservation, University of South Africa. The aim of the over-arching project is to develop an understanding of the ecological dynamics associated with the decomposition of wild animal carcasses in a grassland ecosystem (Melville, 2018). In this project a multi-faceted experimental study was conducted to monitor the rate of decomposition of a typical ungulate carcass during the growing season in grassland vegetation, to determine the composition and concentration of soil nutrient inputs from the decomposition of an ungulate carcass in grassland vegetation, to determine the effect of the decomposition of an ungulate carcass on the faunal component of the ecosystem, and to determine the effect of the decomposition of an ungulate carcass on the floral component of the ecosystem.

This particular project was created to investigate the effect of carcass decomposition on soil nutrient composition at various stages of decomposition, as well as its influence on the growth of *Gazania rigens* (L.).

1.2 Problem statement

Studies by Barton *et al*. (2013), Benninger *et al.* (2008) and Carter and Tibbett (2010), have all shown that animal carcasses have the potential to improve soil quality for plant growth. These studies have mostly been carried out in different regions of the world, but not in southern Africa, where the climatic conditions as well as the soil types are different. Further to these, the diet of the animals is dependent on the vegetation on which they feed, which in turn determines what they add to the soil upon their death. The effect that these carcasses have on the soil varies with animal type, soil type, and the accessibility of the carcass to scavengers. No study that assesses the impacts of carcasses on soil has been documented in southern Africa. There is therefore a need to investigate the changes in soil nutrient composition caused by animal carcasses.

Plant growth traits and development depend on the nutrients available in soils (Barbier, Prizenberg, Reymons, Salt & Stich, 2010; Clarkson & Hanson, 1980; Sinclair, 1992). Minerals are classified into two main groups based on the amounts

required by plants – micro-nutrients (boron and zinc) in relatively small amounts and macro-nutrients (potassium and phosphate), which constitutes between 1,000 – 15,000 $\mu g/g^{-1}$ plant dry weight (Buchanan, Gruissem & Jones, 2002; Marschner, 1995). The effect of different soil nutrients and concentrations leaching at various stages from a decomposing carcass on plant growth traits and development is unknown. Therefore, there is a need to investigate the effects of changes in availability of micro- and macro-nutrients in soils on plant growth at various stages of carcass decomposition to understand how it affects leaf development, flower formation and root growth.

Peat moss, currently being used to a large extent in the cultivation of ornamental plants, is an unsustainable organic growth medium, as it has a negative impact on the environment, due to harvesting peat moss from marshes, which takes 500 years to form (Noyes, 2021). Alternative organic growth media need to be investigated and developed to replace peat moss as growth medium of choice. Therefore, environmentally friendly alternatives need to be studied by making use of recycled plant material such as bagasse and coir incorporated with organic waste material from animal carcasses.

This study was based on identified information gaps and will contribute to the body of knowledge.

1.3 Research aims and objectives

1.3.1 Research aim

The main aim of this study was to investigate the influence of carcass decomposition leachants on soil properties, how these soil properties influence plant growth and how it can be applied to the green industry.

1.3.2 Research objectives

- To determine the effect of decomposing carcasses in a grassland on the surrounding soil nutrient composition and the extent thereof.
- To determine whether accessibility to a decomposing carcass by scavengers has any effects on the nutrient influx into soil around the carcass.

- To determine whether the effect that a decomposing carcass has on the changes in soil nutrients vary with time after death of the animal.
- To determine if the growth and development of *G. rigens* are affected by soil nutrient concentrations in a CDI.
- To establish whether the growth and development of *G. rigens* on soil collected from around carcasses vary with different stages of carcass decomposition.

1.4 Hypotheses

- A decomposing carcass has an effect on soil nutrients and influences indigenous plant growth (H1).
- A decomposing carcass has no effect on soil nutrients and does not influence indigenous plant growth (H0).

1.5 Research rationale

The increasing concern and environmental awareness of the unsustainability of some organic growth media, such as peat moss, for the cultivation of ornamental plants have led to researchers investigating and developing more environmentally sound alternatives. An understandable focus is placed on renewable materials from agricultural, industrial and municipal waste streams to be used as growth media for the cultivation of plants. The disposal of organic material derived from agricultural, industrial and municipal waste streams, such as animal waste from abattoirs, presents an environmental problem, and its reuse as growth medium for plants can provide a convenient solution (Bandaw & Herago, 2017). Growth media must provide a suitable biological and chemical environment in which plants can access nutrients effectively, develop, grow and reach maturity.

Available information on the effects of the decomposition of carcasses on plant growth is limited. Most research on the decomposition of non-living organic matter is subjected to the decomposition of plant biomass and faecal origin; research of carcass decomposition is limited and often poorly understood (Benbow *et al*., 2016), especially in southern Africa. Many forensic studies have been conducted reporting

the changes in soil nutrients beneath decomposing cadavers (Barton, Evans, Higgins, Quaggiotto & Strong, 2019), but not of wild animal carcasses.

This project was established to identify if leached carcass liquids could serve as a suitable growth media enhancer for ornamental plant growth. This project can also identify at what stage of carcass decomposition organic animal waste can be added into growth media to be beneficial to plant growth. This study has identified if nutrients found in soils from decomposing carcasses have benefited plant growth, and at what stage and, which nutrients are utilised by plants.

References

Ackermann, Gail, Amir, A., Bibat, A., Bucheli, Sibyl R., Carter, D.O., Gebert, M.J., Gilbert, J.A., Haarmann, D., Humphrey, G.C., Hyde, Embriette R., Knight, R., Larsen, P., Lauber, C., Lax, S., Lynne, A.M., Metcalf, Jessica L., Nicholas, Catherine, Petrosino, J.F., Reed, S.A., Sangwan, N., Song, S.J., Thompson, L.R., Van Treuren, W., Weiss, Sophie & Xu, Z.Z. 2016. Microbial community assembly and metabolic function during mammalian corpse decomposition. *Science*, 351(6296): 158-162.

Bandaw, T. & Herago, T. 2017. Review on abattoir waste management. *Global Veterinaria,* 19(2): 517-524.

Barbier, H., Prizenberg, Aina E., Reymons, M., Salt, D.E. & Stich, B. 2010. Relationships between growth, growth response to nutrient supply, and ion content using a recombinant inbred line population in *Arabidopsis*. *Plant Physiology,* 154: 1361–1371.

Barton, P.S., Cunningham, S.A., Farrell, M., Macdonald, B.C.T., Manning, A.D. & Tuomia, Seija. 2014. Carrion decomposition causes large and lasting effects on soil amino acid and peptide flux. *Soil Biology and Biochemistry,* 69: 132-140.

Barton, P.S., Cunningham, S.A., Lindenmayer, D.B. & Manning, A.D. 2013. The role of carrion in maintaining biodiversity and ecological processes in terrestrial ecosystems. *Oecologia,* 171: 761–772.

Barton, P.S., Evans, M.J., Higgins, A., Quaggiotto, Maria M., & Strong, C. 2019. Nutrient and moisture transfer to insect consumers and soil during vertebrate decomposition. *Food Webs*, 18: 110.

Benbow, M.E., Tarone, A.M. & Tomberlin, J.K. (eds.) 2016. *Carrion ecology, evolution, and their applications*. CRC Press, New York. 608 pp.

Benninger, Laura A., Carter, D.O, & Forbes Shari L. 2008. The biochemical alteration of soil beneath a decomposing carcass. *Forensic Science International,* 180: 2–3.

Bloodnick, E. 2018. *Fundamentals of growing media* [online]. Premier Horticulture Ltd. Available on the Internet at: https://www.pthorticulture.com/en/training-center/fundamentals-of-growing-media/ (Accessed 15 October 2019).

Brown, P.H. & White, P.J. 2010. Plant nutrition for sustainable development and global health. *Annals of Botany,* 105(7): 1073–1080.

Buchanan, B.B., Gruissem, W. & Jones, R.J. 2002. *Biochemistry and molecular biology of plants*. John Wiley & Sons, West Sussex, UK. 1341 pp.

Carter, D.O., Yellowlees, D. & Tibbett, M. 2007. Cadaver decomposition in terrestrial ecosystems. *Naturwissenschaften,* 94: 12–24.

Carter, D.O. & Tibbett, M. 2010. Cadaver Decomposition and soil: Processes. *Soil Analysis in Forensic Taphonomy,* 6: 29-51.

Clarkson, D.T. & Hanson, J.B. 1980. The mineral nutrition of higher plants. *Annual Review of Plant Physiology,* 31: 239–298.

Fitzhenry, L.B. 2016. *Yields and meat quality attributes of wild fallow deer (Dama dama) in South Africa*. MSc. Dissertation, Stellenbosch University, Stellenbosch. 130 pp.

Growing Media Europe. 2016. *About growing media* [online]. Growing Media Europe. Available on the Internet at: https://www.growing-media.eu/news-1 (Accessed 15 October 2019).

Hoffman, L.C., Kidd, M., Laubscher, Liesel L. & Mostert, A.C. 2009. Meat quality of kudu (*Tragelaphus strepsiceros*) and impala (*Aepyceros melampus*): Carcass yield, physical quality and chemical composition of kudu and impala Longissimus dorsimuscle as affected by gender and age. *Meat Science,* 83: 788–795.

Marschner, H. 1995. *Mineral nutrition of higher plants, Ed 2*. Academic Press, London. 889 pp.

McMahon, Mary. 2022. *What are ornamental plants?* [online]. Home Questions Answered. Available on the internet at: https://www.homequestionsanswered.com/what-are-ornamental-plants.htm#:~:text=Ornamental%20plants%20are%20plants%20which%20are%20grown%20for,many%20people%20feel%20that%20this%20is%20value%20enough. (Accessed 19 May 2023).

Melville, H.A. 2018. *Death in the long grass: The ecological implications of carcass decomposition in a Southern African grassland.* Applied Behavioural Ecology and Ecosystems Research Unit, Florida. 33 pp.

newPlantsandFlowers. 2019. *Gazania new day strawberry shortcake mixture – photo: PanAmerican Seed* [online]. newPlantsandFlowers. Available on the Internet at: http://www.newplantsandflowers.com/new-days-for-gazania/gazania-new-day-strawberry-shortcake-mixture-photo-panamerican-seed/ (Accessed 16 May 2019).

Noyes, Amber. 2021. *Peat moss: What it is and how to use it in your garden* [online]. Gardening Chores. Available on the internet at: https://www.gardeningchores.com/author/ambernoyes/ (Accessed 18 May 2022).

Sinclair, T.R. 1992. Mineral nutrition and plant growth response to climate change. *Journal of Experimental Botany,* 43: 1141–1146.

Van Heerden, A. & Hoffman, L.C. 2018. *Profiling the meat quality of blue wildebeest C. taurinus* [online]. Expand your Game 2018 Conference. University of Stellenbosch. Available on the Internet at: https://www.wrsa.co.za/wp-content/uploads/2018/04/A-VAN-HEERDEN-WRSA.pdf (Accessed 15 July 2019).

CHAPTER 2
LITERATURE REVIEW

2.1 Introduction

This chapter discusses the various aspects relating to this study; the elements affecting carcass decomposition, temporal variations of carcass fluid composition, the effect of carcass composition on soil properties, biological properties, the implications of soil changes on plant growth parameters, the use of carcass-contaminated soils for plant growth, and a conclusion to this chapter.

2.2 Decomposition of animal carcasses

Death is inevitable in all living organisms. Animals may die from diseases, predation, or starvation. The process of carcass decomposition links all organisms to the redistribution and recycling of energy, and nutrients returning to the biosphere (Barton, Benbow, Bump, Evans, Foster, Pechal & Quaggiotto, 2019a). According to Carter, Yellowlees and Tibbett (2007), terrestrial decomposition of carcasses undergoes six stages of decomposition, from fresh, to bloated, active decay, advanced decay, and dry remains. Apart from internal carcass abiotic and biotic decomposition processes, the primary contributors to carcass decomposition are invertebrates such as microbes, and vertebrates such as scavengers (Benbow, Crippen, Mondor, Tarone, Tomberlin & Tremblay, 2012). During the decomposition process soft carcass tissue degrades, carcass remains liquefy and disintegrate, nutrient-rich liquids are leached into surrounding soils, leading to cadaver decomposition island (CDI) (Benbow, Tarone & Tomberlin, 2016). Several factors are known to affect carcass decomposition, as explained in the following sections.

2.2.1 Carcass mass and size

In an experiment conducted by Bushing, Carter, Higley, Johnson and Spicka (2011) on pig (*Sus scrofa*) carcasses of various mass (1 kg, 20 kg, 40 kg and 50 kg), it was found that pig carcasses with a mass of 20 kg decomposed more rapidly than the larger carcasses with a weight of between 40-50 kg within a six-day period. They concluded that carcass mass can affect the rate of decomposition, which reflects

an interaction between carcass volume and blow fly (*Calliphora vomitoria*) colonisation.

The same pattern was observed by Becker, Myburgh, Steyn and Sutherland (2013) in that large pig carcasses decompose 2.82 times slower than smaller pig carcasses, concluding that carcass size influences decomposition rates. According to Adrusi and Rahim (2018) in their study conducted on various animal species of varying sizes, such as plantain squirrels (*Callosciurus notatus*), chickens (*Gallus gallus*), and toads (*Duttaphrynus melanostictus*), all the carcasses went through five stages of decomposition at different times, which were related to the carcass sizes. To complete the five stages of decomposition, chickens completely decomposed after ten days compared to plantain squirrels which decomposed after eight days, and toads which completely decomposed after six days. According to Braack (1987), larger carcass sizes can accommodate massive numbers of fly larvae compared to smaller carcasses; the mass of maggot numbers maintains the internal heat of the carcass, which accelerates decomposition rates.

MacMohan and Parmeter (2009) reported that body coverings, such as feathers or thick skins, which determine the wet and dry mass losses, influence the decomposition rate of carcasses. In an experiment conducted by Matuszewski, Konwerski, Frątczak and Szafałowicz (2014) on the effect of body mass and clothing on the decomposition of pig carcasses (5-15 kg; 15.1-30 kg; 35-50 kg and 55-70 kg), they found that carcass mass revealed significant differences concerning specific gross processes in decomposition, whereas clothes had little effect. They also found that putrefaction was more efficient in larger carcasses, which manifested in the earlier onset and longer duration of bloating; however, active decay was less efficient and at a lower rate, resulting in slower mass loss and advanced decay. It was found that the average rate of active decay showed a significant, logarithmic increase with an escalation in carcass mass, but only in carcasses with active decay due to larval blowfly activity, followed by larval *Necrodes littoralis* activity, commonly found in the decomposition of medium/large to large carcasses. The lower efficiency of active decay in larger carcasses is the result of various processes, influenced by many biotic and abiotic factors.

2.2.2 Carcass mass and CDI contribution

Bushing *et al*. (2011) found in their research that 1 kg pig carcasses were associated with a slower release of Ninhydrin-reactive nitrogen (NRN) into grave soil but at a greater concentration of NRN per kg carcass. They concluded that carcass mass influenced the rate and release of NRN into grave soil and concluded that neonatal carcasses require a different equation than larger carcasses when using grave soil chemistry to estimate the post-mortem interval (PMI). The impact of a CDI is spatially related to the size of the decomposing species (Barton, Benbow *et al.*, 2019a). For example, a meerkat (*Suricata suricatta*), for instance, will leach much smaller volumes of liquids into the surrounding soils compared to an elephant (*Loxodonta africana*), which will leach a far greater volume of liquids into surrounding CDI soils (Mateo-Tomás, Olea & Sánchez-Zapata, 2019).

2.3 Animal diet

In life the intestines of animals, such as pigs (*Sus scrofa*) for instance, contain bacteria, protozoans, and nematodes that after the death of pigs, will consume the intestinal walls and nearby organs, which begins at the onset of decomposition (Australian Museum, 2022); however, any toxins animals consume will affect decomposition. According to Elrakabawi (2021), in his study conducted on six healthy adult rabbits (*Oryctolagus cunicullus L*.), three died after ingesting zinc phosphide. The three rabbit carcasses did not decompose after 17 days as did the three control rabbits with no poison, nor did the poisoned rabbits decompose after 30 days due to the zinc phosphide being toxic to the insects; the insects were unable to digest the poisoned rabbits as zinc phosphide is toxic; being commonly used as an insecticide and rodenticide.

2.4 Climatic factors

There are many factors which affect carcass decomposition; temperature and rainfall have the greatest influence on carcass decomposition and the rate at which carcasses decompose (Mateo-Tomás *et al*., 2019). The season in which animals die will affect the species of insects which consume the carcasses, as some insects are active in warm spring or summer temperatures that are more favourable to their existence, and warmer temperatures increase the rate of decomposition. Some

species of arthropods may be active in winter (Benbow *et al*., 2016); however, generally cold temperatures decrease the rate of decomposition. Moisture and rainfall assist in maintaining the moisture within the decomposing carcass; without the moisture content being maintained the carcasses would be desiccated. In moist conditions, organisms decompose carcasses at faster rates due to the increased presence of micro-organisms. High temperatures reduce moisture which may lead to mummification of carcasses (Mateo-Tomás *et al*., 2019).

2.5 Temporal variation of carcass fluid composition

There are six temporal phases of decomposition: fresh, bloat, active, advance, dry decay and remains (Mateo-Tomás *et al*., 2019). According to Forbes and Carter (2015), after an animal dies, known as fresh phase directly after death, no fluids are produced or purged; however, blood pH decreases (Benbow *et al*., 2016), internal bacteria consume the carcass starting in the intestine, release methane, hydrogen sulphide, and carbon dioxide gasses causing the carcass to bloat, forcing fluids, proteins, carbohydrates, lactic acid, propionic acid, butyric acid, ethanol, butanol, acetate, and butyrate out of cells and blood vessels and into the body cavity. This bloating phase occurs within four to ten days (Australian Museum, 2022). No liquids are released into the surrounding soils during the bloating phase. Active decay follows the bloating phase, which leads to putrefaction and liquefaction of carcass tissue, proteins, fats, and body fluids. Moisture-laden, nutrient-rich fluids found in carcasses, such as nitrogen (N), potassium (K) and sodium (Na), are purged from the carcasses into the surrounding soils. This process may take 10 to 20 days, depending on the size of the carcass (Benbow *et al*., 2016). The dry decay phase may take up to 50 days to several months for the carcass to completely dry out, depending on the soils and climatic conditions, and if the carcasses have been removed or consumed by scavengers (Australian Museum, 2022; Mateo-Tomás *et al.,* 2019).

2.6 Effect of carcass decomposition on soil properties

2.6.1 Physio-chemical properties

Soil colour indicates the mineral content of soils derived from parent rocks and organic matter. Parent rocks containing iron and manganese, weather and oxidize

with their colours changing to yellow or red and black respectively (Lynn & Pearson, 2000). Changes in soil texture and soil colour from carcass decomposition have not been documented in literature as having any effect from carcass leachates; however, many field observations have reported soils appearing darker due to the high moisture content after purging of decomposing carcass liquids (Bump, Frey, Frossard, Morris, Risch & Schütz, 2020).

Organic matter in soils is mostly derived from once-living terrestrial plants and animal tissue that have decomposed through various microbial processes (Frischie, Hopwood, Lee-Mäder & May 2021). The processes of carcass decomposition contribute to soil organic matter (OM) through the release of decomposition fluids into the surrounding soils (Mateo-Tomás *et al*., 2019). Kim, Kim, Kim, Shin & Yoon (2017) conducted a study on pig (*Sus scrofa*) carcasses, using animals affected by foot-and-mouth disease in Korea. Four months after placing the carcasses into burial sites, results indicated that OM content within the burial sites was substantially higher (OM 12.7%) compared to areas outside of carcass burial sites (OM 0.61%). The soil OM content increases did not have any adverse effects on the environment in the long term.

Soil pH is an important characteristic that indicates levels of acidity or alkalinity. Soil pH is mostly affected by mineral composition of parent rocks, soil texture and organic matter (Queensland Government, 2016). Soil pH is regarded as neutral if pH values are 6.5-7.5, alkaline if more than 7.5, very alkaline if more than 8, acidic if less than 6.5, very acidic if less than 5.5 and extremely acidic if less than 4 (Queensland Government, 2016). A study by Koenig, Le Bayon, Mitchell, Seppey and Szelecz (2018) on pig (*Sus scrofa*) carcasses indicated that soil pH values increased significantly from carcass decomposition compared to sites with no pig carcasses due to the influx of ammonium-ions from the decomposing carcasses.

Electrical conductivity (EC) values indicate the flow of current and salinity of soils. Soil minerals and soil texture affect soil EC as soils with high contents of sand particles conduct less electrical currents (USDA, 2014). In a study conducted by Barton, Dawson, Reboldi, Strong, Ueland and Wallman (2020) on pig (*Sus scrofa*) and human carcasses in Australia, the EC values were higher in soils directly under

the carcasses than soils outside the CDI as no lateral spread was reported. Scavengers did not move any of the carcasses, which could account for the high EC value directly under the carcasses and the lack of lateral spread (Barton *et al*., 2020).

2.6.2 Chemical properties

According to Mateo-Tomás *et al*. (2019), carcass decomposition leaches nutrient-rich liquids into soils compared to decomposing plant matter which has much less nitrogen and phosphorus concentrations. Animal carcasses also decompose faster than plant matter, due to the abundant presence of bacteria and fungi, arthropod species, and saprophagous insects (Al-Dali, Fouda, Hammad, Kabadaia & Zeariya, 2015). Carter *et al*. (2007) report that nitrogen leaching from carcasses into soils can contribute five times more nitrogen levels and ten times more soil moisture content than plant matter. Plant matter, however, is available to micro-organisms over a longer period compared to carcasses.

Animals contain macro- and micro-nutrients N, K, P, S, Na, Ca, and Mg, which will leach out once the animals have died and started to decompose (MacMohan & Parmenter, 2009). The degree to which nutrients will leach out of the carcasses into soils depends on the carcass species, and size of the animals as smaller animals decompose faster than larger animals (MacMohan & Parmenter, 2009).

MacMohan and Parmenter (2009) also reported that soil N increased the most underneath decomposing carcasses when compared to increases of K, S, Na, Mg, and P and soil Ca, which fluctuated. MacMohan and Parmenter (2009) indicated that Ca losses were possibly due to wind-blown limestone from surrounding outcrops, that resulted in fluctuating Ca levels of the decomposing carcasses.

In a study conducted by Barton, Cunningham, Farrell, Macdonald, Manning and Tuomi (2014), significant increases in soils C and soil N were reported in CDI soils underneath kangaroo carcasses (*Macropus giganteus*) in week 12 and week 24 after placement of the kangaroo carcasses compared to the control site without carcasses. Barton *et al*. (2014) also indicated that an average of 4.4 kg N m^{-2} of nitrogen was added to the soils directly beneath the kangaroo carcasses. Nutrient

influx, especially N from decomposing pig (*Sus scrofa*) carcasses, was also reported by Koenig *et al*. (2018) in their study in weeks 2 and 3 after placement of carcasses, during the active decay stage, due to degradation of proteins, lipids, and carbohydrates within the carcasses.

Barton *et al*. (2020) reported in their comparative study of pigs and humans that total P levels were higher in soils under carcasses compared to sites outside of the CDI; it was also reported that P levels were higher under pigs compared to humans, due to the differences in the decomposition and soil processes beneath the human and pig carcasses. According to Towne (2000), in his study conducted on bison (*Bison bison*), cattle (*B. taurus*), and deer (*Odocoileus virginianus*) carcasses, P levels were significantly higher in adult and juvenile carcasses than in soils outside the CDI sites one year after the placement of carcasses, indicating that the influx of P from carcass decomposition does affect soil nutrients.

2.6.3 Biological properties

Decomposing carcasses attract and contribute to a diverse presence of microbial species, such as anaerobic bacterial decomposers, especially in the internal organs (Benbow *et al*., 2016), and arthropods which remove soft carcass tissue and depend on certain bacteria for their development (Kalischuk-Tymensen, Lysyk, Perotti, Yanke & Selinger, 2001; Mateo-Tomás *et al.,* 2019). According to Benbow *et al*. (2016), microbial diversity depends on the decomposing carcass species, season in which the animal dies, and climatic conditions which affect the presence of carcass consumer species, and are mostly interlinked communities that support each other or may overlap each other during the carcass decomposition phases. Decomposition fluids leach ammonia-rich elements, phosphorus, and potassium into the surrounding CDI soils, and the altered nitrogen and pH levels of soils promote the abundance of soil microbes (Ackermann, Amir, Bibat, Bucheli, Carter, Gebert, Gilbert, Haarmann, Humphrey, Hyde, Knight, Larsen, Lauber, Lax, Lynne, Metcalf, Nicholas, Petrosino, Reed, Sangwan, Song, Thompson, Van Treuren, Weiss & Xu, 2016).

2.7 Soil nutrients affecting plant growth characteristics

Thirteen of the essential nutrients required by plants for optimal growth and development are obtained from the soil. Plants require macro- and micro-nutrients in specific amounts to ensure optimal plant growth and development. Over- and under-availability may influence plant development and growth negatively. Plants require macro-nutrients nitrogen (N), phosporus (P), potassium (K), magnesium (Mg), sulphur (S) and calcium (Ca) in large quantities and micro-nutrients in smaller amounts. Each of these nutrients has a distinct function and role, necessary for the development and growth of plants (AGO Labs, 2019).

2.7.1 Macro-nutrients

2.7.1.1 Nitrogen (N)

Literature indicates that plants require N for leaf development and chlorophyll production (Ersek, 2012). Nitrogen is one of the nutrients plants require in abundance; however, organic N accounts for 98% that is unavailable N to plants, and only 2% as mineralised N is available to plants (Carson & Phillips, 2021). Without sufficient N, older plant leaves turn yellow-green or yellow first; if deficiencies continue, the whole plant may turn yellow (Mahler, 2004). Natural source of N in soils is derived from organic matter (Fernandez & Kaiser, 2021); the influx of nutrient-rich decomposition liquids, especially N (Barton, *et. al*., 2019b), is expected to elevate leaf growth.

2.7.1.2 Phosphorus (P)

According to Ersek (2012) and Williams (2019), P is a macro-nutrient, which assists with the growth of roots. Mahler (2004) indicated that plants are dependent on a soil pH 5.5 to 6.5 to absorb P. Phosphorus is also an essential nutrient for the development of flowers in plants (AGO Labs, 2019) and is important in seed formation (Khan, 2018). Small amounts of soil P is predominantly found in organic matter, which plants absorb in earlier stages of plant growth (Modi & Prajapati, 2012). With influxes of carcass decomposition liquids into soils, especially an influx of P (Barton, *et. al*., 2019b), it is anticipated that it will improve and enhance bud formation, flower production, and seed heads of *Gazania rigens* plants.

2.7.1.3 Potassium (K)

Potassium is an essential nutrient required by plants for root growth and development, and deficiencies may also lead to stunted plant growth (Tajer, 2016). Additionally, K also assists plants in photosynthesis (Kaiser & Rosen, 2018). According to Phoslab (2013), K assists plants in producing well-developed flowers, deficiencies lead to chlorosis, the appearance of leaf scorching, and leaf tips curl. It is anticipated that influx of K from decomposing carcasses liquids may influence root growth and flower development.

2.7.1.4 Calcium (Ca)

Calcium (Ca) is a secondary macro plant nutrient that promotes the growth of young roots. (Ersek, 2012; Williams, 2019). Deficiencies in Ca lead to stunted plant growth, poor development of roots, leaves and flowers (Age Old Organics, 2016). In sandy soils, Ca is leached due to water flowing through sandy soils faster compared to clay soils which have higher levels of Ca content (Age Old Organics, 2016). Additions of Ca from carcass decomposition fluids (Carter & Tibbett, 2010), even if occasional, should influence root growth positively.

2.7.2 Micro-nutrients

Micro-nutrients are required by plants in small amounts (Lohry, 2007). Amongst other nutrients required by leaves for chlorophyll production are the micro-nutrients iron (Fe) and copper (Cu), and for photosynthesis Fe, Cu and manganese (Mn) (Khan, 2018; Williams, 2019). A deficiency in soils of magnesium (Mg) and zinc (Zn) will hamper seed formation (Ersek, 2012; Williams, 2019). Deficiencies in Cu and Fe may lead to stunted plant growth and flowers may not develop (Khan, 2018; Lohry, 2007). Deficiencies of Zn may lead to poor flower formation in plants (Khan, 2018). In a study conducted by Rajawat, Rathore, Sarvanan, Singh and Singh (2017) in India to establish if micro-nutrients had an effect on flowering and budding of broccoli (*Brassica oleracea var. italica*), it was reported that Mn and Zn significantly increased the number of leaves and bud yields. It is hoped that an influx of micro-nutrients from decomposing carcass fluids may improve and enhance bud formation, seed and flower production.

2.8 Physio-chemical properties of soils on plant growth

2.8.1 pH

Soil pH indicates the acidity and alkalinity levels of soils; it also determines the availability of nutrients to plants. Soil nutrients might be available in abundance; however, plant growth might be affected by unfavourable pH levels (Ward, 2022). Soils are considered acid if pH values are between 7 and 0, alkaline if pH values are between 7 and 14, and neutral at pH 7 (Ward, 2022). According to Ward (2022), optimum ranges for most plant growth is between a pH 5.5 to 7, although some plant species may prefer either slightly acidic or slightly alkaline soils. Bryant (2017) reports that Ca, K, Mg and Cu drain away in very acidic soils with pH 3.0 to 5.0, and P and Fe are less available in alkaline soils with pH 7.1 to 9. In a study conducted by Ambrosini, Caronni, Citterio, Gentili and Montagnani (2018) on common ragweed *(Ambrosia artemisiifolia* L.), the results indicated that plants were shorter, and leaves developed at a slower rate when grown in soils with a pH 7, compared to plants grown in soils with a pH 5 and pH 6. Plants did not produce flowers when grown in soils with a pH 7. It is anticipated that plant growths of *G. rigens* will not be hampered by altered soil pH levels from the influx of carcass decomposition liquids, especially considering that *G. rigens* are hardy plants.

2.8.2 EC

EC indicates the total amount of salts in soils, which affects plants' ability to absorb water; most plants are salt intolerant (Spectrum Technologies, 2022). High levels of sodium (Na) and chloride (Cl) ions in soil result in high soil salinity concentrations. In high saline soil conditions, high levels of K are absorbed by plants, leading to K toxicity and stunted growth (Bhatla & Lal, 2018). In a study conducted by Amanullah & Ahmed (2016) on *Gazania harlequin*, it was reported that high saline soil solutions had negative effects on fresh shoot length, shoot weights, root length, flower sizes, number of flowers, number of leaves and, plant height, especially at levels of NaCI 100 ppm. Leached decomposition carcass liquids may result in elevated soil EC levels, which may also have a negative effect on *G. rigens* plant growth.

2.8.3 CEC

CEC indicates soils' ability to hold nutrients, which determines soil fertility (Efretuei, 2016). Soils with high CEC values have higher water-holding capacity and higher ability to hold cations for soils to be abundant in calcium (Ca), magnesium Mg) and potassium (K), which are more available to plants for optimum growth, than soils with low CEC values with low water-holding capacity and are deficient in cations (Efretuei, 2016; Lines-Kelly, 1993). CEC values are the lowest at soil pH 3.5 to 4; sandy soils also have low CEC values compared to clay soils, and decomposed organic matter has the highest CEC value (Lines-Kelly, 1993). With the addition of organic matter from decomposing carcass liquids (Barton, *et. al*., 2019a), soil CEC should increase, thereby plant growth of *G. rigens* should also benefit from increased CEC values.

2.9 Effect of carcass decomposition fluids on plant growth and development

It has been reported in literature that soil properties and moisture content change with the influx of liquids from decomposing carcasses. It was further noted that the plants underneath and in close vicinity to the decomposing carcass die instantly, due to the presence of ammonias which smother and kill surrounding vegetation. However, it was noted that pioneer species grew vigorously within a year of the carcass liquids entering the soil environment (Figure 2.1) (Benbow *et al*., 2016). In a study conducted by Barton, Bump, Cunningham, Evans, Manning and McIntyre (2016) on the effects of plant growth from the addition of kangaroos (*Macropus giganteus*) carcasses, soil phosphorus was eight times higher in CDI soils than control soils without carcasses. Barton *et al*. (2016) also reported that the growth of plants had increased over a five-year period within the CDI sites, due to high soil P levels.

Figure 2.1: *Carcass fluids form a cadaver decomposition island (CDI) indicating dead plants around the carcass*
Source: Carter et al. (2007)

In a study conducted by Koenig *et al*. (2018) on pigs (*Sus scrofa*), after placement of carcasses, within 15-59 days, significant increases of soil nitrogen, phosphorous and potassium were reported. Barton *et al*. (2019b) also reported in their study on European rabbit (*Oryctolagus cuniculus*) carcasses that soil nitrogen increased by 40% after placement of carcasses; phosphorus and phosphate increased up to 20 days after placement of the rabbit carcasses. MacMohan and Parmenter (2009) indicated in their study of nutrients released into soils by decomposing mule deer (*Odocoileus hemionus*), dog (*Canis familiaris*), jackrabbit (*Lepus townsendii*), ground squirrel (*Spermophilus armatus*), sparrow (*Amphispiza belli*), and frog (*Lithobates pipiens*) that occasional increases in soil K, S, Na, Mg, P, and Ca levels fluctuated after placement of carcasses. All the nutrients leached into the soils from decomposing carcasses should be beneficial to plant growth.

2.10 Use of carcass contaminated soil for plant growth

Soils from around decomposing carcasses of wild animals have previously not been used for ornamental plant growth; however, literature has suggested that cows can be decomposed by making use of dedicated fenced-off composting sites in which specific recipes should be used within the piles to enhance microbial growth to assist the decomposition process (Guthrie, Ross & Rozeboom, 2013). The advantage of composting dead animals is to reduce waste and create nutrient-rich humus, nutrients otherwise not available for plants, suitable for the green industry

as a growth enhancer in growth media for ornamental plants. Carcasses could be added with green and dry plant waste materials to produce nutrient-rich composts. Waste management from chicken farm remains, tree felling industries and municipal green waste could be combined to process compost for the ornamental horticultural industry, thereby reducing multiple avenues of waste to utilise in compost production. Large sites are required to produce compost using large animals to process into compost. Foot-and-mouth and avian influenza diseased animals have a negative impact on other animals and humans, as reported by Kim *et al.* (2017); however, high temperature achieved in compost heaps would destroy harmful pathogens. Composting areas in such instances should be barricaded to exclude dogs or other scavenging animals from digging up compost heaps containing decomposing animal carcasses, and impermeable platforms should be created to prevent decomposing liquids from diseased animals from contaminating groundwater. Furthermore, composting areas should not be located near human settlements due to smell pollution to humans.

2.11 Conclusion

Decomposition of carcasses is influenced by many factors, such as environmental conditions, temperature and rainfall, size of the carcasses, types of soils and geographical locations, and microbial and insect activities. It is indicated in literature that the influx of carcass decomposition fluids into surrounding soils creates nutrient-dense islands (CDI) which increase the nutrient volumes during carcass decomposition. Micro-organisms play a major role in the carcass decomposition process, as well as making N available to plants. Plants are smothered and die with the initial influx of leached carcass liquids within the CDI and then recover after some time with indications of vigorous growth once nutrients are converted into organic forms.

References

Ackermann, Gail, Amir, A., Bibat, A., Bucheli, Sibyl R., Carter, D.O., Gebert, M.J., Gilbert, J.A., Haarmann, D., Humphrey, G.C., Hyde, E.R., Knight, R., Larsen, P., Lauber, C., Lax, S., Lynne, A.M., Metcalf, Jessica L., Nicholas, Catherine, Petrosino, J.F., Reed, S.C., Sangwan, N., Song, S.J., Thompson, L.R., Van Treuren, W., Weiss, S. & Xu, Z.Z. 2016. Microbial community assembly and metabolic function during mammalian corpse decomposition. *Science*, 351(6269): 158-162.

Adrusi, Madinah & Rahim, Nor A.A. 2018. Insect succession and decomposition patterns of carcasses in Sarawak, Malaysian Borneo. *Journal of Sustainability Science and Management*, 13(2): 69-84.

Age Old Organics. 2016. *Calcium (Ca): Essential for Plant Growth and Development* [online]. Age Old Organics. Available on the Internet at: https://www.ageold.com/calcium-ca-essential-for-plant-growth-and-development/#:~:text=Calcium%20was%20deemed%20an%20essential%20element%20for%20plant,the%20formation%20and%20stability%20of%20the%20cell%20walls. (Accessed 25 February 2022).

AGO Labs, 2019. *Macronutrients in plants* [online]. AGQ Labs USA. Available on the Internet at: https://www.agqlabs.us.com/macronutrients-in-plants/ (Accessed 23 February 2022).

Al-Dali, A.G., Fouda, M.A., Hammad, K.M., Kabadaia, M.M. & Zeariya, M.G.M. 2015. Forensic - insect succession and decomposition patterns of dog and rabbit carcasses in different habitats. *Journal of Entomology and Zoology Studies*, 3(5): 473-482.

Amanullah, S. & Ahmed, I. 2016. Assessment of NaCl stress on morphological behavior of *Gazania*. *International Journal of Agronomy and Agricultural Research*, 9(6): 94-111.

Ambrosini, R., Caronni, Sarah, Citterio, Sandra, Gentili, R. & Montagnani, C. 2018. Effect of soil pH on the growth, reproductive investment and pollen allergenicity of *Ambrosia artemisiifolia* L. *Frontiers in Plant Science*, 9: 1335.

Australian Museum. 2022. *Stages of decomposition* [online]. The Australian Museum. Available on the Internet at:

https://australian.museum/learn/science/stages-of-decomposition/ (Accessed 14 February 2022).

Barton, P.S., Benbow, M.E., Bump, J.K., Evans, M.J., Foster, Claire N., Pechal, Jennifer L. & Quaggiotto, Maria M. 2019a. Towards Quantifying Carrion Biomass in Ecosystems. *Trends in Ecology & Evolution*, 2558: 12.

Barton, P.S., Bump, J.K., Cunningham, S.A., Evans, M.J., Manning, A.D. & McIntyre, Sue. 2016. Substantial long-term effects of carcass addition on soil and plants in a grassy eucalypt woodland. *Ecosphere*, 7(10): 01537.

Barton, P.S., Cunningham, S.A., Farrell, M., Macdonald, B.C.T., Manning, A.D. & Tuomi, S. 2014. Carrion decomposition causes large and lasting effects on soil amino acid and peptide flux. *Soil Biology & Biochemistry*, 69: 132-140.

Barton, P.S., Dawson, B.M., Reboldi, Anna, Strong, C., Ueland, M. & Wallman, J.F. 2020. Soil chemical markers distinguishing human and pig decomposition islands: A preliminary study. *Forensic Science, Medicine and Pathology*, 16: 605–612.

Barton, P.S., Evans, E.M., Higgins, A., Quaggiotto, Maria M., & Strong, C. 2019b. Dynamic soil nutrient and moisture changes under decomposing vertebrate carcasses. *Biogeochemistry*, 146: 71–82.

Becker, P.J., Myburgh, J., Steyn, M. & Sutherland, A. 2013. The effect of body size on the rate of decomposition in a temperate region of South Africa. *Forensic Science International*, 231(1–3): 257-262.

Benbow, E.M., Crippen, T.L., Mondor, E.B., Tarone, A., Tomberlin, J.K. & Tremblay, M.N.M. 2012. The ecology of carrion decomposition. *Nature Education Knowledge*, 3(10): 21.

Benbow, M.E., Tarone, A.M. & Tomberlin, J.K. (ed.) 2016. *Carrion ecology, evolution, and their applications*. CRC Press, New York. 608 pp.

Bhatla, Satish C. & Lal, M.A. 2018. *Plant physiology, development and metabolism*. Springer Nature Singapore Pte Ltd. Singapore. 1237 pp.

Braack L.E.O. 1987. Community dynamics of carrion-attendant arthropods in tropical African woodland. *Oecologia,* 72: 402–409.

Bryant, Sophie. 2017. *How does soil pH affect plant growth? How can I measure soil pH?* [online]. The Laboratory People. Available on the Internet at: https://camblab.info/how-does-soil-ph-affect-plant-growth-how-can-i-measure-soil-ph/ (Accessed 25 February 2022).

Bump, J.K., Frey, B., Frossard, A., Morris, A.W., Risch, Anita C. & Schütz, M. 2020. Effects of elk and bison carcasses on soil microbial communities and ecosystem functions in Yellowstone, USA. *Functional Ecology*, 34(9): 1933-1944.

Bushing, Jennifer, Carter, D.O., Higley, L.G., Johnson, Reyna & Spicka, Ashley. 2011. Carcass mass can influence rate of decomposition and release of ninhydrin-reactive nitrogen into gravesoil. *Forensic Science International*, 209 (1–3): 80-85.

Carson, Jennifer & Phillips, Lori. 2021. *Soil nitrogen supply* [online]. Soil Quality Pty Ltd. Available on the Internet at: https://www.soilquality.org.au/factsheets/soil-nitrogen-supply (Accessed 25 October 2021).

Carter, D.O. & Tibbett, M. 2010. Cadaver decomposition and soil: Processes. *Soil Analysis in Forensic Taphonomy*, 6: 29-51.

Carter, D.O., Tibbett, M. & Yellowlees, D. 2007. Cadaver decomposition in terrestrial ecosystems. *Naturwissenschaften*, 94(1): 12–24.

Efretuei, A. 2016. *The soils cation exchange capacity and its effect on soil fertility* [online]. Permaculture Research Institute. Available on the Internet at: https://www.permaculturenews.org/2016/10/19/soils-cation-exchange-capacity-effect-soil-fertility/ (Accesses 25 February 2022).

Elrakabawi, M.S. 2021. *The influence of phosphide poisoning on carcass decomposition and insect succession: A winter study in Cairo, Egypt*. MSA. thesis, October University for Modern Sciences and Arts, Cairo, Egypt. 76 pp.

Ersek, Kaitlyn. 2012. *The 6 essential nutrients for healthy plants* [online]. Holganix. Available on the Internet at: https://www.holganix.com/blog/the-6-essential-nutrients-for-healthy-plants#:~:text=1%20Nitrogen%3A%20Nitrate%20%28the%20form%20of%20nitrogen%20that,cell%20walls%20help%20the%20plant%20resist%20disease.%20 (Accessed 16 February 2022).

Fernandez, F.G., & Kaiser, D.E. 2021. *Understanding nitrogen in soils* [online]. University of Minnesota. Available on the Internet at: https://extension.umn.edu/nitrogen/understanding-nitrogen-soils#organic-n-sources-760712 (Accessed 25 February 2022).

Forbes S.L. & Carter D.O. 2015. *Processes and mechanisms of death and decomposition of vertebrate carrion*. In: Benbow E.M., Tomberlin J.K. & Tarone A.M. (eds). *Carrion ecology, evolution, and their applications.* CRC Press, Boca Raton, 512 pp.

Frischie, Stephanie, Hopwood, Jennifer, Lee-Mäder, E. & May, Emily. 2021. *Farming with soil life: A handbook for supporting soil invertebrates and soil health on farms*. The Xerces Society for Invertebrate Conservation. Portland, OR. 128 pp.

Guthrie, T., Ross, D. & Rozeboom, D. 2013. *Carcass composting – a guide to mortality management on Michigan cattle farms*. Michigan State University, Extension Bulletin E3197. 7 pp.

Kaiser, D.E. & Rosen, C.J. 2018. *Potassium for crop production* [online]. University of Minnesota. Available on the Internet at: https://extension.umn.edu/phosphorus-and-potassium/potassium-crop-production#manure-602513 (Accessed 25 February 2022).

Kalischuk-Tymensen, L., Lysyk, T., Perotti, M., Yanke, L. & Selinger, L. 2001. Growth and survival of immature *Haematobia irritans* (Diptera: *Muscidae*) is influenced by bacteria isolated from cattle manure and conspecific larvae. *Journal of Medical Entomology*, 38: 180–187.

Khan, M.S. 2018. *Role of macro and micro nutrients and their deficiency symptoms in plants* [online]. AgriHunt. Available on the Internet at: https://agrihunt.com/articles/fertilizer-industry/role-of-macro-and-micro-nutrients-and-their-deficiency-symptoms-in-plants/ (Accessed 25 February 2022).

Kim, K-R, Kim, K-H, Kim, Y-N, Shin, D-C & Yoon, J-H. 2017. Management of animal carcass disposal sites using a biochar permeable reactive barrier and fast growth tree (*Populus euramericana*): A field study in Korea. *Sustainability*, 9: 457.

Koenig, Isabelle, Le Bayon, Renée-Claire, Mitchell, E.A.D., Seppey, C.V.W. & Szelecz, I. 2018. Soil chemistry changes beneath decomposing cadavers over a one-year Period. *Forensic Science International*, 286: 155–165.

Lines-Kelly, Rebecca. 1993. *Cation exchange capacity* [online]. NSW Department of Industry. Available on the Internet at: https://www.dpi.nsw.gov.au/agriculture/soils/guides/soil-nutrients-and-fertilisers/cec (Accessed 25 February 2022).

Lohry, R. 2007. *Micronutrients: Functions, sources and application methods*. CCA Conference Proceedings, Indiana.

Lynn, W.C. & Pearson, M.J. 2000. *The color of soil*, [online]. The Science Teacher. Natural Resources Conservation Service Soils. Available on the Internet at: https://www.nrcs.usda.gov/wps/portal/nrcs/detail/soils/edu/?cid=nrcs142p2_054286 (Accessed 16 February 2022).

MacMohan J.A. & Parmenter, R.R. 2009. Carrion decomposition and nutrient cycling in a semiarid shrub–steppe ecosystem. *Ecological Monographs*, 79(4): 637–661.

Mahler, R.L. 2004. *Nutrients plants require for growth*. University of Idaho, CIS 1124.

Mateo-Tomás, Patricia, Olea, P.P. & Sánchez-Zapata, J.A. (ed.). 2019. *Carrion ecology and management*. Springer Nature, Switzerland. 281 pp.

Matuszewski, S., Konwerski, S., Frątczak, K. & Szafałowicz, M. 2014. Effect of body mass and clothing on decomposition of pig carcasses. *International Journal of Legal Medicine*, 128: 1039–1048.

Modi, H.A. & Prajapati, Kalavati. 2012. The importance of potassium in plant growth – a review. *Indian Journal of Plant Sciences*, 1(02-03): 177-186.

Phoslab. 2013. *How does potassium help plants grow?* [online]. Phoslab Environmental Laboratories. Available on the Internet at: https://www.phoslab.com/how-does-potassium-help-plants-grow/ (Accessed 25 February 2022).

Queensland Government. 2016. *Soil pH* [online]. The State of Queensland. Available on the Internet at:

https://www.qld.gov.au/environment/land/management/soil/soil-properties/ph-levels (Accessed 19 August 2021).

Rajawat, K.S., Rathore, J.S., Sarvanan, S., Singh, G. & Singh, Gajendra. 2017. Effect of different micronutrients on plant growth, yield and flower bud quality of broccoli *(Brassica oleracea var. italica*). *Current Agriculture Research Journal*, 5(1): 108-115.

Spectrum Technologies. 2022. *If plants could talk...about EC* [online]. Spectrum Technologies, Inc. Available on the Internet at: https://www.specmeters.com/newsletter/if-plants-could-talk-vol-2/ (Accessed 25 February 2022).

Tajer, A. 2016. *What's the function of potassium (K) in plants?* [online]. Greenway Biotech Inc. Available on the Internet at: https://www.greenwaybiotech.com/blogs/gardening-articles/whats-the-function-of-potassium-k-in-plants (Accessed 25 February 2022).

Towne, E.G. 2000. Prairie vegetation and soil nutrient responses to ungulate carcasses. *Oecologia*, 122: 232–239.

USDA. 2014. *Soil electrical conductivity* [online]. USDA Natural Resources Conservation Service. Available on the Internet at: https://www.nrcs.usda.gov (Accessed 7 September 2021).

Ward, Eileen. 2022. *Gardening: The pH of your soil can affect plant growth and health* [online]. Naples News Daily. Available on the Internet at: https://archive.naplesnews.com/community/gardening-the-ph-of-your-soil-can-affect-plant-growth-and-health-ep-1071358042-331319431.html/ (Accessed 10 January 2022).

Williams, R. 2019. *Plant nutrients explained: Everything you ever need to know* [online]. Epic Gardening. Available on the Internet at: https://www.epicgardening.com/plant- nutrients/ (Accessed 16 February 2022).

CHAPTER 3
RESEARCH METHODS

3.1 Introduction

This chapter outlines the study area, experimental setup, soil samples collection processes and techniques and methods used for soil analysis, plant growth experiments, preparation of soils for potting, experimental layout for plant research, plant maintenance, monitoring of plant growth and harvesting, soil data analysis and plant data analysis.

3.2 Description of study area

This project was conducted in Telperion Nature Reserve in Mpumalanga, South Africa. Telperion is located in Nkangala District Municipality of Thembisile Hani Local Municipality (25°41'35.20"S 29° 0'7.01"E) at the borders between the Gauteng and Mpumalanga provinces. It is located 57 km from Bronkhorstspruit via the N4 national highway, and 44 km from Emalahleni (Figure 3.1). This Nature Reserve forms part of the Ezemvelo Nature Reserve. Whereas Ezemvelo is accessible to the general public, Telperion is a private reserve used for research purposes.

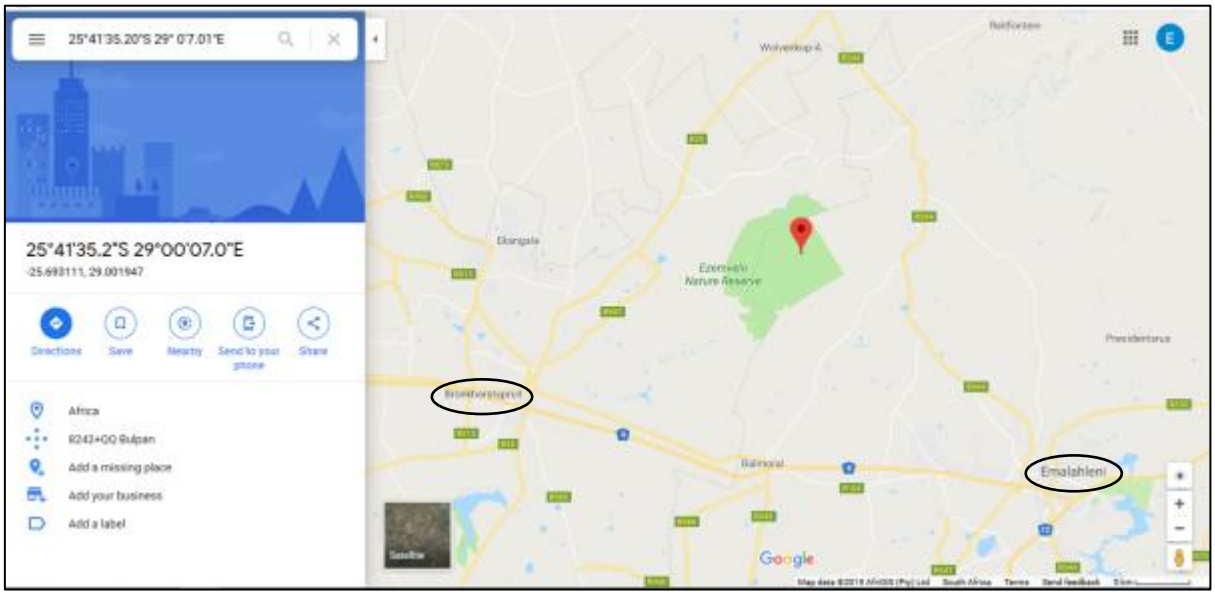

Figure 3.1: *Location of Telperion Nature Reserve, Mpumalanga*

Source: Google, 2019

Telperion Nature Reserve covers an area of approximately 9,000 hectares and is situated in the Rand Highveld Grassland (GM 11) of the Mesic Highveld Grassland Bioregion (Mucina & Rutherford, 2006). Dominant grass species characteristic of this vegetation type includes *Diheteropogon amplectens, Elionurus muticus, Themeda triandra* and *Tristachya leucothrix* (Mucina & Rutherford, 2006). The topography of Telperion consists of undulating plains with extruding quartzite rocky outcrops (Mucina & Rutherford, 2006). The perennial Wilge River runs along the western boundary in a northerly direction. Dystrophic or mesotrophic red soils are the predominant soils found on the Reserve (Macfadyen & Reilly, 2013).

The altitude of the area ranges between 1,240 m to 1,500 m above sea level (Swanepoel, 2006). The annual summer rainfall period is from October to March, with an average of 650 mm per annum. In summer months, temperatures range between a minimum of 14°C and a maximum of 26°C, whereas in winter months temperatures range between a minimum of 4°C and a maximum of 18°C. Frost may occur from May to August (Coetzee, 2012).

3.3 Experimental setup

Ten large senescent adult male blue wildebeest (*Connochaetes taurinus*) of similar size (200-250 kg) were made available for this research project by the owners of Telperion Nature Reserve through the annual culling programme. A contracted hunter was hired to kill the ten *C. taurinus* by means of a single rifle shot to their heads using a monolithic solid bullet with no lead. The carcasses were loaded manually onto an open-back vehicle, transported directly from the location where they were killed to pre-determined sample sites (Figure 3.2), all within 30 minutes of being shot. For this project, five *C. taurinus* were placed into 2.5 m x 1.6 m x 700 mm steel grid cages, with 10 mm x 15 mm mesh, to exclude vertebrate scavengers, and five *C. taurinus* carcasses were pegged onto 2.5 m x 1.6 m steel grid platforms, with 10 mm x 15 mm mesh, that were accessible to vertebrate scavengers. The purpose of the steel grid was to facilitate weighing of carcasses while pegging served to keep the animal carcass in place.

A before-and-after design with ten sample sites, using two different treatments; five caged carcasses and five pegged carcasses were used for this study. Sample sites were located at least 1 km apart to establish spatial independence (Figure 3.2). All the carcasses were placed in the same vegetation type; grassland biome, some of the carcasses were placed on flat areas, and areas with slight slopes. Summer rainfall was experienced during the time of the soil collection periods at Telperion.

Figure 3.2: *Locations for the placement of ten carcass sites*
Source: Melville, 2018

3.4 Soil sample collection

Soil samples were collected from each of the ten sites before positioning of *C. taurinus* carcasses, after placement of carcasses, and at various stages of carcass decomposition to establish how soil parameters around the carcass changed with time and the effect of these changes on the growth of indigenous ornamental plant *G. rigens*. From each of the ten sites, three soil samples weighing ±5 kg were collected at a depth of 0 – 30 cm using a 90 mm diameter soil auger. The three soil samples taken at each experimental site were homogenised to form a composite sample representative of each site. The homogenised soil samples were air-dried, sieved through a 2-3 mm mesh and divided into two portions; one portion was used for soil analysis and the other portion for plant growth experiment. Soil samples collected before placement of carcasses were labelled as week one (W1), whereas soil samples collected at three-week intervals after placement of caged and pegged

carcasses were labelled as week two (W2), week three (W3) and week four (W4). Soil samples were collected around the edges of the steel grid cages and steel grid platforms, where carcass decomposition leachates appeared. The effects that influx of carcass fluids has on soil properties are not known. Soil samples were therefore collected at these different stages of carcass decomposition to determine the extent to which they affected soil properties. Figure 3.3 presents a graphical abstract depicting the experimental procedure from collection of soil samples after placement of carcasses to plant growth of *G. rigens*.

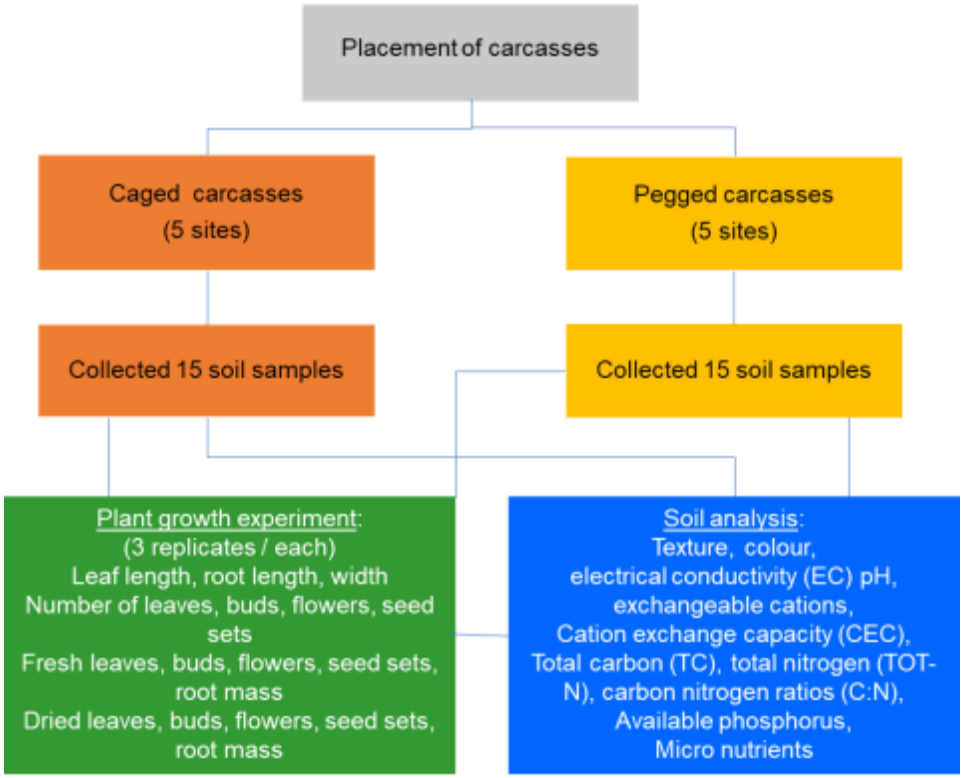

Figure 3.3: *Schematic presentation of the experimental procedure*

3.5 Soil samples analysis

All soil samples were analysed for soil colour, texture, electrical conductivity (EC), pH, exchangeable cations, cation exchange capacity (CEC), total carbon (TC), total nitrogen (TN), carbon and nitrogen ratios (C:N), organic matter content (OM), available phosphorus, and micro-nutrients at the CAES laboratories at Unisa. Details of the protocols used for analysing the soil samples are presented below.

3.5.1 Determination of soil colour

To determine the colour of soils at each site, a Munsell Soil Colour Chart was used (Munsell Color, 2019). Each dry soil sample was placed onto a clear plastic sheet and moved over the different colour chips on the Munsell Colour Charts until the soil samples matched a chip on the charts. The value, chroma and hue of the matching colour chip were noted and recorded as the colour of the soil. Figure 3.4 indicates the charts secured onto a flat surface with the soil samples on the clear plastic sheet.

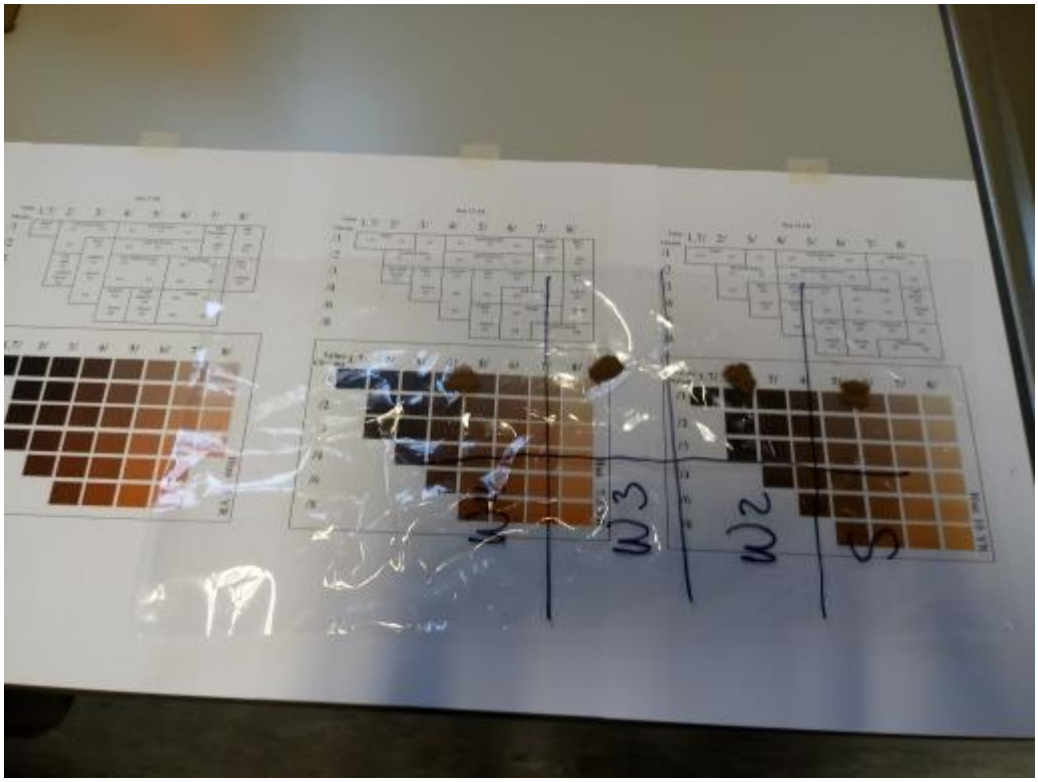

Figure 3.4: *Munsell soil colour charts used to determine soil sample colours (Kullander, E. Roodepoort, March 2021)*

3.5.2 Determination of soil texture

To determine the weight percent of sand, silt and clay contained in the soil samples, the hydrometer method was used. The hydrometer method is based on Stoke's Law (van Reeuwijk, 2002) which determines the rate of sedimentation of particles in a suspension of constant density through gravitational force. In this method, 100 ml of sodium hexametaphosphate ($Na(PO_3)$) was added to 50 g of each soil sample in separate 250 ml beakers to disperse the soil particles into single grains. The

suspensions were swirled and left to settle overnight. Thereafter, each suspension was transferred to separate 1,000 ml measuring cylinders and filled up to the mark with distilled water. The suspension was then mixed thoroughly by shaking end over end and placed on a stable platform and the temperature of the suspension was taken. A hydrometer was immediately placed into the suspension, and a reading was taken 40 seconds after the cylinders were placed on a flat surface. This was repeated three times. This reading represented the density of a suspension containing silt and clay as sand particles are believed to have settled by this time (EE Library, 2019). Figure 3.5 shows the texture experiment set-up.

Figure 3.5: *Soil texture experiment using hydrometer*
(Kullander, E., Roodepoort, December 2019)

The suspension was again homogenised and allowed to stand undisturbed for two hours, after which the hydrometer was again gently dropped into the suspension and another reading taken. This reading represented the density of a suspension of clay. No corrections for temperature were required as the temperature of the suspension was 20°C throughout the experiment. The percentages of sand, silt and clay in each soil sample were calculated using equations 1 – 3.

$$\%\ clay = \frac{\text{corrected hydrometer reading after 2 hours}}{\text{mass of sample (50 g)}}\ x\ 100 \quad (1)$$

$$\%\ silt = \frac{\text{corrected hydrometer reading after 40 seconds}}{mass\ of\ sample\ (50\ g)}\ 100 - \%\ clay \quad (2)$$

$$\%\ sand\ =\ 100\ \%\ -\ (\%\ silt\ +\ clay) \quad (3)$$

3.5.3 pH and electrical conductivity (EC)

The potentiometric method was used to determine the pH and EC of the soil samples in a 1:2.5 soil:distilled water suspension, following the method of Marsh, Maynard, Morrison, Neary, Palmer, Pastorek & Schumacher (1995); and van Reeuwijk (2002). Fifty millilitres (50 ml) of distilled water were added to 20 g of each soil sample weighed into separate 100 ml wide-mouth bottles. Thereafter, the bottles were capped and placed on a reciprocating shaking machine for one hour at 120 rpm. The bottles were then removed and allowed to stand for 30 minutes. The pH probe of a benchtop multimeter was immersed into the upper part of the solution and a pH reading was taken when the reading was stable. This was repeated three times and the mean reported was as the pH of each sample. Figure 3.6 shows the pH experiment with the probes immersed into the upper portion of the solution. To determine the EC of the soil samples, the same suspension used for measuring pH was used according to Fourie (2019). After measuring the pH, the soil/distilled water suspension was allowed to stand for another hour, after which an EC probe was immersed into the suspension to take the EC readings, using the same benchtop multimeter.

Figure 3.6: *Electrode immersed into upper portion of solution (Kullander, E., Roodepoort, March 2021)*

3.5.4 Exchangeable bases and cation exchange capacity (CEC)

To determine the CEC and quantity of exchangeable bases in the soils, the ammonium acetate extraction technique, which measures cations displaced by ammonium acetate, was used (Marsh *et al*., 1995; van Reeuwijk, 2002). Thirty milllitres (30 ml) of 1M (molecule) ammonium acetate was added to 5 g of soil in 100 ml plastic wide-mouth bottles. The bottles were capped and placed on a reciprocating shaking machine overnight at 62 rpm. The next morning, the bottles were removed from the shaker, and the supernatant decanted into centrifuge tubes (Figure 3.7). The tubes were then placed into a centrifuge and centrifuged for 5 min at 3,500 rpm, the supernatant was decanted into another tube and stored. Another 20 ml of 1M ammonium acetate was added in the sample in the centrifuged tube and centrifuged again for another 5 minutes. The supernatant was again decanted into the bottle containing the first supernatant for each respective sample and used for analyses of exchangeable bases Na, Mg, Ca, and K. A Schimadzu ICP-OES

(inductively coupled plasma optical emission spectrometer) was used for the determination of the concentration of the exchangeable bases in the supernatant. The sum of the values obtained for the exchangeable bases in each sample was then used to determine the CEC of the sample.

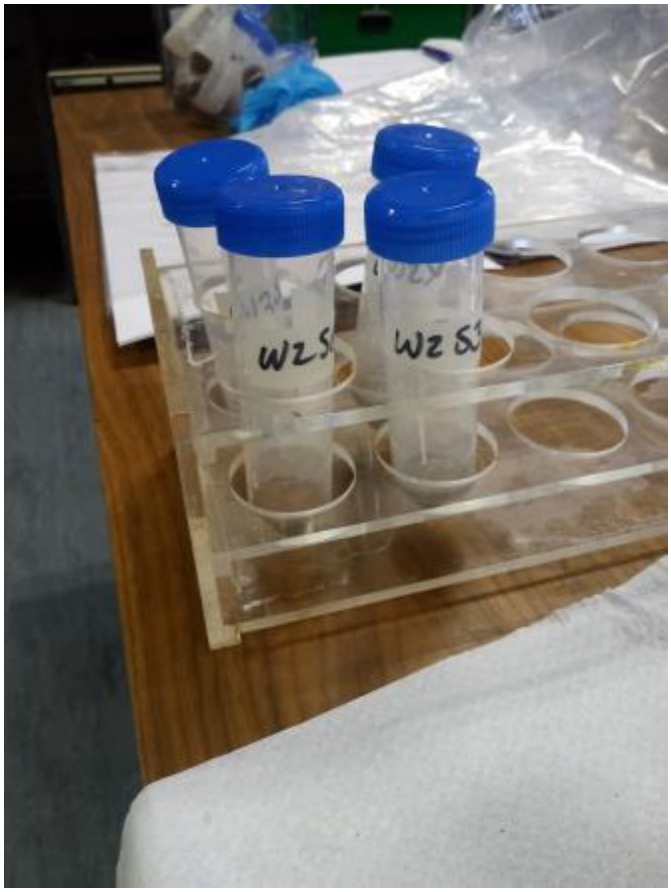

Figure 3.7: *Centrifuge tubes containing decanted NH_4OAc solution from samples (Kullander, E., Roodepoort, March 2020)*

3.5.5 Organic matter content (OM)

To determine the OM content in the soil samples, the modified Walkley-Black method was used, which involved a wet oxidation of OM with sulphuric acid, potassium dichromate, and titration with ferrous ammonium sulphate following Marsh *et al*. (1995); and Tabatabai (1996). In this procedure,10 ml of 1N $K_2Cr_2O_7$ was dispensed into 0.5 g of each soil sample weighed into 500 ml conical flasks. In a fume hood, 20 ml of concentrated H_2SO_4 was slowly added to each of the soil mixtures and allowed to stand for 30 minutes. A volume of 200 ml distilled water followed by 10 ml H_3PO_4 and 5 ml redox indicator (barium diphenylamine sulphate)

was then added to each of the flasks. Ferrous ammonium sulphate was then used to titrate the mixture until it turned to turquoise green. Figure 3.8 shows the set-up for the OM experiment. The following equation was used to calculate the content of OM in each sample.

$$\% OC = \underline{0.396} \times [vol\ of\ K_2Cr_2O_7 \times Normality] - [Vol\ of\ (Fe(NH_4)_2(SO_4)_2 \times Normality] \times mcf \times mass\ of\ soil$$

$$\% Organic\ matter = 1.72 \times \% organic\ carbon\ (OC).$$

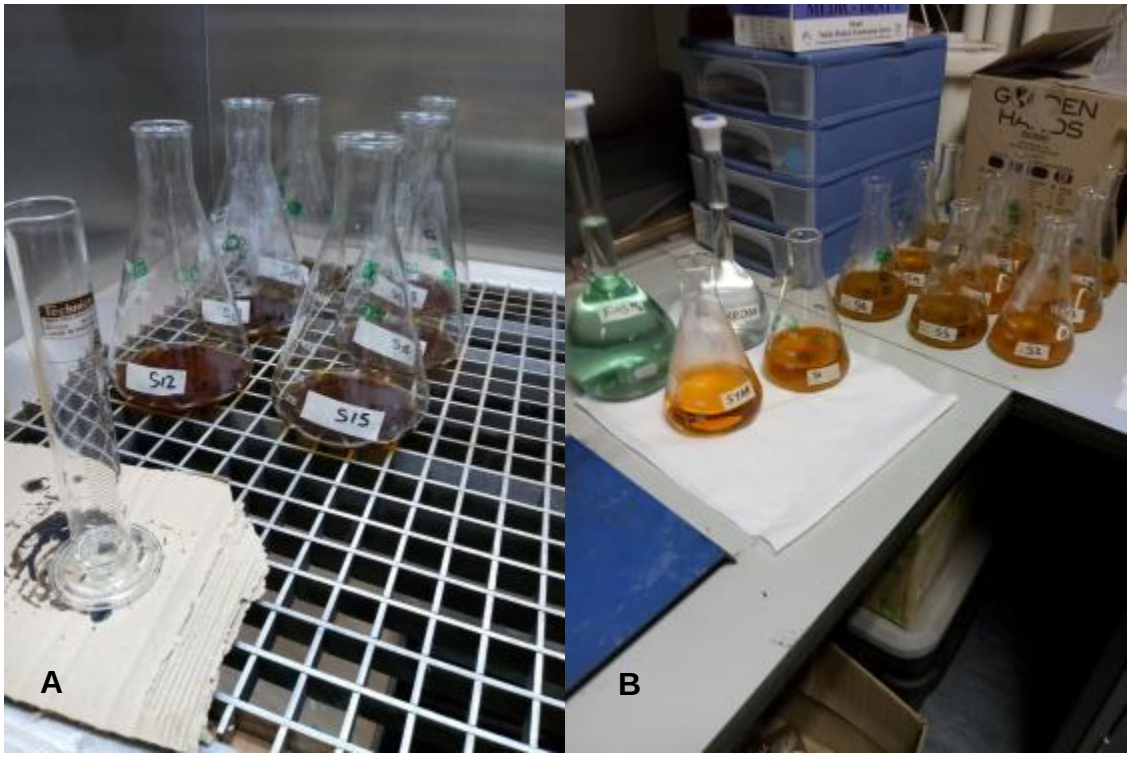

Figure 3.8: *Organic matter analysis: A indicates oxidation of OM, B indicates flask with distilled water added.*
(Kullander, E., Roodepoort, October 2019)

3.5.6 Available phosphorus

To determine the quantities of available phosphorus in soil, the Olsen, Cole, Watanabe and Dean (1954) extraction method was used (van Reeuwijk, 2002). In this method, 100 ml of sodium bicarbonate ($NaHCO_3$) solution was added to 5 g of soil in 250 ml wide-mouth plastic bottles. The bottles were capped and placed on a reciprocating shaking machine for 30 minutes at 102 rpm. Three millilitres (3 ml) of each soil suspension and two blanks, five standard phosphorus concentrations

were then each pipetted into separate test tubes and 3 ml of mixed reagent comprising 50 ml of 4 *M* H_2SO_4, 15 ml of NH_4 molybdate solution, 30 ml of ascorbic acid solution, 5 ml of KSb-tartrate solution and 200 ml distilled water, was slowly added to each test tube using a pipette. After the solution had stood for an hour, the absorbance of each sample was measured on a UV-VIS spectrophotometer at a wavelength of 882 nm. Figure 3.9 shows the standard series and soil sample solutions analysed in the spectrophotometer.

The absorbance of the standards was then used to determine the concentration of P in each sample. P concentration in the soil was determined as indicated in the following equation:

$$P\ (mg/kg\ soil) \quad = \frac{(a-b)x\ 100\ x\ 1000\ x\ mcf}{1000\ s\ gr} = \frac{(a-b)x\ 100\ x\ mcf}{}$$

Where *a* = mg/l P in sample extract

b = ditto in blank

s = sample weight in gram

Conversion factor for reporting;

mcf = moisture correction factor

Figure 3.9: *Standard series and soil sample solutions and spectrometer (Kullander, E., Roodepoort, March 2020)*

3.5.7 Micro-nutrients

To determine the concentrations of micro-nutrients including Fe, Cu, B, and Zn in the soil samples, they were extracted with 0.05 M EDTA (Jilani, 2017). Hundred millilitres (100 ml) of 0.05 M EDTA solution was added to 10 g of soil in 200 ml plastic wide-mouth bottles, thereafter the capped bottles were placed into a reciprocating shaking machine overnight at 62 rpm. Each soil-EDTA suspension was then decanted into separate centrifuge tubes. The soil was rinsed twice with 20 ml of EDTA solution, and after each rinse, the suspension was centrifuged at 3,500 rpm for 5 minutes and decanted into the same respective tube. The clear solution was analysed for micro-nutrients using an ICP-OES (inductively coupled plasma optical emission spectrometer). Figure 3.10 shows the placement of tubes in a bench-top centrifuge in the initial sample preparation (Figure 3.10 A) before being analysed in an ICP-OES machine (Figure 3.10 B).

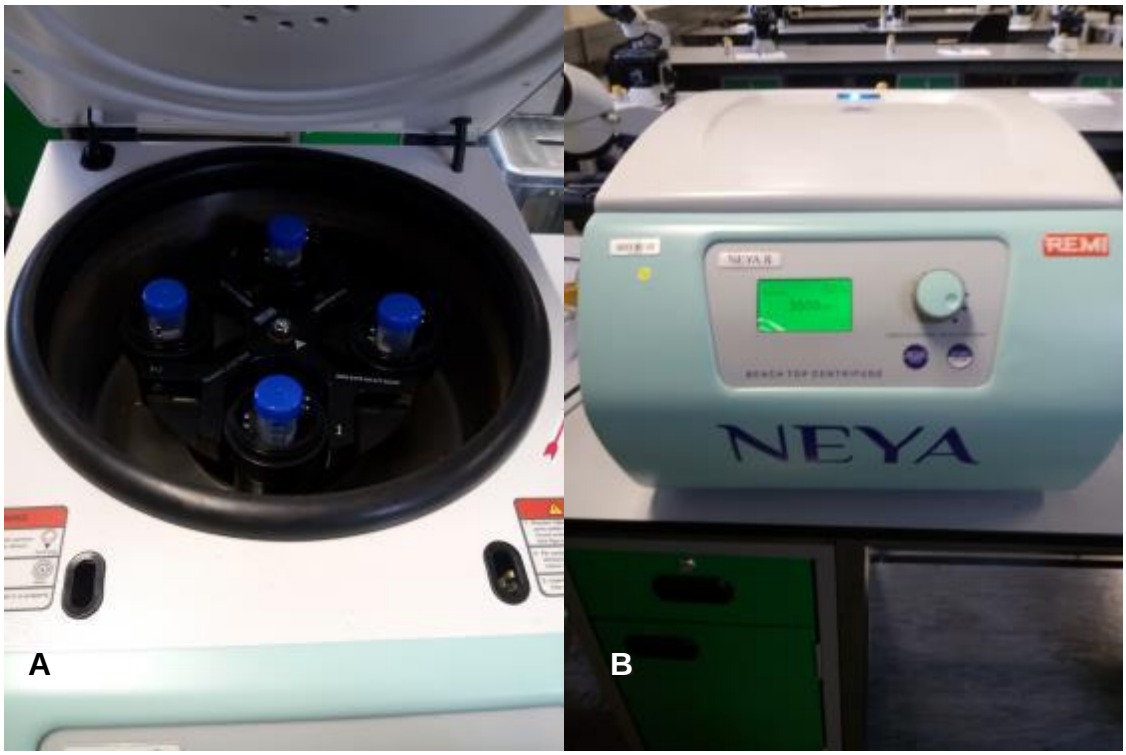

Figure 3.10: *Placement of tubes in bench-top centrifuge (Kullander, E., Roodepoort, March 2020)*

3.5.8 Determination of total carbon and nitrogen

To determine the quantities of total carbon and nitrogen in the samples, a LECO TRUMac CNS was used (LECO Corporation, 2010; Marsh *et al*., 1995). This is an

automated method that required no sample preparation. From each soil sample, 0.5 g was weighed into ceramic boats and placed on the sample magazine of the LECO TRUMac analyser. The samples were then fed into the furnace of the equipment which was set at 1,300°C. After every eighth soil sample, a standard containing 0.2 g glycine 1000 was placed in a ceramic boat as a quality control step. The readings for total C and N in the samples were then produced by the equipment. These values were then used to determine the C:N ratio of each sample. Figure 3.11 shows soil samples in ceramic boats for eventual analysis in the LECO analyser.

Figure 3.11: *Ceramic boats containing soil samples (A) for analysis in the LECO analyser (B).*
(Kullander, E., Roodepoort, March 2019)

3.6 Plant growth experiment

This phase of the project involved determining the effect of soil nutrient influxes from decomposing carcasses on the growth of the indigenous ornamental plant *G. rigens*. The plants were grown in soils collected from around the carcasses at various stages of decomposition to determine the effects of the influx of decomposing carcasses in soil on plant growth. All organic material present in the soil samples, such as insects, exuvia puparia, plant roots, stones etc., were removed as, according to Carter *et al.* (2007), all organic matter found in soils has

an impact on plant growth. The two experimental treatment groups that made up the study consisted of soils from sites with caged carcasses, and soils from sites with pegged carcasses before placement of carcasses, and from caged and pegged sites after placement of carcasses. Soil samples were collected at three-week intervals from each of these sites at various stages of decomposition. The soils samples were collected next to the steel grid cages and platforms where carcass decomposition leachates were found. Three replicates of pots from each homogenised soil sample were used from each site and each treatment of caged and pegged carcasses for growth experiments, with a total of 120 pots containing the specific soil collected from specific experimental sample plots.

The portion of the soil composite reserved for the plant growth experiments was transferred into 120 cleaned fluted pots measuring 200 mm in diameter x 130 mm height x 100 mm bottom diameter. The fluted pots were placed onto 160 mm diameter x 20 mm deep trays to collect residue water which seeped through soils once watered (Figure 3.12). Each pot was filled evenly to the same level with the specific soil samples collected, the weight of soil in each pot was on average 1, 571 kg. No additional fertilizer or additives were administered. The soil in the pots was moistened with 200 ml harvested rainwater prior to transplanting of seedlings to minimise transplant shock. Harvested rainwater was collected on UNISA greenhouses and stored in multiple 5000L water storage tanks for use in the greenhouses. Plant propagation was conducted using a completely randomised design (CRD) (Annexure A). Microsoft Excel was used to randomise pot layout (White, 2010) as indicated in Annexure A. In W1 before placement of carcasses, there were five caged and five pegged carcass sites from which soils were collected; each homogenised soil sample was divided into three pots, a total of 30 pots filled with soil. This was repeated again in W2, W3 and W4 after placement of carcasses, a total of 120 pots filled with soil.

Figure 3.12: *Cleaned fluted pots with trays*
(Kullander, E., Roodepoort, May 2019)

3.6.1 Agronomic properties of research plant: *Gazania rigens*

A total of 120 *G. rigens* five-week-old seedlings grown from seed, were acquired from Nu-leaf Nursery, in Honeydew, Gauteng (Figure 3.13). According to Joffe and Oberholzer (2012), *G. rigens* (Asteraceae), are indigenous ornamental, perennial, evergreen, hardy plants which are drought and heat tolerant. It requires full sun, well-drained soils, and can withstand growing in containers (Solomon, 2017). Optimal growing temperatures for *G. rigens* range from a minimum of 12°C at night and a maximum of 26°C during the day, in the early phases of growth (Ball Horticultural Company, 2010). Temperatures of 38°C should not be exceeded as the plants mature (Rinaldi, 2013). Taking these aspects into consideration, *G. rigens* was selected as experimental plant.

Figure 3.13: *Five-week-old G. rigens seedlings in growing tray (Kullander, E., Roodepoort, May 2019)*

3.6.2 Plant maintenance

The five-week-old *G. rigens* seedlings of same size, leaf length of 8 cm and leaf quantity of four leaves, were transplanted into the 120 pots containing the moistened experimental soil. The original growth media that the seedlings were planted in was washed off before they were planted into the experimental soils, ensuring that the seedlings came directly into contact with the experimental soil from the start of the experiment (Figure 3.14). Care was taken to minimise transplant shock. Seedlings were planted individually directly into dibber pre-made planting holes in the moistened soils. The pre-made planting hole depths were correlated with the root length of the seedlings in the original growing trays. *G. rigens* plant growth experiments were conducted in CAES greenhouse zone 3 at Unisa's Florida Campus in Roodepoort.

The plants were watered twice weekly, initially with 100 ml rainwater, and thereafter as the plants increased in size up to 250 ml of rainwater. Records of bi-weekly (Mondays and Fridays) minimum and maximum temperatures were recorded using

a minimum and maximum mercury thermometer, average minimum temperature recorded was 7.7ºC and average maximum temperature recorded was 31.7ºC. Observations were made of growth rates as depicted by plant height, and leaf numbers, colour changes of leaves, dead leaves, pests and diseases found, development of buds, opening of florescence, and setting of seeds during each watering visit. Data was recorded on an Excel spreadsheet.

Figure 3.14: *Original growth media was washed off plants to maximise contact with experimental soils*
(Kullander, E., Roodepoort, May 2019)

3.6.3 Monitoring of plant growth and harvesting

At the end of the three-month growth period all plants were harvested individually. The diameter of the root ball of each plant was measured, as well as the length of the longest root by making use of a flexible measuring tape. The root ball was washed, and the wet weight determined by making use of a digital scale before it was placed in a labelled brown paper bag to dry out for 15 days at room temperature. The dry weight was determined after the 15-day period by making use of a digital scale.

Leaf growth was determined by measuring the length of the three longest leaves. Leaf lengths were measured from the leaf apex to the base of the petiole along the leaf midrib with a flexible measuring tape. The number of leaves was counted, and the number of dead leaves noted. All leaves were harvested at the end of the three months, placed in a labelled paper bag, weighed to determine the wet weight and left for 15 days to dry out. After the 15-day period, the dried leaves were weighed again to determine the dry weight of the leaves.

The number of flower buds, inflorescences and seed heads for each plant was counted and harvested separately for each individual plant, placed in separate brown paper bags, weighed and air dried for 15 days, after which it was weighed again to determine the dry weight.

3.7 Data analysis

3.7.1 Soil data

Data was recorded on Microsoft Excel, and analysed using SPSS (JMP v15) as follows:

- To determine how decomposing carcass treatments in a grassland affect nutrients in soils and to what extent. One-Way ANOVA Pooled t, Tukey HSD and Wilcox Test analyses were used to compare soil data from different carcass sites to determine the extent to which nutrient input from the different carcasses into the soil varied.
- To determine the effect that a decomposing carcass had on the soil nutrients and how this varied with time after death of the animal, a Two-Way ANOVA with LS Means Tukey HSD analysis was used.

3.7.2 Plant data analysis

Quantitative data obtained from the plant growth experiment, such as measurements, weights and quantities of the various plant organs produced and formed under the certain experimental conditions, were analysed and evaluated. Data was recorded on Microsoft Excel, and analysed using SPSS (JMP v15) as follows:

- To establish whether the growth and development parameters of *G. rigens* were affected by soils collected around the caged and pegged carcasses, a One-Way ANOVA with Pooled t, Tukey HSD and Wilcox Test analyses were used.
- To determine if the growth and development of *G. rigens* was affected by soil nutrient concentrations in a cadaver decomposition island (CDI), a Two-Way ANOVA was used to compare soil nutrients and plant growth parameters.

3.8 Ethical considerations in the study

Ethical practices relating to the Telperion environment, disposal of chemicals and soils used at the Florida Unisa campus for soil analysis testing and soils for plant growth were as follows:

- Soil samples were collected around *C. taurinus* carcasses, next to the steel grid cages and platforms, where carcass leachants were found, from Telperion by means of an auger, holes created were back filled with surrounding soil to leave the environment as undisturbed as possible.
- Soil samples used for analysis and plant growth were stored in plastic bags sealed with cable ties, placed in a locked storage unit at the Unisa greenhouse area.
- Soils used for analysis at the Unisa laboratory were disposed of in biowaste bins provided at Unisa and removed from the campus by an external service provider; Oricol.
- Reagents required for soil analysis were locked away and stored in designated cupboards at the Florida Unisa laboratory.
- Chemicals used for soil analysis, as well as the soils used to execute the analysis, were placed separately into appropriate containers provided by Oricol for different types of waste and labelled with the chemical content. Oricol removed and disposed of waste upon request. Waste chemicals used for soil analysis were not emptied down any laboratory drains, ensuring that they did not enter the municipal network.
- Water which percolated through the experimental soils for plant growth was caught in saucers to ensure that no carcass-contaminated water polluted the greenhouses.

- Soils used in the Unisa greenhouse for plant growth were disposed of in Unisa gardens with other soils for composting purposes as they did not contain toxic or harmful chemicals such as pesticides, herbicides or fungicides.
- For personal safety, a lab coat, gloves and a mask were worn during soil analysis and plant growth experiments. Colleagues performing the same duties and who performed pest control measures wore additional safety shields and ventilation masks.

3.9 Conclusion

The study area of Telperion was an ideal site to conduct the experiments and to extract sufficient soil samples to analyse. Soil analysis was conducted successfully according to prescribed methods. The experimental set-up and plant growth period, plant organ harvesting and capturing of data were conducted according to relevant methods established from literature. All ethical considerations were implemented.

References

Ball Horticultural Company, 2010. *New Day F1 Series Gazania* [online]. Ball Horticultural Company. Available on the Internet at: https://www.panamseed.com/media/Culture/PAS/GazaniaNewDay.pdf (Accessed 27 February 2019).

Carter, D.O., Yellowlees, D. & Tibbett, M. 2007. Cadaver decomposition in terrestrial ecosystems. *Naturwissenschaften*, 94: 12–24.

Coetzee, Charleen. 2012. *The effect of vegetation on the behaviour and movements of Burchell's Zebra, Equus burchelli (Gray 1824) in the Telperion Nature Reserve, Mpumalanga, South Africa*. MSc. Dissertation, University of Pretoria, Pretoria. 174 pp.

EE Library. 2019. *Sedimentation analysis - Stokes Law* [online]. Elementary Engineering Library. Available on the Internet at: https://www.elementaryengineeringlibrary.com/civil-engineering/soil-mechanics/sedimentation-analysis-stokes-law (Accessed 12 October 2021).

Fourie, M. 2019. *What can electrical conductivity tell us about our soil?* [online]. Trace and Save. Available on the Internet at: http://traceandsave.com/what-can-electrical-conductivity-tell-us-about-our-soil/ (Accessed 6 March 2019).

Google. 2019. *25°41'35.20"S 29° 0'7.01"E* [online]. AfriGIS (Pty) Ltd. Available on the Internet at: https://www.google.com/maps/place/25%C2%B041'35.2%22S+29%C2%B000'07.0%22E/@-25.7296347,28.8227119,10.75z/data=!4m5!3m4!1s0x0:0x0!8m2!3d-25.6931111!4d29.0019472 (Accessed 11 June 2019).

Jilani, A.A. 2017. Effect of Ca EDTA and Fe EDTA on micro and macro nutrients uptake by black gram. *IOSR-JCE*, 19(2): 66-69.

Joffe, Pitta. & Oberholzer, T. 2012. *Creative Gardening with Indigenous Plants*. 2nd ed. Briza Publications, Pretoria. 391 pp.

LECO Corporation. 2010. *Total/organic carbon and nitrogen in soils* [online]. LECO Corporation Available on the Internet at: http://www.leco.co.za/wp-content/uploads/2012/02/CN2000_SOILS_TOTAL_ORGANIC_CARBON_NITROGEN_203-821-165.pdf (Accessed 20 February 2019).

Macfadyen, D.N. & Reilly, B.K. 2013. The dynamics of Buprestidae (Coleoptera) in rocky Highveld grasslands of Ezemvelo Nature Reserve, Gauteng, South. *The Coleopterists Bulletin,* 67(1): 7-15.

Marsh, M., Maynard, D.G., Morrison, I.K., Neary, A.J., Palmer, C.J., Pastorek, L. & Schumacher, B.A. 1995. Laboratory methods for soil and foliar analysis in long-term environmental monitoring programs. *United States Environmental Protection Agency*, 600(R-95): 077.

Melville, H.A. 2018. *Death in the long grass: The ecological implications of carcass decomposition in a Southern African grassland.* Applied Behavioural Ecology and Ecosystems Research Unit, Florida. 33 pp.

Mucina, L. & Rutherford, M.C. (ed.). 2006. *The vegetation of South Africa, Lesotho and Swaziland Strelitzia 19*. South African National Biodiversity Institute, Pretoria. 808 pp.

Munsell Color. 2019. *How to read a munsell color chart* [online]. Munsell Color. Available on the Internet at: https://munsell.com/about-munsell-color/how-color-notation-works/how-to-read-color-chart/ (Accessed 21 February 2019).

Olsen, S.R., Cole, C.V., Watanabe, F.S. & Dean, L.A. 1954. Estimation of available phosphorus in soils by extraction with sodium bicarbonate. *US Department of Agriculture Circular*, 939 :19.

Rinaldi, Hilary. 2013. *High heat flowers for hot summer areas* [online]. WM Media. Available on the Internet at: http://www.weekendgardener.net/garden-plants/high-heat-flowers-080908.htm (Accessed 27 February 2019).

Solomon, Lorraine. 2017. *Gazania* [online]. Kumbula Nursery. Available on the Internet at: https://kumbulanursery.co.za/plants/gazania (Accessed 9 January 2019).

Swanepoel, Barbara A. 2006. *The vegetation ecology of Ezemvelo Nature Reserve, Bronkhorstspruit, South Africa*. MSc. dissertation, University of Pretoria, Pretoria. 138 pp.

Tabatabai, M.A. 1996. *Soil organic matter testing: An overview* [online]. Alliance of Crop, Soil, and Environmental Science Societies. Available on the Internet at: https://dl.sciencesocieties.org/publications/books/abstracts/sssaspecialpubl/soilorganicmatt/1?access=0&view=pdf (Accessed 6 March 2019).

Van Reeuwijk, L.P. 2002. *Procedures for soil analysis*. 6th ed. International Soil Reference and Information Centre. The Netherlands. 119 pp.

White, R. 2010. *Randomizing a list of students using Excel* [online]. YouTube ZA. Available on the Internet at: https://www.youtube.com/watch?v=lajWM28JCHE&feature=youtu.be (Accessed 30 April 2019).

CHAPTER 4
SOIL ANALYSIS RESULTS AND DISCUSSIONS

4.1 Introduction

This chapter presents the results and discussions of soil analysis that were done in this study. The analyses carried out included soil texture, colour, electrical conductivity (EC) and pH, organic matter (OM), exchangeable bases; sodium (Na), magnesium (Mg), calcium (Ca) and potassium (K), cation exchange capacity (CEC), total nitrogen (TN), total carbon (TC) and C:N ratios, available phosphorus (P), and micro-nutrients; copper (Cu), iron (Fe) and zinc (Zn).

4.2 Soil properties

4.2.1 Soil texture

Soil texture varied from site to site within the study area. The percentage of the different particles in soils from around caged carcass sites was on average 84.47% sand, 12.18% silt and 3.35% clay, and in soils from around the pegged carcass sites 88.37% sand, 8.88% silt and 2.75% clay. The predominant soil texture at all ten sites was sand and loamy sand (Table 4.1). Soil texture is an important and stable soil property which influences several soil characteristics (Agriculture Victoria, 2020). It influences soil porosity, regulates soil water holding capacity, gives roots the ability to penetrate soils so that plants can access water and nutrients, and influences nutrient retention (Magdoff & van Es, 2021). Soils with high proportions of sand are reported to have poor water holding capacity, with larger air spaces which allow water to drain rapidly via gravity, thereby making this water unavailable to plant roots. Sandy soils are also nutrient deficient, as nutrients do not adhere to coarse sand particles. They also dry out faster and are susceptible to compaction, which restricts plant root penetration (Bruand, Hartmann & Lesturgez, 2005). According to Ashton, Binley, Dodd, Gao, Hawkesford, Hedden, Hodgkinson, Jin, Phillips, Ren, Shen, Watts and Whalley (2016), plant roots penetrate deeper into sandy soils to obtain water and nutrients through pore spaces, which are usually abundant in sandy soils. Sandy soils display low levels of organic carbon, cation exchange capacity, and high levels of nutrient leaching (Albrecht, Bernoux,

Blanchart, Brauman, Chotte, Feller, Ganry, Hien, Manlay, Masse, Sall & Villenave, 2005). Nutrient-rich decomposition liquids and organic matter seep through sandy soils rapidly, which may affect their degradation by soil microbes, and might in turn prohibit decomposition to some extent (Benbow *et. al*., 2016). Loamy sand soils, on the other hand, are often rich in humus, have a higher water holding capacity than sandy soils, retain moisture and nutrients, roots penetrate more densely, thereby having more access to nutrients and water (Magdoff & van Es, 2021). Plants are therefore expected to perform better on sandy loam, than on loamy soils.

Table 4.1: Temporal soil texture of each site and treatment

SN	Site number	Treatment	Soil texture	SN	Site number	Treatment	Soil texture
1	W1S1	Caged	loamy sand	22	W3S3	Pegged	sand
2	W1S3	Pegged	loamy sand	23	W3S4	Caged	loamy sand
3	W1S4	Caged	sandy loam	24	W3S6	Pegged	sand
4	W1S6	Pegged	loamy sand	25	W3S8	Pegged	loamy sand
5	W1S8	Pegged	loamy sand	26	W3S9	Caged	sand
6	W1S9	Caged	loamy sand	27	W3S11	Caged	sand
7	W1S11	Caged	loamy sand	28	W3S12	Pegged	sand
8	W1S12	Pegged	sand	29	W3S13	Caged	sand
9	W1S13	Caged	sand	30	W3S15	Pegged	sand
10	W1S15	Pegged	sand	31	W4S1	Caged	sand
11	W2S1	Caged	sand	32	W4S3	Pegged	sand
12	W2S3	Pegged	sand	33	W4S4	Caged	loamy sand
13	W2S4	Caged	sandy loam	34	W4S6	Pegged	sand

14	W2S6	Pegged	sand	35	W4S8	Pegged	sand
15	W2S8	Pegged	loamy sand	36	W4S9	Caged	sand
16	W2S9	Caged	sand	37	W4S11	Caged	loamy sand
17	W2S11	Caged	loamy sand	38	W4S12	Pegged	sand
18	W2S12	Pegged	sand	39	W4S13	Caged	sand
19	W2S13	Caged	sand	40	W4S15	Pegged	sand
20	W2S15	Pegged	sand	41	W4S13	Caged	sand
21	W3S1	Caged	sand	42	W4S15	Pegged	sand

Although soil texture is regarded as a stable property, Ndimele (2018) indicated that contaminations from oil spillages can affect soil texture and porosity, as soil particles are merged together by petroleum oil. Liquids penetrating soils from carcass decomposition are not likely to affect soil texture and porosity as those of contaminants from oil spillages. In a study conducted in Ankara, Turkey, over a six-month period, on domestic pigs (*Sus scrofa*) in sandy and loamy soil environments, findings indicated that soil texture affected the rate of decomposition, especially in loamy soils compared to sandy soils (Akcan, Farasat, Karacaoglu, Keten, Namli, Odabas, Sert & Tumer, 2013).

4.2.2 Soil colour

Soil colours varied from site to site, with 40% of the sites having reddish brown soils, 20% yellowish brown, and the remaining being either red, weak red, dark yellowish brown, and dark brown (Table 4.2). Soil colour is a characteristic of soils that reflect the parent material, mineral composition, and organic matter content. Yellow and reddish soils indicate the presence of oxidised ferric iron oxide, whereas brown soils indicate the presence of accumulated organic matter from decomposing plants, animals and soil organisms forming humus over time (Agriculture Victoria, 2021; Lynn & Pearson, 2000). Three weeks after placement of caged and pegged carcasses (W2), the soils appeared darker, especially directly around the decomposing carcass (Figure 4.1 note red arrows). Carter *et al.* (2007) have

reported that carcass liquid increases soil moisture content, which darkens the soil and might explain the darker soil colour observed in this study.

Figure 4.1: *Dark soils observed three weeks after placement of carcasses (Kullander, E., Telperion, February 2019)*

Despite the darkness of the soil, no colour changes were observed in the soils before placement of caged and pegged carcasses (W1) and after carcass placements (W2, W3 and W4) (Table 4.2). The liquids draining from the caged or pegged carcasses at each of the ten sites did not appear to have made any change to the soil colour. It is expected (Benbow *et al*., 2016) that the addition of organic matter to soils around carcasses may result in darkening of the soils due to the increase in humic acid and moisture content (Benbow *et al.*, 2016). A lack of soil colour change observed in soil samples collected from around the carcasses may indicate that the leached decomposition liquids from the carcasses may have been clear and void of colour-causing substances. Sites without any carcasses were control sites not analysed for this study; S2, S5, S7, S10 and S14.

Table 4.2: Soil colour before and after collection periods

Site number	Treatment	Hue	Value	Chroma	True colour
S1	Caged	5YR	4	4	Reddish brown
S3	Pegged	10YR	4	4	Dark yellowish brown
S4	Caged	10YR	5	4	Yellowish brown
S6	Pegged	5YR	4	4	Reddish brown
S8	Pegged	10YR	5	4	Yellowish brown
S9	Caged	5YR	4	4	Reddish brown
S11	Caged	10R	4	4	Weak red
S12	Pegged	2.5YR	4	6	Red
S13	Caged	2.5YR	4	4	Reddish brown
S15	Pegged	10YR	4	3	Dark brown

4.2.3 Soil electrical conductivity (EC)

Soil EC values before placement of carcasses (W1) ranged from 41.1–61.9 µS/cm and from 32.0–64.0 µS/cm in soils from around caged and pegged carcasses respectively, with means of 48.06 µS/cm, and 45.42 µS/cm respectively. After placement of caged carcasses, the mean soil EC values increased to 130.78 µS/cm, then 189.38 µS/cm, and finally 204.14 µS/cm at weeks 2, 3 and 4, respectively. Soils from sites with the pegged carcasses had slightly lower EC values compared to soils from the sites with caged carcasses, with values of 118.8 µS/cm (W2), 158.72 µS/cm (W3), and 156.82 µS/cm (W4). The EC values of soils collected from the caged carcass sites increased from week 2 to week 4, whereas the increase in soil EC around the soil from pegged carcasses sites was noted between week 2 and week 3, with a slight decline in week 4 (Figure 4.2). The differences observed in soil EC values in the soils from one week to the other were significant ($p = 0.0004$).

There are vast differences in EC values reported in literature for soil samples taken from decomposing carcass sites. These differences have been attributed to location, temporal variances, animal size and soil textures. In a study conducted on a red deer (*Cervus elaphus*) carcass over a three-month period in a temperate

grassland area with sandy soils, soil EC values were reported as varied throughout the sampling sites (Krawczynski & Schlaghamerský, 2015). In a study conducted in grassy eucalypt woodland area, located near Canberra, southeast Australia, over a five-year period, soils from kangaroo carcasses (*Macropus giganteus*) indicated mean EC values of 56.76 µS/cm on control sites without carcasses and 62.66 µS/cm around carcasses (Barton *et al*., 2016). These EC values are much lower than what was observed in this study.

The expectation was for EC values in the soils collected from the carcass sites to increase with time for the first few weeks after death because of the addition of organic compounds into the surrounding soils from the decomposing carcasses, which was indeed the case. Soil EC is influenced by the amount of dissolved salts in the soil. According to studies by MacMohan & Parmenter (2009) on various sized and types of carcasses, carcasses contain S, K, Ca, Mg, Cl, carbohydrates, and bicarbonates which leach into soils during decomposition. The leached carcass liquids may have contained soluble salts that were added to the soil, contributing to the increased EC values in the surrounding soils (Brau, Heo, Jalaludin, Shamsudin & Yong, 2019).

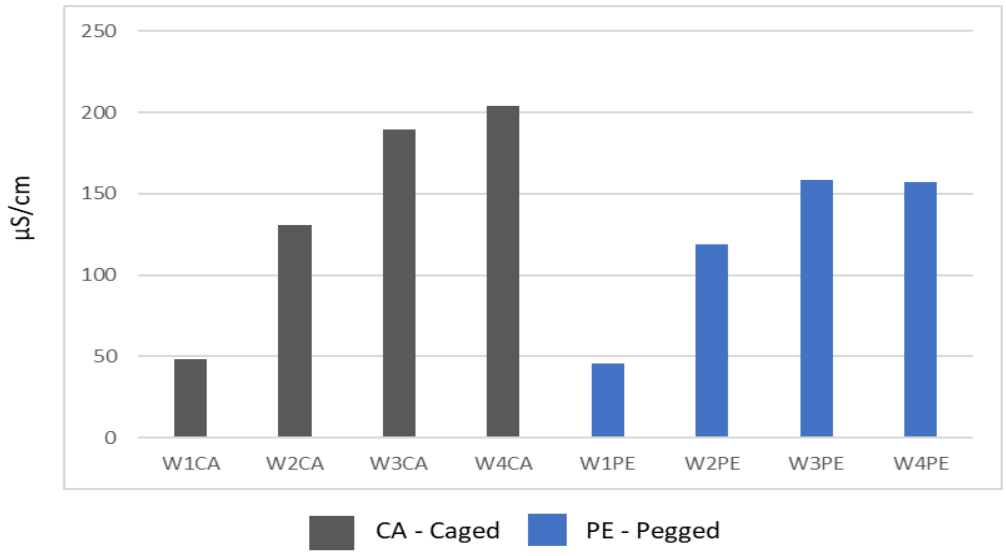

Figure 4.2: *Electrical conductivity over four-week collection periods and treatments*

Dutton, Post, Puth, Rosi & Subalusky (2020) reported in a study conducted in Kenya on the bones of the Serengeti wildebeest (*C. taurinus*), that it takes 78-119 days for bone matter to decompose. Field observations indicated dry carcasses devoid of

microbial activity in W4; however, increased EC values, especially in soil collected from caged carcasses sites, may be attributed to the release of organic matter from microbial activity within the surrounding soils or from the bones which may still have been decomposing and contained decomposing fluids with saline solutions to elevate the EC values in week 4. The lower EC values of pegged carcasses is not unknown, further studies need to be conducted to explain this phenomenon further. Soil EC values are used to assess salt content or salinity in soils. EC values of less than 1 µS/m are regarded as non-saline, whereas values greater than 1 µS/m are regarded as saline soils with a high content of soluble salts, which could become sodic affecting soil drainage. High EC values have a negative effect on nitrogen cycling and decomposition with a decrease in soil nitrogen levels (USDA, 2014).

4.2.4 Soil pH

The pH of the soil samples collected from around the caged carcass sites before placement of carcasses (W1) ranged from 4.49–5.38, with an average pH of 4.86, whereas the pH of soils around the pegged carcass sites before placement of carcasses (W1) ranged from 4.53–5.2, with an average of 4.81. The mean soil pH values around both the caged and pegged carcasses increased after placement of the carcasses to 5.74 and 5.73, respectively from week 2 (W2). During the third week (W3), soil pH values continued to increase around the caged carcasses (5.88), but decreased slightly (5.67) around the pegged carcass, with soil pH around both the caged and pegged carcasses decreasing in week 4 (W4) to 5.31 and 5.34, respectively (Figure 4.3). In a study conducted on kangaroos (*Macropus giganteus*) in a grassy eucalypt woodland area, over a five-year period, mean pH levels of 5.29 on a kangaroo carcass site, and 5.38 on a control site without a kangaroo carcass were reported (Barton *et al.*, 2016). The results obtained in this study, therefore, compare with those reported by other researchers in similar studies. Soil pH in the soils collected from the caged carcasses was higher than those in samples collected from areas with pegged carcasses; however, the differences were insignificant ($p = 0.9$).

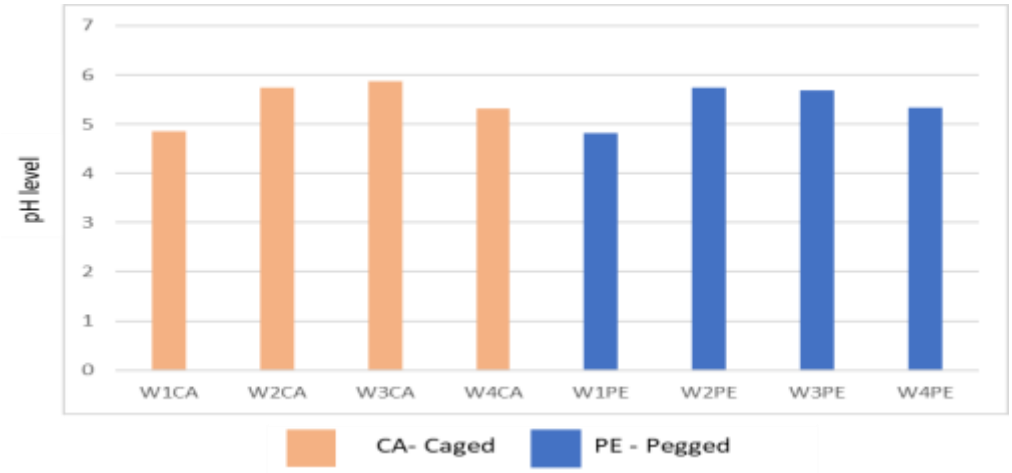

Figure 4.3: *Temporal variation of soil pH in areas around pegged and caged carcasses*

Soil samples before placement of carcasses were acidic; this could be attributed to a high organic matter content already present in the soils (Bickelhaupt, 2020). In this study organic matter was present in the soils before placement of caged carcasses, with a mean organic matter content of 0.484% for the caged and 0.466% for the pegged sites at week one (W1). After placement of carcasses, leached liquids from the carcasses could have reduced the acidic levels of the soils before increasing the acidity in W4. A similar pH (8.2–8.5) pattern was also observed by Haslam & Tibbett, (2009) in their study of sheep (*Ovis aries*). The differences observed in the pH of the soil samples from one week to the other, in both caged and pegged carcasses, were significant ($p = 0.006$), especially in the second week (W2). The expectation is for pH content in soils around carcasses to increase with time for the first few weeks after death as a result of the addition of organic compounds into the surrounding soils from the decomposing carcasses. As microbial processes begin to break down organic matter to other compounds, the pH starts to decrease. Field observations during sampling indicated that very little decomposition occurred one week after the death of the animals, which could explain the observed increase in soil pH around the carcasses only from W2. The increase in soil pH around the caged and pegged carcasses in W2, and W3 in pegged carcasses, could be attributed to leaching of fluids and humic acids in the porous sandy soils, and after W4, the soils began reverting to their original acidic state. In a forensic science study of pig carcasses (*Sus scrofa*) in a woodland environment in Ontario, Canada, soil pH was reported to have fluctuated

considerably, with a significant increase over a three-week period (pH 7.7), followed by a significant decrease on day 30 (pH 6.8) (Benninger, Carter & Forbes, 2008). This study's initial increase and subsequent decrease in pH values correlate with that of Benninger *et. al.* (2008).

4.2.5 Soil organic matter

Organic matter (OM) content in soils collected before placement of caged carcasses (W1) ranged from 0.229–0.610% (mean 0.484% for the 5 caged sites) and before placement of pegged carcasses (W1) 0.074-0.909% (mean 0.466% for the five pegged sites). The OM content of soils collected from around both the caged and pegged carcasses increased from week 1 to week 4 (W2, W3 and W4) after placement. The mean OM content of soils collected after placement of caged carcasses increased to 0.99% in W2, 1.955% in W3 and 2.051% in W4, whereas after placement of pegged carcasses mean soil OM content collected around the carcasses increased to 0.831% in W2, 1.621% in W3 and 1.754% in W4. The differences observed in OM % in the samples from one week to the other, were significant ($p = 0.00$). In a study conducted over a three-year period in South Korea using 3,147 slaughtered pigs (*Sus scrofa*) with foot-and-mouth disease buried in a 400 m² area, mean soil OM values of 0.61% were recorded outside the burial site and 12.7% inside the burial site (Kim, Kim, Kim, Shin & Yoon, 2017). The increase in organic matter content of the soils reported by Kim *et al*. (2017) correlates with the findings of this study. In another study conducted in Germany over a two-year period on a badger (*Meles meles*) carcass on sandy soil, no significant changes in OM content were observed (Gu, Klonowski, Krawczynski, Rössler & Wiegleb, 2015), which could be attributed to the length of the study and size of the carcass compared to this study.

Organic matter content in soils collected around the pegged carcass was slightly lower compared to soils from around the caged carcasses, but the differences were insignificant ($p = 0.3$). The temporal pattern in soil OM content was similar where OM content in the soils increased with time in soils around the pegged and caged carcasses (Figure 4.4). The lower OM content in soils collected from the pegged carcasses could be attributed to the variation of slope where the carcasses were

placed, which could have caused more run-off from the pegged than the caged carcass sites.

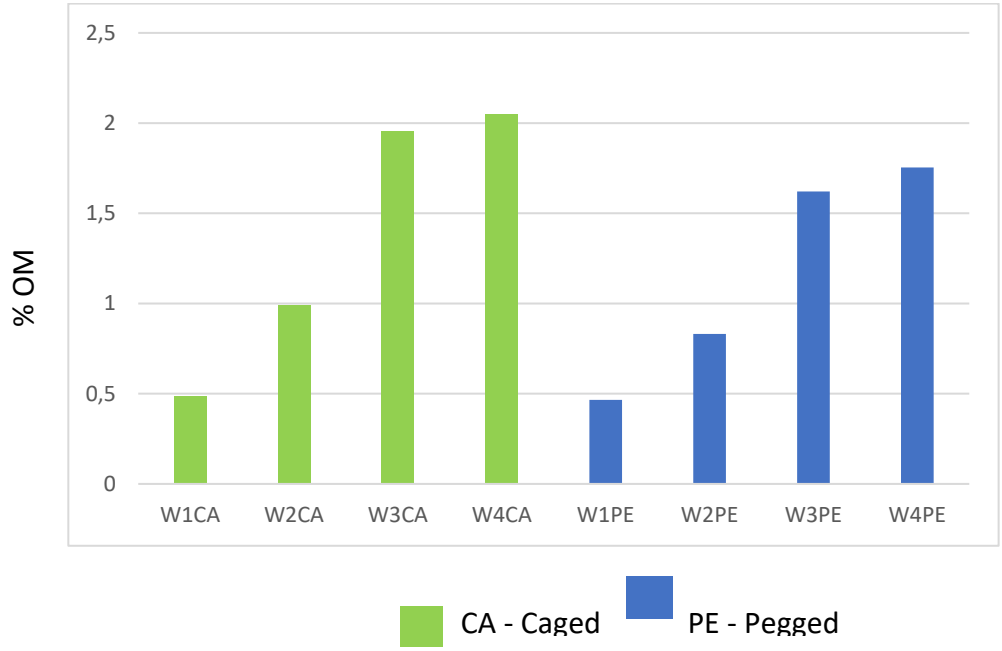

Figure 4.4: *Organic matter % temporal changes and treatments*

The expectation was for OM % in soils around caged carcasses to increase more than pegged carcasses with time for the first few weeks after death, as a result of scavenger removal of carcasses in pegged sites. However, there was very little disturbance of the pegged carcasses, and so the amount of organic matter added to the soils around the pegged carcasses was most likely similar to what was added to the soils around the caged carcasses, hence the similarity in the patterns of OM content. The results of this study indicate that animal carcasses play a significant role in the patterns of OM in soils around where they are found. Organic matter is important in soil as it improves soil structure, increases resistance to compaction, improves water holding capacity, releases nutrients into the soil as it decomposes, aids in nutrient retention and availability, as soil phosphorus and nitrogen for plant absorption in the form of humus, is responsible for soil carbon sequestration from the atmosphere, enhances rainwater infiltration and prevents soil erosion (Bergtold & Sailus, 2020; James Lind Institute, 2019). Organic matter also protects the soil from evaporation, crusting, regulates soil temperatures, stimulates seed germination, root development, plant growth, and soil microbial activity and

biodiversity (Benites & Bot, 2005). Carcasses could therefore contribute positively to plant growth in areas where they are found due to the addition of soil OM.

4.2.6 Exchangeable bases

Mean values of soil exchangeable calcium (Ca), potassium (K), magnesium (Mg) and sodium (Na) before placement of carcasses were higher than mean values after placement of carcasses (Figure 4.5). Soil exchangeable Ca mean values were the highest among the four exchangeable bases for both treatments before the placement of carcasses. The amount of exchangeable Ca in soils from around the caged carcasses was highest (0.34 meq/100 g soil) compared to pegged carcass (0.21 meq/100 g soil). A decrease in mean soil exchangeable Ca values was observed from W2 (0.05 meq/100 g soil) to W4 (0.04 meq/100 g soil) after placement of carcasses around both caged and pegged areas. This temporal pattern was not observed for exchangeable K, Mg or Na in W2 to W4. However, decreases in mean K, Mg or Na values were observed to a lesser degree after placement of caged and pegged carcasses compared to Ca changes (Figure 4.5). Decreases in exchangeable base values were significant from one week to another for all exchangeable bases (Ca, K ($p = 0$), Mg ($p = 0.00001$), Na ($p = 0.0016$)); however, differences between exchangeable bases in soils collected from around caged and pegged treatments were insignificant: Ca ($p = 0.2$), K ($p= 0.5$), Mg ($p = 0.3$), Na ($p= 0.7$). According to MacMohan & Parmenter (2009), typical nutrient composition in mule deer (*Odocoileus hemionus*) carcasses is Ca 3.09%, K 0.953%, Mg 0.091% and Na 0.388% in a semi-arid, shrub–steppe environment. They also found that soil nutrients increase from leached fluids of mule deer over a 39-month period. In this study a decrease in exchangeable bases was recorded, especially after placement of carcasses; the decrease in exchangeable bases may have been affected by the rains experienced at Telperion or possibly by an increase in micro-organism activity from influx of nutrients into the soils and by plant growth in which plants absorbed more nutrients in the surrounding soils at Telperion.

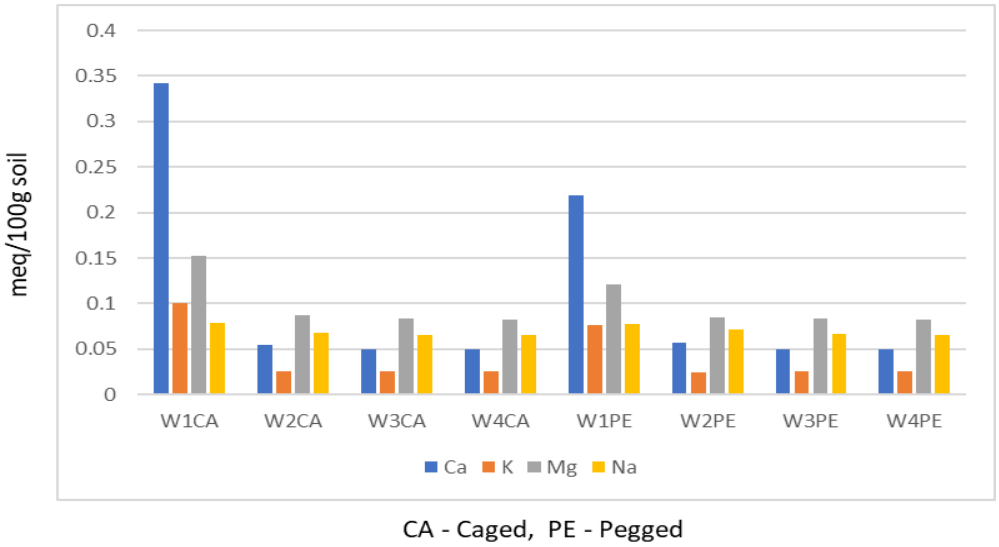

Figure 4.5: *Exchangeable bases; calcium (Ca), potassium (K), magnesium (Mg) and sodium (Na) temporal variations in four-week collection periods and treatments*

According to Fan, Goyne, Kabrick & Meinert (2011), exchangeable bases are affected by slope position and water movement within the soil which could cause their leaching. The caged and pegged carcasses were placed into their locations during the raining season which may explain the decreases in exchangeable bases throughout all the ten sites. Field observations indicated a return of plant growth, especially around the unrestricted areas around metal platforms that the carcasses were placed onto, which could also explain the decrease in macro-nutrients as they were absorbed by the plants (Figure 4.6). According to studies by Bernard, De Bruyn, Phillips & Taylor (2020), nematode population increased significantly in soils leached with liquid from six adult beaver (*Castor canadensis*) carcasses in a humid subtropical region over an 11-month period, and contributed to a decrease in soil exchangeable bases. The decrease in exchangeable bases could also be attributed to the abundant presence of soil nematode in this study.

Figure 4.6: *Plant growth around dried remains of caged carcass (Kullander, E., Telperion, March 2019)*

4.2.7 Cation exchange capacity (CEC)

Cation exchange capacity (CEC) values in soils collected before placement of carcasses ranged from 2.81-4.02 meq/100 g (mean 3.37 meq/100 g) for caged carcasses (W1), and 2.74-3.73 meq/100 g (mean 3.29 meq/100 g) for pegged carcasses (W1). After placement of caged carcasses, soil CEC values increased in W2 (mean values 4.72 meq/100 g) and decreased again in W3 (mean value 3.62 meq/100 g) and W4 (mean value 3.56 meq/100 g). The differences observed in CEC in the samples from one week to the other were insignificant ($p = 0.63$). The trend of the soil CEC increase around caged carcases was not matched in soils collected around pegged carcasses. After placement of pegged carcasses, CEC values increased slightly in W2 (mean value 3.34 meq/100 g) and in W3 remained the same, with a slightly greater increase observed in W4 (mean value 3.60 meq/100 g) compared to W3, but not to the extent observed in soils collected from around caged carcasses (Figure 4.7). These patterns indicate that the carcasses had an influence on CEC of the soils directly around the carcasses, especially in W2 for caged carcasses and in W4 for pegged carcasses after placement of

carcasses. This could be attributed to the increased soil pH values and especially the addition of organic matter content from the leached carcass fluids. Soils containing organic matter and a neutral soil of pH7 will have a higher CEC value than a soil with pH5. As OM matter increased, so too did CEC values, which in this study indicates that the addition of organic matter from decomposing carcasses had a positive effect on the CEC soil pH and OM increases contributed positively to CEC values. CEC values in the soils collected from the caged carcass samples were higher than those in samples collected from areas with pegged carcasses; however, the differences were insignificant ($p = 0.4$).

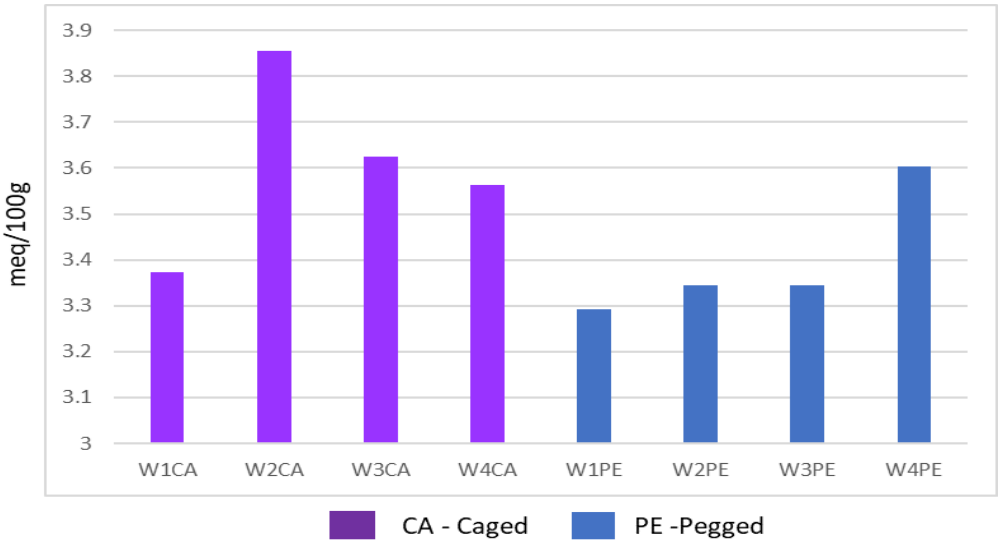

Figure 4.7: *Cation exchange capacity over four-week collections periods and treatments*

CEC indicates the capacity of soils to retain non-acidic base nutrients; calcium (Ca), magnesium (Mg) and potassium (K) and other cations, making them available to plants when needed. It is dependent on parent rocks from which soils are developed, is affected by soil pH, soil organic matter content and texture. The CEC values obtained in these soils reflect the texture, OM content and pH of the soils. As pH values increase (less acid), so does CEC due to the availability of more negatively charged sites, especially on OM and soil minerals which contain pH dependent charges. The CEC values were relatively low and are not likely to make a significant contribution to the retention of nutrients by the soils.

4.2.8 Soil total carbon, total nitrogen and C:N ratios

Total carbon % (TC) content in soils collected from around caged carcass sites ranged from 0.56-0.99% (mean 0.76%) before placement of carcasses (W1), and in soils from pegged carcass sites from 0.47-0.91% (mean 0.68%) before placement of carcasses (W1). Total carbon content in soils from around both caged and pegged carcasses increased in W2 after placement of carcasses with values of 0.91% (mean) for soils from around caged carcasses and 0.77% (mean) for soils from around pegged carcasses. Decreases in soil TC content were observed for W3 (mean 0.84%) and W4 (mean 0.73%) in soils from around caged carcasses, and in W3 for soils from around pegged carcasses (mean 0.71%). An increase was, however, observed in W4 for soils from around pegged carcasses (mean 0.84%) (Figure 4.8). The differences observed in soil TC content in the soil samples from one week to the other were insignificant ($p = 0.7$). Soil TC content in the soils collected from the caged carcass was higher than those in soils from around the pegged carcasses. However, the differences were insignificant ($p = 0.7$).

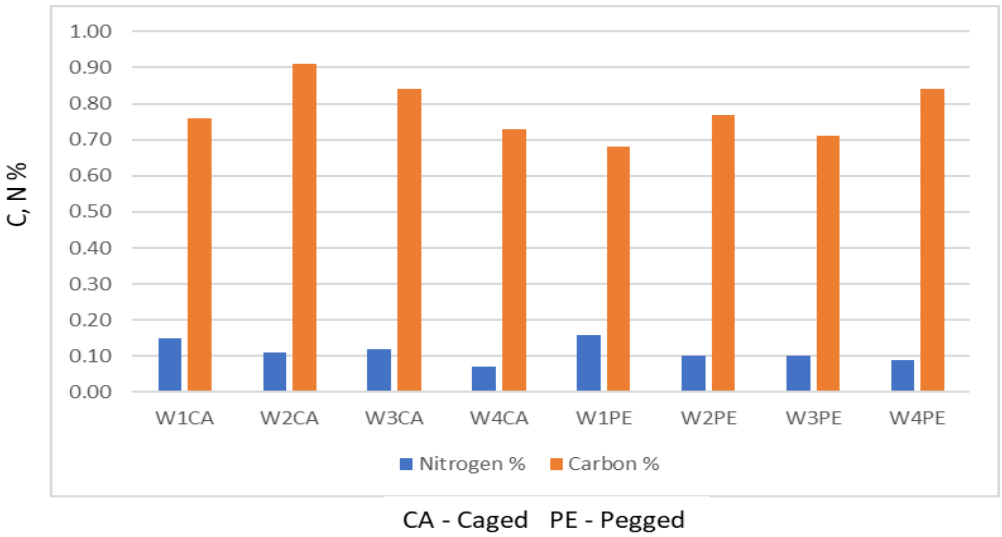

Figure 4.8: *Temporal differences for total nitrogen % and total carbon % content for caged and pegged treatments*

Total nitrogen % (TN) content in soils collected from around caged carcasses ranged from 0.07-0.20% (mean 0.15%) before placement of carcasses in week 1 (W1), and in soils from around pegged carcasses sites from 0.06-0.21% (mean 0.16%) before placement of carcasses in W1. From W1 to W2 soil TN content decreased after placement of carcasses around both caged (mean 0.11%) and

pegged (mean 0.10%) carcasses. Soil TN content around caged carcasses increased in W3 to 0.12% (mean), and decreased in W4 to 0.07% (mean); however, in soils around pegged carcasses, TN decreased to 0.10% (mean) in W2 and W3, and again in W4 to 0.09% (mean) (Figure 4.8). The differences observed in soil TN content from one week to the other were significant ($p = 0.006$). Soil TN contents in the soils collected from the caged carcasses were in some instances higher than those in samples collected from areas with pegged carcasses; however, the differences were insignificant ($p = 0.98$).

In a study conducted on seven bison (*Bison bison*) carcasses and twelve elk (*Cervus canadensis*) carcasses in Yellowstone National Park, USA, both in grasslands and shrublands over a two-year period, soil C mean values were recorded as 2.85 to 29.31%, and N mean values were recorded as 0.24 to 1.91% (Bump, Frey, Frossard, Morris, Risch & Schütz, 2020). In another study conducted over a five-year period near Canberra, southeast Australia, on twelve kangaroos (*Macropus giganteus*) carcasses, in a box-gum grassy woodland area, the study reported mean values of 3.22% for TC a decrease from 2.88% on sites with no carcasses, and mean values of 0.25% for TN, a decrease from 0.22% and a mean C:N ratio of 13.00, a decrease from 13.08 (Barton *et al.*, 2016). Both studies conducted on bison and kangaroos vary from each other and do not correlate with this study. Typically, a live pig (*Sus scrofa*) of 2 months of age will contain a C:N ratio of 7.7, and N of 26 g/kg of this will leach into soils (Carter & Tibbett, 2008). N levels typically found in a carcass of a mule deer *(Odocoileus hemionus*) are 10.40%. In a study conducted in Wyoming, USA, in a shrub-steppe, on a mule deer (*O. hemionus*), 39 months after death of the animal, 63.76 g of N was released into the soils (MacMohan & Parmenter, 2009). The increase in N content in soils around carcasses in this study is therefore not unexpected.

C:N ratio in soils collected from around carcasses ranged from 2.76-9.67 (mean 5.82) and from 2.84-10.5 (mean 5) before placement of caged and pegged carcasses respectively (W1). Soil C:N ratio increased around both caged (mean 8.5) and pegged carcasses (mean 7.72) from W1 to W2, but in W3 a decrease in soil C:N ratio was observed around caged (mean 6.97) and pegged (mean 6.81) carcasses. An increase in soil C:N ratio was again observed in W4 around caged

(mean 9.92) and pegged (mean 9.17) carcasses (Figure 4.9). The differences observed in soil C:N ratios in the soil samples from one week to the other were significant (p = 0.001). Soil C:N ratios in the soils collected from the caged carcass samples were higher than those in samples collected from areas with pegged carcasses; however, the differences were insignificant (p = 0.49).

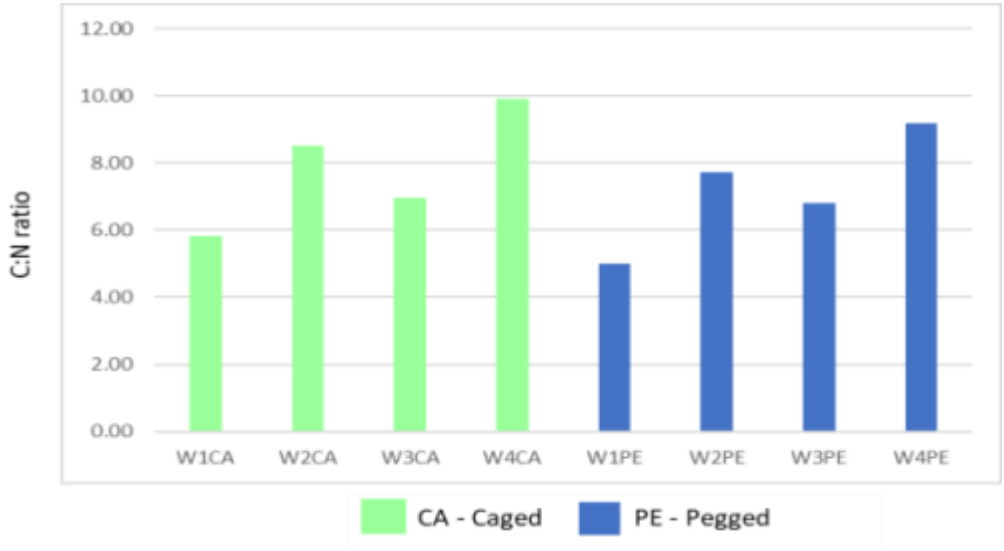

Figure 4.9: *Temporal differences for C:N ratios for caged and pegged treatments*

Carbon and nitrogen are key components of OM, and they play a significant role in soil microbial activities (Miller, 2000). Optimum levels of C:N ratios of 8:1 are required in soils for micro-organisms to accelerate decomposition and to survive; micro-organisms require a C:N ratio content of 24:1 on which to feed. High soil C:N ratios lead to slower decomposition rates and low C:N ratios lead to faster N loss, whereby plants will absorb soil N more rapidly, reducing soil N reserves. (Advance Cover Crops, 2021; Akratos, Tekerlekopoulou, Vasiliadou & Vayenas, 2017). The results for soil C:N ratios observed in this study indicate that the carcasses increased the C:N ratios of the soils to near optimum values for microbial activities, which would contribute positively to soil health.

4.2.9 Soil phosphorus (P)

Before placement of carcasses, available phosphorus (P) content in soils ranged from 0.83–8.63 mg/kg (mean 4.12 mg/kg) in sites where caged samples were to be placed, and from 1.77–6.69 mg/kg (mean 3.84 mg/kg) in soils around the sites

where pegged carcasses were to be placed. Soil P content around both caged and pegged carcasses increased from W1 to W3 (Figure 4.10). In soils around the caged carcasses in W2, mean available soil P content was 5.58 mg/kg, and increased in W3 to 8.18 mg/kg. In soils around the pegged carcasses, W2 and W3 mean available soil P was 4.19 mg/kg, and 5.21 mg/kg, respectively, also showing an increase over time. In W4, however, a decrease in available soil P was recorded around both caged (mean 5.47 mg/kg) and pegged (mean 4.77 mg/kg) carcasses. The differences observed in soil P content in the samples from one week to the other were insignificant (p = 0.25). Soil P content in soil collected from the caged carcass samples was higher than those in samples collected from areas with pegged carcasses; however, the differences were insignificant (p = 0.25).

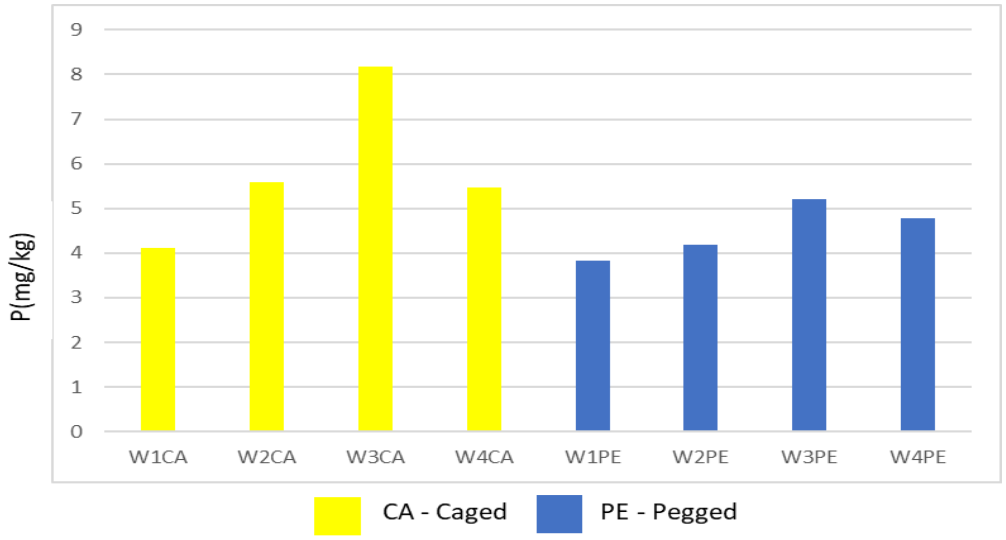

Figure 4.10: *Phosphorus temporal and treatment differences*

In a study conducted over a five-year period in Konza Prairie Research Natural Area, 10 km south of Manhattan, USA, bison (*Bison bison*), cattle (*B. taurus*), and deer (*Odocoileus virginianus*) carcasses (p = 0.05) increased soil P values significantly by between 100 and 200 mg/g (Towne, 2000). In another study conducted near Neuchâtel, Switzerland, in a spruce (*Picea abies*) forest on three pig carcasses (*Sus scrofa domesticus*) over a one-year period, mean soil P values recorded were 284.29 mg/g around carcasses, and 24.39 mg/g on empty control sites (Koenig, Le Bayon, Mitchell, Seppey & Szelecz, 2018), which correlate with this study. The decrease in available soil P content over time around animal carcasses has also been reported in other studies. According to MacMohan &

Parmenter (2009), in a study conducted in Wyoming, USA shrub–steppe terrain, mule deer (*Odocoileus hemionus*) carcasses contain 2.26% P; P loss from the mule deer carcass was 100% after 39 months, soil P changes after 15 months were 0 g, P increased to 13.05 g after 27 months, and increased further to 41.10 g after 39 months. There is a correlation to this study in that soil P values increased from W2 after placement of both caged and pegged carcasses. Phosphorus is a macro-nutrient essential for plant growth. Increase in available P in soils from around decomposing animal carcasses strongly indicates that these carcasses could serve as nutrient-rich islands which could sustain vegetation growth, providing a source of forage for other herbivores.

4.2.10 Micro-nutrients

Mean values of copper (Cu), iron (Fe) and zinc (Zn) in the soils did not increase or decrease at the same rates after placement of carcasses, nor was a difference noticed between soils from sites with caged and soils from sites with pegged carcasses. Before placement of carcasses in W1 soils collected from around areas designed to have caged carcasses contained 2.79-16.46 mg/kg Cu (mean= 9.34 mg/kg), whereas areas around sites designed to have pegged carcasses contained 2.33-15.05 mg/kg Cu (mean= 12.76 (mg/kg). Cu content in soils from both caged and pegged carcasses increased from W2 to W3 and decreased in W4 (Figure 4.11). The differences observed in Cu content in soil samples from one week to the other were significant ($p = 0.005$); the addition of leached carcass liquids containing Cu increased the soil Cu values over time. Copper content in the soils from the caged carcasses was lower than those in soil samples collected from areas with pegged carcasses; however, the differences were insignificant ($p = 0.33$).

Iron (Fe) content in soils around sites where caged carcasses were to be placed ranged from 2.94-248.38 mg/kg, with a mean of 125.05 mg/kg, whereas in soils from sites designed to have pegged carcasses, Fe content ranged from 3.83-340.22 mg/kg, with a mean 141.13 mg/kg. Fe content for both caged and pegged carcasses decreased in W2, increased in W3 and decreased again in W4 (Figure 4.11). The differences observed in Fe content in the samples from one week to the other were insignificant ($p = 0.9$). Fe content in the soils collected from the caged carcass

samples was higher than those in soil samples collected from areas with pegged carcasses; however, the differences were insignificant (p = 0.9).

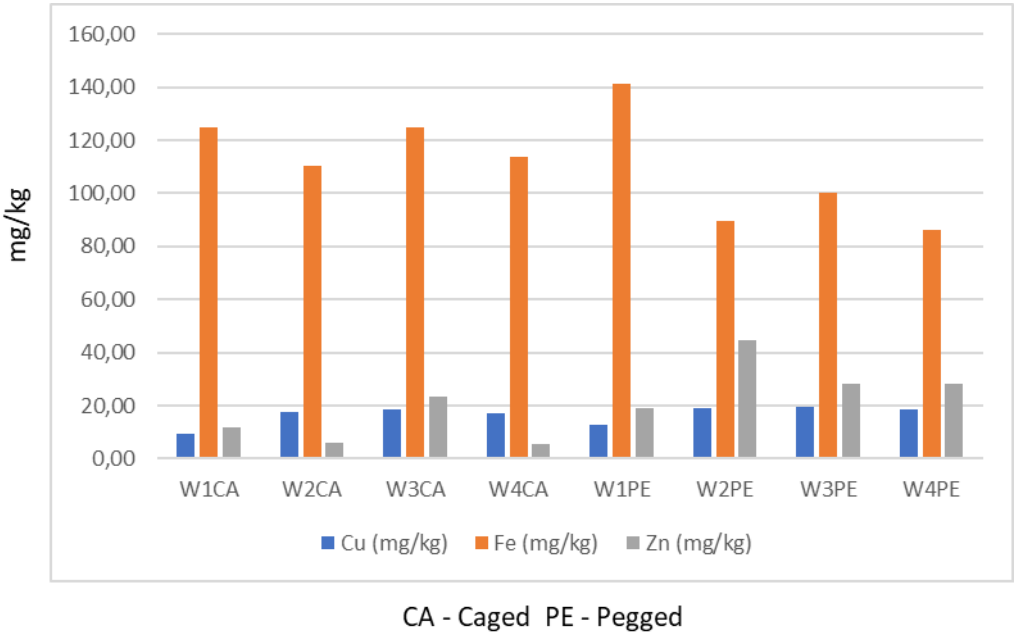

Figure 4.11: *Micro-nutrients Cu, Fe and Zn temporal changes and treatment differences*

Zinc (Zn) content in the soils before placement of carcasses ranged from 3.44-29.21 mg/kg, with a mean of 11.73 mg/kg in sites designed to have caged carcasses, whereas in sites where pegged carcasses were to be placed, the Zn content in the soils ranged from 1.19–35.86 mg/kg, with a mean 19.24 mg/kg. Zn content in soils around the caged carcasses decreased in W2, increased in W3 and decreased in W4, whereas for soils form sites with pegged carcasses, Zn increased in W2, decreased in weeks 3 and 4 (Figure 4.11). The differences observed in Zn content in the soil samples from one week to the other were insignificant (p = 0.36). Zinc content in the soils collected from the pegged carcass samples were higher than those in soil samples collected from soil with caged carcasses; the differences were significant (p = 0.004).

Carcasses usually contain these micro-nutrients as they are part of the animals' diet. Studies on young lamb carcasses show that they typically contain significantly high levels of Cu, Fe and Zn, according to Breidenstein, Cross, Field, Johnson, Lin & Randecker (1988), which could account for increased levels of these micro-

nutrients in soils around where they are found. The levels of Cu, Fe and Zn in the soils collected from the carcasses in this study are therefore not unexpected. The decrease in the levels of these nutrients in the soils could be attributed to absorption by soil micro-organisms and plants, which compete for these nutrients in the soils, as reported by Colombo, Cesco, He, Palumbo & Pinton (2014).

4.3 Summary

Nutrient islands were created after placement of both *C. taurinus* carcasses, which initially had a negative impact on plant growth; however, after the carcasses had completely dried out after a three-month period, plant growth returned. Observations of the areas directly under the metal platforms with the decomposing carcasses, indicated that these areas were completely void of vegetation at the beginning of the decomposition stage when liquids leached from the carcasses; plants also re-emerged under the metal cages after three months. Scavenger activity at Telperion was minimal, which could account for the absence of any differences reported in nutrient content in soils around the caged and pegged carcasses. Liquid inputs from decomposing carcasses indicated that soil texture and soil colour were not affected by decomposition fluids of *C. taurinus*, compared to the sites of soils collected before placement of carcasses. Soil EC increased over time in both caged and pegged treatments especially after placement of carcasses. Decomposition of caged and pegged carcasses had a positive influence on soil pH, as the soils became more neutral, which is a more favourable growing environment for plants. Leached carcass liquids contributed positively to soil OM after placement of both caged and pegged carcasses.

Values of soil exchangeable bases calcium (Ca), potassium (K), magnesium (Mg) and sodium (Na) decreased after placement of caged and pegged carcasses, especially Ca, indicating that the decomposition liquids had a negative effect on soils. There was no significant change in CEC values over time from W1 to W4, nor any significant differences between the soils collected from caged or pegged sites. Soils collected from caged and pegged sites indicated that leached carcass liquids did not contribute significantly to soil C content; however, temporal differences were reported for total N, with a significant decline in soil N over time after placement of carcasses. Soil C:N ratios increased, especially after placement of both carcass

treatments. P inputs from both decomposing carcasses had no significant contributions to soils over time or in either carcass treatments. The soil micro-nutrient Cu indicated a significant increase, especially after placement of both caged and pegged carcass treatments. Zn in soils collected from pegged carcasses was the only carcasses which reported significant differences in treatments; soils collected from caged carcasses reported lower increases after placement of carcasses. Fe did not make any contribution to soils collected from either caged or pegged treatments.

References

Advance Cover Crops. 2021. *Understanding the carbon: nitrogen ratio (C:N)* [online]. Advance Cover Crops. Available on the Internet at: https://advancecovercrops.com/resources-advanced-cover-crops/carbon-nitrogen-ratio/ (Accessed 20 October 2021).

Agriculture Victoria. 2020. *Soil texture* [online]. Victorian Resources Online. Available on the Internet at: http://vro.agriculture.vic.gov.au/dpi/vro/vrosite.nsf/pages/soilhealth_texture_prac (Accessed 26 July 2021).

Agriculture Victoria. 2021. *Soil colour* [online]. Victorian Resources Online. Available on the Internet at: http://vro.agriculture.vic.gov.au/dpi/vro/vrosite.nsf/pages/soilhealth_colour (Accessed 15 July 2021).

Akcan, R., Farasat, S., Karacaoglu, E., Keten, A., Namli, Ayten, Odabas, A.B., Sert, O. & Tumer, A.R. 2013. Effects of different types of soil on decomposition: An experimental study. *Legal Medicine*, 15: 149–156.

Akratos, C.S., Tekerlekopoulou, Athanasia G., Vasiliadou, Ioanna A. & Vayenas, D.V. 2017. *Cocomposting of olive mill waste for the production of soil amendments*. Olive Mill Waste, 161-182 pp.

Albrecht, A., Bernoux, M., Blanchart, E., Brahman, A., Chotte, J.L., Feller, C., Ganry, F., Hien, E., Manlay, R., Masse, D., Sall, S. & Villenave, C. 2005. Organic matter and biofunctioning in tropical sandy soils and implications for its management. In *Management of Tropical Sandy Soils for Sustainable Agriculture Conference*. Thailand.

Ashton, R.W., Binley, A., Dodd, I.C., Gao, W., Hawkesford, M.J., Hedden, P., Hodgkinson, L., Jin, K., Phillips, A.L., Ren, T., Shen, J., Watts, C.W. & Whalley, W.R. 2016. Deep roots and soil structure. *Plant, Cell & Environment*, 39(8): 1662–1668.

Barton, P.S., Bump, J.K., Cunningham, S.A., Evans, M.J., Manning, A.D. & McIntyre, Sue. 2016. Substantial long-term effects of carcass addition on soil and plants in a grassy eucalypt woodland. *Ecosphere*, 7(10): e01537.

Benbow, M.E., Tarone, A.M. & Tomberlin, J.K. 2016. *Carrion ecology, evolution, and their applications*. Taylor & Francis Group, Florida. 608 pp.

Benites, J. & Bot, A. 2005. *The importance of soil organic matter: Key to drought-resistant soil and sustained food and production*. Food and Agriculture Organization of the United Nations, Italy. 80 pp.

Benninger, Laura A., Carter, D.O. & Forbes, Shari L. 2008. The biochemical alteration of soil beneath a decomposing carcass. *Forensic Science International,* 180: 70–75.

Bergtold, J. & Sailus, M. (eds.) 2020. *Conservation tillage systems in the Southeast: Production, profitability and stewardship*. Sustainable Agriculture Research and Education (SARE), USA. 308 pp.

Bernard, E.C., DeBruyn, Jennifer M., Phillips, G. & Taylor, L.S. 2020. Soil nematode functional diversity, successional patterns, and indicator taxa associated with vertebrate decomposition hotspots. *PLoS ONE*, 15 (11): e0241777.

Bickelhaupt, D. 2020. *Soil pH: What it means* [online]. ESF SUNY College of Environmental Science and Forestry. Available on the Internet at: https://www.esf.edu/pubprog/brochure/soilph/soilph.htm (Accessed 3 September 2021).

Brau, E., Heo, C.C., Jalaludin, N.H., Shamsudin, N.N. & Yong, S.K. 2019. Changes in soil nutrients (ammonia, phosphate and nitrate) associated with rat carcass decomposition under tropical climatic conditions. *Soil Research*, 57(5): 482.

Breidenstein, B.C., Cross, H.R., Field, R.A., Johnson, H.K., Lin, K.C. & Randecker, V. 1988. Mineral composition of lamb carcasses from the United States and New Zealand. *Meat Science*, 24(1): 47-59.

Bruand, A., Hartmann, C. & Lesturgez, G. 2005. Physical properties of tropical sandy soils: A large range of behaviours. In *Management of Tropical Sandy Soils for Sustainable Agriculture Conference*. Thailand.

Bump, J.K., Frey, B., Frossard, A., Morris, A.W., Risch, Anita C. & Schütz, M. (2020). Effects of elk and bison carcasses on soil microbial communities and ecosystem functions in Yellowstone, USA. *Functional Ecology*, 34(9): 1933-1944.

Carter, D.O., Yellowlees, D. & Tibbett, M. 2007. Decomposition in terrestrial ecosystems. *Naturwissenschaften,* 94: 12–24.

Carter, D.O. & Tibbett, M. 2008. *Soil analysis in forensic taphonomy: Chemical and biological effects of buried human remains*. CRC Press, Boca Raton. 29-52 pp.

Colombo, C., Cesco, S., He, J.Z., Palumbo, G. & Pinton, R. 2014. Review on iron availability in soil: Interaction of Fe minerals, plants, and microbes. *Journal of Soils Sediments*, 14: 538–548.

Dutton, C.L., Post, D.M., Puth, Linda M., Rosi, Emma J. & Subalusky, Amanda L. 2020. A river of bones: Wildebeest skeletons leave a legacy of mass mortality in the Mara River, Kenya. *Frontiers in Ecology and Evolution*, 8: 31.

Fan, Z., Goyne, K.W., Kabrick, J.M., & Meinert, D. 2011. Landscape determinants of exchangeable calcium and magnesium in Ozark Highland Forest soils. *Soil Science Society of America Journal,* 75(1): 164–180.

Gu, Xiaoying, Klonowski, B., Krawczynski, R., Rössler, Annett & Wiegleb, G. 2015. Influence of a badger carcass on soil chemistry and Collembola*. Acta Societatis Zoologicae Bohemicae*, 79: 235–241.

Haslam, Tamsin C.F. & Tibbett, M. 2009. Soils of contrasting pH affect the decomposition of buried mammalian (*Ovis aries*) skeletal muscle tissue. *Journal of Forensic Sciences*, 54(4): 900-904.

James Lind Institute. 2019. *The importance of soil organic matter* [online]. James Lind Institute, School of Agribusiness. Available on the Internet at: https://www.jliedu.com/blog/importance-soil-organic-matter/ (Accessed 5 October 2021).

Kim, K-R, Kim, K-H, Kim, Y-N, Shin, D-C & Yoon, J-H. 2017. Management of animal carcass disposal sites using a biochar permeable reactive barrier and fast growth tree (*Populus euramericana*): A field study in Korea. *Sustainability*, 9: 457.

Koenig, Isabelle, Le Bayon, Renée-Claire, Mitchell, E.A.D., Seppey, C.V.W. & Szelecz, I. 2018. Soil chemistry changes beneath decomposing cadavers over a one-year Period. *Forensic Science International*, 286: 155–165.

Krawczynski, R. & Schlaghamerský, J. 2015. Does carcass decomposition affect soil-dwelling enchytraeids? *Soils Organisms*, 87(2): 91-100.

Lynn, W.C. & Pearson, M.J. 2000. *The color of soil* [online]. USDA, Natural Resources Conservation Services. Available on the Internet at: https://www.nrcs.usda.gov/wps/portal/nrcs/detail/soils/edu/?cid=nrcs142p2_054286 (Accessed 26 July 2021).

Magdoff, F. & van Es, H. 2021. *Building soils for better crops ecological management for healthy soils*. 4th ed. Sustainable Agriculture Research & Education, United States. 394 pp.

MacMohan J.A. & Parmenter, R.R. 2009. Carrion decomposition and nutrient cycling in a semiarid shrub–steppe ecosystem. *Ecological Monographs*, 79(4): 637– 661.

Miller, C. 2000. *Carbon-nitrogen ratio: Understanding chemical elements in organic matter* [online]. Eco Farming Daily, Acres, U.S.A. Available on the Internet at: https://www.ecofarmingdaily.com/build-soil/soil-inputs/minerals-nutrients/carbon-nitrogen-ratio/ (Accessed 18 October 2021).

Ndimele, P.E. (ed). 2018. *The Political Ecology of Oil and Gas Activities in the Nigerian Aquatic Ecosystem*. Elsevier, United Kingdom. 467 pp.

Towne, E.G. 2000. Prairie vegetation and soil nutrient responses to ungulate carcasses. *Oecologia*, 122: 232–239.

USDA. 2014. *Soil electrical conductivity* [online]. USDA Natural Resources Conservation Service. Available on the Internet at: https://www.nrcs.usda.gov (Accessed 7 September 2021).

CHAPTER 5
RESULTS AND DISCUSSIONS ON PLANT GROWTH EXPERIMENT

5.1 Introduction

This chapter presents the results and discussions on the growth and development of *G. rigens (L.)* in soils collected from around decomposing carcasses of *C. taurinus.* It explains and investigates how the influx of nutrients from the carcasses at various stages of decomposition (W2, W3, and W4) influenced the growth and development of the plants. Plant growth traits and development assessed included leaf length, leaf number, flowering and rooting, as well as wet and dry weight of plant organs.

5.2 Effects on the leaves of *G. rigens*

Leaves are appendicular organs on plant stems responsible for vital physiological activities such as photosynthesis, respiration, transpiration, and synthesis and supply of growth regulators (Adams, Bahadur, Krishnamurthy & Venkatasubramanian, 2015). Leaf size is largely determined by the behaviour of the cell cyclic arrest front (AF), cell division and enlargement (Adams *et al*., 2015). Nitrogen is a key nutrient required for optimal leaf growth in plants, and a nitrogen deficiency will lead to yellow older leaves (A1 Organics, 2019). The changes observed in various parameters of the leaves of *G. rigens* grown in soils collected from around decomposing carcasses of *C. taurinus* are presented below.

5.2.1 Leaf length

Leaf lengths of the three longest leaves from each plant were measured at the start and again at the end of the experimental period (three months). The average leaf lengths of plants grown in soils collected from around both caged and pegged carcasses over the three-month period of carcass decomposition increased. The longest average leaf length was measured in W3 (12.3 cm) for plants grown in soils from around pegged carcasses, whereas the average longest leaf length for plants grown in soils collected from around caged carcasses was observed in W2 (11.7 cm from the initial leaf length of 8 cm) (Figure 5.1). The differences observed in

average leaf lengths of *G. rigens* grown in soils collected from one week to the other after carcass placement were significant (p = 0.006), especially from W1 to W2 and W3. Average leaf lengths of plants grown in soils collected from the pegged carcass sites were longer than those of plants grown in soils collected from the caged carcasses; however, the differences were insignificant (p = 0.18).

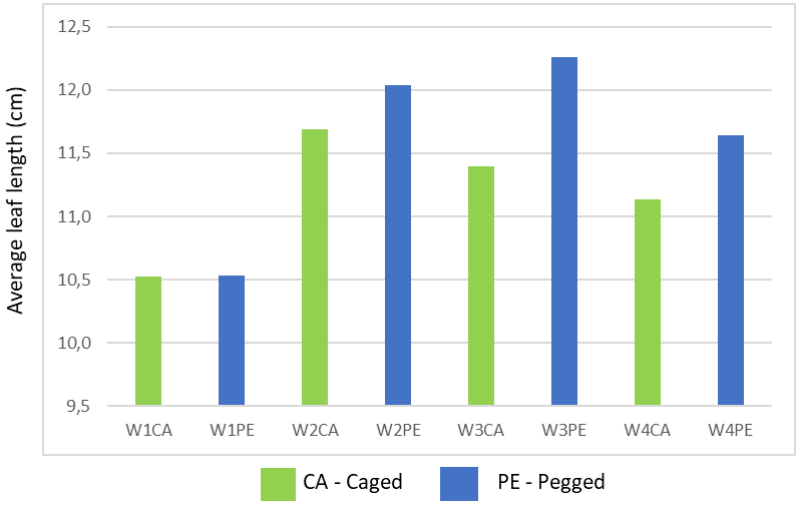

Figure 5.1: *Average leaf lengths measured at the end of the three-month growth period, for the two different treatments caged (CA) and pegged (PE) and four soil collection weeks*

Leaf development is affected by photosynthesis, available soil nutrients, especially carbon and water, cell division aided by auxins, and cell expansion which affects leaf size and length (Anozie, Chen, Morales, Sharkey, Weise & Weraduwage, 2015; Beemster, De Vos & Kalve, 2014). Phosphate is a nutrient essential for photosynthesis, energy conservation, and carbon metabolism; potassium is essential for cell expansion, and nutrient deficiencies would lead to reduced growth (Barbier, Prinzenberg, Reymond, Salt & Stich, 2010). Average leaf lengths of plants performed better in the soils collected after placement of carcasses compared to the plants grown in soils collected before placement of carcasses.

5.2.2 Number of leaves

The average number of leaves (live and dead) of each experimental plant for each treatment (caged and pegged carcass sites) and soil collection W1 (before carcass placements and after carcass placements W2, W3 and W4) was counted at the end

of the three-month growing period. The average number of leaves (live and dead) for the duration of the experiment for each of the four soil collection periods and two treatments are presented in Figure 5.2 and Figure 5.3, respectively. The average number of live leaves counted in W1 of plants grown in soils collected around caged carcass sites increased from 25.3 leaves to 47.9 leaves in W2 and 47.5 in W3. The highest average number of leaves counted was in W3 in plants grown in soils collected around pegged carcasses (50.1 leaves), which was nearly double what was counted in W1 (25.7) (Figure 5.2). The average number of leaves for plants grown in soils from around both caged and pegged carcasses increased significantly from W1 to W4 (Figure 5.2) ($p = 0.00005$). Average leaf numbers for plants grown in soils collected from around the pegged carcasses were higher than those in plants grown in soils from around caged carcasses, but the differences were insignificant ($p = 0.82$). The phenomenon of these results is clarified in the discussions in chapter 6 (6.2.2) in which the number of leaves correlates with soil findings.

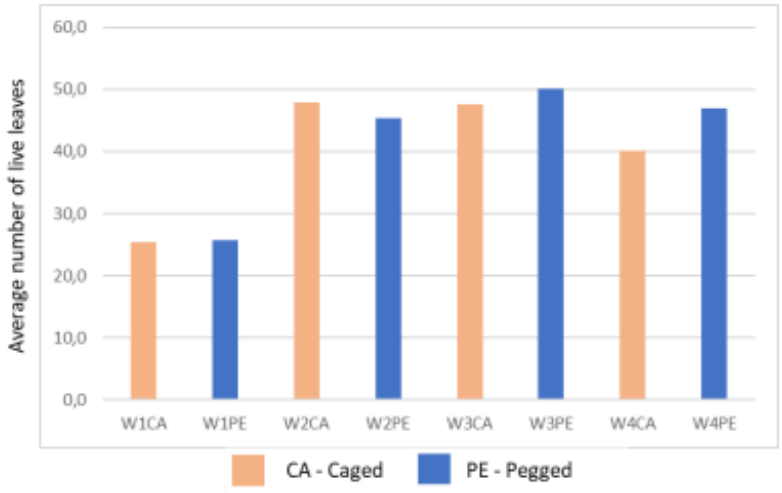

Figure 5.2: *Average number of live leaves counted at the end of the three-month growth period for the two different treatments caged (CA) and pegged (PE) and four soil collection weeks*

The highest average number of dead leaves counted was from plants grown in soils collected around caged carcasses in W3 (9.4) and W3 in plants grown in soils collected around pegged carcasses (9.3) (Figure 5.3). A decline in the average number of dead leaves per plant was recorded in W4 in the soils collected around the caged carcass areas (7.5) and W4 in soils collected around the pegged carcass areas (8.4). The differences observed in dead leaves from W1 to the other weeks

were significant (p = 0.007), especially in W3. The average number of dead leaves in the plants grown in soils from around the caged *C. taurinus* carcass was fractionally higher than those in plants grown in soils from around the pegged carcasses. However, the differences were insignificant (p = 0.87).

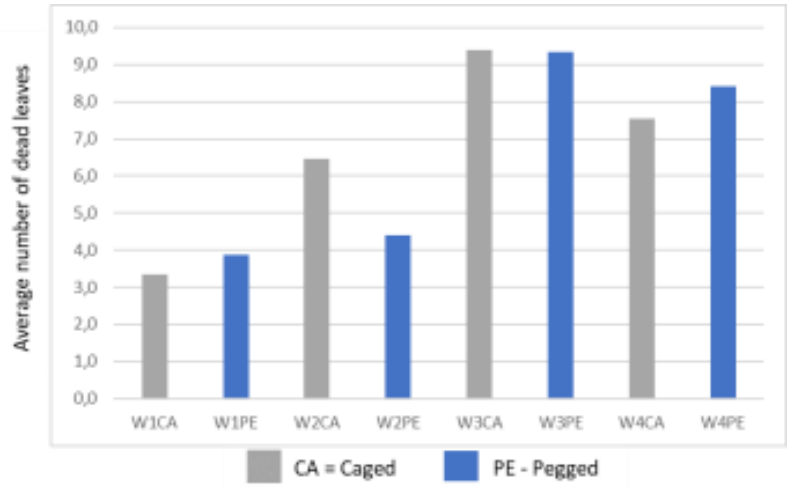

Figure 5.3: *Average number of dead leaves counted at the end of the three-month growth period for the two different treatments (caged (CA) and pegged (PE)) and four soil collection weeks*

5.2.3 Fresh and dry leaf weights

The highest average fresh leaf weight was measured in plants grown in soils collected during W3 around both caged carcasses (22.0 g) and pegged carcasses (23.1 g), whereas the average dry weight for plants grown in soils from the two different sites over the four weeks were constant at ±9 g (Figure 5.4). The differences observed in average fresh and dry leaf weights from W1 to W4 were statistically significant (p = 0), especially between W2 and W3. The differences in weights compared to W1 could be attributed to plants taking up more nutrients available in soils after the placement of carcasses. The average fresh and dry leaf weights for plants grown in the soils collected from pegged carcass sites were higher than those for plants grown in soils from caged carcass sites, but the differences were also insignificant (p = 0.68 fresh and p = 0.91 dry). Figure 5.5 indicates the photographic observations of the least number of leaves of a single plant and the pot with the most number of leaves recorded before the plant organs were harvested. The findings of fresh and dry leaf weights have been discussed in chapter 6 (6.2.3) as they correlate with the findings of soil results.

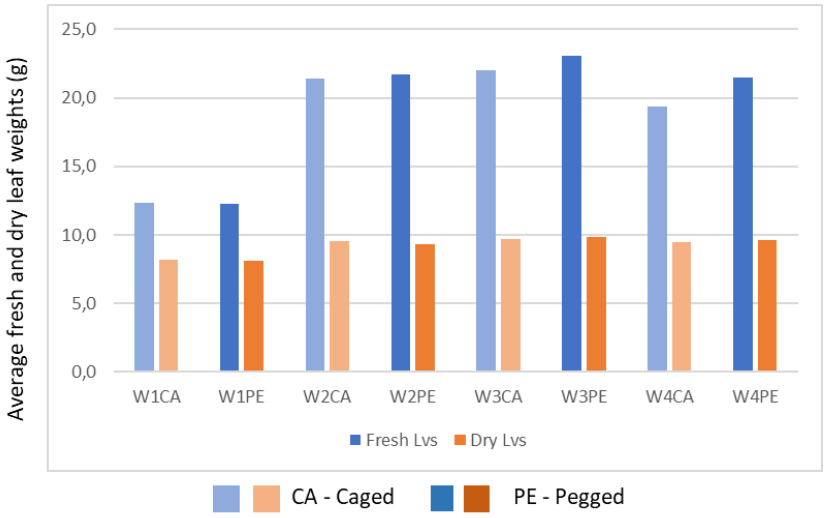

Figure 5.4: *Average fresh and dry leaf weights after a three-month growth period for the two different treatments caged (CA) and pegged (PE) and four soil collection weeks*

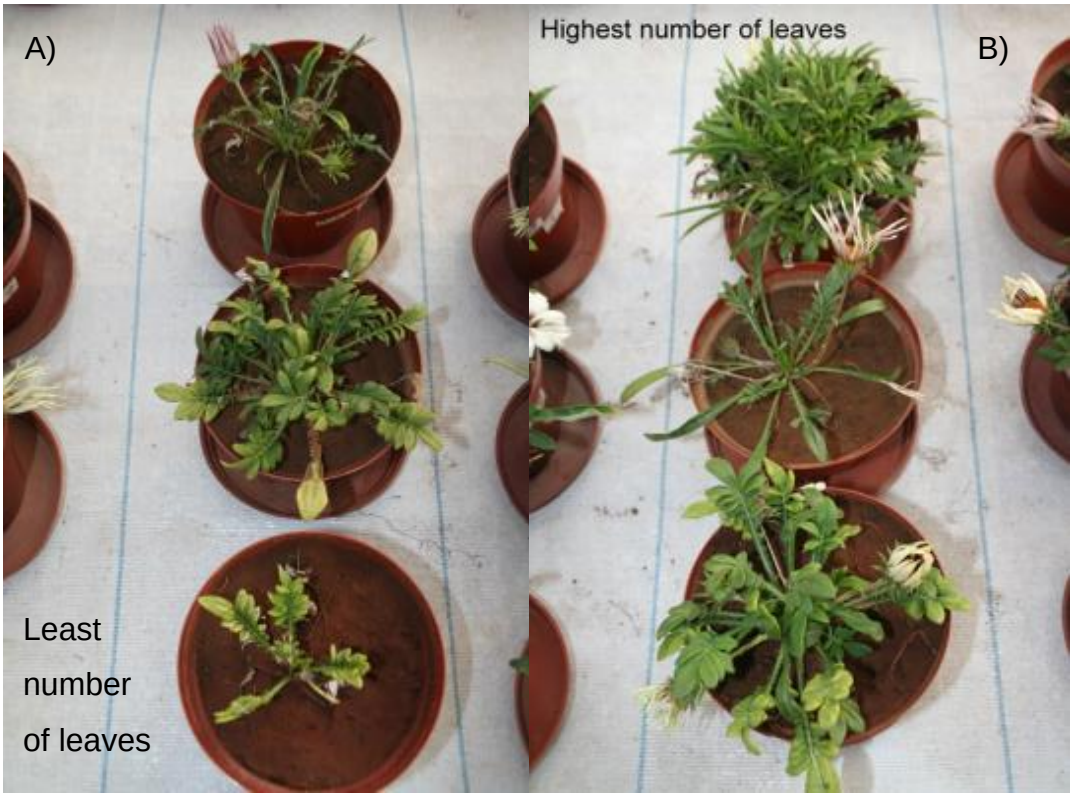

Figure 5.5: *Least number of leaves of G. rigens (A) grown in soils collected from around pegged C. taurinus carcasses in W4, and highest number of leaves (B) from around caged C. taurinus carcasses in W3, at the end of the three-month growth period*

(Kullander, E., Florida, July 2019)

5.3 Effects on flowering

Flowers are characteristically the reproductive organs of angiosperms and flower formation is affected by the amount and various nutrients available in soils. Phosphorus is essential for flowering and fruiting, whereas calcium deficiencies lead to premature shedding of buds and blossoms, and boron deficiencies lead to the death of terminal buds causing lateral buds to develop, also known as the “witches’ broom” effect (Manjula, 2017). Nitrogen and phosphorus are nutrients required by plants to promote flowering (A1 Organics, 2019). The flowering of *G. rigens* was affected by the two different soils (soils collected from around pegged carcasses and soils collected from around caged carcasses). The observations on flowering are presented in the following section.

5.3.1 Number of flower buds

Figure 5.6 depicts the average number of flower buds produced for the three-month experimental period for the two different treatments (CA, PE) and four soil collection periods. An increase in bud formation from an average of 1.2 in plants grown in soils collected around pegged carcasses in W1 to the highest average number of buds produced in plants grown in soils collected around pegged carcasses in W3 (5.9 buds) was observed. The average number of buds produced by plants grown in soils collected from around the caged *C. taurinus* carcasses after placement of carcasses was more or less the same, with values of 3.2, 3.9 and 3.6, respectively for plants grown in soils collected during W2, W3 and W4. The differences observed in the average bud formation in plants grown in soils collected from W1 before carcass placement to W4 after carcass placement were significant ($p = 0.0004$). The average number of buds formed in plants grown in soils collected from around the pegged carcass was higher than those in plants grown in soils collected from around the caged carcasses, but the differences were insignificant ($p = 0.44$). The discussions of the number of flower buds have been correlated with soil findings in chapter 6 (6.3.1).

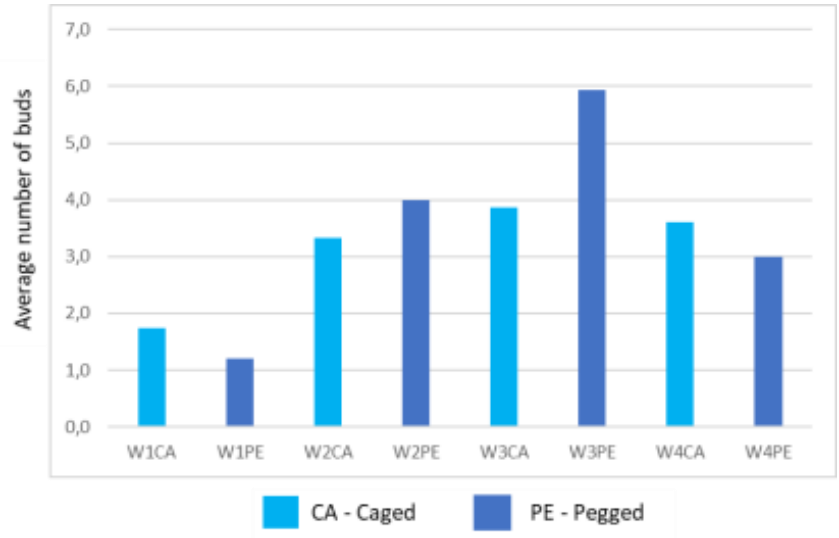

Figure 5.6: *Average number of buds produced at the end of the three-month growth period for the two different treatments caged (CA) and pegged (PE) and four soil collection weeks*

5.3.2 Number of flowers

It is evident in Figure 5.7 that the highest average number of open flowers was observed in plants grown in soils collected around caged carcasses in W3 (1.6), whereas the lowest average number of open flowers was observed in plants grown in soils collected around caged carcasses in W4 (0.6). A constant increase in open flowers was noted in plants grown in soils collected in W1 to W4 around pegged carcasses (0.7 to 1.1), whereas a spike in flower production was noted in plants grown in soils collected in W3 around caged carcasses (Figure 5.8), where an average of 1.6 flowers was recorded. The differences observed in fresh flowers from soils collected from one week to the other around the carcasses were insignificant ($p = 0.26$). The average number of fresh flowers in *G. rigens* grown in soils from around the caged carcasses was higher than those of plants grown in soils collected from around the pegged carcasses. However, the differences were insignificant ($p = 0.64$). The number of flowers correlate with soil findings which are discussed in chapter 6 (6.3.2).

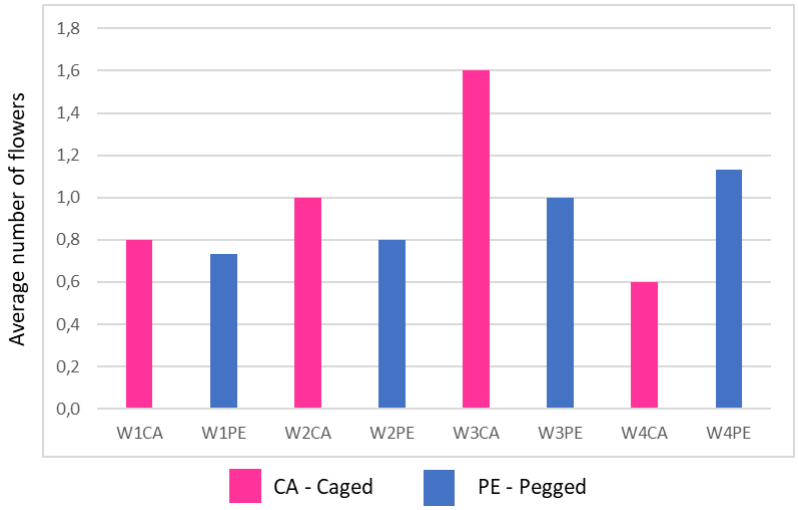

Figure 5.7: *Average number of fresh flowers produced at the end of the three-month growth period for the two treatments caged (CA) and pegged (PE) and four soil collection weeks*

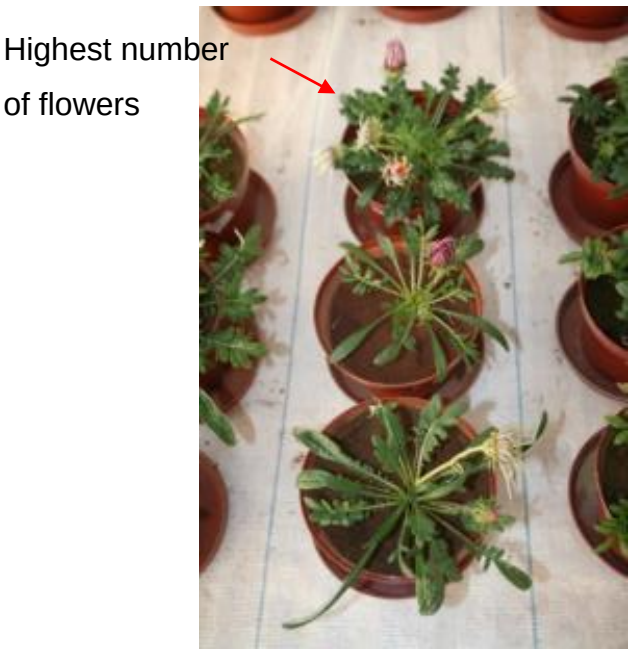

Figure 5.8: *Highest number of flowers in a single pot at the end of three-month growth period in third collection week (W3) of caged treatment*
(Kullander, E., Florida, July 2019)

5.3.3 Number of seed heads

A spike in the average number of seed heads counted was observed for plants grown in soils collected around pegged carcasses in W3 (2.6) (Figure 5.9), which correlates with the highest number of buds produced. The differences observed in

the average number of seed heads produced by plants grown on soils collected in W1 to W4 were insignificant (p = 0.26). The average number of seed heads produced by *G. rigens* plant grown in soils collected around the pegged carcasses was higher than those of plants grown in soils collected around caged carcass sites; however, the differences were insignificant (p = 0.64). A clarification and explanation of the number of seed head results can be found in chapter 6 (6.3.3), which correlates with soil findings.

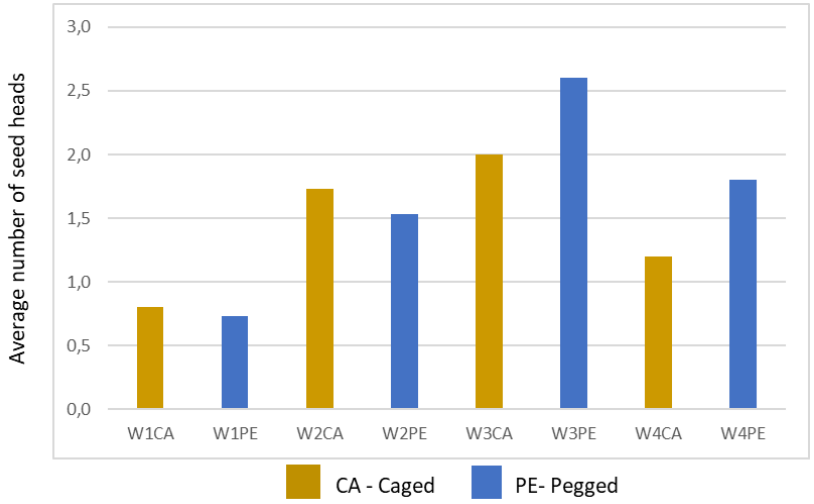

Figure 5.9: *Average number of seed heads produced at the end of the three-month growth period for the two different treatments caged (CA) and pegged (PE) and four soil collection weeks*

5.3.4 Comparison between fresh weight of buds, flowers and seed heads, and dry weight of buds, flowers and seed heads

Fresh weights of buds, flowers and seed heads present the live freshly harvested flowering plant organs, whereas the dry weight of buds, flowers and seed heads represents the dry weight of the flowering plant organs after air drying. The difference between the fresh weights and dry weights depicted cell water content of the plant organs (Kim & Park, 1993). Buds refer to the flowers which had started forming and not opened into flowers at the time of plant organ harvesting at the end of the three-month growth period. The highest average fresh weight for buds, flowers and seed heads was recorded in plants grown in soils collected in W3 around caged (14.2 g) and pegged carcasses (13.5 g). The average dry weight for buds, flowers and seed heads recorded was similar throughout; however, those in

plants grown in soils collected in W2 around caged carcasses (8.5 g) and in W3 around pegged carcasses (8.6 g) were the highest. The average fresh weight for buds, flowers and seed heads of plants grown in soils collected around caged and pegged carcasses increased in W2 and W3. The average dry weight for buds, flowers and seed heads increased in plants grown in soils collected around caged carcasses in W2 and in W3 (Figure 5.10). The differences observed in fresh and dry weight for buds, flowers and seed heads for plant grown in soil collected from W1 to the other collection weeks were significant for fresh weight ($p = 0.0003$), especially those grown in soil collected in W2 and W3, and insignificant for dry weight ($p = 0.57$). The average fresh weight for buds, flowers and seed head for plants grown in soils collected around the pegged carcasses was higher than those in plants grown in soils collected around caged carcasses, and the average dry weight was higher for plants grown in soils collected around caged carcasses than those grown in soils collected around pegged carcasses, although the differences were insignificant for fresh ($p = 0.87$) and dry weight ($p = 0.57$). The discussion and comparison between fresh weight of buds, flowers and seed heads, and dry weight of buds, flowers and seed heads can be in chapter 6 (6.3.4), as the findings correlate with the results of soil outcomes.

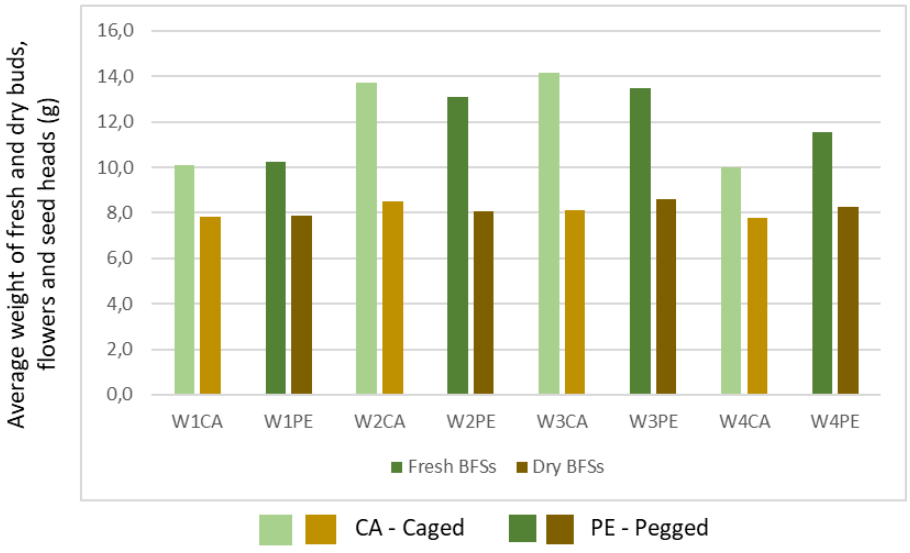

Figure 5.10: *Average fresh and dry weights for buds, flowers and seed heads over three-month growth period, for the four soil collection weeks and two treatments caged (CA) and pegged (PE)*

5.4 Roots

Roots are important plant organs as they are responsible for the anchorage of plants, as well as supplying plants with water, nutrients and hormones (Fageria & Moreira, 2011). The root growth of plants is controlled genetically but is also influenced by environmental factors and the availability of nutrients. Root growth is mainly measured in terms of root density, length and weight. The dry weight is often better related to yield than to root length or density. Increased nutrient supply in soil may decrease root length but increase root weight. Roots with adequate nutrient supplies may also have more root hairs than nutrient-deficient roots (Fageria & Moreira, 2011). According to A1 Organics (2019), phosphorus and calcium are essential nutrients for root development and growth. Phosphorus deficiency inhibits primary root growth.

5.4.1 Root length and width

The highest average *G. rigens* root lengths recorded were 12 cm in plants grown in soils collected around caged carcasses in W1, and 11.5 cm in plants grown in soils collected around pegged carcasses. A steady decline in average root length was recorded in plants grown in soils collected in W1 to W4 around both caged and pegged carcasses; W4CA 10.2 cm and W3PE and W4PE 10.7 cm (Figure 5.11). The differences observed in average *G. rigens* root lengths grown in soils collected from W1 to the other weeks were insignificant ($p = 0.46$). Root lengths for plants grown in soils collected around the caged carcasses were higher than those for plants grown in soils collected around pegged carcasses; however, the differences were also insignificant ($p = 0.84$). Correlation between root length and width findings and soil results has been discussed in chapter 6 (6.4.1).

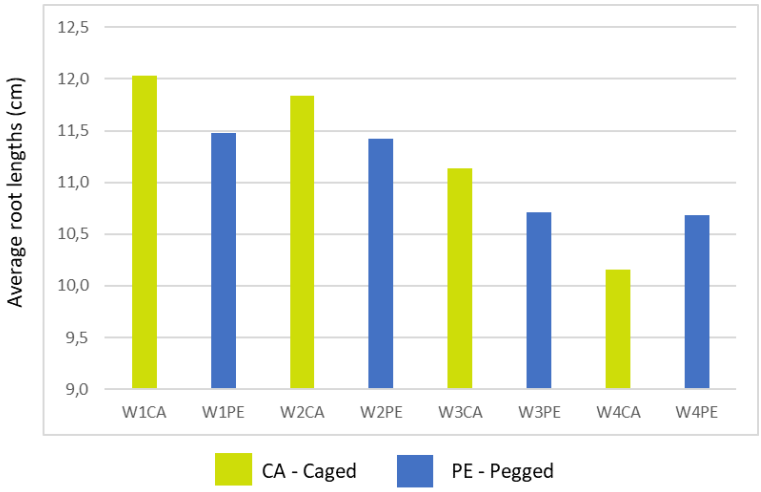

Figure 5.11: *Average root lengths at the end of the three-month growth period, for the four collection weeks and two treatments caged (CA) and pegged (PE)*

The widest average root ball width was recorded for *G. rigens* grown in soils collected around caged carcasses in W1 (4.8 cm), and 4.9 cm for *G. rigens* grown in soils collected around pegged carcasses in W2. Average root ball widths for plants grown decreased in plants grown in soils collected around caged carcasses in W2 to W4, and for plants grown in soils collected around pegged carcasses, the decrease was observed in plants grown in soils collected in W3 and W4 (Figure 5.12). The differences observed in average root ball widths from W1 to the other weeks were insignificant ($p = 0.38$). Average root ball widths for plants grown in soils collected around the pegged carcass were higher than those in plants grown in soils collected around caged carcasses, but the differences were insignificant ($p = 0.50$).

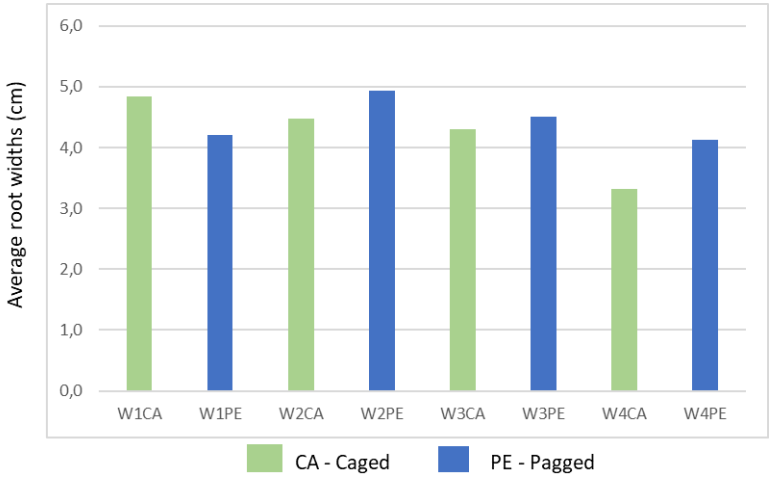

Figure 5.12: *Average root widths at end of three-month growth period, in four soil collection weeks and two treatments caged (CA) and pegged (PE)*

5.4.2 Fresh and dry root weights

The highest average fresh root weights were recorded for *G. rigens* grown in soils collected around caged carcasses in W2 (8.2 g) and in soils collected around pegged carcasses in W2, W3 and W4 (8.0 g). Average *G. rigens* fresh root weights declined as the decomposition of the carcasses increased. The highest *G. rigens* average dry root weights were recorded in W2 for soils collected around caged carcass and W3 for soils collected around pegged carcass (Figure 5.13). The difference observed in the average fresh and dry root weights from one week to the other was insignificant (p = 0.17 fresh and p = 0.27 dry). Although average fresh and dry root weights for plants grown in the soils collected around the pegged carcass plants were higher than those in plants grown in soils collected around the caged carcass, the differences were insignificant (p = 0.34 fresh and p = 0.67 dry). Fresh and dry root weights and soil outcomes have been discussed in chapter 6 (6.4.2), as there is a correlation between the two findings.

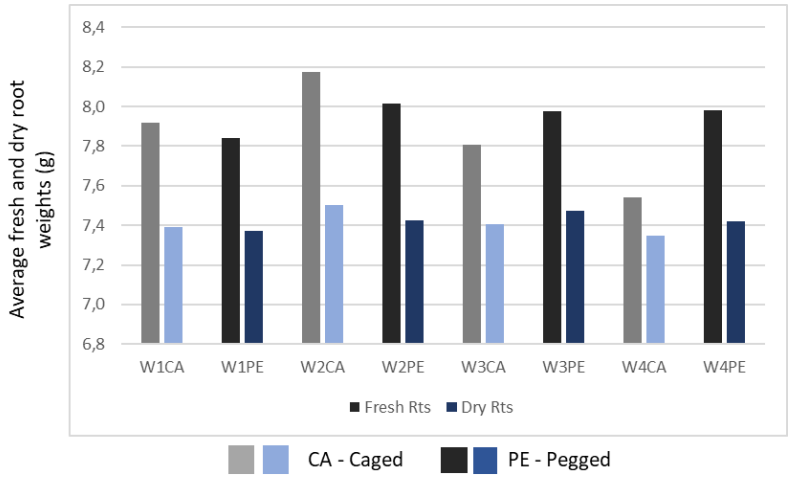

Figure 5.13: *Average fresh and dry root weights at the end of three-month growth period in four soil collection weeks and two treatments caged (CA) and pegged (PE)*

No other studies have been conducted using soils collected around decomposing carcasses, which leached decomposing liquids into soils, to grow *G. rigens*. According to Carter *et al*. (2007), in a study carried out around pig (*Sus scrofa*) carcasses, the established plants within the carcass decomposition islands (CDI) died after decomposing pig carcass liquids leached into the soil; 80 days after the death of the pig plant growth increased.

5.5 Summary

Overall, in this study, above ground plant parts indicated an increase in growth, average number, weight and length of plant parts, indicating a positive effect from soils collected around carcasses on plant growth. However, below ground parts; root length and width indicated a negative effect from soils collected around decomposing carcasses.

Average leaf lengths, number of fresh and dead leaves, and fresh and dry leaf weights all indicated significant changes in weeks after growing in soils collected at various stages of carcass decomposition around caged and pegged carcasses placed in grasslands in Telperion Nature Reserve. However, treatments of caged and pegged carcasses had no effect on any leaf characteristics observed.

The average number of buds, and fresh weights of buds, flowers and seed heads indicated significant changes over time in plants grown in soils collected at various stages of carcass decomposition around caged and pegged carcasses. No significant differences were observed between the caged and pegged treatments in the average number of buds, and fresh weights of buds, flowers and seed heads.

All other parameters were insignificant. Average root lengths and widths, and fresh and dry root weights all indicated insignificant temporal and treatment changes (Annexure A).

References

Adams, S.J., Bahadur, B., Krishnamurthy, K.V. & Venkatasubramanian, P. (eds.). 2015. Origin, Development and Differentiation of Leaves. *Plant Biology and Biotechnology*, 1: 153-175.

Anozie, Fransisca C., Chen, J., Morales, A., Sharkey, T.D., Weise, S.E. & Weraduwage, Sarathi M. 2015. The relationship between leaf area growth and biomass accumulation in *Arabidopsis thaliana*. *Frontiers in Plant Science*, 6: 167.

A1 Organics. 2019. *Nutrients – What plants need and why!* [online]. A1 Organics. Available on the Internet at: https://a1organics.com/education/nutrients-what-plants-need-and-why/ (Accessed 14 December 2021).

Barbier, H., Prinzenberg, Aina E., Reymond, M., Salt, D.E. & Stich, B. 2010. Relationships between growth, growth response to nutrient supply, and ion content using a recombinant inbred line population in *Arabidopsis*. *Plant Physiology*, 154: 1361-1371.

Beemster, G.T.S., De Vos, D. & Kalve, S. 2014. Leaf development: a cellular perspective. *Frontiers in Plant Science*, 5: 362.

Carter, D.O., Yellowlees, D. & Tibbett, M. 2007. Cadaver decomposition in terrestrial ecosystems. *Naturwissenschaften*, 94: 12–246.

Fageria, N.K. & Moreira, A. 2011. The role of mineral nutrition on root growth of crop plants. *Advances in Agronomy*, 110: 251-331.

Kim, D. & Park, I. 1993. Significance of fresh weight to dry cell weight ratio in plant cell suspension cultures. *Biotechnology Techniques*, 7: 627–630.

Manjula, V.N. 2017. *Soils, plant nutrition and nutrient management*. University of Missouri Extension, USA. 12 pp.

CHAPTER 6
EFFECTS OF SOIL NUTRIENTS ON PLANT GROWTH RESULTS

6.1 Introduction

This chapter discusses how inputs from *C. taurinus* carcasses into soils influenced the growth of *G. rigens*. It presents a discussion on the relationship between the various soil properties and the various growth parameters of *G. rigens* that were investigated in this study. The chapter concludes by presenting implications of carcasses leaching into soils on ornamental plants.

6.2 Leaves

6.2.1 Leaf length

For leaf lengths to grow optimally, sufficient soil P is required (Fonseca, McC. Overton, McDonald & Westoby, 2003). In this study a significant relationship was observed between pH and mean average leaf length of *G. rigens* ($p = 0.02$) (Table 6.1). Before placement of carcasses (W1), the recorded soil pH levels were acidic (4.8), which affected leaf length negatively, as the shortest leaf length was recorded in plants grown in soil collected during W1. After placement of the carcasses (W2, W3), the soils became less acidic, with pH levels being closer to neutral. This increase in pH had a positive effect on leaf lengths, with leaf lengths increasing. In W4, the soil pH became slightly more acidic, which again affected leaf lengths slightly negatively. The relationship between leaf length and soil pH may be an indirect one. According to Ward (2022), in soils with pH values between 5.5 to 7.0, plants will grow optimally, as soil nutrients are more accessible to plants, which explains why in this study, the leaves were longer (12.3 cm) when the soil pH was less acidic enlarging the leaf area for photosynthesis. The growth of *G. rigens* in the experimental soils was enhanced as a result of the circumneutral pH levels of the soils caused by leachate from the carcasses. Despite leaves requiring N for leaf length growth (Sundblad, 2022), this study reported a positive influence from an increase in pH values on leaf lengths, N or P was not reported to have a significant relationship on average leaf length.

Table 6.1: Soil nutrient effects of soil properties on plant growth parameters

Plant growth parameters	Soil nutrient	Correlation Coefficient	Significance
Leaves			
Average leaf lengths	pH	0.41	p = 0.02
Average number of leaves	pH	0.36	p = 0.04
	EC	0.47	p = 0.02
	P (mg/kg)	0.36	p = 0.02
	Cu (mg/kg)	-0.53	p = 0.002
Average fresh leaf weights	pH	0.47	p = 0.003
	EC	0.58	p = 0.001
	P (mg/kg)	0.36	p = 0.01
	N %	0.34	p = 0.04
	Cu (mg/kg)	-0.34	p = 0.04
Average number of dead leaves	pH	0.74	p = 0.00001
	EC	0.67	p = 0.0005
Flowering			
Average number of buds	CEC	-0.36	p = 0.01
Average number of flowers	OM %	0.60	p = 0.02
	C %	0.39	p = 0.02
Average number of seed heads	pH	0.51	p = 0.003
	EC	0.48	p = 0.01
Average fresh buds, flowers, seed head weights	EC	0.43	p = 0.04
	C %	0.37	p = 0.02
	N %	0.39	p = 0.04
	Cu (mg/kg)	-0.41	p = 0.03
	Zn (mg/kg)	0.45	p = 0.02
Roots			
Average root lengths	C:N ratio	-0.48	p = 0.04
	Fe (mg/kg)	0.47	p = 0.004
	Mg (meq/100 g)	0.55	p = 0.04
Average root widths	Fe (mg/kg)	0.33	p = 0.04
Average fresh root weights	Cu (mg/kg)	-0.41	p = 0.03

6.2.2 Number of leaves

The number of leaves produced by *G. rigens* was affected significantly by soil pH ($p = 0.04$), EC ($p = 0.02$), P (mg/kg) ($p = 0.02$) and Cu (mg/kg) ($p = 0.002$) (Table 6.1). The average number of leaves increased to 50.1 after placement of carcasses as soil pH levels became more alkaline (5.678) and as EC levels increased (158,72). Soil EC was at its peak during W4 after placement of both caged and pegged carcasses and plants grown in soils collected during this week showed fewer number of leaves, indicating an inverse relationship with soil EC. Soil EC gives an idea of the salinity status of soils. Most plants would not grow well in soils with a high salt content, and the same was observed for *G. rigens.* Amanullah & Ahmed, (2016) also reported a decline in the number of leaves in their study of *Gazania harlequin;* however, Ahmed, Hameed, Nadeem, Riaz, Siddique, Tariq & Younis (2014) reported that moderate levels of soil salinity are tolerated by *G. harlequin*. Phosphorus and Cu are nutrients required by plants to grow optimally, especially for photosynthesis and respiration (Bloodnick, 2021; Silva & Uchida, 2000). The introduction of these nutrients into the soils as a result of fluids seeping from the decomposing carcasses would have contributed to an overall increase in the nutrient status of the soils, hence the increase in the number of leaves in the plant studied and a possible increase in photosynthesis. The role of carcass fluids in the improvement of plant growth is reflected in the increased number of leaves with an increase in P and Cu content of the soils surrounding the ten carcasses.

6.2.3 Fresh leaf weights

Plant biomass is generally affected by several factors, but soil nutrient availability is one of the most important, according to Chatzistathis & Therios (2013). Average fresh leaf weights in this study showed a significant direct relationship with soil pH, EC, P (mg/kg), N% and Cu (mg/kg) (Table 6.1). According to Chatzistathis & Therios (2013), P, Cu and N are some of the nutrients necessary for plant growth. Before placement of carcasses (W1), soil P (mg/kg) content and the average leaf weights had the lowest values of 3.84 (mg/kg) and 12.3 g, respectively, as no input from carcass leachates was made into soils at this stage. After placement of carcasses, especially in W3, the highest average fresh leaf weights and P levels were recorded, indicating that P increases had a positive effect on fresh leaf weights. The highest

soil N content was recorded in W1 before placement of carcasses, and plants grown in soils collected during this period had lower leaf weights. This could be attributed to the acidic status of the soils prior to carcass placement, which would have reduced the availability of the soil N, and consequently its uptake by the plants, which would have contributed to the low fresh biomass of the plants. According to Heath (2019), acidic soils hinder bacteria from breaking down OM, which needs nitrogen. According to Murungi, Silas & Wanjau (2012), in their study of fruit trees, exotic trees and indigenous trees in Kenya, N is available to plants at a soil pH of 6-8, P is available to plants at a soil pH of 6.5-7.5, and Cu decreases in solubility at high pH levels. Dufault & Melton (1991) reported an increase in fresh leaf weight as soil N levels increased from 25 to 225 mg/litre. In this study, increased soil Cu (mg/kg) levels due to influx of Cu contained in fluids from the decomposing carcass during W2 to W4 also had a positive effect on fresh leaf weights. As the Cu levels increased to 19,59 mg/kg, so too did the weight of the fresh leaves. According to Korgaokar, Markana, Soni & Vyas (2016), in their study of the effects of Cu on fresh leaf weights of bean plants, the opposite was recorded; as Cu levels increased, leaf weights decreased significantly, especially in Cu additions which were threefold that of the control, indicating toxicity. Copper is an essential element for plant growth as it affects photosynthesis, which determines plant biomass. At high concentrations, Cu could be toxic to plants, depending on the plant species. Ghazaryan, Ghazaryan, Movsesyan & Watts (2019) reported in their study on Cu contaminated soils in Armenia that 16 different plants reported to have moderate Cu tolerance (up to 1,000 mg/kg), tolerant (up to 3,000 mg/kg) and highly tolerant (more than 3,000 mg/kg). The positive correlation between Cu and leaf weights could indicate that the soil Cu levels, which had increased as a result of the leached carcass fluids, were tolerable by the *G. rigens* plants. Plants grown in the soils collected in W1 before the placement of caged and pegged carcasses had the lowest average fresh leaf weights reported. Soils collected after the placement of carcasses (W2 to W4) indicated an increase in the Cu content. The plants grown in soils collected around carcasses during these periods indicated increases in fresh leaf weights correlating with the increase in the soil Cu content. According to Huang, Hui, Ratkowsky, Shi, Su & Wang (2019) leaves, including their size, number, and weight, are important organs for respiration and plants to photosynthesize energy, to ensure a plants survival. The impact of heavier leaf weights in specific *G. rigens* may indicate that

the photosynthesis and respiration functions are more optimal for superior growth, compared to their counterparts grown in soils from other sites of this study.

6.2.4 Number of dead leaves

The number of dead leaves was significantly affected by soil pH (p = 0.00001), and EC (p = 0.0005) (Table 6.1). The highest average number recorded for dead leaves for plants grown in soils collected around caged (9.4) and pegged (9.3) treatments was reported in W3 after placement of carcasses, and the lowest number recorded was in W1 before placement of both carcasses (3,3 CA, 3,9 PE); high EC levels may have contributed to the number of dead leaves. Soil EC levels before placement of caged and pegged carcasses (W1) recorded the lowest values (48,06 CA, 45,42 PE), with the lowest number of dead leaves recorded in plants grown in the soil collected during W1, indicating that low salinity levels had a positive effect on the number of dead leaves. High saline levels in soils after placement of carcasses (W3 and W4) affected the average number of leaves which died, especially in W2 and W3 of plant growth. According to Getter (2013), high EC levels result in a physiological plant drought, restricting root water uptake, which may have led to leaves dying at higher numbers in W3 and W4, furthermore, a higher number of dead leaves in *G. rigens* will result in reduced photosynthesis resulting in poor plant growth.

6.3 Flowering

6.3.1 Number of flower buds

This study reported that the average number of flower buds was significantly affected by CEC (p = 0.01) (Table 6.1). The higher the CEC, the more the soil will be able to hold essential plant nutrients (Brown & Lemon, 2022). The positive correlation between flowering and soil CEC is not unexpected. According to Kumar (2018), K is typically a required nutrient for flower bud formation, and P for flower bud development (Kelly, 2018). As CEC increased, so too did the availability of soil nutrients. Two weeks after placement of carcasses, soil CEC levels increased, as did the number of flower buds. Soil CEC had a positive effect on the formation of flower buds. According to North Country Organics (2022), sandy soils, as found predominantly in Telperion, contain low levels of organic matter (OM) and record

low CEC levels. The low soil CEC levels reported in W1 before placement of both caged (3,37 meq/100g) and pegged (3,85 meq/100g) carcasses could explain the low number of flower buds produced in plants grown in soils collected in W1 (1,7 CA,1,2 PE). When compared to W2 to W4, the CEC increased as well as the average number of flower buds produced by plants grown in soils collected around pegged and cage carcasses, indicating that there was a positive relationship between CEC increases and average number of flower buds produced. There is a correlation between the influx of soil nutrients from decomposing carcass liquids and the influence it had on increasing the number of flower buds in *G. rigens* of this study.

6.3.2 Number of flowers

The average number of flowers in this study was significantly affected by OM % ($p = 0.02$), and C % ($p = 0.02$) (Table 6.1). As with the number of buds, K and P are essential nutrients required for flower development in plants (Kelly, 2018; Kumar, 2018). Soil OM contains macro- and micro-nutrients, including C (Magdoff & van Es, 2021) necessary for flowering. There is therefore a correlation between flowering and OM. Soil OM had a positive effect on the number of flowers produced; as soil OM increased from W1, so did the number of flowers produced, especially in plants grown in soil collected at W3CA (1,6). In W1 before placement of both carcasses, soil OM levels were low (0,48% CA, 0,47% PE), as were the average number of fresh flowers produced in plants grown in soils collected during W1 (0,8). When compared to plants grown in soils collected in W2 to W4 with a high C, the average number of flowers produced increased, indicating that there was a positive relationship between the two, due to the high OM levels making carbon more available to plants. Soil C increased the most in W2CA and W4PE after placement of carcasses, which also had a positive effect on the average number of flowers produced when compared to W1 before placement of both carcasses. This indicates that the addition of both carcasses had a positive effect on soil C and the average number of flowers produced by *G. rigens*. The soils enriched by C and OM from the influx of leached decomposition liquids had a positive effect on flower development after placement of carcasses (W2 to W4). According to Grant (2021), plants grow optimally in the presence of soil C and organic matter (Traunfeld, 2020). Busato, Façanha, Peres, Santos & Zandonadi (2013) report that OM is vital in plant

development and growth, and C also plays a positive role in the development of flowers, as stated by McIntosh (2020), as flower growth starts with strong and healthy plant roots. The impact of the addition of carcass decomposition fluids had a strong relationship on *G.rigens* in this study to produce more flowers.

6.3.3 Number of seed heads

The average number of seed heads was directly affected by pH ($p = 0.003$), and inversely by soil EC ($p = 0.01$) (Table 6.1). According to Liles (2021), seed head development is primarily influenced by P, especially in soils with pH between 5.5-7. Soil pH indicated an increase in alkalinity from W2 (5.7) to W3 (5.9) and a slight drop in W4 (5.3) after placement of carcasses in both test areas, compared to W1 (4.9 CA, 4.8 PE) before placement of carcasses. The pH increases were mirrored in the average number of seed heads produced in plants grown in soils collected before and after placement of carcasses. EC had a positive effect on the number of seed heads produced, thus a positive impact on *G. rigens* is a potential for more offspring and survival of the species. EC also had a positive influence on soil pH influencing the availability of soil nutrients to plants, indicated by the average fresh leaf weights obtained in this study. EC was at its highest after placement of both CA in W4 (204.14) and PE carcasses in W3 (156.82), which negatively affected the number of seed heads produced; however, a correlation can be drawn between the acidic soils compared to the number of seed heads produced before placement of carcasses in W1, in that nutrients were not available to plants in acidic soils. According to Ambrosini, Caronni, Citterio, Gentili & Montagnani (2018), plants grown in soil pH4 had a negative effect on development of *Viola tricolor* seeds, which decreased by 33%. Reduced seed heads were reported in the presence of increased EC values in W4 after placement of both caged (1.2) and pegged (1.8) carcasses compared to before placement of carcasses in W1.

6.3.4 Fresh flower buds, flowers, seed head weights

Five elements affected the average weights of fresh buds, flowers and seed heads significantly: EC ($p = 0.04$) inversely, C % ($p = 0.02$) directly, N % ($p = 0.04$) directly, Cu (mg/kg) ($p = 0.03$) directly, and Zn (mg/kg) ($p = 0.02$) directly (Table 6.1). The

average weight of fresh flower buds, flowers and seed heads grown in soils collected around carcasses increased from W1 (10,1 CA, 10,2 PE) to W3 (14,2 g CA, 13,5 g PE). Soil EC also increased gradually after placement of both CA and PE carcasses, indicating that moderate saline increases did not affect the average flowering weights adversely; however, soil EC in W4 reported the highest soil EC increases, which affected fresh flower weights negatively. In a study conducted by Amanullah & Ahmed (2016) on *G. harlequin* in Pakistan, their findings indicated that fresh shoot weights decreased as salinity levels increased, affecting plant weights negatively, and fresh shoot weights were the highest in control soils with no saline solutions added. This study correlates with the findings, especially in W4 after placement of carcasses. The impact of high soil EC values affects *G. rigens* adversely. The production of fresh flower buds, flowers and seed head of higher weight indicate that more seeds are being produced as well as that the viability of seeds maybe higher ensuring more offspring and survival of the species.

Soil C increased after placement of carcasses from W1 which correlates with the average weight of fresh flower organs, indicating a positive impact on fresh flower organ weights after placement of both CA and PE carcasses compared to before placement of carcasses.

Soil N recorded the highest levels in W1 before placement of carcasses in both test areas (0.15% CA, 0.16% PE), which had a negative effect on average fresh flower organ weights, as soil N levels dropped in W2 to W4 after placement of carcasses, and the average fresh flower organ weights increased, suggesting lower nitrogen levels had a positive effect and impact on the fresh flower organ weights of *G. rigens*. There may have been an increase in soil micro-organisms after placement of carcasses which depleted the N levels. Bai, Chen, Hu, Lan, Saleem, Wu & Xing (2019) conducted a study in a semi-arid grassland on the Mongolian Plateau in China, in which it was reported that nitrogen enrichment of soils increased the number of soil organisms, indicating that micro-organisms require N for survival. The loss of soil N after placement of carcasses could have been due to the increased activity of micro-organisms and not due to rainfall. The decline in soil N, especially in W4, could also have resulted from the new plant growth observed at

all the carcass sites, which could have further reduced the soil N levels to that of W4.

A positive correlation was reported between Cu (mg/kg) in soils and the average fresh flower organ weights; as the Cu increased, so too did the fresh flower organ weights increased in W2 and W3 after placement of both caged and pegged carcasses. In W1 before placement of carcasses, both values of Cu (9.34 mg/kg CA, 12.76 mg/kg PE) and average flower organ weight recorded the lowest values 10.1 g CA, 10.2 g PE), indicating that the influx of decomposition carcass liquids had a positive effect and impact on flower organ weight of *G. rigens*.

An influx of Zn (mg/kg) into soils had an overall positive impact on the average fresh flower organ weights, which also increased especially after placement of pegged carcasses compared to before placement of carcasses.

The performance of plants' average weight of fresh buds, flowers and seed heads was overall positively affected by the addition of leached liquids from decomposing caged and pegged carcasses compared to before placement of both carcasses in W1. As can be seen from this study, there is not a single soil element which played a positive role in the average fresh weights of flowering organs, but rather multiple soil parameters due to the influx of leached liquids from decomposing caged and pegged carcasses. The increased average weights of fresh buds, flowers and seed heads have a positive affect on the survival of *G rigens* by contributing to the vitality of the seeds ensuring a larger offspring.

6.4 Roots

6.4.1 Root length and width

According to Herrera, López-Bucio & Shane (2015) and AgriSight (2022), roots require N, P B, and Fe for root development. There was clearly a correlation between Fe and root growth in this study, which supports what has been reported in literature by Herrera *et al.* (2015) that Fe is required for optimal root development. In another study conducted by Jin, Niu & Zhang (2014) on *Arabidopsis thaliana* (thale cress), root length and width were not influenced by low Mg; however, over

time they were inhibited by high Mg levels. This does not correlate with the findings of this study. *G. rigens* root lengths were significantly affected by C:N ratio ($p = 0.04$) inversely, Fe (mg/kg) ($p = 0.004$) positively, and Mg (meq/100 g) ($p = 0.04$) positively, whereas the width of the root ball was affected by Fe (mg/kg) ($p = 0.04$) inversely (Table 6.1). A decrease in root lengths was reported from plants grown in soils collected around carcasses in W1 to W4. This suggests that a decrease in soil Fe (mg/kg) and Mg (mg/kg) may have had a mirrored impact on root lengths after placement of both CA and PE carcasses. Soil C:N ratios increased in W2 and W4 after placement of both CA and PE carcasses from W1 before placement of carcasses, which affected root length negatively, especially in W4 after placement of caged and pegged carcasses. C:N ratios affected Fe (mg/kg) levels as a reversed pattern was reported in C:N ratios and Fe (mg/kg) levels, which may have affected root lengths positively after placement of both CA and PE carcasses. Literature does not indicate any correlation between soil C:N ratios or soil Fe. Root widths were generally negatively affected by a decrease in Fe (mg/kg) levels after placement of carcasses, suggesting that Fe is a required nutrient for the development of abundant plant root widths. According to Feng, Liu, Sun & Zhao (2017), soil Fe deficiencies affect root development, auxin and nitric oxide are important regulators of root growth under low levels of Fe, they also indicated that different plants require different levels of Fe for root development. Fe was abundant before placement of carcasses in W1 (125,05 mg/kg CA, 141,13 mg/kg PE), as were the root widths compared to after placement of both CA and PE carcasses, suggesting that the levels Fe levels reported in this study were beneficial for *G. rigens* root growth. A well developed root system ensures an efficient water and nutrient uptake by the plant that enhances growth and survival.

6.4.2 Fresh root weights

Copper (mg/kg) ($p = 0.03$) significantly affected the average fresh root weights positively (Table 6.1). As soil Cu increased, so too did the average weights of fresh roots increase, suggesting that overall, the influx of decomposition fluids containing Cu affected fresh root weights positively after placement of caged and pegged carcasses compared to W1 before placement of carcasses, enhancing plant growth and survival. In a study conducted by Korgaokar *et al*. (2016) on *Glycine max* (soybean)*, Vigna unguiculata* (cowpea) and *V. aconitifolia* (moth bean), fresh root

weights gradually decreased after increasing copper concentrations from 0.4-1.6 mM in the three plant treatment groups. A study conducted by Chen, Li, Liu, Lu, Peng, Shafi, Wu, Yan & Ye (2014) on *Phyllostachys pubescens* (Moso bamboo), indicated that most of the plants died after an application of 400 µM Cu was administered to the plants, which was toxic to them. In comparison to this study, levels of Cu (mg/kg) 19,59 did not affect the roots fresh weights adversely after placement of both CA and PE carcasses.

6.5 Implications of carcass fluids on the growth media for ornamental crops

Based on the results obtained in this study, soils contaminated with leached fluids from decomposing carcasses could improve the performance of soils as growth media for ornamental crops. The *G. rigens* plant organs which showed the most improvement from the leached decomposition liquids after placement of both the caged and pegged carcasses, were the average leaf lengths, average number of leaves, average fresh leaf weights enhancing photosynthesis and respiration ensuring better growth and survival of plants. The average number of fresh buds, average number of fresh flowers and average fresh weights of buds, flowers and seed heads also increased ensuring a bigger offspring and survival of the species. Plants with vigorous growth indicated by leaf lengths and the number of leaves and which produce an abundance of flowers and buds are beneficial for the horticultural commercial industry. Most nutrients leached into the soils by the decomposing carcasses played a positive role in the growth of *G. rigens*. Further studies could be done on other nutrients not analysed in this study to determine what other nutrients would benefit the growth of *G. rigens* plants and what nutrients the plants did utilise for development. Rates of seed germination are another area which could be studied in soils from decomposition fluids of carcasses.

6.6 Summary

Almost all soil nutrients analysed in this study influenced *G. rigens* plant organ's growth, except K, Na and Ca. According to Murungi *et al*. (2012), very low soil pH values can contribute to deficiencies in K and Ca, which could explain why K and Ca did not influence plant growth in this study. In this study pH values increased to more alkaline levels, which had positive effects on plant growth. Soil elements pH

and EC influenced leaves and seed heads positively after placement of both carcasses, especially leaf lengths, the number of leaves and the number of seed heads increased. Another abundant soil nutrient that featured significantly was Cu. It influenced the number of leaves and leaf weights positively, both growth parameters increased in numbers and weights after the influx of leached decomposition liquids into the soils. In general leaf lengths, number of leaves and fresh leaf weights reported similar rates of increases from W2 to W3, indicating that leached liquids had a positive effect on leaf growth. Cu and Zn had a positive influence on flower organ weights, whereas Cu, Fe and Mg had an influence on roots. Fe and Mg had a positive influence on root lengths, and the growth of root length was mirrored by Fe and Mg inputs. As the nutrients decreased, so too did the root lengths. Cu had a positive impact on fresh root weights; as the nutrient levels increased so too did the root weights. According to Murungi *et al*. (2012), Cu, Fe and Zn decrease in solubility at high soil pH values. In this study soil pH values did not exceed 5.88, hence the minerals influenced the flower organ weights and roots. Plant growth of *G. rigens* was affected positively by leached carcass liquid inputs temporally, after placement of carcasses and not by the two treatments of caged and pegged carcasses.

References

AgriSight. 2022. *Boron – a critical nutrient required for root growth and potassium uptake* [online]. The Mosaic Company. Available on the Internet at: https://www.cropnutrition.com/resource-library/boron-a-critical-nutrient-required-for-root-growth-and-potassium-uptake (Accessed 21 January 2022).

Ahmed, I., Hameed, M., Nadeem, M., Riaz, A., Siddique, M.I., Tariq, U. & Younis, A. 2014. Anatomical changes induced by NaCl stress in root and stem of *Gazania harlequin* L. *Agricultural Communications*, 2(3): 8-14.

Amanullah, S. & Ahmed, I. 2016. Assessment of NaCl stress on morphological behavior of Gazania. *International Journal of Agronomy and Agricultural Research*, 9(6): 94-111.

Ambrosini, R., Caronni, Sarah, Citterio, Sandra, Gentili, R. & Montagnani, C. 2018. Effect of soil pH on the growth, reproductive investment and pollen allergenicity of *Ambrosia artemisiifolia* L. *Frontiers in Plant Science*, 9: 1335.

Bai, Y., Chen, D., Hu, S., Lan, Z., Saleem, M., Wu, Y. & Xing, W. 2019. Direct and indirect effects of nitrogen enrichment on soil organisms and carbon and nitrogen mineralization in a semi-arid grassland. *Functional Ecology*, 33(1): 175–187.

Bloodnick, E. 2021. *Role of copper in plant culture* [online]. Pro-Mix. Available on the Internet at: https://www.pthorticulture.com/en/training-center/role-of-copper-in-plant-culture/ (Accessed 11 January 2022).

Brown, Kathrine & Lemon, J. 2022. *Cations and cation exchange capacity* [online]. Soil Quality Pty Ltd. Available on the Internet at: https://www.soilquality.org.au/factsheets/cation-exchange-capacity (Accessed 2 February 2022).

Busato, J.G., Façanha, A.R., Peres, L.E.P., Santos, M.P. & Zandonadi, D.B. 2013. Plant physiology as affected by humified organic matter. *Theoretical and Experimental Plant Physiology*, 25(1): 12-25.

Chatzistathis, T. & Therios, I. 2013. *How soil nutrient availability influences plant biomass and how biomass stimulation alleviates heavy metal toxicity in soils: The cases of nutrient use efficient genotypes and phytoremediators, respectively, biomass now - cultivation and utilization* [online]. IntechOpen. Available on the Internet at: https://www.intechopen.com/chapters/44413 (Accessed 1 February 2022).

Chen, J., Li, S., Liu, D., Lu, K., Peng, D., Shafi, M., Wu, J., Yan, W. & Ye, Z. 2014. Effect of copper toxicity on root morphology, ultrastructure, and copper accumulation in Moso bamboo (*Phyllostachys pubescens*). *Zeitschrift fur Naturforschung. C, Journal of Bioscience*, 69(9-10): 399-406.

Dufault, R.J. & Melton, Regina R. 1991. Nitrogen, phosphorus, and potassium fertility regimes affect tomato transplant growth. *HortScience*, 26(2): 141-142.

Feng, F., Liu, J., Sun, H. & Zhao, Q. 2017. The interaction between auxin and nitric oxide regulates root growth in response to iron deficiency in rice. *Frontiers in Plant Science*, 8: 2169.

Fonseca, C.R., McC. Overton, J., McDonald, P.G. & Westoby, M. 2003. Leaf-size divergence along rainfall and soil-nutrient gradients: Is the method of size reduction common among clades? *Functional Ecology*, 17: 50 – 57.

Getter, Kristin. 2013. *pH and EC issues still a problem in some greenhouses* [online]. Michigan State University. Available on the Internet at: https://www.canr.msu.edu/news/ph_and_ec_issues_still_a_problem_in_some_greenhouses (Accessed 11 January 2022).

Ghazaryan, Karen, Ghazaryan, N., Movsesyan, H. & Watts, Beatriz A. 2019. Copper phytoremediation potential of wild plant species growing in the mine polluted areas of Armenia. *Environmental Pollution*, 249: 491-501.

Grant, Amy. 2021. *Do plants use carbon: Learn about the role of carbon in plants* [online]. Gardening Know How. Available on the Internet at: https://www.gardeningknowhow.com/garden-how-to/soil-fertilizers/role-of-carbon-in-plants.htm (Accessed 20 January 2022).

Heath, A. 2019. *How does acidic soil affect nitrogen availability?* [online]. Easierwithpractice. Available on the Internet at:

https://easierwithpractice.com/how-does-acidic-soil-affect-nitrogen-availability/ (Accessed 12 February 2022).

Herrera, L.F.R., López-Bucio, J. & Shane, M.W. 2015. Nutritional regulation of root development. *Wiley Interdisciplinary Reviews: Developmental Biology*, 4(4): 431-43.

Huang, W., Hui, C., Ratkowsky, D.A., Shi, P., Su, J., & Wang, P. 2019. Leaf fresh weight versus dry weight: Which is better for describing the scaling relationship between leaf biomass and leaf area for broad-leaved plants? *Forests*, 10: 256.

Jin, G.L., Niu, J.F. & Zhang, Y.S. 2014. Root development under control of magnesium availability. *Plant Signaling & Behavior*, 9: 9.

Kelly, Jasey. 2018. *Which fertilizer element encourages flowering growth in plants?* [online]. SFGate. Available on the Internet at: https://homeguides.sfgate.com/fertilizer-element-encourages-flowering-growth-plants-76453.html (Accessed 19 January 2022).

Korgaokar, S.S., Markana, T.V., Soni, K.V. & Vyas, M.K. 2016. Effect of heavy metals on biomass and growth rate of selected pulses. In the *International Conference on Research & Entrepreneurship*. India.

Kumar, V.R.A. 2018. *Explained: 14 nutrients required by plants for best growth & development* [online]. Krishi Jagran. Available on the Internet at: https://krishijagran.com/featured/explained-14-nutrients-required-by-plants-for-best-growth-development/ (Accessed 12 January 2022).

Liles, Valerie. 2021. *Elements & uses of NPK fertilizer* [online]. SFGate. Available on the Internet at: https://homeguides.sfgate.com/elements-uses-npk-fertilizer-71006.html (Accessed 19 January 2022).

McIntosh, Jamie. 2020. *How to choose the right flower fertilizer type* [online]. The Spruce. Available on the Internet at: https://www.thespruce.com/choose-right-flower-fertilizer-1315828 (Accessed 2 February 2022).

Magdoff, F. & van Es, H. 2021. *Building soils for better crops ecological management for healthy soils*. 4th ed. Sustainable Agriculture Research & Education, United States. 394 pp.

Murungi, Jane I., Silas, N.E. & Wanjau, R.N. 2012. The pH of leaf water extracts and amount of acid required lowering the pH of leaf water extracts to 5.0. *American International Journal of Contemporary Research*, 2(11): 72-78.

North Country Organics. 2022. *Cation exchange capacity (CEC)* [online]. North Country Organics. Available on the Internet at: https://norganics.com/index-2/technical-articles/cation-exchange-capacity-cec/ (Accessed 11 January 2022).

Silva J.A. & Uchida, R. (eds). 2000. *Plant nutrient management in Hawaii's soils, approaches for tropical and subtropical agriculture*. College of Tropical Agriculture and Human Resources, University of Hawaii at Manoa. 31-55 pp.

Sundblad, Donna. 2022. *Nutrients needed for plants to grow* [online]. LoveToKnow Media. Available on the Internet at: https://garden.lovetoknow.com/wiki/Nutrients_Needed_for_Plants_to_Grow (Accessed 10 January 2022).

Traunfeld, J. 2020. *Organic matter and soil amendments* [online]. University of Maryland. Available on the Internet at: https://extension.umd.edu/resource/organic-matter-and-soil-amendments (Accessed 20 January 2022).

Ward, Eileen. 2022. *Gardening: The pH of your soil can affect plant growth and health* [online]. Naples Daily News. Available on the Internet at: https://archive.naplesnews.com/community/gardening-the-ph-of-your-soil-can-affect-plant-growth-and-health-ep-1071358042-331319431.html/ (Accessed 10 January 2022).

CHAPTER 7
CONCLUSION

7.1 Introduction

This chapter presents a concluding overview for each research objective, makes recommendations, and summarises the contributions and influences of carcass leach on soils and growth of the indigenous ornamental *G. rigens* plants.

7.2 Research objectives and summary of findings

7.2.1 Soil analysis

The objectives of this study were to determine how decomposing carcasses in a grassland affect nutrients in soils and to what extent, and to determine whether the effect that a decomposing carcass had on the changes in soil nutrients varied with time after death of the animal. This study found that the influx of decomposition carcass liquids had no effect on soil texture or colour; however, the influx of decomposition fluids significantly increased soil EC, pH, OM, exchangeable bases; sodium (Na), magnesium (Mg), calcium (Ca) and potassium (K), TN, C:N ratios, and micro-nutrient Cu. These soil parameters increased with time after placement of carcasses; however, the other soil properties analysed, CEC, TC, P and Zn, also indicated slight increases. Fe indicated decreases in values; however, the changes over time were not significantly affected after placement of all carcasses.

Another objective of this study was to determine whether accessibility to a decomposing carcass by scavengers had any effect on the nutrient influx into soil around the carcasses. In this study, very few scavengers were present to make a marked difference between caged and pegged carcasses, even though one dried out pegged carcass was moved by a warthog (*Phacochoerus africanus*) at the end of the soil collection period. Therefore, the two soils from around treatments of caged and pegged carcasses reported no significant differences in the nutrient contents of soils.

It can be concluded that the influx of certain decomposition nutrients had a significant influence on soil nutrients over time. No difference was reported in caged or pegged treatments.

7.2.1.1 Recommendations for implementation for soil analysis

Many studies have been conducted on pigs (*Sus scrofa*) for forensic purposes, to determine different stages of decomposition, and in the northern hemisphere. Further studies could be carried out in southern Africa on different soil types and in the different biomes in different seasons to establish differences to this study and the effects from different decomposing animals, large and small. Having more studies such as this one could add more value in a southern African context for soil analysis and plant growth studies.

7.2.2 Plant growth analysis

For plant growth, one of the research objectives was to establish whether the growth and development of *G. rigens* in soil collected from around carcasses vary with the different stages of carcass decomposition. The results obtained in this study show that the average leaf lengths, average number of leaves, average number of dead leaves, average weight of fresh leaves, average weight of dry leaves, average number of buds, and average weights of fresh buds, flowers and seed heads of *G. rigens* reported a marked difference after placement of both caged and pegged carcasses in W2. Growth parameters which were not significantly affected by the different stages of carcass decomposition were the average number of flowers, average number of seed heads, average root lengths, average root widths, average fresh root weights, average dry root weights, average dry buds, and average number of flowers, despite changes reported after placement of carcasses.

7.2.2.1 Recommendations for implementation for plant growth analysis

Suggestions for further research could include analysing exactly what nutrients plants have absorbed from the soils into the different plant organs, including analysing boron in soils, as literature indicates that boron also plays an important role in plant development. Research could be enhanced to determine what macro-

and micro-nutrients plants absorb from CDI soils at various carcass decomposition stages using ICP-OES analysis methods of the plant's different organs.

Other ornamental plants species and edible plants could be grown in the same manner or at later stages of decomposition to determine the sustainability of such methods on various plants.

7.2.3 Effects of soil nutrients on plant growth

The final objective for this study was to determine if the growth and development of *G. rigens* was affected by soil nutrients and soil characteristics in a CDI. Certain soil nutrients and soil characteristics positively affected plant growth from W2 in this study. In leaves, the average leaf lengths were positively affected by pH, the average number of leaves were positively influenced by pH, Cu and P, and negatively affected by EC. The average fresh leaf weights were positively affected by P, pH and Cu; however, negatively affected by N and EC. The average number of dead leaves was positively affected by pH, especially in W1 before placement of caged and pegged carcasses, and in W2, after placement of carcasses when the lowest number of dead leaves was recorded, and negatively by EC after placement of caged and pegged carcasses, especially in W3, when the highest number of dead leaves was recorded.

In the flowering plant organs, the average number of buds produced by *G. rigens* in this study was positively affected by CEC, the average number of flowers produced was positively affected by OM and C. The average number of seed heads produced was positively affected by pH and negatively affected by EC; according to literature excess soil salinity causes poor yields in plants. The average fresh weights of buds, flowers and seed heads were positively affected by N, C, Cu, Zn; however, negatively affected by EC, all after placement of caged and pegged carcasses. In roots, the average root length was positively affected by Mg; however, negatively affected by the C:N ratio and Fe; average root ball width was negatively affected by Fe, and average root weights were positively affected by Cu after placement of caged and pegged carcasses.

As can be seen in this study, all plant growth parameters reported changes after placement of both caged and pegged carcasses. Plant growth was not entirely affected by the traditional norms of NPK (nitrogen, phosphorus, potassium), which could be attributed to the nutrients not being available to the plants in organic form.

7.2.3.1 Recommendations for implementation on the effects of soil nutrients on plant growth

Results of this study could be used to determine what other nutrients would be suitable for different ornamental plant growth for specific growth parameters. Even though there is ample literature on plant growth available, there have been a few nutrients not covered in literature found in this study, such as Fe decreasing in soils over time and influencing root ball width negatively.

Notwithstanding that there are regulations governing animal waste used as an alternative source of plant nutrients, it is clear from this study that decomposing carcass liquids recycle energy and nutrients back into soils. If studies could be conducted to determine at which point decomposing carcasses no longer pose a hazard to soils, plants or humans, it would be an advantage to make use of animal waste for plant growth, as is evident from this study that carcasses have had a positive effect on plant growth. For example, chicken carcasses could be incorporated into compost to decompose, which would contribute positively to recycling waste back into the environment. Further research could determine at what stage of decomposition carcasses could be used to add to growth media. Observations at Telperion during soil collection periods indicated that the initial onset indicated plant death days after the placement of carcasses.

7.3 Summary of contributions

Other researchers have conducted many studies on different stages of decomposition, identifying below ground organisms underneath decomposing carcasses, some effects of decomposition liquids and nutrients on soils, weight of animals during life versus nutrients released into soils and remaining carcass weights, plant death after influx of decomposition fluids and forensic studies of cadavers' effects on surrounding soils. This study builds upon the body of

knowledge, indicating additional information for nutrients of decomposing carcasses released into soils.

No other study has been conducted on the growth of ornamental plants in soils containing leached liquids of decomposing carcasses, which further contributes to the body of a knowledge gap, especially to the horticulture industry.

Publications of animal decomposition are mostly related to either nature conservation or forensic studies, as well as publications related to plant growth media and nutrients beneficial to the commercial industry of plants. Growing plants directly from decomposing liquids of animals is novel, although not entirely unique, as plants can be grown with bone, fish or blood meal. This study has indicated the benefits that leached decomposition liquids have on the growth of *G. rigens,* which further raises the question could animal waste from abattoirs or chicken farms be used in the production of growth media for the ornamental plant growth industry, to turn animal waste into a useable product.

A positive contribution of this study clearly indicates that the application of leached carcass decomposition liquids to collected soils did not pose a detrimental effect to ornamental plant growth over time.

7.4 Hypotheses

A decomposing carcass has an effect on soil nutrients and influences indigenous plant growth (H1).

References

A1 Organics. 2019. *Nutrients – what plants need and why!* [online]. A1 Organics. Available on the Internet at: https://a1organics.com/education/nutrients-what-plants-need-and-why/ (Accessed 14 December 2021).

Ackermann, Gail, Amir, A., Bibat, A., Bucheli, Sibyl R., Carter, D.O., Gebert, M.J., Gilbert, J.A., Haarmann, D., Humphrey, G.C., Hyde, Embriette R., Knight, R., Larsen, P., Lauber, C., Lax, S., Lynne, A.M., Metcalf, Jessica L., Nicholas, Catherine, Petrosino, J.F., Reed, S.A., Sangwan, N., Song, S.J., Thompson, L.R., Van Treuren, W., Weiss, Sophie & Xu, Z.Z. 2016. Microbial community assembly and metabolic function during mammalian corpse decomposition. *Science*, 351(6296): 158-162.

Adams, S.J., Bahadur, B., Krishnamurthy, K.V. & Venkatasubramanian, P. (eds.). 2015. Origin, Development and Differentiation of Leaves. *Plant Biology and Biotechnology*, 1: 153-175.

Adrusi, Madinah & Rahim, Nor A.A. 2018. Insect succession and decomposition patterns of carcasses in Sarawak, Malaysian Borneo. *Journal of Sustainability Science and Management*, 13(2): 69-84.

Advance Cover Crops. 2021. Understanding the carbon: citrogen catio (C:N) [online]. Advance Cover Crops. Available on the Internet at: https://advancecovercrops.com/resources-advanced-cover-crops/carbon-nitrogen-ratio/ (Accessed 20 October 2021).

Age Old Organics. 2016. *Calcium (Ca): Essential for plant growth and development* [online]. Age Old Organics. Available on the Internet at: https://www.ageold.com/calcium-ca-essential-for-plant-growth-and-development/#:~:text=Calcium%20was%20deemed%20an%20essential%20element%20for%20plant,the%20formation%20and%20stability%20of%20the%20cell%20walls. (Accessed 25 February 2022).

AGO Labs, 2019. *Macronutrients in plants* [online]. AGQ Labs USA. Available on the Internet at: https://www.agqlabs.us.com/macronutrients-in-plants/ (Accessed 23 February 2022).

AgriSight. 2022. *Boron – a critical nutrient required for root growth and potassium uptake* [online]. The Mosaic Company. Available on the Internet at: https://www.cropnutrition.com/resource-library/boron-a-critical-nutrient-required-for-root-growth-and-potassium-uptake (Accessed 21 January 2022).

Ågren G.I., Bird, J.A., Clarholm, Marianne, Gärdenäs, Annemieke I., Hallin, Sara, Ineson, P., Kätterer, T., Knicker, H., Näsholm, T., Nilsson S.I., Ogle, S., Paustian, K., Persson, T. & Stendahl, J. 2011. Knowledge gaps in soil carbon and nitrogen interactions – from molecular to global scale. *Soil Biology & Biochemistry*, 43: 702-717.

Agriculture Victoria. 2020. *Soil texture* [online]. Victorian Resources Online. Available on the Internet at: http://vro.agriculture.vic.gov.au/dpi/vro/vrosite.nsf/pages/soilhealth_texture_prac (Accessed 26 July 2021).

Agriculture Victoria. 2021. *Soil colour* [online]. Victorian Resources Online. Available on the Internet at: http://vro.agriculture.vic.gov.au/dpi/vro/vrosite.nsf/pages/soilhealth_colour (Accessed 15 July 2021).

Ahmed, I., Hameed, M., Nadeem, M., Riaz, A., Siddique, M.I., Tariq, U. & Younis, A. 2014. Anatomical changes induced by NaCl stress in root and stem of *Gazania harlequin* L. *Agricultural Communications*, 2(3): 8-14.

Akcan, R., Farasat, S., Karacaoglu, E., Keten, A. Namli, Ayten, Odabas, A.B., Sert, O. & Tumer, A.R. 2013. Effects of different types of soil on decomposition: An experimental study. *Legal Medicine*, 15: 149–156.

Akratos, C.S., Tekerlekopoulou, Athanasia G., Vasiliadou, Ioanna A. & Vayenas, D.V. 2017. *Cocomposting of olive mill waste for the production of soil amendments*. Olive Mill Waste, 161-182 pp.

Albrecht, A., Bernoux, M., Blanchart, E., Brahman, A., Chotte, J.L., Feller, C., Ganry, F., Hien, E., Manlay, R., Masse, D., Sall, S. & Villenave, C. 2005. Organic matter and biofunctioning in tropical sandy soils and implications for its management. In *Management of Tropical Sandy Soils for Sustainable Agriculture Conference*. Thailand.

Al-Dali, A.G., Fouda, M.A., Hammad, K.M., Kabadaia, M.M. & Zeariya, M.G.M. 2015. Forensic - insect succession and decomposition patterns of dog and rabbit carcasses in different habitats. *Journal of Entomology and Zoology Studies*, 3(5): 473-482.

Amanullah, S. & Ahmed, I. 2016. Assessment of NaCl stress on morphological behavior of *Gazania*. *International Journal of Agronomy and Agricultural Research*, 9(6): 94-111.

Ambrosini, R., Caronni, Sarah, Citterio, Sandra, Gentili, R. & Montagnani, C. 2018. Effect of soil pH on the growth, reproductive investment and pollen allergenicity of *Ambrosia artemisiifolia* L. *Frontiers in Plant Science*, 9: 1335.

Anozie, Fransisca C., Chen, J., Morales, A., Sharkey, T.D., Weise, S.E. & Weraduwage, Sarathi M. 2015. The relationship between leaf area growth and biomass accumulation in Arabidopsis thaliana. *Frontiers in Plant Science*, 6: 167.

Ashton, R.W., Binley, A., Dodd, I.C., Gao, W., Hawkesford, M.J., Hedden, P., Hodgkinson, L., Jin, K., Phillips, A.L., Ren, T., Shen, J., Watts, C.W. & Whalley, W.R. 2016. Deep roots and soil structure. *Plant, Cell & Environment*, 39(8): 1662–1668.

Australian Museum. 2022. *Stages of decomposition* [online]. The Australian Museum Available on the Internet at: https://australian.museum/learn/science/stages-of-decomposition/ (Accessed 14 February 2022).

Bai, Y., Chen, D., Hu, S., Lan, Z., Saleem, M., Wu, Y. & Xing, W. 2019. Direct and indirect effects of nitrogen enrichment on soil organisms and carbon and nitrogen mineralization in a semi-arid grassland. *Functional Ecology*, 33(1): 175–187.

Ball Horticultural Company, 2010. *New day F1 series Gazania* [online]. Ball Horticultural Company. Available on the Internet at: https://www.panamseed.com/media/Culture/PAS/GazaniaNewDay.pdf (Accessed 27 February 2019).

Bandaw, T. & Herago, T. 2017. Review on abattoir waste management. *Global Veterinaria,* 19(2): 517-524.

Barbier, H., Prizenberg, Aina E., Reymons, M., Salt, D.E. & Stich, B. 2010. Relationships between growth, growth response to nutrient supply, and ion content using a recombinant inbred line population in *Arabidopsis*. *Plant Physiology,* 154: 1361–1371.

Barton, P.S., Benbow, M.E., Bump, J.K., Evans, M.J., Foster, Claire N., Pechal, Jennifer L. & Quaggiotto, Maria M. 2019a. Towards quantifying carrion biomass in ecosystems. *Trends in Ecology & Evolution*, 2558: 12.

Barton, P.S., Bump, J.K., Cunningham, S.A., Evans, M.J., Manning, A.D. & McIntyre, Sue. 2016. Substantial long-term effects of carcass addition on soil and plants in a grassy eucalypt woodland. *Ecosphere*, 7(10): 01537.

Barton, P.S., Cunningham, S.A., Farrell, M., Macdonald, B.C.T., Manning A.D. & Tuomia, Seija. 2014. Carrion decomposition causes large and lasting effects on soil amino acid and peptide flux. *Soil Biology and Biochemistry*, 69: 132-140.

Barton, P.S., Cunningham, S.A., Lindenmayer, D.B. & Manning, A.D. 2013. The role of carrion in maintaining biodiversity and ecological processes in terrestrial ecosystems. *Oecologia*, 171: 761–772.

Barton, P.S., Dawson, B.M., Reboldi, Anna, Strong, C., Ueland, M. & Wallman, J.F. 2020. Soil chemical markers distinguishing human and pig decomposition islands: a preliminary study. *Forensic Science, Medicine and Pathology*, 16: 605–612.

Barton, P.S., Evans, E.M., Higgins, A., Quaggiotto, Maria M. & Strong, C. 2019b. Dynamic soil nutrient and moisture changes under decomposing vertebrate carcasses. *Biogeochemistry*, 146: 71–82.

Barton, P.S. Evans, M.J., Higgins, A., Quaggiotto, Maria M. & Strong, C. 2019. Nutrient and moisture transfer to insect consumers and soil during vertebrate decomposition. *Food Webs*, 18: 110.

Becker, P.J., Myburgh, J., Steyn, M. & Sutherland, A. 2013. The effect of body size on the rate of decomposition in a temperate region of South Africa. *Forensic Science International*, 231, (1–3): 257-262.

Benbow, E.M., Crippen, T.L., Mondor, E.B., Tarone, A., Tomberlin, J.K. & Tremblay, M.N.M. 2012. The ecology of carrion decomposition. *Nature Education Knowledge*, 3(10): 21.

Beemster, G.T.S., De Vos, D. & Kalve, S. 2014. Leaf development: a cellular perspective. *Frontiers in Plant Science*, 5: 362.

Benbow, M.E., Tarone, A.M. & Tomberlin, J.K. (ed.) 2016. *Carrion ecology, evolution, and their applications*. CRC Press, New York. 608 pp.

Benites, J. & Bot, A. 2005. *The importance of soil organic matter: Key to drought-resistant soil and sustained food and production*. Food and Agriculture Organization of the United Nations, Italy. 80 pp

Benninger, Laura A., Carter, D.O. & Forbes, Shari L. 2008. The biochemical alteration of soil beneath a decomposing carcass. *Forensic Science International* 180: 70–75.

Bergtold, J. & Sailus, M. (ed.) 2020. *Conservation tillage systems in the Southeast: Production, profitability and stewardship*. Sustainable Agriculture Research and Education (SARE), USA. 308 pp.

Bernard, E.C., De Bruyn, Jennifer M., Phillips, G. & Taylor, L.S. 2020. Soil nematode functional diversity, successional patterns, and indicator taxa associated with vertebrate decomposition hotspots. *PLoS ONE*, 15 (11): e0241777.

Bickelhaupt, D. 2020. *Soil pH: What it means* [online]. ESF SUNY College of Environmental Science and Forestry. Available on the Internet at: https://www.esf.edu/pubprog/brochure/soilph/soilph.htm (Accessed 3 September 2021).

Bloodnick, E. 2018. *Fundamentals of growing media* [online]. Premier Horticulture Ltd. Available on the Internet at: https://www.pthorticulture.com/en/training-center/fundamentals-of-growing-media/ (Accessed 15 October 2019).

Bloodnick, E. 2021. *Role of copper in plant culture* [online]. Pro-Mix. Available on the Internet at: https://www.pthorticulture.com/en/training-center/role-of-copper-in-plant-culture/ (Accessed 11 January 2022).

Bhatla, Satish C. & Lal, M.A. 2018. *Plant Physiology, Development and Metabolism*. Springer Nature Singapore Pte Ltd. Singapore. 1237 pp.

Braack L.E.O. 1987. Community dynamics of carrion-attendant arthropods in tropical African woodland. *Oecologia,* 72:402–409.

Brau, E., Heo, C.C., Jalaludin, N.H., Shamsudin, N.N. & Yong, S.K. 2019. Changes in soil nutrients (ammonia, phosphate and nitrate) associated with rat carcass decomposition under tropical climatic conditions. *Soil Research*, 57(5); 482.

Breidenstein, B.C., Cross, H.R., Field, R.A., Johnson, H.K., Lin, K.C. & Randecker, V. 1988. Mineral composition of lamb carcasses from the United States and New Zealand. *Meat Science*, 24(1): 47-59.

Brown, Kathrine & Lemon, J. 2022. *Cations and Cation Exchange Capacity* [online]. Soil Quality Pty Ltd. Available on the Internet at: https://www.soilquality.org.au/factsheets/cation-exchange-capacity (Accessed 2 February 2022).

Brown, P.H. & 2 White, P.J. 2010. Plant nutrition for sustainable development and global health. *Ann Bot*. 105(7): 1073–1080.

Bruand, A., Hartmann, C. & Lesturgez, G. 2005. Physical properties of tropical sandy soils: A large range of behaviours. In *Management of Tropical Sandy Soils for Sustainable Agriculture Conference*. Thailand.

Bryant, Sophie. 2017. *How does soil pH affect plant growth? How can I measure soil pH?* [online]. The Laboratory People. Available on the Internet at: https://camblab.info/how-does-soil-ph-affect-plant-growth-how-can-i-measure-soil-ph/ (Accessed 25 February 2022).

Buchanan, B.B., Gruissem, W. & Jones, R.J. 2002. *Biochemistry and Molecular Biology of Plants*. John Wiley & Sons, West Sussex, UK. 1341 pp.

Bump, J.K., Frey, B., Frossard, A., Morris, A.W., Risch, Anita C. & Schütz, M. 2020. Effects of elk and bison carcasses on soil microbial communities and ecosystem functions in Yellowstone, USA. *Functional Ecology*, 34(9): 1933-1944.

Busato, J.G., Façanha, A.R., Peres, L.E.P., Santos, M.P. & Zandonadi, D.B. 2013. Plant physiology as affected by humified organic matter. *Theoretical and Experimental Plant Physiology*, 25(1): 12-25.

Bushing, Jennifer, Carter, D.O., Higley, L.G., Johnson, Reyna & Spicka, Ashley. 2011. Carcass mass can influence rate of decomposition and release of ninhydrin-reactive nitrogen into gravesoil. *Forensic Science International*, 209(1–3): 80-85.

Carson, Jennifer & Phillips, Lori. 2021. *Soil nitrogen supply* [online]. Soil Quality Pty Ltd. Available on the Internet at: https://www.soilquality.org.au/factsheets/soil-nitrogen-supply (Accessed 25 October 2021).

Carter, D.O. & Tibbett, M. 2010. Cadaver decomposition and soil: Processes. *Soil Analysis in Forensic Taphonomy*, 6: 29-51.

Carter, D.O., Yellowlees, D. & Tibbett, M. 2007. Cadaver decomposition in terrestrial ecosystems. *Naturwissenschaften*, 94(1): 12–24.

Chatzistathis, T. & Therios, I. 2013. *How soil nutrient availability influences plant biomass and how biomass stimulation alleviates heavy metal toxicity in soils: The cases of nutrient use efficient genotypes and phytoremediators, respectively, biomass now - cultivation and utilization* [online]. IntechOpen. Available on the Internet at: https://www.intechopen.com/chapters/44413 (Accessed 1 February 2022).

Chen, J., Li, S., Liu, D., Lu, K., Peng, D., Shafi, M., Wu, J., Yan, W. & Ye, Z. 2014. Effect of copper toxicity on root morphology, ultrastructure, and copper accumulation in Moso bamboo (*Phyllostachys pubescens*). *Zeitschrift fur Naturforschung. C, Journal of Bioscience*, 69(9-10): 399-406.

Clarkson, D.T. & Hanson, J.B. 1980. The mineral nutrition of higher plants. *Annual Review of Plant Physiology,* 31: 239–298.

Coetzee, Charleen. 2012. *The effect of vegetation on the behaviour and movements of Burchell's Zebra, Equus burchelli (Gray 1824) in the Telperion Nature Reserve, Mpumalanga, South Africa*. MSc. Dissertation, University of Pretoria, Pretoria. 174 pp.

Colombo, C., Cesco, S., He, J.Z., Palumbo, G. & Pinton, R. 2014. Review on iron availability in soil: interaction of Fe minerals, plants, and microbes. *Journal of Soils Sediments*, 14: 538–548.

Dufault, R.J. & Melton, Regina R. 1991. Nitrogen, phosphorus, and potassium fertility regimes affect tomato transplant growth. *HortScience*, 26(2): 141-142.

Dutton, C.L., Post, D.M., Puth, Linda M., Rosi, Emma J. & Subalusky, Amanda L. 2020. A river of bones: Wildebeest skeletons leave a legacy of mass mortality in the Mara River, Kenya. *Frontiers in Ecology and Evolution*, 8: 31.

EE Library. 2019. *Sedimentation analysis - Stokes Law* [online]. Elementary Engineering Library. Available on the Internet at: https://www.elementaryengineeringlibrary.com/civil-engineering/soil-mechanics/sedimentation-analysis-stokes-law (Accessed 12 October 2021).

Efretuei, A. 2016. The soils cation exchange capacity and its effect on soil fertility [online]. Permaculture Research Institute. Available on the Internet at: https://www.permaculturenews.org/2016/10/19/soils-cation-exchange-capacity-effect-soil-fertility/ (Accessed 25 February 2022).

Elrakabawi, M.S. 2021. *The influence of phosphide poisoning on carcass decomposition and insect succession: A winter study in Cairo, Egypt*. MSA. thesis, October University for Modern Sciences and Arts, Cairo, Egypt. 76 pp.

Ersek, Kaitlyn. 2012. The 6 essential nutrients for healthy plants [online]. Holganix. Available on the Internet at: https://www.holganix.com/blog/the-6-essential-nutrients-for-healthy-plants#:~:text=1%20Nitrogen%3A%20Nitrate%20%28the%20form%20of%20nitrogen%20that,cell%20walls%20help%20the%20plant%20resist%20disease.%20 (Accessed 16 February 2022).

Fageria, N.K. & Moreira, A. 2011. The role of mineral nutrition on root growth of crop plants. *Advances in Agronomy*, 110: 251-331.

Fan, Z., Goyne, K.W., Kabrick, J.M. & Meinert, D. 2011. Landscape determinants of exchangeable calcium and magnesium in Ozark Highland Forest soils. *Soil Science Society of America Journal,* 75(1): 164–180.

Feng, F., Liu, J., Sun, H. & Zhao, Q. 2017. The interaction between auxin and nitric oxide regulates root growth in response to iron deficiency in rice. *Frontiers in Plant Science*, 8: 2169.

Fernandez, F.G. & Kaiser, D.E. 2021. *Understanding nitrogen in soils* [online]. University of Minnesota. Available on the Internet at: https://extension.umn.edu/nitrogen/understanding-nitrogen-soils#organic-n-sources-760712 (Accessed 25 February 2022).

Fitzhenry, L.B. 2016. *Yields and meat quality attributes of wild fallow deer (Dama dama) in South Africa*. MSc. Dissertation, Stellenbosch University, Stellenbosch. 130 pp.

Fonseca, C.R., McC. Overton, J., McDonald, P.G. & Westoby, M. 2003. Leaf-size divergence along rainfall and soil-nutrient gradients: Is the method of size reduction common among clades? *Functional Ecology*, 17: 50 – 57.

Forbes S.L. & Carter D.O. 2015. *Processes and mechanisms of death and decomposition of vertebrate carrion*. In: Benbow E.M., Tomberlin J.K. & Tarone A.M. (eds). *Carrion ecology, evolution, and their applications*. CRC Press, Boca Raton, 512 pp.

Fourie, M. 2019. *What can electrical conductivity tell us about our soil?* [online]. Trace and Save. Available on the Internet at: http://traceandsave.com/what-can-electrical-conductivity-tell-us-about-our-soil/ (Accessed 6 March 2019).

Frischie, Stephanie, Hopwood, Jennifer, Lee-Mäder, E. & May, Emily. 2021. *Farming with soil life: A handbook for supporting soil invertebrates and soil health on farms*. The Xerces Society for Invertebrate Conservation. Portland, OR. 128 pp.

Getter, Kristin. 2013. *pH and EC issues still a problem in some greenhouses* [online]. Michigan State University. Available on the Internet at: https://www.canr.msu.edu/news/ph_and_ec_issues_still_a_problem_in_some_greenhouses (Accessed 11 January 2022).

Ghazaryan, Karen, Ghazaryan, N., Movsesyan H. & Watts, Beatriz A. 2019. Copper phytoremediation potential of wild plant species growing in the mine polluted areas of Armenia. *Environmental Pollution*, 249: 491-501.

Grant, Amy. 2021. *Do plants use carbon: Learn about the role of carbon in plants* [online]. Gardening Know How. Available on the Internet at: https://www.gardeningknowhow.com/garden-how-to/soil-fertilizers/role-of-carbon-in-plants.htm (Accessed 20 January 2022).

Google. 2019. *25°41'35.20"S 29° 0'7.01"E* [online]. AfriGIS (Pty) Ltd. Available on the Internet at: https://www.google.com/maps/place/25%C2%B041'35.2%22S+29%C2%B000'07.0%22E/@-25.7296347,28.8227119,10.75z/data=!4m5!3m4!1s0x0:0x0!8m2!3d-25.6931111!4d29.0019472 (Accessed 11 June 2019).

Growing Media Europe. 2016. *About growing media* [online]. Growing Media Europe. Available on the Internet at: https://www.growing-media.eu/news-1 (Accessed 15 October 2019).

Gu, Xiaoying, Klonowski, B., Krawczynski, R., Rössler, Annett & Wiegleb, G. 2015. Influence of a badger carcass on soil chemistry and Collembola. *Acta Societatis Zoologicae Bohemicae*, 79: 235–241.

Guthrie, T., Ross, D. & Rozeboom, D. 2013. *Carcass composting – a guide to mortality management on michigan cattle farms*. Michigan State University, Extension Bulletin E3197. 7 pp.

Haslam, Tamsin C.F. & Tibbett, M. 2009. Soils of contrasting pH affect the decomposition of buried mammalian (*Ovis aries*) skeletal muscle tissue. *Journal of Forensic Sciences*, 54(4): 900-904.

Heath, A. 2019. *How does acidic soil affect nitrogen availability?* [online]. Easierwithpractice. Available on the Internet at: https://easierwithpractice.com/how-does-acidic-soil-affect-nitrogen-availability/ (Accessed 12 February 2022).

Herrera, L.F.R., López-Bucio, J. & Shane, M.W. 2015. Nutritional regulation of root development. *Wiley Interdisciplinary Reviews: Developmental Biology*, 4(4): 431-43.

Hoffman, L.C., Kidd, M., Laubscher, Liesel L. & Mostert, A.C. 2009. Meat quality of kudu (*Tragelaphus strepsiceros*) and impala (*Aepyceros melampus*): Carcass yield, physical quality and chemical composition of kudu and impala Longissimus dorsimuscle as affected by gender and age. *Meat Science,* 83: 788–795.

Huang, W., Hui, C., Ratkowsky, D.A., Shi, P., Su, J., & Wang, P. 2019. Leaf fresh weight versus dry weight: Which is better for describing the scaling relationship between leaf biomass and leaf area for broad-leaved plants? *Forests*, 10: 256.

James Lind Institute. 2019. *The importance of soil organic matter* [online]. James Lind Institute, School of Agribusiness. Available on the Internet at: https://www.jliedu.com/blog/importance-soil-organic-matter/ (Accessed 5 October 2021).

Jilani, A.A. 2017. Effect of Ca EDTA and Fe EDTA on micro and macro nutrients uptake by black gram. *IOSR-JCE*. 19 (2): 66-69.

Jin, G.L., Niu, J.F. & Zhang, Y.S. 2014. Root development under control of magnesium availability. *Plant Signaling & Behavior*, 9: 9.

Joffe, Pitta & Oberholzer, T. 2012. *Creative gardening with indigenous plants*. 2nd ed. Briza Publications, Pretoria. 391 pp.

Kaiser, D.E. & Rosen, C.J. 2018. *Potassium for crop production* [online]. University of Minnesota. Available on the Internet at: https://extension.umn.edu/phosphorus-and-potassium/potassium-crop-production#manure-602513 (Accessed 25 February 2022).

Kalischuk-Tymensen, L., Lysyk, T., Perotti, M., Yanke, L. & Selinger, L. 2001. Growth and survival of immature *Haematobia irritans* (Diptera: *Muscidae*) is influenced by bacteria isolated from cattle manure and conspecific larvae. *Journal of Medical Entomology*, 38: 180–187.

Kelly, Jasey. 2018. *Which fertilizer element encourages flowering growth in plants?* [online]. SFGate. Available on the Internet at: https://homeguides.sfgate.com/fertilizer-element-encourages-flowering-growth-plants-76453.html (Accessed 19 January 2022).

Khan, M.S. 2018. Role of macro and micro nutrients and their deficiency symptoms in plants [online]. AgriHunt. Available on the Internet at: https://agrihunt.com/articles/fertilizer-industry/role-of-macro-and-micro-nutrients-and-their-deficiency-symptoms-in-plants/ (Accessed 25 February 2022).

Kim, K-R, Kim, K-H, Kim, Y-N, Shin, D-C & Yoon, J-H. 2017. Management of animal carcass disposal sites using a biochar permeable reactive barrier and fast growth tree (*Populus euramericana*): A field study in Korea. *Sustainability*. 9: 457.

Kim, D. & Park, I. 1993. Significance of fresh weight to dry cell weight ratio in plant cell suspension cultures. *Biotechnology Techniques*, 7: 627–630.

Koenig, Isabelle, Le Bayon, Renée-Claire, Mitchell, E.A.D., Seppey, C.V.W. & Szelecz, I. 2018. Soil chemistry changes beneath decomposing cadavers over a one-year Period. *Forensic Science International*, 286: 155–165.

Korgaokar, S.S., Markana, T.V., Soni, K.V. & Vyas, M.K. 2016. Effect of heavy metals on biomass and growth rate of selected pulses. In the *International Conference on Research & Entrepreneurship*. India.

Krawczynski, R. & Schlaghamerský, J. 2015. Does carcass decomposition affect soil-dwelling enchytraeids? *Soils Organisms*, 87(2): 91-100.

Kumar, V.R.A. 2018. *Explained: 14 nutrients required by plants for best growth & development* [online]. Krishi Jagran. Available on the Internet at: https://krishijagran.com/featured/explained-14-nutrients-required-by-plants-for-best-growth-development/ (Accessed 12 January 2022).

LECO Corporation. 2010. *Total/organic carbon and nitrogen in soils* [online]. LECO Corporation. Available on the Internet at: http://www.leco.co.za/wp-content/uploads/2012/02/CN2000_SOILS_TOTAL_ORGANIC_CARBON_NITROGEN_203-821-165.pdf (Accessed 20 February 2019).

Lines-Kelly, Rebecca. 1993. *Cation exchange capacity* [online]. NSW Department of Industry. Available on the Internet at: https://www.dpi.nsw.gov.au/agriculture/soils/guides/soil-nutrients-and-fertilisers/cec (Accessed 25 February 2022).

Liles, Valerie. 2021. *Elements & uses of NPK fertilizer* [online]. SFGate. Available on the Internet at: https://homeguides.sfgate.com/elements-uses-npk-fertilizer-71006.html (Accessed 19 January 2022).

Lohry, R. 2007. *Micronutrients: Functions, sources and application methods*. CCA Conference Proceedings, Indiana.

Lynn, W.C. & Pearson, M.J. 2000. *The color of soil* [online]. USDA, Natural Resources Conservation Services. Available on the Internet at: https://www.nrcs.usda.gov/wps/portal/nrcs/detail/soils/edu/?cid=nrcs142p2_054286 (Accessed 26 July 2021).

Macfadyen, D.N. & Reilly, B.K. 2013. The dynamics of Buprestidae (Coleoptera) in Rocky Highveld grasslands of Ezemvelo Nature Reserve, Gauteng, South. *The Coleopterists Bulletin,* 67(1): 7-15.

MacMohan J.A. & Parmenter, R.R. 2009. Carrion decomposition and nutrient cycling in a semiarid shrub–steppe ecosystem. *Ecological Monographs*, 79(4): 637–661.

Magdoff, F. & van Es, H. 2021. *Building soils for better crops ecological management for healthy soils*. 4th ed. Sustainable Agriculture Research & Education, United States. 394 pp.

Mahler, R.L. 2004. *Nutrients plants require for growth*. University of Idaho, CIS 1124.

Manjula, V.N. 2017. *Soils, plant nutrition and nutrient management*. University of Missouri Extension, USA. 12 pp.

Marschner, H. 1995. *Mineral nutrition of higher plants, Ed 2*. Academic Press, London. 889 pp.

Marsh, M., Maynard, D.G., Morrison, I.K., Neary, A.J., Palmer, C.J., Pastorek, L. & Schumacher, B.A. 1995. Laboratory methods for soil and foliar analysis in long-term environmental monitoring programs. *United States Environmental Protection Agency*, 600(R-95): 077.

Mateo-Tomás, Patricia, Olea, P.P. & Sánchez-Zapata, J.A. (ed.). 2019. *Carrion ecology and management*. Springer Nature, Switzerland. 281 pp.

Matuszewski, S., Konwerski, S., Frątczak, K. & Szafałowicz, M. 2014. Effect of body mass and clothing on decomposition of pig carcasses. *International Journal of Legal Medicine*, 128: 1039–1048.

McIntosh, Jamie. 2020. *How to choose the right flower fertilizer type* [online]. The Spruce. Available on the Internet at: https://www.thespruce.com/choose-right-flower-fertilizer-1315828 (Accessed 2 February 2022).

McMahon, Mary. 2022. *What are ornamental plants?* [online]. Home Questions Answered. Available on the internet at: https://www.homequestionsanswered.com/what-are-ornamental-plants.htm#:~:text=Ornamental%20plants%20are%20plants%20which%20are

%20grown%20for,many%20people%20feel%20that%20this%20is%20value%20enough. (Accessed 19 May 2023).

Melville, H.A. 2018. *Death in the long grass: The ecological implications of carcass decomposition in a Southern African grassland.* Applied Behavioural Ecology and Ecosystems Research Unit, Florida. 33 pp.

Miller, C. 2000. Carbon-nitrogen ratio: Understanding chemical elements in organic matter [online]. Eco Farming Daily, Acres, U.S.A. Available on the Internet at: https://www.ecofarmingdaily.com/build-soil/soil-inputs/minerals-nutrients/carbon-nitrogen-ratio/ (Accessed 18 October 2021).

Modi, H.A. & Prajapati, Kalavati. 2012. The importance of potassium in plant arowth – a review. *Indian Journal of Plant Sciences*, 1(02-03): 177-186.

Mucina, L. & Rutherford, M.C. (ed.). 2006. *The vegetation of South Africa, Lesotho and Swaziland Strelitzia 19*. South African National Biodiversity Institute, Pretoria. 808 pp.

Munsell Color. 2019. *How to read a Munsell color chart* [online]. Munsell Color. Available on the Internet at: https://munsell.com/about-munsell-color/how-color-notation-works/how-to-read-color-chart/ (Accessed 21 February 2019).

Murungi, Jane I., Silas, N.E. & Wanjau, R.N. 2012. The pH of leaf water extracts and amount of acid required lowering the pH of leaf water extracts to 5.0. *American International Journal of Contemporary Research*, 2(11): 72-78.

Ndimele, P.E. (ed). 2018. *The political ecology of oil and gas activities in the Nigerian aquatic ecosystem*. Elsevier Inc., United Kingdom. 467 pp.

newPlantsandFlowers. 2019. *Gazania new day strawberry shortcake mixture – photo: PanAmerican Seed* [online]. newPlantsandFlowers. Available on the Internet at: http://www.newplantsandflowers.com/new-days-for-gazania/gazania-new-day-strawberry-shortcake-mixture-photo-panamerican-seed/ (Accessed 16 May 2019).

North Country Organics. 2022. *Cation exchange capacity (CEC)* [online]. North Country Organics. Available on the Internet at: https://norganics.com/index-2/technical-articles/cation-exchange-capacity-cec/ (Accessed 11 January 2022).

Noyes, Amber. 2021. *Peat moss: What it is and how to use it in your garden* [online]. Gardening Chores. Available on the internet at: https://www.gardeningchores.com/author/ambernoyes/ (Accessed 18 May 2022).

Olsen, S.R., Cole, C.V., Watanabe, F.S. & Dean, L.A. 1954. Estimation of available phosphorus in soils by extraction with sodium bicarbonate. *US Department of Agriculture Circular*, 939: 19.

Phoslab. 2013. *How does potassium help plants grow?* [online]. Phoslab Environmental Laboratories. Available on the Internet at: https://www.phoslab.com/how-does-potassium-help-plants-grow/ (Accessed 25 February 2022).

Queensland Government. 2016. *Soil pH* [online]. The State of Queensland. Available on the Internet at: https://www.qld.gov.au/environment/land/management/soil/soil-properties/ph-levels (Accessed 19 August 2021).

Rajawat, K.S., Rathore, J.S., Sarvanan, S., Singh, G. & Singh, Gajendra. 2017. Effect of different micronutrients on plant growth, yield and flower bud quality of broccoli *(Brassica oleracea* var. *italica*). *Current Agriculture Research Journal*, 5(1): 108-115.

Rinaldi, Hilary. 2013. *High heat flowers for hot summer areas* [online]. WM Media. Available on the Internet at: http://www.weekendgardener.net/garden-plants/high-heat-flowers-080908.htm (Accessed 27 February 2019).

Sinclair, T.R. 1992. Mineral nutrition and plant growth response to climate change. *Journal of Experimental Botany,* 43: 1141–1146.

Silva J.A. & Uchida, R. (eds). 2000. *Plant nutrient management in Hawaii's soils, approaches for tropical and subtropical agriculture*. College of Tropical Agriculture and Human Resources, University of Hawaii at Manoa. 31-55 pp.

Solomon, Lorraine. 2017. *Gazania* [online]. Kumbula Nursery. Available on the Internet at: https://kumbulanursery.co.za/plants/gazania (Accessed 9 January 2019).

Spectrum Technologies. 2022. *If plants could talk...about EC* [online]. Spectrum Technologies, Inc. Available on the Internet at: https://www.specmeters.com/newsletter/if-plants-could-talk-vol-2/ (Accessed 25 February 2022).

Sundblad, Donna. 2022. *Nutrients needed for plants to grow* [online]. LoveToKnow Media. Available on the Internet at: https://garden.lovetoknow.com/wiki/Nutrients_Needed_for_Plants_to_Grow (Accessed 10 January 2022).

Swanepoel, Barbara A. 2006. *The vegetation ecology of Ezemvelo Nature Reserve, Bronkhorstspruit, South Africa*. MSc. dissertation, University of Pretoria, Pretoria. 138 pp.

Tabatabai, M.A. 1996. *Soil organic matter testing: An overview* [online]. Alliance of Crop, Soil, and Environmental Science Societies. Available on the Internet at: https://dl.sciencesocieties.org/publications/books/abstracts/sssaspecialpubl/soilorganicmatt/1?access=0&view=pdf (Accessed 6 March 2019).

Tajer, A. 2016. *What's the function of potassium (K) in plants?* [online]. Greenway Biotech Inc. Available on the Internet at: https://www.greenwaybiotech.com/blogs/gardening-articles/whats-the-function-of-potassium-k-in-plants (Accessed 25 February 2022).

Towne, E.G. 2000. Prairie vegetation and soil nutrient responses to ungulate carcasses. *Oecologia*, 122:232–239.

Traunfeld, J. 2020. *Organic matter and soil amendments* [online]. University of Maryland. Available on the Internet at: https://extension.umd.edu/resource/organic-matter-and-soil-amendments (Accessed 20 January 2022).

USDA. 2014. *soil electrical conductivity* [online]. USDA Natural Resources Conservation Service. Available on the Internet at: https://www.nrcs.usda.gov (Accessed 7 September 2021).

Van Heerden, A. & Hoffman, L.C. 2018. *Profiling the meat quality of blue wildebeest C. taurinus* [online]. Expand your Game 2018 Conference. University of Stellenbosch. Available on the Internet at: https://www.wrsa.co.za/wp-

content/uploads/2018/04/A-VAN-HEERDEN-WRSA.pdf (Accessed 15 July 2019).

Van Reeuwijk, L.P. 2002. *Procedures for soil analysis*. 6th ed. International Soil Reference and Information Centre. The Netherlands. 119 pp.

Ward, Eileen. 2022. *Gardening: The pH of your soil can affect plant growth and health* [online]. Naples Daily News. Available on the Internet at: https://archive.naplesnews.com/community/gardening-the-ph-of-your-soil-can-affect-plant-growth-and-health-ep-1071358042-331319431.html/ (Accessed 10 January 2022).

White, R. 2010. *Randomizing a list of students using Excel* [online]. YouTube ZA. Available on the Internet at: https://www.youtube.com/watch?v=lajWM28JCHE&feature=youtu.be (Accessed 30 April 2019).

Williams, R. 2019. *Plant nutrients explained: Everything you ever need to know* [online]. Epic Gardening. Available on the Internet at: https://www.epicgardening.com/plant-nutrients/ (Accessed 16 February 2022).

Annexure A: A schematic presentation of the random pot layout in the greenhouse

Random Pot Layout							
Grid #		Internal thermometer					
S13	W4S12-2	W2S12-3	S6-3		S8-2	S10-3	W4S15-3
S12	S2-2	W4S6-2	S3-1		W2S13-1	W3S6-2	W2S9-1
	S7-1	S14-2	W3S4-1		S3-3	W4S15-2	W4S6-1
S11	W4S4-1	W2S6-3	W2S3-2		W2S1-1	W2S8-1	W2S11-2
	W3S3-3	S9-3	W3S9-3		W3S1-2	W3S1-1	W3S4-3
S10	S8-1	W4S9-3	S5-1		W2S6-1	S4-2	W4S1-3
	W2S6-2	W3S15-2	W3S13-3		W3S12-1	S15-1	W3S4-2
S9	S1-2	W4S1-2	W4S8-1		W2S15-3	W4S8-3	S12-1
	W3S11-3	S1-1	W4S11-2		W4S13-1	W2S11-3	S5-3
S8	W2S9-2	W2S4-1	W4S6-3		S4-1	S9-2	W4S4-3
	W3S15-1	W3S11-2	S6-1		S15-3	W3S8-1	S7-3
S7	S4-3	W3S6-3	S7-2		W3S9-1	W3S8-3	W4S12-1
S6	S11-3	S12-2	W4S9-1		W2S11-1	W2S12-1	S12-3
	W4S13-2	S8-3	W4S9-2		S6-2	W3S3-2	S2-3
S5	W2S4-2	S13-3	W4S1-1		W3S13-1	S2-1	W3S11-1
	S13-2	W2S12-2	W3S3-1		S13-1	W2S3-3	W3S15-3
S4	W2S9-3	S5-2	S10-2		S3-2	W3S9-2	W2S1-3
	W2S13-2	S15-2	W4S3-3		W2S1-2	W3S12-3	W4S3-1
S3	S9-1	W3S12-2	W2S15-1		S10-1	W4S3-2	W2S13-3
	W2S15-2	W4S11-1	W4S4-2		W3S1-3	W4S13-3	W4S12-3
S2	W4S15-1	W4S11-3	W2S8-2		S1-3	S14-1	
	S14-3	W3S6-1	W2S8-3		W3S13-2	W2S4-3	
S1	W3S8-2	S11-1	W4S8-2		W2S3-1	S11-2	
		Vent				Greenhouse door	

Key;	
S1-15	Control groups
	S = Telperion site numbers 1-15 of control, caged and pegged carcass sites
W2	Week 2 of soil collection period
W3	Week 3 of soil collection period
W4	Week 4 of soil collection period
1,2,3	Indicates repitition numbers

Annexure B: Average plant growth parameters by week and treatments of caged and pegged

WEEK Treatment	Leaf Length	# Leaves	Dead Lvs	Buds	Flowers	Seed sets	Root Length	Root Width	Fresh Lvs	Fresh BFSs	Fresh Rts	Dry Lvs	Dry BFSs	Dry Rts
W1CA	10,5	25,3	3,3	1,7	0,8	0,8	12,0	4,8	12,3	10,1	7,9	8,2	7,8	7,4
W1PE	10,5	25,7	3,9	1,2	0,7	0,7	11,5	4,2	12,3	10,2	7,8	8,1	7,9	7,4
W2CA	11,7	47,9	6,5	3,3	1,0	1,7	11,8	4,5	21,4	13,7	8,2	9,6	8,5	7,5
W2PE	12,0	45,4	4,4	4,0	0,8	1,5	11,4	4,9	21,7	13,1	8,0	9,3	8,0	7,4
W3CA	11,4	47,5	9,4	3,9	1,6	2,0	11,1	4,3	22,0	14,2	7,8	9,7	8,1	7,4
W3PE	12,3	50,1	9,3	5,9	1,0	2,6	10,7	4,5	23,1	13,5	8,0	9,8	8,6	7,5
W4CA	11,1	40,2	7,5	3,6	0,6	1,2	10,2	3,3	19,4	10,0	7,5	9,5	7,8	7,3
W4PE	11,6	47,0	8,4	3,0	1,1	1,8	10,7	4,1	21,5	11,6	8,0	9,6	8,2	7,4

Annexure C: Turn-it-in Receipt

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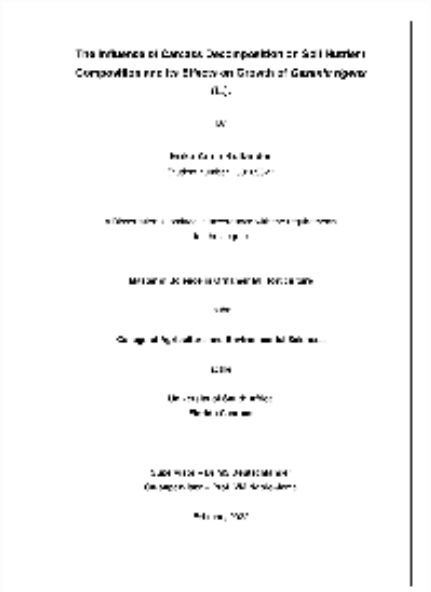

Annexure D: Language Editor's Declaration

l 'Avenir Consulting

Monica Botha
T/a l'Avenir Consulting
PO Box 32945
WAVERLEY
0135

Cellular: 083 269 0757
E-mail: monicabo@lantic.net

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This serves to confirm that I have edited and proofread the dissertation entitled

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MONICA BOTHA
28 February 2022