



**The impact of fly ash on the growth rate of maize (*Zea mays L.*) and the potential for
fly ash to be used commercially in agriculture.**

by

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DECLARATION

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ABSTRACT

South Africa's coal-fired power plants (CFPPs) produce about 34.4 million tons of fly ash (FA) per annum, as a by-product of the coal combustion process, which is stockpiled adjacent to these CFPPs. This FA contains traces of heavy metals (HMs), as a result it is a potential environmental hazard. The purpose of this study was, therefore, to establish the effects of ('FA') on the growth of maize (*Zea mays L.*), as well as its potential commercial use in agriculture due to its nutrient content, instead of maintaining the status quo. Another aim of the study was also to determine the phytoextraction abilities of the HMs (Mn, Cr, Ba and Mo) from contaminated soils by the maize plants. The study was conducted by planting maize seeds in five soil substrates with 0 %, 20 %, 40 %, 55 % and 60 % FA treatment, on mass basis. The ANOVA indicated that there were significant differences when comparing most of the mean stem length, number of leaves, root length and the mean mass of total plant dry biomass, across all the FA treatments, after 122 days of growth. The mean stem lengths after 122 days of growth were 86.67 ± 20.55 cm, 148.33 ± 13.87 cm, 154.17 ± 18.10 cm, 121.17 ± 14.99 cm and 149.50 ± 24.20 cm for maize plants grown in 0 % , 20 % , 40 % , 55 % and 60 % FA treatments, respectively. The mean number of leaves were 12 ± 1.87 , 16 ± 2.34 , 18 ± 2.83 , 12 ± 1.87 and 14 ± 1.79 across all treatments, respectively. The key finding was that the growth of these plants improved with the amount of FA in the soil, with the plants that were grown in the 40 % FA treatment yielding the best results. The same observation was made with the root length and the mean masses of total plant biomass (roots, stems and leaves). Another important finding was that the accumulation of the HMs (Mn and Cr) in the plants increased, across the FA treatments, while their concentrations in the soil decreased across the FA treatments. The accumulation of Ba and Mo in the plants also increased, across the FA treatments, while Ba remained slightly constant and Mo increased in the soil. This indicated that the maize plants can potentially remediate Mn and Cr contaminated soils, through phytoextraction, but are retained in the root system.

Key words: Fly Ash, Maize plant growth, Heavy Metals, Phytoextraction

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1. INTRODUCTION

1.1 Overview

In terms of section 24 of the Constitution of the Republic of South Africa, Act No. 108 of 1996 (the Constitution), as amended, states that “everyone has the right:

- a) *to an environment that is not harmful to their health or wellbeing; and*
- b) *to have an environment protected, for the benefit of present and future generations, though reasonable legislative and other measures that:*
 - i. *prevent pollution and ecological degradation;*
 - ii. *promote conservation; and*
 - iii. *secure ecologically sustainable development and use of natural resources while promoting justifiable economic and social development”.*

Anthropogenic interventions must be done such that the environment and its biodiversity remain capable of providing services to all its current and future beneficiaries. This is achievable by ensuring that there is sustainable development, through the reasonable use of natural resources, including plants and animals, while satisfying the socio-economic needs of the human population (Gomiero, 2016). In summary, sustainable development is a concept, which promotes the exploration and utilization of natural resources in a manner that will preserve the environment, its ecology and its services for current and future beneficiaries, while ensuring sound economic, social and technological development (Ferreira *et al.*, 2020).

Anthropogenic land use may also lead to the degradation of soil quality and subsequently its capability to maintain function within the ecosystem and land-use boundaries (Doran & Zeiss, 2000, Gomiero, 2016). Doran and Zeiss (2000) and Gomiero, 2016 further highlights that agricultural human interventions resulted in the degeneration of 40% of global arable land due to erosion, excessive cultivation, air pollution, salinity modification, overgrazing, mining, land clearing and desertification, as a result of a population of 2.5 billion people. According to the United Nations Environment Programme (UNEP) (as cited in Mani, Osborne and Cleaver, 2020, p 979), “the amount of degraded land in South Africa is estimated to be 60%”.

It is necessary to ensure that human interventions, which are deemed necessary for survival, are done in a sustainable manner such that the environment and its ecological services are not compromised in the short and long terms. This includes ensuring that the ecosystem and its services are used in a sustainable manner, such that the needs of living beings, for example, human beings, whose livelihood depends on the cultivation of crops, like maize are not compromised. Maize is an essential staple food, after wheat and rice, in most developing

countries, including Africa (Golob *et al.* (as cited in Aladesanmi *et al.*, 2019)), and its growth depends on good soil quality, a healthy environment, its ecology and services. Soil, together with water and air provide the fundamental resources for sustaining the ecosystem, its services, along with human and plant wellbeing (Bunemann *et al.*, 2018).

Simpson *et al.* (2019), states out of the nine provinces of South Africa, the Mpumalanga Province (MP) is the second largest and it possesses about half of potential arable land in South Africa. The Department of Forestry, Fisheries and Environment (DFFE) (as cited in Adisa *et al.*, 2019, p 174), estimates that “46.2 % of South Africa’s gross value of field crops is attributed to maize production”. Adisa *et al.* (2019, p 174) points out that “in 2017, 87 % of the total maize that was produced in South Africa was from MP, KwaZulu-Natal (KZN), Free State (FS), and the North West (NW) Provinces, of which, MP’s contribution was 20 %”.

In MP, the requirement for cultivated crops, including maize, coexists with the need for its abundant coal resources, as well as its many coal-fired power plants (CFPPs), which are strategically positioned close to these mines (Simpson *et al.*, 2019). Simpson *et al.* (2019) also highlights that there is a competition for space and water resource between industry and agriculture, which requires a solution between the two sectors.

Marschner (2012); Singh and Schulzer, (2015), point out that apart from the water requirement and its quality, there are 14 essential chemical elements or nutrients, which are naturally absorbed by plants in different amounts, via roots, from the soil; and these include some toxic metals. Other interrelated chemical properties of soil that influence fertility are its cation exchange capacity (CEC) and the pH (Tanzi *et al.*, 2018; Marschner, 2012). These concepts are discussed in detail in the literature review section. Singh and Schulze (2015); Basu *et al.* (2009) classified these elements as follows:

- a) Primary or macronutrients such as Calcium (Ca), Nitrogen (N), Phosphorus (P), and Potassium (K),
- b) Secondary nutrients such as Magnesium (Mg), Sulphur (S); and
- c) Micronutrients e.g., Boron (B), Chlorine (Cl), Copper (Cu), Iron (Fe), Manganese (Mn), Molybdenum (Mo), Nickel (Ni) and Zinc (Zn).

In addition, plant growth is also depended on three more elements, namely Carbon (C), Oxygen (O) and Hydrogen (H), which are sourced from water and air (Marschner, 2012; Singh and Schulzer, 2015). Djaman *et al.* (2022); O’Keeffe (2009), all mentioned that the development, growth and yield of maize plants are also dependent on the plant density, planting patterns, tillage, water management, soil types, soil hydrological and chemical properties and

management practices. Water required for maize yield is provided through rainfall and/or irrigation, depending on access to water sources such as rivers, lakes, dams, etc (Ibrahim and Ibrahim, 2020).

The dynamics of the absorption of nutrients by plants relative to their availability in the soil plays an important role in plant development and growth, therefore, it is important to understand the role of these nutrients, as well as the effects of their deficiency or toxicity on maize plants (Oberholzer-Grain SA, 2020). These minerals are absorbed by plants in different forms and they perform different functions in plant metabolism, as such they are categorised into four distinct groups (Marschner, 2012) and will be discussed later in the document. Arnon and Stout (as cited in Marschner, 2012, p 3), proposed the following three criteria that must be met in determining the essentiality of these minerals:

- a) *“The lifecycle of a specific plant cannot be complete due to deficiency of a specific element;*
- b) *An element’s functions in plant development must not be replaceable; and*
- c) *The element must have a specific function in the metabolism of the plant.”*

The soil also becomes depleted of essential nutrients if cultivation is continuous without any input of fertilizers and it is important that they are replenished in order to maintain soil quality and fertility for current land use and for future generations. According to O’Keeffe (2009), the deficiency of various nutrient in maize plants, identified through a variety of visible defects on the physical structure of the maize plant. The essential nutrients such as N, P and K (NPK) play different roles in the development and growth of maize plants, therefore a deficiency in any of NPK will exhibit specific defects on the structure of these plants (Marschner, 2012; O’Keeffe, 2009). For example, the midrib section of older leaves tends to turn yellow due to lack of N; whereas, the lack of P results in reddish-purple lower leaves; while insufficient K can lead to a lack of chlorophyll, stunted growth; lack of defence against diseases, amongst others. (O’Keeffe, 2009).

The Food and Agriculture Organisation of the UN (FAO), defines soil fertility as the “soil’s capability to maintain plant growth and reproduction by provision of enough key plant nutrients and soil water in an environment that is free of toxic substances”. However, in conventional farming, soil fertility is enhanced by continuous use of chemical fertilisers, however excessive use can result in negative impacts, such as soil acidification, water and air pollution, chemical burn of crops and the depletion of minerals in soil through leaching (Chandini *et al.*, 2019).

The use of FA in agricultural soil was also found to be eco-friendly and cost-saving compared to conventional fertilisers (Panday and Singh, 2010; Srivastava *et al.*, 2016). According to

Ayanda *et al.* (2012); Basu *et al.* (2009); Reynold-Classsen *et al.* (2018) and Yao *et al.* (2015), these CFPPs produce fly ash (FA), as a by-product of combustion, which contains essential macro and micronutrients for plants, for example elements such as Ca, Fe, Mg, Mn, P, K, Na, S and Zn. The treatment of soil with FA also improved the soil texture, porosity, soil pH and the water retention capacity. (Basu *et al.*, 2009; Panda and Biswal, 2018; Srivastava *et al.*, 2016).

According to Basu *et al.* (2009); and Yao *et al.* (2015), FA can be a potential substitute for lime, which is normally used for remedying acidic soil. The reason being that the Carbon (C) element content in lime is eventually released into the atmosphere as carbon dioxide, thus making the use of FA as a substitute to be eco-friendly, since it contains less C. Please note that this topic is outside the scope of this current study.

The use of FA in the agricultural sector is limited due to traces of potentially toxic heavy metals (HMs) such as Al, Arsenic (As), Barium (Ba), Chromium (Cr), Mercury (Hg) and Mo (Basu *et al.*, 2009). Some HMs, are essential for plant development and growth, while some are toxic, not biodegradable and as a result they accumulate in soils and may be absorbed and translocated by plants (Silva *et al.*, 2021).

Several studies that were conducted, by; Niu *et al.* (2021); Parab *et al.* (2012), and Srivastava *et al.* (2016) discovered the positive effects on plant growth and fresh mass of edible plant parts, following the addition of limited FA. Basu *et al.* (2009); Murugan and Vijayarangam (2013); Panda *et al.* (2015) and Rajakumar and Patil (2019) also reported improved plant height, cob length, mass of the plant biomass and seed yield on maize plants, which were grown in FA treated soil.

1.2 Motivation

In South Africa, maize is sometimes planted in farming fields surrounding Eskom's CFPPs, which produce about 34.4 million tons of FA per annum, as a by-product, from coal combustion processes (Reynolds-Clausen & Singh, 2017). These CFPPs do not only produce FA that is stockpiled nearby, but they also emit gaseous pollutants such as NO_x, and SO₂ and other particulates, through the flue gas stacks, resulting in atmospheric pollution over a distance from the source point (Conradie *et al.*, 2016; Kok *et al.*, 2021). The distribution and concentration of these emitted elements and particulate matter also varies due to distance and season (Conradie *et al.*, 2016). These chemical elements and particulate matter eventually get deposited on the soil, which includes maize fields, through wet and/or dry deposition (Conradie *et al.*, 2016).

The deposition of toxic elements is not only limited to emissions from CFPP flue gas stacks, but

can happen in dust from the FA dumpsites, thus distributing FA components, including HMs over large distances from the dumpsite (Munawer *et al.*, 2017). Wand *et al.* (2020) further stated that FA may not be favourable to the environment and its ecosystem services, in particular soil quality, because of its potential to leach, radioactivity and toxicity. These findings are findings differ from those studies described in section 1.1, in which FA was found to be beneficial to soil quality and maize plant growth.

The key question here is whether maize plants can grow in soils contaminated with FA due to its metal and metalloid content. According to Wuana and Okieimen (2010), maize plants have the potential to translocate heavy metals from the stem to their foliar parts, thus making these plants a good candidate for phytoextraction.

1.3 Aim and Objectives

The aim of this study is to evaluate the effects that the direct application of FA on soil had on maize plant growth. The other focus of the study is to determine whether maize plants can phytoextract HMs from the FA treated soil, without negatively affecting the plants.

The key concepts in this research project are soil quality, acidity, alkalinity, cation exchange, essential plant nutrients and non-nutrients. Therefore, the objectives are to evaluate:

- a) The effect of FA and its HMs on the soil pH and CEC, as well as the ability of maize plants to absorb essential nutrients (N, P, K, Ca and Mg) from the soil;
- b) The effects of FA on the growth of maize, compared to the maize plants that grew in the soil that had 0% FA; and
- c) The ability of maize plants to partially decontaminate the soil, through phytoextraction.

2. LITERATURE REVIEW

The purpose of this study was to determine whether soils treated with fly ash (FA) have an impact on the growth of maize plants and also to evaluate the concentration of plant nutrients and heavy metals (HM) that were absorbed by the maize roots; and stem and leaves (above ground material/biomass AGM/AGB) combined. This literature review focuses on:

- a) Soil Quality;
- b) Soil chemical and physical properties;
- c) Maize plant growth analysis;
- d) Water requirements during the growth of maize plants;
- e) FA properties;
- f) Potential impacts of FA and its HM content on plant growth,
- g) FA as a potential fertiliser; and
- h) Phytoextraction.

2.1 Soil Quality

Doran & Zeiss (2000) (as cited in Tahat *et al.* 2020, p 1), defines soil quality as “its ability to continue to sustain all forms of life, including human needs, as well as land use, through its dynamic biodiversity and ecological services; while also maintaining good water and air quality”. Soil quality is “the capacity of a reference soil to function within natural or managed ecosystem boundaries; to sustain plant and animal productivity; maintain or enhance water quality; and support human health and habitation”. Human life depends on good soil quality as an important delivery mechanism for ecosystem goods and services like carbon storage, water regulation and plant production (Neina, 2019).

Soil quality or its functional capacity entails two aspects namely (Seifu and Elias, 2018):

- a) Its inherent properties, which change minimally as influenced by the climate, topography, parent material, biota and time. These properties include soil texture, type of clay, depth of bedrock and drainage class; and
- b) Its dynamic properties, which are affected by anthropogenic and natural disturbances over time such as cropping, livestock farming, landslides etc., examples of these properties are organic matter content, biological activity, aggregate stability, aggregate size, infiltration and soil fertility.

Soil quality also includes its physical, chemical and biological properties, which mainly determines its fertility (Seifu and Elias, 2018). According to Bunemann *et al.* (2018), soil quality also entails its ability to sustain biological productivity; maintain the quality of the environment;

protect watersheds; promote plant growth and animal wellbeing, by offering protection against pollutants such as organic waste, and agricultural and industrial chemicals, such as those contained in FA.

2.2 Soil Chemistry

Soil is ideally made up of 25 % water, 25 % air, 45 % minerals and 5 % organic matter (humus), where the minerals and organic matter account for much of the solid structure of the soil, whereas the water and air are in the pore space (Manjula, 2017). The soil has multiple functions, amongst others, being able to provide a healthy environment for plant growth, through the provision of the following groups of mineral elements (Singh & Schulze, 2015; Basu *et al.*, 2008):

- a) Primary or macronutrients such as N, P, K, and Ca;
- b) Secondary nutrients such as Mg, S; and
- c) Micronutrients e.g., B, Cl, Cu, Fe, Mn, Mo, Ni and Zn.

In general, the majority of these nutrients are absorbed by plants as ions (Marschner, 2012), through cation exchange between the roots and the soil solution. Plant root hairs secrete exudates that contain positively charged H ions in exchange for positively charged nutrients such as Ca^{+2} , K^{+} , Mg^{+2} , etc., which are mostly contained in the soil structure but can also be found in soil solution (Franklin, 2014). Soil components such as some clays and humus generally have negatively charged surfaces, which attracts positively charged nutrients. It is the interaction between the plant roots and the soil solution, which is part of the soil, where the nutrients are mobile and eventually get assimilated by the plant roots through cation exchange.

Cation Exchange Capacity and Extractable Cations

This ability of the soil to host sufficient cations is referred to as cation exchange capacity ('CEC'), which is the amount of cations in the soil solution that are able to be attached to the surface of the negatively charged soil particles (Beyers *et al.*, 2005). Similarly, soil particles have to a lesser extent, positively charged surfaces that are able to attract anions that are in soil solution. Higher CEC values imply that there is an increased ability for cation exchange between the soil solution and the plant's cation binding sites, which promotes the absorption of nutrients by plant root systems. Various plant species have different CECs', which are also affected by the pH of the soil solution, for example in monocotyledonous species such as maize, the CEC decreases due to increased protons in the cation binding sites (Marschner, 2012).

According to Rayment (2016), many fertile soils have CEC values ranging from 15 to 25 cmol kg^{-1} , while values above 40 cmol kg^{-1} are too high and too low at 4 cmol kg^{-1} and below. These

figures represent the total CEC for extractible cations, such as Ca^{2+} , Mg^{2+} , K^+ , Na^+ , NH_4^+ , etc. which are present in the soil substrate, noting that they have different molecular weights.

It must also be noted that soil particles are negatively charged and the number of these charges may depend on the texture of the soil, i.e. sand, silt, clay, or humus (organic matter), pH and pKa. These factors will control the CEC values. These negatively charged soil surface particles tend to repel negatively charged nutrients, leaving them in the soil solution where they are susceptible to leaching (Manjula, 2017; Singh & Schutte, 2015).

The soil from the Highveld region of South Africa is said to have textures of loamy sands, clay loams and clay (Haarhoff *et al.*, 2020). Each type of soil has different characteristics, including CEC values, with loamy sands having CEC ranging between 5 to 10 cmol kg^{-1} ; clay loams ranging from 15 to 30 cmol kg^{-1} and clay at more than 30 cmol kg^{-1} (Sonon, Kissel & Saha 2017). These figures are corroborated by the mean CEC values that were reported in a study by Adam *et al.* (2021), which were 6.73 cmol kg^{-1} for loamy sand, 38.71 cmol kg^{-1} for silty clay loam and 44.71 cmol kg^{-1} for clay. FA increases the alkalinity of soil due to its Ca content (Ayanda *et al.*, 2018; Basu *et al.*, 2009; Chandrakar *et al.*, 2018) and subsequently the soil pH due the alkalinity of Ca (Basu *et al.* (2008), Srivastava *et al.* (2016), Panda and Biswal (2018)).

Soil Acidity

Another chemical property of soil that influences its CEC and thus fertility is the pH, which is a measure of its acidity or its alkalinity on a scale of 1 to 14 and the soil pH is influenced by the amount of H proton (H^+) activity in the soil solution (Tanzi *et al.*, 2018). The pH range that is ideal for most crop plant growth ranges between 5.5 and 8. Acidic solutions which are below 5.5 are likely to have low CEC, whereas CEC will increase with higher pH values (Ketterings *et al.*, 2007). Low pH also creates a favourable environment for the increased availability of Aluminium oxides, which outcompetes P, Mn and Fe absorption. Neina (2019), states that soil pH affects biological, chemical and physical properties of soils and in turn plant growth and yield.

According to Tandzi *et al.* (2018), plant growth, in particular the yield of maize can be reduced by up to 69 % due to low pH or acidic soils. Courtman *et al.* (2012) reported that most regions of Gauteng and Mpumalanga have pH ranging from 5.6 to 6.4; with some parts of Eastern Mpumalanga having pH of 5 and lower possibly due to acid rain and sulphur deposition. Most of the Western and Northern Cape are alkaline with pH of 7.5 and higher (Courtman *et al.*, 2012)

2.3 Plant Nutrient Requirement

Plants generally absorb a lot of primary nutrients, and they are usually the first to be depleted in the soil solution, whereas secondary and micronutrient concentrations deplete slowly. Plants absorb nutrients from the soil as ions, acids and/or chelates through the interaction between the roots of a plant and the soil solution (Marschner, 2012). The chemical characteristics of these mineral elements determine how they are absorbed by the roots (Marschner, 2012):

- a) Group 1: C, H, O, N, and S are absorbed from the soil solution as ions or gases from the atmosphere as CO_2 , HCO_3^- , H_2O , O_2 , NO_3^- , NH_4^+ , N_2 , SO_4^{2-} , SO_2 etc.; Nutrients in this group have different roles in plant growth (Marschner, 2012), however, they are all the main parts of plant organic material, in the form of amino acids, proteins, enzymes and nucleic acids. They are absorbed mainly through reduction oxidation (redox) as the major nutrients that ensures plant growth.
- b) Group 2: They are absorbed by plants as inorganic anions, with P absorbed as a phosphate, B as boric acid or borate and Si as silicic acid, which are formed through esterification with alcohol groups. Their function is to promote mobility through energy transfer;
- c) Group 3: K, Sodium (Na), Ca, Mg, Mn and Cl are absorbed as ions from the soil solution and they have differing functions, which are essential for plant cell development; and
- d) Group 4: Fe, Cu, Zn and Mo as ions or organic chelates, which plant roots absorb from the soil solution to promote the transportation of electrons through valency (Marschner, 2012).

Other factors, which affect maize plant growth and yield are the plant density, measured in plant population per hectare (ha^{-1}) and the spacing of the planting rows in maize fields (Haarhoff *et al.*, 2020 and du Plessis, 2003). Plant density affects the growth and yield due to the demand for nutrients and water by each plant, hence fields with low plant density might have low risk to the plant growth compared to those with high plant density (Haarhoff *et al.*, 2020 and du Plessis, 2003). These effects also differ from region to region because of different climates, as well as whether the plants are rain-fed or irrigation-fed, mainly from river streams or dams (Ibrahim and Ibrahim, 2020). Kima *et al.* (2020) and Nangia *et al.* (2018) (as cited in Ibrahim and Ibrahim, 2020) point out that rain-fed agriculture is most widely used in crops, such as maize, particularly in rural areas. The disadvantage with rain-fed agriculture is that it results in low productivity due to erratic rainfall during the growth period, often having to be supplemented with irrigation (Ibrahim and Ibrahim, 2020).

The growth of maize plants is dependent on a number factors, some of which were introduced above. Plant growth can also be limited by the capability of plants to assimilate them, under certain environmental conditions, while some toxic elements, can be absorbed by plants even

though they are not necessary for plant growth (Marschner, 2012). Maize plants, amongst other higher growing plants, are selective in the absorption of ions and the concentration of nutrients tends to decrease when the plants are grown in confined spaces (Marschner, 2012), like plastic planting bags, as it is in this study.

According to Marschner (2012), selective absorption may occur such that K, P and nitrate concentration in the soil solution decreases at a higher rate, while the concentration of Na and sulphates increases due to the plants absorbing more water. Petral-Videa *et al.* (as cited in Arif *et al.*, 2016), emphasise that the concentration gradient of HMs such as Cu, Fe, Mn and Zn influences their absorption, by plants, including selectivity.

According to Oberholzer, 2020, optimal production of any crop including maize depends on sufficient water and macro nutrients such as N, P and K as well as micronutrients such as Mg, S, Ca, Zn, and Mn. N enhances photosynthesis, growth, reproduction and maintenance of genetic material, whereas P does the same including respiration, cell division, growth of roots, flowering and plant maturity. In addition, K strengthens the stalk, increases resilience in drought and cold, and contributes to the production of proteins, sugar, starch and oils. Table 1 below illustrates the approximate concentrations of nutrients that a maize plant requires to grow fully and their variation indicates the level of their essentiality (Marschner, 2012):

Table 1: Average concentrations of mineral elements in plant shoot dry matter sufficient for adequate growth (source: Marschner 2012)

Element	Symbol	$\mu\text{mol g}^{-1}$ dry mass	mg kg^{-1}
Molybdenum	Mo	0.001	0.1
Nickel	Ni	0.001	0.1
Copper	Cu	0.1	6
Zinc	Zn	0.3	20
Manganese	Mn	1.0	50
Iron	Fe	2.0	100
Boron	B	2.0	20
Chlorine	Cl	3.0	100
Sulphur	S	30	1 000
Phosphorus	P	60	2 000
Magnesium	Mg	80	2 000
Calcium	Ca	125	5 000
Potassium	K	250	10 000
Nitrogen	N	1 000	15 000

These nutrients are also essential for seed germination, plant emergence and subsequent growth to full maturity, but there are other factors that play a role, such as moisture, temperature, oxygen, sowing depth, plant population (O'Keeffe, 2009).

The sowing depth of seeds (ideally, 4 to 5 cm), the timing of the planting of maize seeds needs to be considered carefully because low soil temperatures and high moisture content can affect germination (Djaman *et al.*, 2022; Song, Jin & He. 2019 and O'Keeffe, 2009). O'Keeffe, (2009), further stated that plant growth is retarded at temperatures below 10 °C and stabilises at temperatures above 30 °C, however, the plant growth rate increases with increments in temperature from 10 °C to 30 °C.

The rate of germination and emergence of plants from the soil also requires sufficient water moisture and oxygen, whereas soils saturated with water or waterlogged soils or very dry soils can impede germination and subsequently plant emergence (O'Keeffe, 2009). Song, Jin and He (2019) observed similar results, and further determined that insufficient watering after planting seeds, lowers other plant photosynthesis traits, resulting in irreversible damage such as, reduced leaf area index (LAI), chlorophyll content, reproduction and biomass at later growth stages. The soil water moisture makes the plant seed softer, which enables the seed to absorb the much needed oxygen from the soil solution and at this stage the nutrients within the maize kernel/seed endosperm are sufficient to sustain the plant for the first couple of days before emergence; and thereafter, once developed, the root system takes over the function (O'Keeffe, 2009 and Zhao *et al.*, 2011).

The number of maize roots, length, root dry matter, ability to absorb nutrients, nutrient concentration and water mass flow towards the roots are some of the factors that influence the absorption of these nutrient by the plant roots (Marschner, 2012). Other aspects that affect maize growth are the cultivation techniques and the weather. Djaman *et al.* (2022) and O'Keeffe (2009), cite plant density, planting patterns, tillage, water management, soil types, soil hydrological and chemical properties and management practices as the main contributing factors that influence maize growth and the yield of its grain.

According to Amos and Walters (as cited in Hauck *et al.*, 2015), various studies stated that the root to shoot ratio (RS) differs at the different maize phenological stages. The RS values of 2:3 (67 %), 1:3 (33 %) and 1:5 (20 %) were suggested for emergence stage (day 6), flowering (day 60 plus) and physiological maturity (days 118 to 125), respectively. Magaia *et al.* (2016)

2.4 Plant Growth

Figure 1 below illustrates the growth stage of maize plants and it was used as a guide for observing the growth of maize plants, during the experiment, from the plant emergence stage, with the collection of measurable data of the stem length and number of leaves commencing on day 24 after planting. It depicts the growth stages and even though it is specific for field grown maize, it also covers, in principle, the maize grown in pots in this experiment. It is important to note that these development and growth of maize is divided into two stages, namely “vegetative” stage, denoted by the letter “V”, with the successive number representing the number of leaves at a point in time; and the reproductive stage, “R” (Zhao *et al.*, 2011 and O’Keeffe, 2009). In brief the stages are as follows (Zhao *et al.*, 2011 and O’Keeffe, 2009):

Vegetative Stages

- a) VE – Emergence approximately six days after planting;
- b) V2 – Two leaves fully emerged at about 14 days after planting, plant height at 10 cm;
- c) V5 – Five leaves, tassel and ear initiation, with plant height of about 30 cm after 26 days of planting;
- d) V8 – Eight leaves, with plant height of 60 cm after 36 days of planting;
- e) V12 to V16: Active growth and cob development, between 51 and 66 days after planting, and there is more water or moisture content required - there is a critical need for water between V14 and R3.

Reproductive Stages

- a) R1 – Development of silk and commencement of pollination at approximately 76 days after planting, with plant height at about 270 cm and critical water requirements;
- b) R2 to R3 – Kernel development at about 76 to 90 days after planting, with critical water requirements
- c) R3 to R4 – Grain filling stage occurs at 97 to 110 days after planting
- d) R5 to R6 – Plants reached physiological maturity and the end of biomass gain at about 118 to 125 days after planting and are ready for harvesting after 160 days.

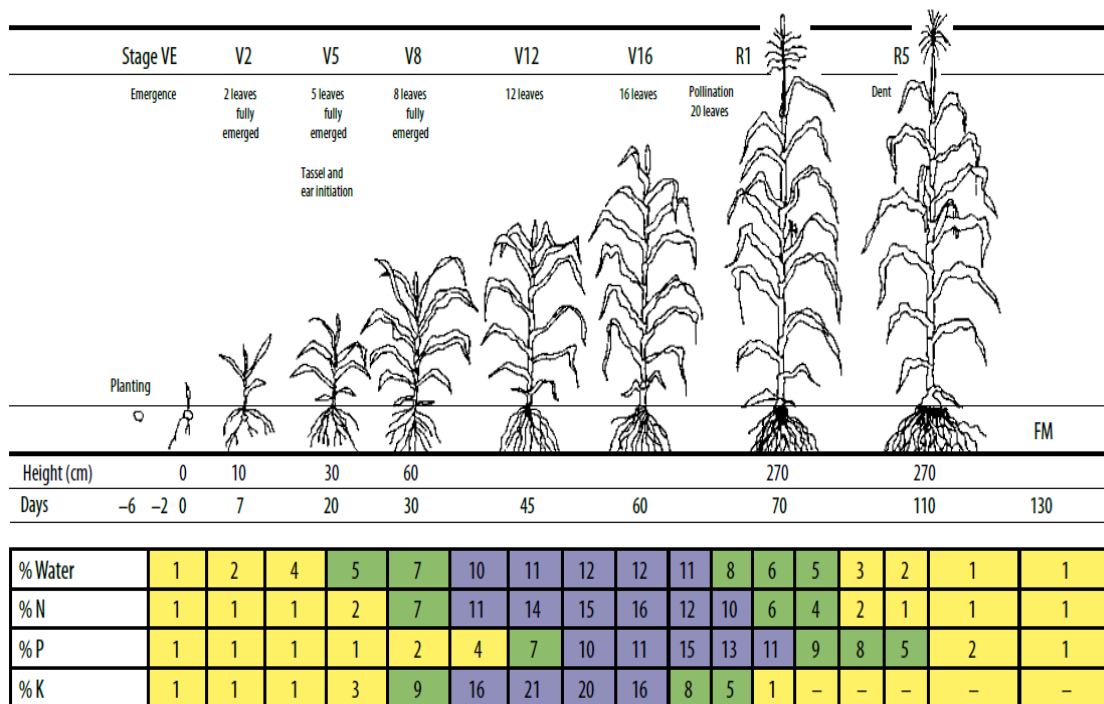


Figure 1: Growth Stages, Water and Nutrient Requirements per Stage (source: O'Keeffe 2009)

Figure 2 represents the weekly NPK requirements for growth of maize plants from germination to full growth after approximately 18 weeks (126 days) from germination. This closely corresponds to Figure 1, where the nutrient requirement increases when the plants develop more leaves and peak at around day 50 where the plants have more than 12 leaves (vegetative stage V12).

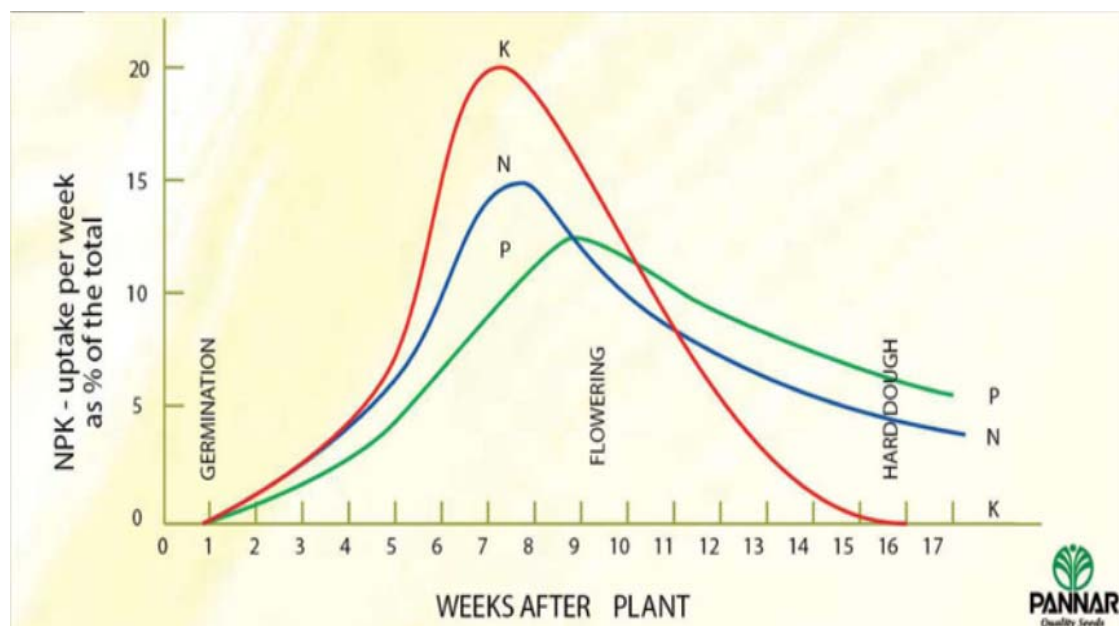


Figure 2: Number of Growth Weeks after Seed Germination (source: Pannar Seeds)

2.5 Maize Plants' Water Requirements

The importance of the availability of sufficient water for plant development and growth was briefly introduced in the preceding section. In this section the focus will be on some specific water requirements for maize production in the field trying to relate their similarities to maize growth under greenhouse conditions. This is with the understanding that greenhouse conditions are limiting, in terms of the available space within a planting pot, when compared with the recommended plant density in the field.

Agricultural production of maize globally, South Africa included, is conducted with two different methods of water supply, namely rain-fed and irrigation-fed maize production (Ibrahim and Ibrahim, 2020). The effectiveness of each method differs from region to region and also by season per region and the rainfall patterns per region (Masereka *et al.*, 2019). Annual rainfall, which also differs by region and season also have an effect on maize production, in particular the planting date since this determines whether the maize seeds germinates and establishment and ultimate yield (Hendricks *et al.*, 2016). All these factors, including the quality of the soil and drought play a role in the choice of the preferred production method, which will provide good maize development and growth; good yield and quality (Masereka *et al.*, 2019 and Hendricks *et al.*, 2016).

The average rainfall for South Africa, which is considered to be semi-arid, is 450-495 mm annum⁻¹, compared to the global average of 860 mm annum⁻¹ (Annandale *et al.*, 2011, Wettstein *et al.* (2017)). The wetter inland regions of South Africa, which covers the Eastern Free State, Gauteng and the Highveld region of the Mpumalanga Province (MP), which under normal circumstance experience annual rainfall between 600 to 700 mm have maize plant densities that range from 25 000 to 50 000 plants ha⁻¹ (2.5 to 5 plants m⁻²) (Haarhoff *et al.*, 2020). Whereas, the humid region of KwaZulu-Natal Province (KZN) has plant densities ranging from 50 000 to 70 000 plants ha⁻¹ (5 to 7 plants m⁻²), with annual rainfall ranging between 700 to 900 mm (Haarhoff *et al.*, 2020). Adisa *et al.* (2019), reported annual rainfall ranging from 500 mm to 900 mm for KZN, whose summer temperatures range between 22 °C and 33 °C, and 16 °C to 25 °C in winter; whereas MP has average annual temperature of about 19 °C and rainfall ranging from 500 mm to 800 mm.

The combination of climate conditions, water scarcity, due to lack of rain, access to natural water courses and/or drought and soil quality determines where and when to plant maize (Mugiyo *et al.*, 2021). Masereka *et al.* (2019) and Adisa *et al.* (2019), also established that drought, minimal and inconsistent rainfall are some of the factors that decreases the growth, development and

yield of maize plants in arid and semi-arid areas. The water requirement for the growth of rain-fed maize plants, in dry land farming, is often affected by the amount of rainfall and the rain patterns within a growth period, and this differs from region to region, hence the timing of planting needs to be considered (Masereka, *et al.*, 2019 and Adisa *et al.*, 2019).

In their study titled, “*Frequency analysis of agricultural drought of maize in Sabie River catchment in South Africa*”, Masereka *et al.* (2019), established that maize production required a minimum average rainfall of about 628 mm when planted during the growth period of December to February, compared to 457 mm for the October to December growth period. This minimum water requirement has to take into account of evapotranspiration (ET) from the maize plant and soil through solar radiation and wind; water runoff, which depends on soil type and landscape slope and deep percolation, which is the movement of water away from the root zone into the deeper layers of the soil (Masereka, *et al.*, 2019). Djaman *et al.* (2018) also concluded, from their study in South Western USA, that water management and planning are essential in agriculture, because climatic conditions, particularly water availability, influences the ET of maize plants, and ultimately maize plant development and growth. Irrigated maize production is necessary for water supply in areas with low rainfall and drought (Hendricks *et al.*, 2016).

Zhao and Nan (2007), estimated that the ET for maize in an arid region of NW China to be 1.09 mm day⁻¹ at initial growth stage; 3.67 mm day⁻¹ at development stage and peaking at 5.49 mm day⁻¹ before declining to 3.33 mm day⁻¹ at the last growth stage. This translated to a total ET of 611.5 mm, which translates to the total amount of water depth required for the entire growth to maturity period. This total ET of 611.5 mm less the rainfall equates to the amount of irrigation required for field maize plants. In the study conducted by Jarman *et al.* (2014) in Douglas, in the Northern Cape Province, the average mid-day temperature was 18.4 °C between January and May and annual rainfall estimated at 339 mm. The mean ET for maize was found to range from 4.79 mm day⁻¹ to 5.13 mm day⁻¹ and flowering was reported to occur after about 75 to 80 days of planting.

Maize plants which grew in smaller confined 20 litre cylindrical planting bags, which contain 10kg of soil substrate, cannot necessarily grow to the same size compared to field grown maize plants over the same period of time. Maize planted in these confined pots in a greenhouse, have limitations for the growth of the root structure. This was not expected to have had a major impact on the growth of the plants because the plants were harvested when it appeared that they were becoming pot bound, as explained further in sections 3.4 and 3.5.

2.6 FA and its Properties

The disposal of FA occupies huge portions of land, in most cases adjacent to agricultural land, which is not environmentally friendly because of its leachability, radioactivity and toxicity (Wang *et al.*, 2020). Most of Eskom's coal power stations are surrounded and/or located near agricultural farms, some of which cultivate maize (*Zea mays*) (Reynolds-Clausen & Singh, 2017), and it is a common diet in some communities in South Africa (Hendricks *et al.*, 2016).

Coal, which consists mainly of C and H as well as various groups of minerals, is the most C intensive fossil fuel that is used worldwide as a source of energy (Hailu *et al.*, 2019). Coal is used, in the generation of electricity in power stations, in a combustion process, which produces a silica and alumina rich FA as a by-product (Hailu *et al.*, 2019). According to Basu *et al.* (2008), power-stations produce tons of class 'F' FA per year, with high costs of disposal, including water, electricity and land". In South Africa, Eskom produces close to 34 million tons of FA and this amount of FA "is about 30 % of Eskom's annual coal consumption, with about 7 % of it is sold to the cement industry, while about 74 % is used by the power-stations for waste water treatment and the remainder is stockpiled from continuous daily production" (Reynolds-Clausen & Singh, 2017). Figure 3 illustrates the ash volumes at a number of power stations in South Africa.

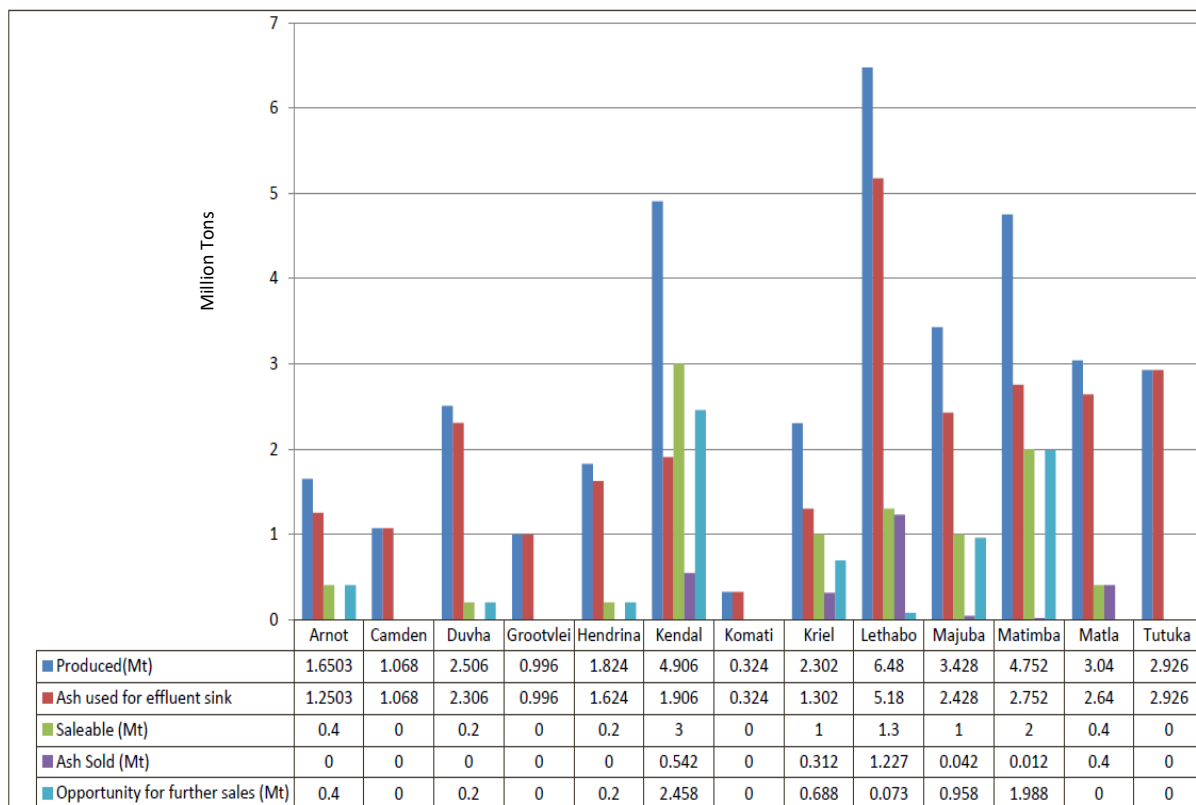


Figure 3: Estimated Ash Volumes per Power Station (Reynolds-Clausen & Singh, 2017)

The major components of FA, are oxides of Silicon (Si), Ca, Al, Mg, Fe, Na, and K (Heyns *et al.*, 2016). It also contains traces of metals and metalloids such as Antimony (Sb), As, B, Cadmium (Cd), Cobalt (Co), Cr, Cu, Hg, Lead (Pb), and Zn (Ayanda *et al.*, 2012; Reynolds-Clausine & Singh, 2017; Heyns *et al.*, 2016).

The United States Environmental Protection Agency ('EPA') considers FA as a non-hazardous by-product (Heyns *et al.*, 2016). It is also part of the Organisation for Economic Cooperation and Development's ('OECD') "Green List" (Basu, *et al.*, 2009), which is meant for waste materials that are not restricted for commercial purposes because they are considered safe for human and environmental health.

In South Africa, FA is classified as a hazardous waste, in terms of the National Environmental Management: Waste Amendment Act (Act No. 26 of 2014) ('NEMWAA'). This is because of its HM contents, which are measured in terms of leachable concentrations ('LC') and total concentrations ('TC') (Reynolds-Clausen & Singh, 2017). Its classification as hazardous waste, limits demand due to costs and long processes involved in obtaining licences for handling hazardous waste from the Department of Forestry, Fisheries and the Environment ('DFFE') (Reynolds-Claudine & Singh, 2017; Heyns *et al.*, 2016).

FA is said to be capable of ameliorating the soil (Yao *et al.* 2015 and Basu *et al.*, 2008) and it also has the ability to enhance plant nutrients in the soil, which assist in enhancing crop growth and yield in less fertile acidic soils (Basu *et al.*, 2008). On the contrary, it has been as established that FA is also capable of decreasing the soil CEC, due to its particle structure (Nalbantoglu and Gucbilmez, 2001; and Nalbantoglu, 2004, as cited in Akbulut and Arasan 2010). Adari and Prasad (2017) and Rajpoot, Kumar and Kumar, (2018), also established that there was a negative correlation between FA and CEC. This was also corroborated by Tulabandula and Jayanth, 2021.

Further, Hailu *et al.* (2019), states that the speciation of elements determines the form in which they exist, e.g. whether they are reducible, oxidizable and residual, water-soluble, exchangeable, acid-soluble. In their research, Hailu *et al.* (2019) concluded that Al, Fe, Zn and Pb, in FA, are highly prone to leaching in acidic solutions, whereas there is increased leaching of Si at both extremes of the pH scale. A contrary finding was that elements like Al, Fe and Zn displayed low leaching capabilities when pH values range between 4 and 7, and stronger leaching capabilities under both high acidic and alkaline conditions (Kania *et al.*, 2019). Leaching, in FA, was observed to decrease at higher liquid-to-solid ratios because of particle

aggregation, whereas it increased with increased contact time and temperature increases from 20 °C to 40 °C (Hailu *et al.*, 2020).

2.7 Potential impacts of FA and its HM content on plant growth

FA is promoted as an eco-friendly and economic agent for that can be used in the agricultural sector to fertilise the soil, and thus improve plant development and growth (Pandey and Singh, 2010; Srivastava *et al.*, 2016). It has been used in several studies using several plants, including maize, to establish its effects on plant growth and yield. This section focuses on several studies, which were conducted to establish the effects of FA on plant development and growth, in general. FA, mainly contains Si, Al and Fe, including essential plant macro-nutrients such as Ca, Mg, Na, K and P, as well as micro-nutrients such as Mn, Zn, Cu, Mo, B, S and Co, but lacks organic C and N (Pandey and Singh, 2010; Parab *et al.*, 2012; Mupambwa *et al.*, 2015; Panda *et al.*, 2015, etc.). Arif *et al.* (2016, p 2) points out that “a supply of small amounts of HMs such as Co, Cu, Cr, Fe, Mg, Mn, Mo, Ni, Se and Zn is important for plant development and growth”

Plant development and growth is dependent on various factors such as the soil quality or fertility, which includes its mineral/nutrient content, soil pH, soil moisture content, soil texture, soil porosity, CEC, climatic conditions etc. It is important, for the purpose of this study, to understand how the treatment of soil substrate with varying amounts of FA affects the development of maize plants. Coupled with this is the understanding that different methods of treating soils with FA might yield different results, yet showing improvement in plant growth up to certain thresholds of FA treatments. For example, this study is based on once-off mixing of the soil with FA, on a mass basis, in planting pots located in a greenhouse.

Enhanced plant development and growth was observed in a greenhouse study conducted by Panda *et al.* (2015) in soils with 0 %, 20 %, 40 %, 60 %, 80 % and 100 % FA treatments. The plants were harvested at days 15, 30 and 45 after planting and analysed for the length of the shoot, roots, fresh mass, dry mass and photosynthetic pigments. Maximum germination was observed in plants that grew in 40% FA treatment, which decreased at FA concentrations beyond that treatment of 60 % yielded optimum results and this positive FA effect on plant growth was reversed, when FA treatments exceeded 80 %. The study revealed a gradual decrease in root length, shoot length, fresh and dry mass of the plant biomass, with higher concentrations of FA in the soil substrate. The confinement of the root system in planting pots, was suspected to be the reason for the reduction of root length over time; and high pH impeded the availability of essential nutrients such as P (Panda *et al.*, 2015).

In another study, Rajakumar and Patil (2019), planted maize seeds in three parts of a field. One part of the field was not treated with FA, while the other two were treated with of 30 tons ha⁻¹ and 40 tons ha⁻¹ of FA, respectively. The germination percentage, plant height, cob length, the mass of the plant biomass, seed yield and stover yield were measured and analysed at the end of each harvest. The germination of seeds was not affected in all the FA treatments. It is further reported that the average stem length in the untreated soil was the lowest at 150 cm, compared to 180 cm for the plants that grew in the field with 30 tons ha⁻¹ FA and 182 cm in 40 tons ha⁻¹ soils. Similarly, there was an increase in the mass of the plant biomass, with increasing FA applications.

According to a study conducted by Murugan and Vijayarangam (2013), using several fruit and vegetable bearing plants, which were planted in a field, indicated an increase in yield when soils were treated with FA, with wheat; mustard, rice and maize having a yield ranging from 6 % to 18 %. A further observation was that plant development was negatively affected, when the soil substrate, in which they were planted, was treated with the amount of FA that exceeded certain thresholds, which differ from plant to plant (Arif *et al.*, 2016).

The aforementioned study is corroborated by Srivastava *et al.* (2016); Niu *et al.* (2021); Parab *et al.* (2012), who stated that the application of FA improved plant growth and fresh mass of edible part materials, and that growth was negatively affected when FA application in soils exceeded certain thresholds depending on the plant species, the type of coal, the country and the soil texture. Mishra and Shukla (as cited in Niu *et al.*, 2021), established that the treatment of soils with FA from deposition under field conditions, increased plant height, metabolism, dry mass of roots, stems, leaves, chlorophylls and carotenoids.

As stated earlier, the soil contains nutrients, in the form of chemical elements, which are essential for plant development and growth and some of these are present in the chemical composition of FA. There are macro-nutrients; micro-nutrients; and some toxic trace HMs in FA, which are not bio-gradable, but rather accumulate and are bio-translocated in different parts of plants (Silva *et al.*, 2021). Marschner (2012), stated that plants characteristically absorb nutrients and non-essential elements selectively from the soil. A limited supply of micro-nutrients, such as those in FA, is essential for maize plant development and growth, whereas their deficiency or toxicity can result in negative results (Adhikary *et al.*, 2010).

These traits could explain the development and growth of the plants due to the dose-response to FA soil treatments with FA. It is important to understand how some of these elements and/or HMs affect plant development and growth. The effects of some of the HMs are described below.

Barium

Barium (Ba) is an alkaline earth trace metal, which is found in igneous, sedimentary rocks and naturally as low soluble minerals such as barite (BaSO_4) and witherite (BaCO_3) (Nogueira *et al.*, 2010; Abreu *et al.*, 2012). According to Nogueira *et al.* (2010), natural soils have concentrations of Ba ranging from 100 to 3000 mg kg^{-1} , with a mean of approximately 425 mg kg^{-1} in the crust of the Earth.

There are no current studies or evidence that Ba affects plant development and growth, however, evidence is there that higher concentrations of Ba in food can be toxic for human and animal consumption (Nogueira *et al.*, 2010; Abreu *et al.*, 2012).

Chromium (Cr)

Increased accumulation and soil enrichment with non-essential HMs such as Cr results in toxicity in plants, which negatively affects total chlorophyll content, photosynthesis, transpiration rate, inter-cellular CO_2 , stomatal conductance, water use efficiency and water use efficiency (Anjum *et al.*, 2016).

It also negatively affects seed germination, the plant height, number of leaves; leaf area; photosynthesis; chlorophyll biosynthesis; stem height and diameter; fresh and dry biomass; plant respiration (CO_2 fixation); enzymatic reactions; energy provision through photophosphorylation; electron transportation; (Anjum *et al.*, 2016). The severity of Cr effects on plants depends on several factors such as Cr concentration, environmental conditions, soil and plant species.

Manganese (Mn)

Manganese is one of the 17 essential micronutrients (Alejandro *et al.*, 2020), and has to be available in small quantities for efficient plant development and growth, and it acts as a catalyst in plant metabolism for antioxidant enzymes (Nozulaidi *et al.*, 2016; Alejandro *et al.*, 2020).

Excessive concentrations of Mn in plant tissue may negatively affect physiological processes such as absorption, translocation, enzymatic activities and oxidation (Nozulaidi *et al.*, 2016). Heavy metals such as Mn, in very small amounts, play an important role in plant development and growth by promoting photosynthesis and the provision of energy for such; photolysis of water molecules and it provides protection for plants against airborne and fungal diseases (Nozulaidi *et al.*, 2016).

A deficiency of Mn also makes plants susceptible to soil-borne diseases as well as fungal leaf

diseases, because Mn plays an essential role in the biosynthesis of lignin, which assists in the resistance to pathogenic infections (Nozulaidi *et al.*, 2016). Other affected processes of plant growth are the production of fatty acids, water and heat stress in leaves; thus decreased plant water retention due to transpiration, as well as water use efficiency. Soils with high pH and partial pressure of O₂, also result the reduced bioavailability of Mn, and subsequently plant nutritional disorders.

Molybdenum

Molybdenum (Mo) is one of few essential microelements that are present in agricultural soils in average ranges between 1 to 2 mg kg⁻¹, and is required for plant development and growth (Bodi *et al.*, 2015). The availability of Mo in soils for plant absorption is influenced by the pH of the soil (Bodi *et al.*, 2015; Adhikary *et al.*, 2010), and the application of lime in acidic soils increases its absorption. Bodi *et al.* (2015) further states that other factors which effect the Mo availability in soils are the concentrations of iron oxides, water content and organic compounds in the soil colloids.

The presence of Mo in plants has been confirmed not to cause any harmful effects on the development and growth of maize. Mo is involved in essential N₂ metabolism processes, such as N₂ fixation and nitrate reduction (Bodi *et al.*, 2015; Rana *et al.*, 2020). Apart from N metabolism processes, Mo is an essential catalyst in reactions of several enzymes, such as phytohormones (Rana *et al.*, 2020). Modi *et al.* (2015), also states that insufficient availability of Mo results in reduced photosynthesis, low sugar and vitamin C content, while it physically manifests itself through rolled up plant leaf edges; narrow edges in young leaves, as well as spots; while older leaves may experience chlorosis, necrosis and stunted growth.

2.8 Phytoextraction

The purpose of this study is also to determine whether maize plants can absorb HMs from contaminated soil, without compromising the plant's development and growth, and subsequently human and animal health. These HMs are not chemically degradable or biodegradable, therefore they accumulate in soils; leach into underground water systems (Ali *et al.*, 2013); and accumulate in plant biomass after absorption from the soil during their growth. There are no cost effective methods of removing these HMs, some of which, such as Fe, Mn, Cu, Zn and Ni, are essential for biochemical and physiological roles in living organisms, but in small amounts; while other HMs, such as Cd, Pb, As Hg and Cr, are non-essential (Ali *et al.*, 2013).

The presence of HMs in soils, above acceptable concentrations, causes harm to both the

environment and human health, unless removed from the soil by any cost effective means such as phytoremediation (Suman *et al.*, 2018; Mahmood, 2010 and Wuana and Okieimen, 2010). Ali *et al.* (2013), suggests phytoremediation as a cost effective and green method of cleaning removing HMs from soils, rather than costly conventional methods, such as on site vitrification, soil excavation and landfill, soil incineration, soil washing, flushing etc. There are different types of phytoremediation processes, namely phytoimmobilization, phytostabilization, phytoextraction, etc., with the latter being promoted as the more economically viable option (Mahmood, 2010; Wuana and Okieimen, 2010; and Ali *et al.*, 2013).

Ali *et al.* (2013); Wuana and Okieimen (2010), concluded in their study on the phytoremedial potential of maize, that maize plants can tolerate HM and have the ability to continuously phytoextract heavy metals from contaminated soils, through their roots and translocate them to the shoots, or they can accumulate around the root systems.

Further, scientific research has highlighted the benefits of using plants and associated microorganisms to remedy compromised soil, thus sustaining ecological systems, their services and human needs (Furini *et al.*, 2015). Furini *et al.* (2015) and Wuana and Okieimen (2010), stated that maize have the potential to ameliorate the soil through phytoextraction.

2.9 The use of FA as a potential fertiliser

Continuous cultivation of land depletes the concentration of nutrients in the soil substrate, due to absorption of these nutrients by plants, which ultimately renders the soil infertile if they are not replenished. Soil infertility is also caused by the deficiency of nutrients, due to leaching or low concentrations of N, P, K, S, Ca, Mg, Na, Mo, Zn and B (Tandzi *et al.*, 2018). Fertilisers are used to enhance the quality of degraded soils in the agricultural sector, through the enrichment of agricultural soil with current commercial chemical fertilisers such as N, P and K enhancers and organic fertilisers such as farm yard manure, cow dung, sewage sludge etc.

In this section we explore several studies that were conducted to establish whether FA can be an overarching fertiliser in the agricultural sector, and if not, what were the recommendations of such studies. This is keeping in mind that FA lacks sufficient content of some of the essential nutrients such as N and P. The composition of its chemical elements and HMs depends on the type of coal used and the combustion process that is utilised by some of the electricity generation power stations.

Pandey and Singh (2010); and Srivastava *et al.* (2016), suggest that the utilisation of FA in agricultural farming is eco-friendly and economical, compared to reliance on commercial

fertilisers. Cheaper and readily available FA has the ability to reduce the mobility of HMs; provide plant macro-nutrients, such as K, P and Ca; as well as micro-nutrients namely, B, Cu, Fe, Mo, Zn etc, while simultaneously reducing the formation of soil crust (Srivastava *et al.*, 2016). Basu *et al.* (2008), further stated that the development, growth and yield of many plants, including maize, is enhanced due to the abundance of Ca, Fe, K, Mg, Na and Zn in FA.

Experimental work was performed in which improvement of soil texture, porosity, pH values, lower bulk density, hydraulic conductivity and higher water retention capacity were observed in soils treated with FA, as a result of the presence of silt sized particulates (Basu *et al.*, 2008, Srivastava *et al.*, 2016, and Panda and Biswal, 2018). Kishor *et al.* (2010), confirmed this in their findings and states that despite a few harmful impacts, FA improves the soil pH, its buffering capacity, increases the essential soil nutrients and can reduce the usage of fertilisers and lime in agriculture.

The usage of lime (Calcium Hydroxide – $\text{Ca}(\text{OH})_2$) in agricultural farming is viewed as a contributor to global warming because of its content, which is subsequently released into the atmosphere as Carbon Dioxide (CO_2) (Basu *et al.*, 2008). FA is cheaper compared to lime; it has a high content of Calcium Oxide (CaO) and other essential plant nutrients, FA is therefore recommended as a replacement to lime in order to reduce CO_2 emissions.

The shortfall with FA is that it cannot completely replace chemical fertilizers and organic manures, due to its lack of some essential elements such as organic N and C (Basu *et al.*, 2008). FA has the ability to increase the soil pH, due to its high CaO content, therefore its increased application in soils would at some point result in high pH 8 to 12, salinity and phytotoxic elements, which will result in reduced bioavailability of some essential nutrients and subsequently impede development of plants (Srivastava *et al.*, 2016).

Mupambwa *et al.* (2015) states that the role of FA, which lacks C and N, as a fertiliser in South Africa can be profitable if its nutrient bioavailability is enhanced and its toxic HM content is reduced. The study by Mupambwa *et al.* (2015) proposes the production of FA based composts, using earthworms, some special microbials and bio-inoculants. The aim being to allow the different agents to augment each other, when applied in certain proportions, with organic compost contributing C and N; FA supplying additional mineral elements; and earthworms and microbes that tolerate toxic HMs acting as catalysts in the process (Mupambwa *et al.*, 2015). In their study, Adhikary *et al.* (2010), also observed good maize plant development, growth and maize grain yield in acidic soils, when NPK fertilisers were applied in combination with the additional supply of micronutrients, such as B, Zn, S, Mn and Mo, in specific ratios.

Several studies propose the utilisation of FA as a fertiliser because it enhances plant development and growth, however, its general lack of C and N as well as its trace HM content is still concerning which can be overcome by applying it together with other fertilisers, in specific proportions, in order to achieve better plant development and growth.

This literature review has covered the key ecosystem services that are essential for the growth and development of maize plants, amongst others, soil quality and its attributes, nutrients and water requirements. The review also covered the understanding of the different phenological stages of the maize plant, including the amount of required essential elements at each stage, in order to realise full healthy plant maturity and yield. The essence of this was to understand how the amendment of the soil with FA would impact these growth and development processes, hence the review of some of the similar studies that were conducted was also covered in this literature review. This further included the effects of some HMs; and whether FA can be used as a soil ameliorant or fertiliser, which informed the purpose of this research experiment and its methodology.

3. RESEARCH DESIGN AND METHODOLOGY

3.1 Study Area

The study area is situated close to the Kusile Power Station (Kusile) in Balmoral, a small farming town within the Emalahleni Local Municipality, which is located in the grassland Highveld region, west of the Drakensburg escarpment, in the Mpumalanga Province (MP Government, 2022). The Highveld region is a summer rainfall region. The rainfall often occurs as afternoon thunderstorms mostly between October and February, whereas the winter season between May and August is often dry and cold (MP Government, 2022). Balmoral is located along the N14 freeway, approximately 78 km east of the capital city, Pretoria, Gauteng Province and it has a latitude and longitude of 25°51'41" South 28°58'42" East, with an elevation of 1456 meters above sea level (Google Earth).

The economic activity in Balmoral and surrounding areas such as Emalahleni is dominated by mining, construction, agriculture and transportation businesses. The choice of the study area, which forms part of an area zoned for mining and agriculture in terms of the Nkangala Industrial Development Plan of 2003, was made on the basis of the presence of agriculture as well as Kusile and its fly ash (FA) dumpsite. According to the Kusile environmental impact assessment report (Ninham, 2007), farmers in the area have maize yields of about 5 tonnes per hectare under rain fed conditions and up to 10 tonnes per hectare when irrigating from the adjacent Wilge River.



Figure 4: Kusile Power Station, Farms Bluekombos (soil) and Noortgerdard (maize seeds)

3.2 Experimental Design

The experiment was conducted in the roof-top greenhouse located in the Oppenheimer Life Sciences Building (OLS), East Campus, University of the Witwatersrand. In order to determine the effects of FA on the growth of maize plants, 45 times 20 litre planting bags, each containing 10kg of soil substrate were prepared. This was done by separating the 45 planting bags into five different FA treatments, each with nine planting bags per FA treatment, as indicated in Table 2 below. The first nine planting bags had 0 % FA treatment, whereas the next, nine each, had 20 % FA, 40 % FA, 55 % FA and 60 % FA added to them on a dry mass basis.

Table 2: Planting Bags Set-up per Harvest

	1 st Harvest			2 nd Harvest			3 rd Harvest		
0%	H1-0-1	H1-0-2	H1-0-3	H2-0-1	H2-0-2	H2-0-3	H3-0-1	H3-0-2	H3-0-3
20%	H1-20-1	H1-20-2	H1-20-3	H2-20-1	H2-20-2	H2-20-3	H3-20-1	H3-20-2	H3-20-3
40%	H1-40-1	H1-40-2	H1-40-3	H2-40-1	H2-40-2	H2-40-3	H3-40-1	H3-40-2	H3-40-3
55%	H1-55-1	H1-55-2	H1-55-3	H2-55-1	H2-55-2	H2-55-3	H3-55-1	H3-55-2	H3-55-3
60%	H1-60-1	H1-60-2	H1-60-3	H2-60-1	H2-60-2	H2-60-3	H3-60-1	H3-60-2	H3-60-3

This was to allow the gathering of enough information, including root length and biomass, by harvesting the maize plants at three different periods, during their growth period, i.e. 60, 77 and 122 days after planting. During each harvest period, 15 bags, which consisted of five different FA treatments with three replicates were selected.

3.3 Soil and Fly Ash (FA) Collection

Fly Ash Samples

For easy handling, twelve 50kg bags of dry FA were collected, from the section of the FA dumpsite at the Kusile Power Station, which is used to store dry FA, as opposed to the adjacent wet FA slurry dam, with the assistance of Eskom employees.

A complete analysis of the physicochemical properties of the FA collected from Kusile Power Station ('Kusile') were requested from Eskom, however, Eskom provided only a waste assessment report, dated 09 November 2017, which was conducted by Aquatico Scientific (Pty) Ltd. The analysis in this report mainly focussed on HMs, and it was conducted according to the provisions of the National Environmental Management: Waste Act, 2008 (Act No. 59 of 2008) ('NEM:WA'), the GG36784, the GNR635 & the 636 National Norms & Standards for Assessment and Disposal of Waste to Landfill (2013).

According to Auquatico (2017), FA samples from Kusile's were analysed according to the Australian Standard Leaching Procedures (AS 4439.1 – 3, 1997-99) as stipulated in the regulations. The analysis included the recording total concentrations ('TC') of HMs; and organic analysis of the solids. Aquatico (2017), states that in terms of GNR 635, FA was thus classed as type 3 (low risk), due to the TC, mg/kg, of HMs being lower than the allowable guideline limits (see Table 3), therefore, it can be safely discarded in Class C type landfills.

Table 3:Waste Assessment - Kusile FA

Variables	Guidelines Limits (mg kg ⁻¹)			Actual TC (mg kg ⁻¹)
	TCT0	TCT1	TCT2	
Antimony, Sb	10	75	300	<10.0
Arsenic, As	5.8	500	2000	<5.80
Barium, Ba	62.5	6250	25000	351
Boron, B	150	15000	60000	<150
Cadmium, Cd	7.5	260	1040	<7.50
Chromium, Cr	46000	800000	-	<1000
Cobalt, Co	50	5000	20000	<50.0
Copper, Cu	16	19500	78000	<16.0
Hexavalent chromium (Cr ⁶⁺)	6.5	500	2000	<0.500
Lead, Pb	20	1900	7600	<20.0
Manganese, Mn	1000	25000	100000	<1000
Mercury, Hg	0.93	160	640	<0.900
Molybdenum, Mo	40	1000	4000	24.5
Nickel, Ni	91	10600	42400	<50.0
Selenium, Se	10	50	200	<10.0
Vanadium, V	150	2680	10720	<100
Zinc, Zn	240	160000	640000	<220
Fluoride, F	100	10000	40000	<4.00
Total Cyanide, CN	14	10500	42000	<0.100
Moisture %	-	-	-	25
Solid %	-	-	-	75
Organic Outsource	-	-	-	-

Soil Samples

The top soil used in this experiment was collected from approximately 20 to 30 cm deep from the agricultural field in the nearby Bluekombos Farm (GPS Coordinates: 25°57'1.43" South, 28°48'57.41" East), which is located along the route 960 (R960) approximately 8,5km South West of Kusile. A sample of the soil was sent for analysis at the Agricultural Research Council (ARC) and the results are discussed in later in results section.

3.4 Preparation of the Treatments and Planting

A dose-response experiment was conducted between October 2020 and March 2021, in which dried soil was mixed with FA in increments from 0%; 20 %; 40 %; 55 % and 60 % FA treatments, on a dry mass basis. The seeds, which were used for planting in this study, were the Starke Ayres Zama maize seed variant (509279), which were obtained from Farm Noortgerdard (GPS Coordinates: 25°58'5.99" South, 28°50'25.95" East) South West of Kusile. To ensure successful germination and establishment, a minimum of two seeds were planted in each of the planting bags.

These planting bags were placed in the greenhouse located at the rooftop of the Oppenheimer Life Sciences (OLS) building at Wits, which according to its climate logs, had the minimum (mostly between 5 to 6 am), maximum (afternoon) and mean temperatures and relative humidity (RH) contained in Tables 4 and 5.

Table 4: Greenhouse Temperature Data (source: Greenhouse Climate Logs)

Month	Minimum Temperature, °C	Maximum Temperature, °C	Mean Temperature, °C
October 2020	11.4	46.3	20.71
November 2020	14.9	35.30	21.34
December 2020	14.9	57.8	22.85
January 2021	15	34.9	21.53

Table 5: Greenhouse RH Data (source: Greenhouse Climate Logs)

Month	Minimum RH	Maximum RH	Average RH
October 2020	78.90	97.10	61.63
November 2020	27.70	97.50	71.54
December 2020	27.10	95.80	70.75
January 2021	15	95.90	73.84

Measurements were obtained both non-destructively and destructively. The individual plant height and number of leaves were measured non-destructively at 24, 38, 60, 77 and 122 days after planting and the destructive measurements, such as the individual plant root length and mass of the plant biomass, which were taken at each harvest on days 60, 77 and 122. This study was conducted for approximately 18 weeks, where it was terminated because the plants had become root bound within the planting pots and also to prevent the plants from falling over due to inadequate support by the bound roots.

3.5 Harvesting and sample analyses

Additional measurements were also done during each harvest for root length and dry mass per plant:

- a) the stem length and number of leaves were recorded;
- b) the individual plants were uprooted in each planting bag;
- c) the roots, were gently hand-rinsed in clean water before measuring their longest root as a measure of root length;
- d) all plant biomass was placed in individual paper bags, labelled and oven dried at 60°C for 48 hours, before recording their individual total mass, which is the total of the above ground (stem and leaves) mass and below ground (roots) mass.
- e) Fifteen maize plant samples, which were harvested from the five different FA treatments on day 122, were taken to ARC for laboratory analysis. These consisted of three complete maize plants, with roots, per FA treatment; and
- f) 100g of soil substrate was collected from each pot; placed in a labelled paper bag and oven-dried at 60°C for about 48hours. Five soil samples, i.e. one per FA treatment from the planting bags that were harvested on day 122, were send to the ARC laboratory for physicochemical analysis, including metal content.

The laboratory methods used by the ARC were:

Plant Samples (ARC Procedures)

- a) The individual samples were first milled to less than 0.5 mm;
- b) The concentrations of HMs: Cr, Mn, Mo and Ba in the above ground and below ground biomass was determined using the Agilent Technologies' 7700 Series Inductively Coupled Plasma Mass Spectrometry (ICP-MS). The first step is to digest 0.5 g of the sample with 9 ml of 65 % concentrated Nitric Acid (HNO_3) and 2 ml of 30 % Hydrogen Peroxide (H_2O_4) at 180 °C in a microwave and brought to volume in a 50 ml flask. Aliquots of the digested samples are then diluted 10 times with an ARC Indium standard before the ICP-MS analysis;
- c) The concentrations of Ca, Mg and P in all plant samples was determined using the Aligent Technologies' 700 Series ICP- Optical emission spectrometry (ICP-OES). In this case the 0.5 g of the sample is initially digested with 7 ml of HNO_3 and 3 ml of Perchloric Acid (HClO_4) at 180 °C in a microwave and brought to volume in a 100 ml flask, before analysis.
- d) The total N and C concentrations in plants were determined using the Thermo Scientific's Flash 2000 CHNS-O analyser, which requires 8 to 12 mg of each sample in a tin foil. The process involves Dumas method for dry oxidation, in which the samples are ignited at 950

°C to produce CO₂, N₂ and NO_x.

Soil Samples

- a) The ICP-MS analysis was utilised to determine the concentrations of HMs, in particular, Ba, Cr, Mn and Mo, using EPA 3052- microwave assisted acid digestion. Prior to the ICP-MS analysis, 0.5 g of the soil sample is digested by mixing it with 9 ml of concentrated HNO₃ and 3 ml of concentrated Hydrofluoric acid (HF), before being heated in 180 °C in a microwave;
- b) An Ammonium Acetate solution (1 mol dm⁻³) digestion and distillation was used to determine the CEC and extractable cations Ca, Mg, Na and K, in all soil samples;
- c) Dry Combustion for P, Total N and C; and
- d) The acidity/alkalinity of the soil samples was determined using the pH (H₂O) method.

3.6 Data Analyses

Data collected, including the lab analyses, were subjected to the single factor ANOVA in order to determine if there were significant difference between the variables. This was used together with the statistical descriptive analysis and Tukey post-hoc test (Honest Significant Difference and Q-Test).

4. RESULTS AND INTERPRETATION

The data analyses in this section were subdivided into two sub-sections, in order to test the hypothesis that treating agricultural soil with different fly ash (FA) amounts (mass basis 0 %, 20 %, 40 %, 55 % and 60 %; FA) had a positive impact on the development and growth of maize plants (*Zea mays L.*) grown separately in these treatments. This was also to establish an optimum amount of FA treatment that would not negatively impact plant growth and development. The aim of the experiment is to also determine whether maize plants have phytoextraction abilities for heavy metals (HMs) contained in soils that were treated with FA, without absorbing and accumulating traces of toxic HMs at levels above the regulated total concentration threshold (TCT).

The first section of the results will focus on the physical measurements of the plants (stem length, number of leaves, root length and the mass of the biomass per plant) for each of the plants grown in the different FA treatments. The analyses were based on the data collected on days 24, 38, 60, 77 and 122 of the growth of the maize plants, which were subsequently harvested on days 60, 77 and 122. There were five treatments of 0 %, 20 %, 40 %, 55 % and 60 % FA, each with three replicates and two plants per replicate. The data were statistically analysed to determine the impacts of the treatments at any point in time and across time.

The second section will present the laboratory analyses of the physicochemical properties of the original soil that was collected from the farm, which was never treated with FA, and used for planting in this experiment. These laboratory analyses were also conducted on the soil samples from each treatment, as well as the respective plant biomass samples, which were collected after harvesting at day 122 (approx. 4 months). The experiment was terminated at day 122, because the roots were pot bound and sufficient data had been collected for analyses. The analyses determined the amount of essential nutrients and HMs that were absorbed by the individual plants in different FA treatments and the concentrations remaining in the soil after harvesting at day 122.

Laboratory analysis were conducted on the soils for cation exchange capacity (CEC), pH, Carbon (C), Nitrogen (N), Phosphorus (P); HMs: Barium (Ba), Chromium (Cr), Manganese (Mn), and Molybdenum (Mo) for the five treatments (0 % FA, 20 % FA, 40 % FA, 55 % FA and 60 % FA) after 122 days of plants growing in them. Further analyses were done on the roots, stem and leaves, for elements: C, N, P, Calcium (Ca) and Magnesium (Mg); and HMs: Ba, Cr, Mn, and Mo.

4.1 The analysis of the physical data

This section focuses on the ANOVA and the Q test analysis of the physical data collected from plants across all the treatments (0 %, 20 %, 40 %, 55 % and 60 % FA treatments), on days 24, 38, 60, 77 and 122 after planting the seeds. The first set of data, were the stem lengths and number of leaves during all the days of data collection, and analysed for within each time period across FA treatments and within a treatment across time. The analysis of the root length and mass of the plant biomass was only limited to the data, which could only be collected on days 60, 77 and 122 harvests.

4.1.1 Effects of FA on stem length

Results and Interpretation: Stem length within time across treatments

The statistical analyses of the stem length data, using ANOVA and the Q test indicated that there were significant differences ($p < 0.05$) between some of the stem length data collected across all treatments. In some instance the Q test analysis indicated there were no significant differences ($p > 0.05$) when comparing stem length data from some of the treatments against each other, which makes up about 46 % of all the comparisons from days 24 to 122. The details are discussed further per period in this section.

Figure 5 below, illustrates the growth of the plants based on the means of the stem lengths that were measured on days 24, 38, 60, 77 and 122 of growth, across all treatments.

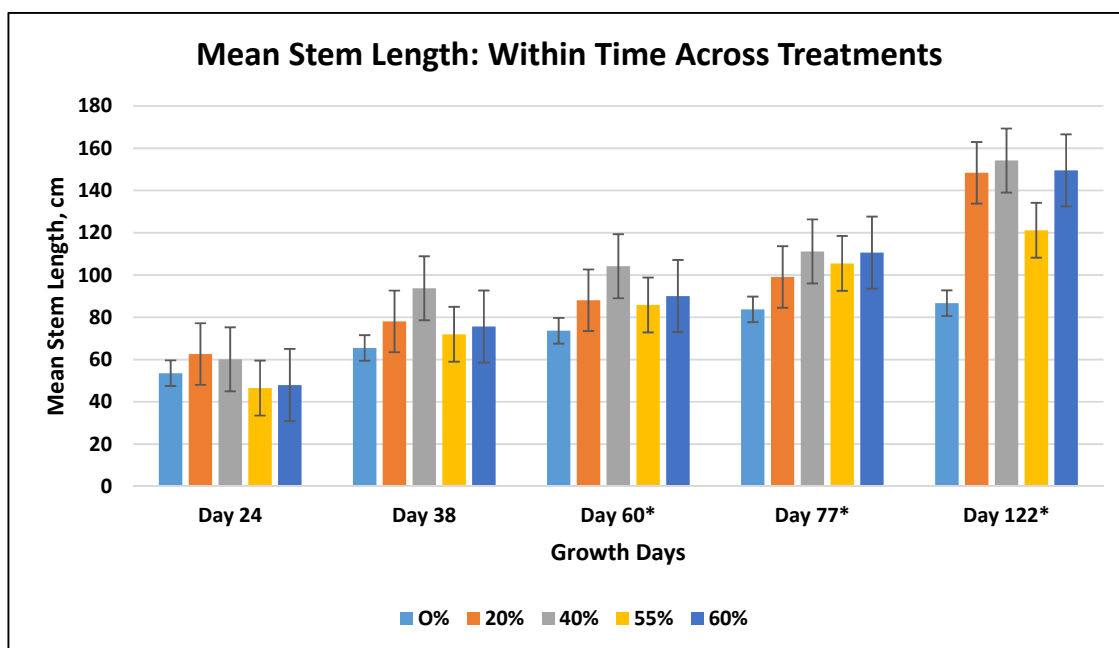


Figure 5: Mean Stem Length within Time Across FA Treatments (NB: The asterisk on Days 60, 77 and 122 indicates that some plants were harvested)

Figure 6 below indicates a decline in the mean stem lengths for the data collected on day 24, with the lengths of the plants that grew in the 20 % and 40 % FA treatments slightly above the trendline, while those from the 0 % and 55 % FA treatments being below the trendline. The mean stem lengths of the plants from the days 38, 60 and 77 also had similar trends with plants from 0 %, 55 % and 60 % being slightly below the trendline. The mean stem lengths from the data collected on day 122 was mostly off the trendline except for 60 % FA treatment; with 20 % and 40 % FA treatment plants above the trendline; while those from the 0 % and 55 % FA treatments were below.

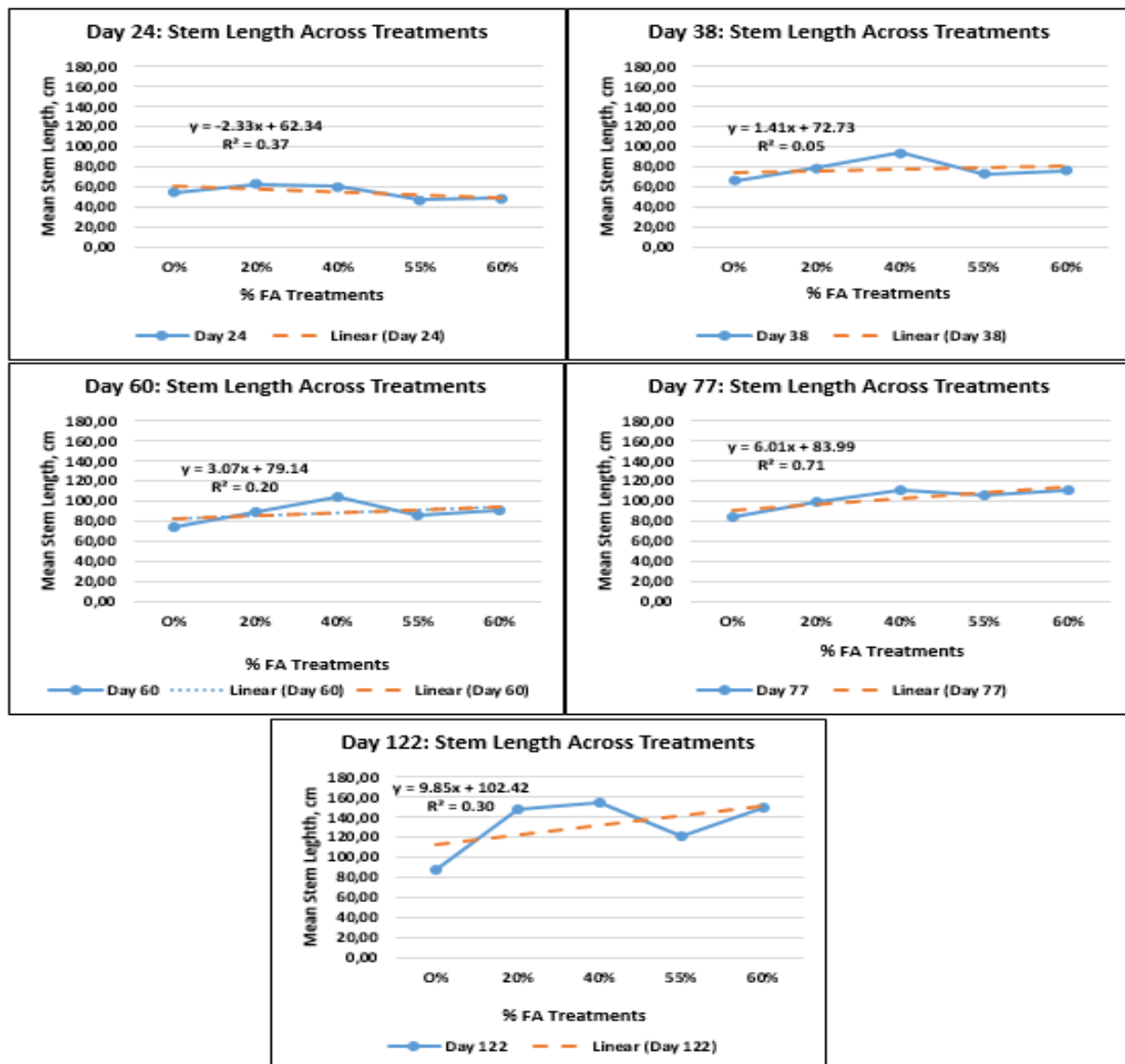


Figure 6: Trendline Analysis - Mean Stem Length Within Time Across Treatments

a) Day 24 Stem Length Data Analysis

The ANOVA indicated that there was a significant difference ($p < 0.05$ - the F-value of 9.37 and P value of 9.03×10^{-05}) between the day 24 stem length data, across all FA treatments. The maize plants that were grown in 20 % FA had the highest mean stem length of 62.61 ± 4.94 cm, followed by 60.11 ± 4.39 cm for 40% FA treatment. These are closer to the estimated stem length of 60 cm at day 30, as reported by O'Keeffe 2009 (see Figure 1 in literature review section). The plants in the remaining treatments had mean stem lengths of 46.50 ± 3.90 , 47.94 ± 7.25 and 53.56 ± 7.22 cm, for plants in 55 %, 60 % and 0 % FA treatments, respectively.

The Q-test analysis reveals that on day 24, there was a significant difference ($p < 0.05$) between the average stem lengths of plants grown in the 0 % FA treatment and those grown in 20 % FA treatment. There was a significant difference ($p < 0.05$) between plants grown in 20 % FA and those grown in 55 % and 60 % FA; and those grown in 40 % FA compared to 55 % and 60 %, which amounts to 50% of the comparisons between FA treatments. The Q test also indicated that there was no significant difference ($p > 0.05$) between the stem lengths of the plants grown in the 0 % FA treatment compared to those grown in 40 %, 55 % and 60 % FA treatment. There was no significant difference ($p > 0.05$) between plants grown in 20 % FA compared to 40 % FA; as well as between 55 % FA and 60 % FA. There is little effect of the FA at early developmental stages.

b) Day 38 Stem Length Data Analysis

The ANOVA indicated that there is a significant difference ($p < 0.05$ - F-value was 16.14, with a P value of 1.18×10^{-06}) between the stem lengths of the maize plants that were growing in the different FA treatments. At day 38, the plants grown in 40 % FA treatment had a higher mean stem length of 93.72 ± 7.24 cm, and 78.06 ± 4.26 cm for plants grown in 20 %. The stem lengths of the plants grown in 0 % FA were shorter at 65.50 ± 6.57 cm, followed by 71.94 ± 5.49 and 75.61 ± 7.80 cm for plants that were grown in 55 % and 60 % FA treatment, respectively. The estimated plant height, at this stage in field conditions, is about 72 cm, when extrapolating using the figures provided by O'Keeffe (2009).

The Q-Test that was performed on the stem length data collected on day 38, reveals the significant difference ($p < 0.05$) between stem length of the plants grown in the 0 % FA and those grown in 20 %, 40 %, and 60 %; 20 % vs 40 %; 40 % vs 55 %; and 40 % vs 60 %, which also amounts to 50 % of the comparisons. The Q test indicated that there is no significant difference between plants grown in the 0 % FA treatment and those in the 55 % FA treatment; 20 % vs 55

% and 60 %; and 55 % vs 60 %.

c) Day 60 Stem Length Data Analysis

Similar to days 24 and 38, the ANOVA indicated a significant difference ($P < 0.05$: F-value of 24.38 and a P value of 2.58×10^{-08}), between groups, across all FA treatments at day 60. The longest mean stem length was 104.17 ± 4.39 cm for maize plant grown in 40 % FA treatment, followed by the plants grown in 60 % FA treatment with a mean stem length of 90.06 ± 5.89 cm. The plants in 0 % FA had the shortest stem mean stem length of 73.61 ± 6.77 cm, followed by 85.83 ± 5.18 cm and 88.06 ± 5.06 cm, for 55 % and 20 % FA treatments, respectively.

The Q-Test results for day 60 were similar to those yielded by day 38 data, except for the 0 % and 55 % treatments, in which there was no significant difference (P value = 0.43, $p > 0.05$) between day 38 stem length data, whereas there was a significant difference (P value = 0.0052, $p < 0.05$) for similar at day 60. In general, 60 % of the comparisons between groups indicated that there was a significant difference ($p < 0.05$) between them.

d) Day 77 Stem Length Data Analysis

The ANOVA indicated that there was a significant difference ($p < 0.05$: F-factor of 17.40 and an calculated of P value of 6.09×10^{-07}) between the stem length at day 77, across all FA treatments. The mean stem length was also at longer at 111.17 ± 4.76 cm for plants grown in 40 % FA treatment, followed by 110.58 ± 9.23 and 105.50 ± 3.78 cm for plants in 60 % and 55 % FA treatments, respectively. The shortest mean stem length was 83.75 ± 8.42 cm for the plants grown in 0 % FA treatment, followed by 99.08 ± 5.22 cm for 20 % FA treatment.

The Q test reveals the significant difference ($p < 0.05$) between the stem length of plants grown in the 0 % FA treatment and that of the plants grown in 20 %; 40 %; 55 %; and 60 % FA treatments. There was a significant difference ($p < 0.05$) between the stem lengths from 20 % FA treatment and both 40 % and 60 % treatments. There was no significant difference ($p > 0.05$) between 20 % vs 55 %; 40 % vs 55 % and 60 %; and 55 % vs 60 % FA treatments. In summary, there was significant difference ($p < 0.05$) for 60 % of the comparisons between groups, while 40 % were not.

e) Day 122 Stem Length Data Analysis

The ANOVA indicated that there was a significant difference ($p < 0.05$: F-value of 13.85 and the p-value of $4.37E-06$) between the stem length at day 122, across all FA treatments. Similarly, the maize plants grown in 40 % FA treatment had the longest mean stem length of $154.17 \pm$

18.10 cm followed by 149.50 ± 24.20 cm and 148.33 ± 13.87 cm for maize plants grown in 60 % and 20 % FA treatments, respectively. The shorter were 86.67 ± 20.55 and 121.17 ± 14.99 cm for plants in 0 % and 55 % FA treatments, respectively. These lengths are also shorter than the expected 270 cm stipulated in the literature, as referred in the preceding paragraphs.

The Q test for the stem lengths at day 122 generally yielded similar results to those at day 77. This was with the exception of 20 % vs 40 %, which was not significantly different (P value = 0.98, $p > 0.05$) for day 77, but significantly different (P value = 0.03, $p < 0.05$) at day 122; 20 % vs 60 % with P values of 1 and 0.044 for days 77 and 122, respectively; and 40 % vs 55 % with P values of 0.04 and 0.58 for days 77 and 122 respectively. The Q test for the day 122 analysis across treatments also yielded results indicating that 50 % of the comparisons were significantly different ($p < 0.05$).

In conclusion, the ANOVA and the Q-Test at a particular time, across treatments, generally indicate a significant difference between the treatments; with plants grown in soils treated with less and more than 40 % FA having shorter stems.

ANOVA Results and Interpretation: Stem length within treatments across time

The statistical analyses of the stem length data, using ANOVA and the Q test indicated that there were significant differences ($p < 0.05$) between some of the stem length data when analysed within treatments across time. The Q test analysis indicated there were no significant differences ($p > 0.05$) when comparing stem length data from some similar treatments against each other across time.

The trends in of the graphs in Figure 7 and 8 indicate an incremental growth within all FA treatments across time, which clearly indicates that prolonged exposure to FA treated soils does not negatively affect these maize plants. This indicates that, although, the maize plants in 0 % FA treatment grew at a steady linear rate, their growth was slower compared to the plants in other FA treatments. The mean stem lengths in 20 %, 40 % and 60 % were almost similar at day 122.

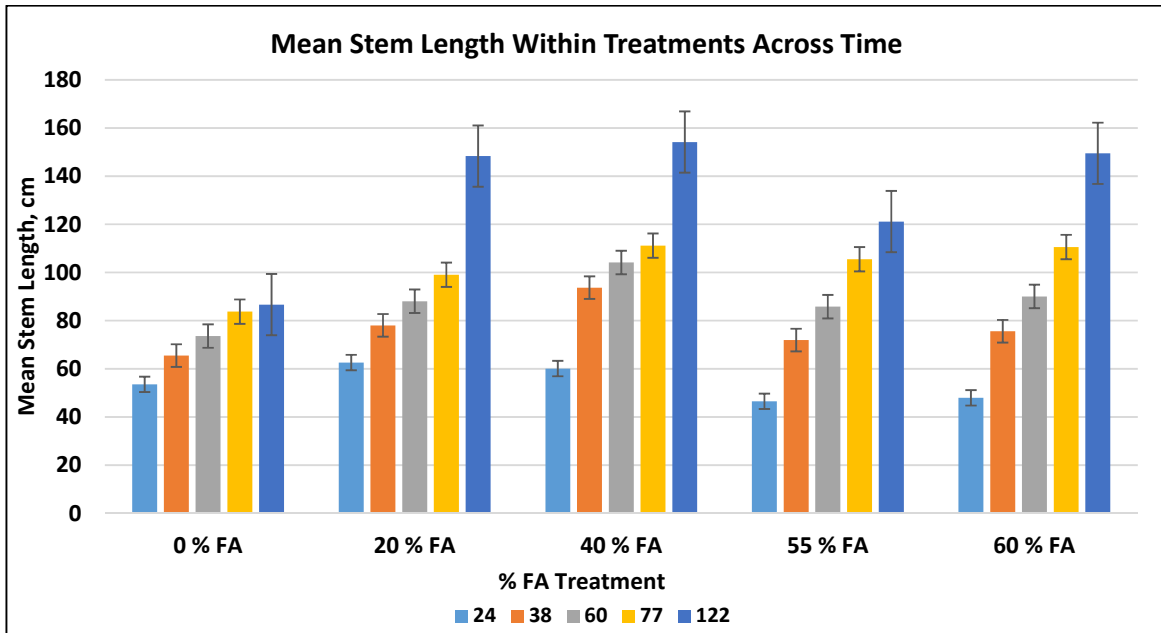


Figure 7: Mean Stem Length within FA Treatments Across Time

The gradients of the trendlines in Figure 8 clearly shows the difference in the growth rates of the maize plants that grew in the different FA treatments. The graph that represents the mean stem length data for the plants that were grown in 0 % FA treatment have a lower gradient of about 15.20 for growth from day 0 to day 122. This is a slower growth rate compared to the maize plants that grew in 20 %, 40 %, 55 % and 60 %, which has gradients of 24.60, 26.70, 22.76 and 27.14, respectively.

It is also observed that there are slight similarities between the growth patterns of the plants grown in 0 % and 55 % treatments. The same is the case for the 20 %, 40 % and 60 % FA treatments, which have almost similar mean stem lengths of 148.33 cm, 154.17 and 149.50 cm, respectively, at day 122. There was a sudden overshoot in the mean stem lengths between days 77 and 122 of 49.71 %, 38.68 % and 35.19 % for plants in 20 %, 40 % and 60 %, respectively, compared to 3.48 % and 14.85 % for 0 % and 55 % FA treatment plants.

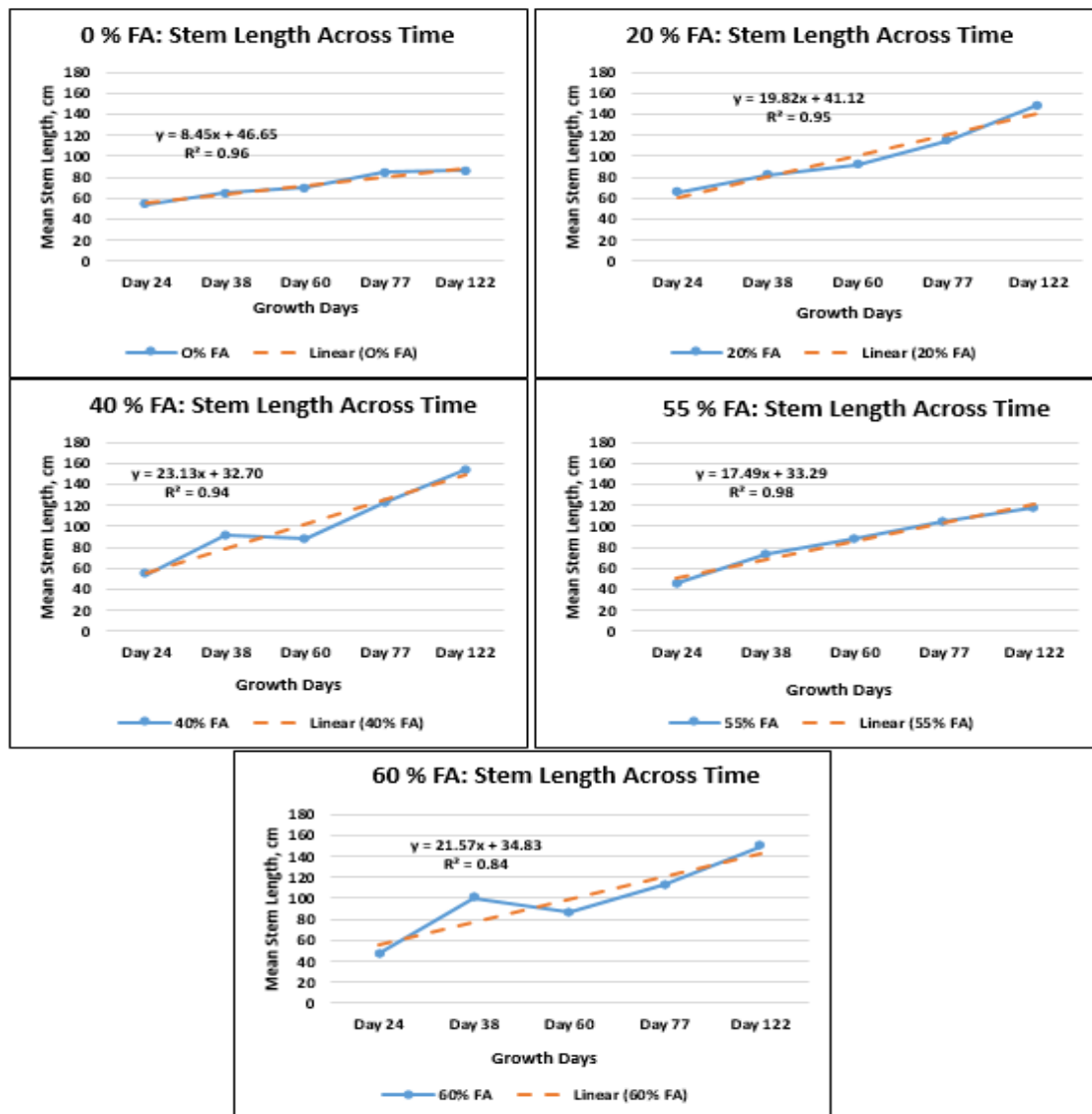


Figure 8: Trendline Analysis - Mean Stem Length within Treatments across Time

a) 0 % FA Treatment

The ANOVA indicated that there was a significant difference ($p < 0.05$: F-value of 8.71 and the p-value of 0.00015) between the stem lengths, across time, of the plants grown in 0 % FA treatment. There was a initial slow growth between days 24 and 38 with the mean stem lengths of 53.56 ± 7.22 cm increasing by 24.90 % to 65.50 ± 6.57 cm, and 73.61 ± 6.77 cm (46.51 %), 83.75 ± 8.42 cm (51.50 %) and 86.67 ± 20.55 cm (18.74 %), between days 38, 60, 77 and 122, respectively. Maize plants reaches transitions from vegetative ("V") stage to reproductive ("R") stage at about day 70 and their physiological growth slows down (O'Keeffe 2009). This could be the reason for the low 18.74 % growth between days 77 and 122, which resulted in a mean stem length of 86.67 ± 20.55 cm at day 122.

The Q test for the stem lengths for 0 % FA treatment indicated that there was a significant difference ($p < 0.05$) between the stem length for day 24 compared to days 60, 77 and 122; and day 38 compared to days 77 and 122. There was no significant difference ($p > 0.05$) at the early growth stages, i.e. between days 24 and 38; days 38 and 60; day 60 compared to days 77 and 122; and days 77 and 122. This amounts to 50 % of the comparisons indicating that there was a significant difference ($p < 0.05$) between the stem length data collected between days 24 and 122 for 0 % FA treatment.

b) 20 % FA Treatment

The ANOVA for the stem lengths of the plants grown in 20 % FA treatment, indicated that there was a significant difference ($p < 0.05$: F-value 110.65 and P value of 1.62×10^{-1}) across time, between the groups. The means stem length of 62.61 ± 4.94 cm, 78.06 ± 4.26 cm, 88.06 ± 5.06 cm, 99.08 ± 5.23 cm and 148.33 ± 13.87 cm for days 24, 38, 60, 77 and 122, respectively. These mean stem lengths represent increases of about 24.67 % between days 24 and 38; 12.81 % between days 38 and 60; 12.52 % from day 60 to day 77; and 49.71 % from day 77 to 122. The growth between days 77 and 122 for 20 % FA treatment across time is contrary to the same time period in the 0 % graph, which seems to suggest a decrease in the stem length.

The Q test also yielded results that indicates that there was a significant difference ($P < 0.05$) between all the days, except for the data for days 38 and 60, which had no significant difference ($P > 0.05$), with a P value of 0.18. The overall outcome of the Q test was that 90 % of the data in the 20 % FA treatment was significantly different ($p < 0.05$).

c) 40 % FA Treatment

The ANOVA for the stem lengths of the plants grown in 40 % FA treatment, indicated that there was a significant difference ($p < 0.05$) across time, with an F-value 78.12 and P value of 9.34×10^{-14} between the groups. The stem length increment pattern in this FA treatment displayed different results compared to those of plants in 0 % and 20 %, with the mean stem length sharply increasing by 55.91 % from 60.11 ± 4.40 cm at day 24 to 93.72 ± 7.24 cm at day 38. This was followed by a slow increase of 11.14 % in the mean stem length to 104.17 ± 4.39 cm at day 60; 6.72 % to 111.17 ± 4.76 cm at day 77; followed by an overshoot of 38.68 % to 154.17 ± 18.10 cm at day 122.

The Q test also indicated that there was a significant difference ($p < 0.05$) between 80 % the groups except day 60 compared to days 38 and 77, which were not significantly different ($p > 0.05$), with P values of 0.33 and 0.70, respectively, which makes up the remaining 20 %.

d) 55 % FA Treatment

The ANOVA for the stem lengths of the plants grown in 55 % FA treatment, indicated that there was a significant difference ($p < 0.05$) across time, with an F-value 81.36 and P value of 5.85×10^{-14} between the groups. The stem length increment pattern in this FA treatment displayed different results compared to those of plants in 0 % and 20 %, with the mean stem length sharply increasing by 54.72 % from 46.50 ± 3.90 cm at day 24 to 71.94 ± 5.49 cm at day 38.

This was followed by slower increases of 19.31.14 % in the mean stem length to 85.83 ± 5.18 cm at day 60; 22.91 % to 105.50 ± 3.78 cm at day 77; and 14.85 % to 121.17 ± 3.78 cm at day 122. This data indicates that growth slowed down towards day 122, which was a similar trend displayed by the plants that grew in 0 % FA treatment, mainly because at this stage of growth, maize plants enter a reproductive stage that is characterised by decreased physiological growth (O'Keeffe 2009). The Q test indicated that there was significant difference ($p < 0.05$) between all the groups.

e) 60 % FA Treatment

The ANOVA for the stem lengths of the plants grown in 60 % FA treatment, indicated that there was a significant difference ($p < 0.05$: F-value 53.74 and P value of 6.44×10^{-12}) across time, between the groups. There was 57.71 % spike in the mean stem length from 47.94 ± 7.25 cm at day 24 to 75.61 ± 7.80 cm at day 38; followed by a reduced growth of 19.10 % to 90.06 ± 5.39 cm at 60; 22.79 % to 110.58 ± 9.23 cm at day 77; and another sharp 35.19 % increase to 149.50 ± 24.20 cm at day 122. The Q test indicated that there was significant difference ($p < 0.05$) between all the groups, except for the mean stem lengths between days 30 and 60.

4.1.2 Effects of FA on the number of leaves

The purpose for this was also to determine whether the different treatments of FA has any impact on the number of leaves per plant over a growth period and whether the development of the leaves of these maize plants was affected by the different FA treatments.

Results and Interpretation: No. of leaves within time across treatments

The statistical analysis of the number of leaves, using ANOVA indicated that there was a significant difference ($p < 0.05$) within a time period across FA treatments and also within each treatment across time. The Q test though indicated that, in the analysis within a time across treatments, 27.5 % were significantly different ($p < 0.05$), whereas the remaining 72.5 % were not

significantly different ($p>0.05$).

Figure 9 below, illustrates the mean number of leaves developed per plant over the growth period spanning from day 24 to day 122. The maize plants are expected to have reached maturity at around day 122 (O’Keeffe, 2009).

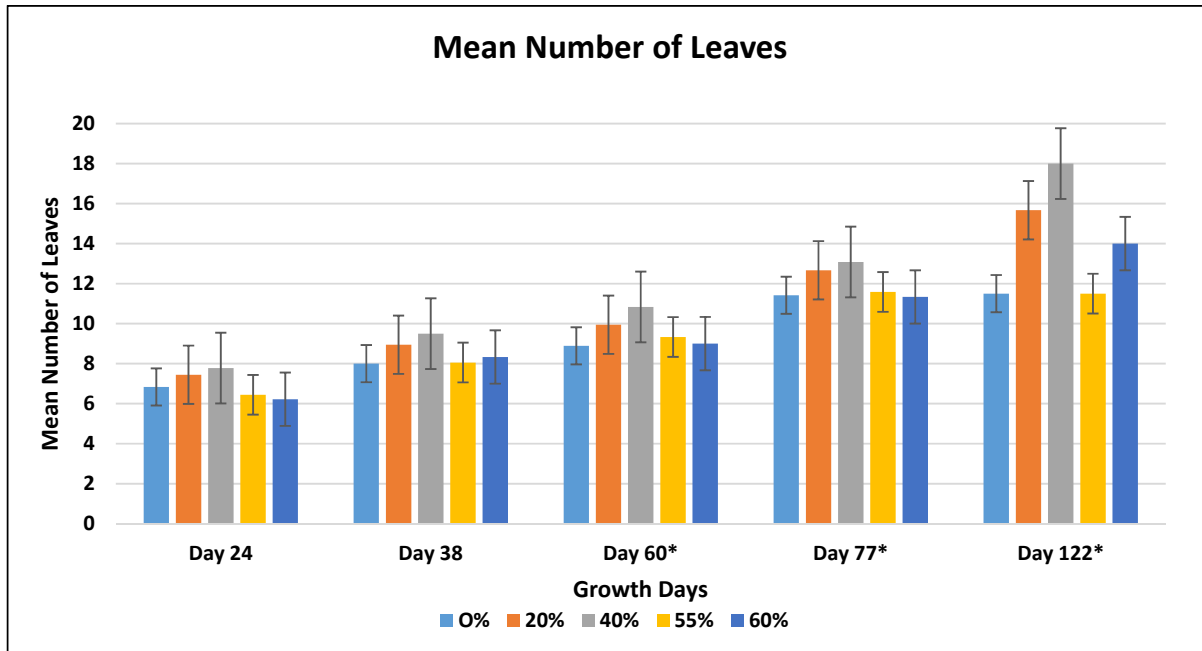


Figure 9: Mean Number of Leaves within Time across FA Treatments (NB: The asterisk on Days 60, 77 and 122 indicates some plants were harvested)

It is evident in Figure 9 and 10 that the number of leaves for each treatment varied with the number of days of growth, which is expected since maize plants develop more leaves as they grow over time. At day 30, maize plants are normally at vegetative stage V5 with about 5 leaves; with stage V8 at about 60 days of vegetative growth and they stop physiological growth with about 20 leaves at the beginning of the reproductive stage (R1) after 70 days, in normal field conditions (O’Keeffe 2009).

In this case, the number of leaves stay constant after day 70 for 0 %, 55 % FA treatments, whereas the number of leaves for the plants in 20 %, 40 % and 60 % FA treatments continue to increase beyond day 70. Another important observation is the gradual increase between 0 % and 40 % FA treatment, followed by a decline beyond 40 % for days 24, 38, 60 and 77; and a decline for 55 % and an increase for 60 % FA treatment at day 122.

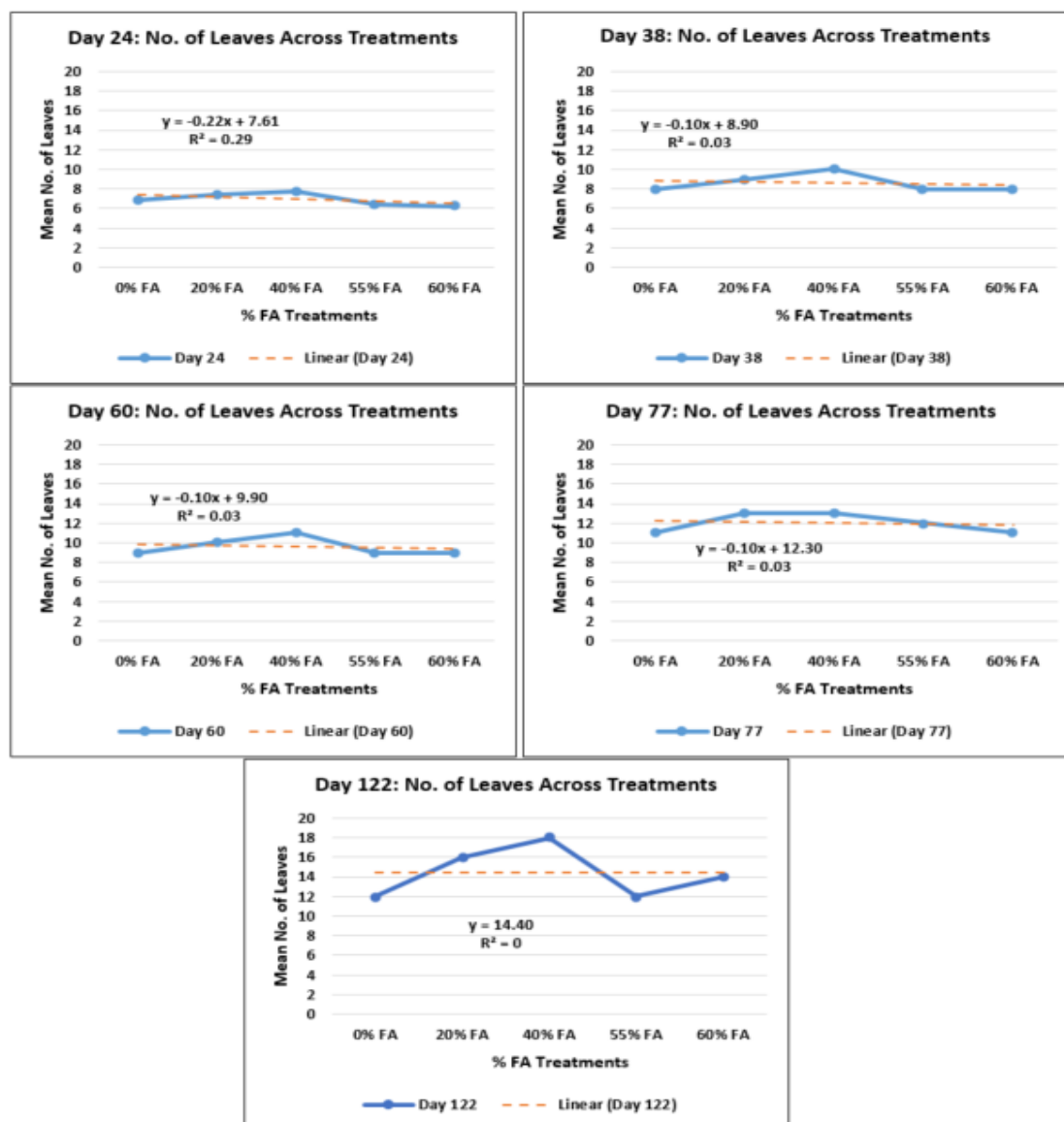


Figure 10: Trendline Analysis - Number of Leaves within a Time across Treatments

a) Day 24 No. of leaves across treatments

The ANOVA results for the mean no. of leaves, indicated that there was a significant difference ($p < 0.05$), with an F-value of 4.39 and P value of 0.00795, across all FA treatments on day 24. The data indicate that the 40 % FA treatments had a mean of 8 ± 1.13 mean no. of leaves. The plants that grew in the 0 % and 20 % FA treatments had 7 ± 0.62 and 7 ± 0.81 mean number of leaves, respectively; followed by 55 % and 60 % FA treatments by about 6 ± 0.40 and 6 ± 0.69 mean no. of leaves, respectively. At this stage the maize plants have normally passed the vegetative stage, V5 (five leaves), which typically commences at around day 20 after germination (O'Keeffe 2009).

The Q-test analysis reveals that on day 24, there was a significant difference ($p < 0.05$) for 20 % of the comparisons of the no. of leaves across FA treatments, which were between plants grown in 40 % FA treatment compared to 55 % and 60 % FA treatments. The remaining 80 % of the comparisons yielded results indicating that there was no significant difference ($p > 0.05$) between plants grown in 0 % and 20 % FA compared all FA treatments; as well as between 55 % FA and 60 % FA. This closely correlates with the stem length analysis within this period, which indicated that 60 % of the comparisons were not significantly different.

b) Day 38 No. of leaves across treatments

The ANOVA results for the mean no. of leaves, indicated that there was a significant difference ($p < 0.05$: F-value of 3.55 and P value of 0.02), across all FA treatments on day 38. The data indicates that the 40 % FA treatments had developed about 10 ± 1.41 mean no. of leaves, followed by 20 % FA treatment plants with 9 ± 0.53 mean no. of leaves. The 0 %, 55 % and 60 % treatments had about 8 ± 0.63 , 8 ± 0.61 and 8 ± 0.67 mean no. of leaves, respectively. At this stage the maize plants have passed the vegetative stage, V8 (eight leaves), which typically commences at around day 30 after germination, followed by V12 at around day 45 (see Figure 1 in literature review section) (O’Keeffe 2009). A linear extrapolation from the literature suggests that day 38, the maize plants would normally have developed about 11 leaves.

The Q-test analysis reveals that on day 38, there was a significant difference ($p < 0.05$) for 20 % of the comparisons of the no. of leaves across FA treatments. These were between plants grown in 40 % FA treatment compared to 0 % and 55 % FA treatments, with P values of 0.034 and 0.044, respectively. The remaining 80 % of the comparisons yielded results indicating that there was no significant difference ($p > 0.05$) between plants across the other treatments.

c) Day 60 No. of leaves across treatments

The ANOVA results for the mean no. of leaves, indicated that there was a significant difference ($p < 0.05$), with an F-value of 24.38 and P value of 2.6×10^{-08} , across all FA treatments on day 60. The data indicates that the 40 % FA treatments had developed about 11 ± 0.96 mean no. of leaves, followed by 20 % FA treatment plants with 10 ± 0.44 ; 0 %, 55 % and 60 % treatments had about 9 ± 0.40 , 9 ± 0.67 and 9 ± 0.42 , respectively. All of these mean no. of leaves at day 60, across the FA treatments are lower than those proposed in literature for the vegetative stage, V16 (16 leaves), which takes place during this time period after planting.

The Q-test analysis reveals that on day 60, there was a significant difference ($p < 0.05$) for 40 % of the comparisons of the no. of leaves across FA treatments. These were between plants grown

in 40 % FA treatment compared to 0 %, 55 % and 60 % FA treatments, with P values of 0.0001, 0.002 and 0.0002, respectively; as well as 0 % compared with 20 % (P value, 0.05). The remaining 60 % of the comparisons yielded results indicating that there was no significant difference ($p>0.05$) between plants across the other treatments.

d) Day 77 No. of leaves across treatments

The ANOVA results for the mean no. of leaves, indicated that there was a significant difference ($p<0.05$), with an F-value of 2.56 and P value of 0.06, across all FA treatments on day 77. The data indicates that the 20 % and 40 % FA treatments' plants had developed about 13 ± 0.88 and 13 ± 1.46 mean no. of leaves, respectively), followed by 55 % FA treatment plants with 12 ± 0.74 . The 0 % and 60 % FA treatments had about 11 ± 0.40 , 11 ± 0.67 and 11 ± 0.42 , respectively. At this stage the maize plants should be past the 1st reproductive stage (R1), in which all plants should have about 20 leaves, stopped growing and have started pollinating.

The Q-test analysis reveals that on day 77, there was no significant difference ($p>0.05$) between all the treatments.

e) Day 122 No. of leaves across treatments

The ANOVA results for the mean no. of leaves, indicated that there was a significant difference ($p<0.05$), with an F-value of 9.88 and P value of 6.2×10^{-06} , across all FA treatments on day 122. The data indicates that the plants grown in 40 % FA treatment had developed about 18 ± 2.83 mean no. of leaves, followed by 20 % FA treatment plants with 16 ± 2.34 . The 60 % treatment plants had about 14 ± 1.79 mean no. of leaves; and both the 0 % and 55 % treatments had 12 ± 1.87 mean no. of leaves, each. Similar to the day 77 data, these mean no. of leaves at day 122 were also below the 20 plus that expected for the R1 stage, as stipulated in the literature (O'Keeffe 2009).

The Q-test analysis reveals that on day 122, there was a significant difference ($p<0.05$) for 50 % of the comparisons of the no. of leaves across FA treatments. These were between plants grown in 40 % FA treatment compared to 0 %, 55 % and 60 % FA treatments, with P values of 0.0002 for 0 % and 55 % FA treatments' plants, and 0.03 60 % FA treatment plants. The remaining 50 % of the comparisons yielded results indicating that there was no significant difference ($p>0.05$) between plants across the other treatments.

In conclusion, the study generally reveals that the FA treatments did not affect the number of the leaves. This is despite the evidence that the stem length was affected, as discussed in the

preceding sections.

Results and Interpretation: No. of leaves within treatments across time

The ANOVA results indicated that there was a significant difference ($p < 0.05$) between the no. of leaves within FA treatments across time. The Q test yielded results indicating that 22 % were not significantly different ($p > 0.05$), while 78 % were significantly different ($p < 0.05$).

Figures 11 and 12 illustrates the number of leaves with each FA treatment across time, as well as the associated trend analyses. There is indication in graphs that the number of leaves increased over time, within each of the FA treatments. The detail of the results for the individual treatments across time are summarised below.

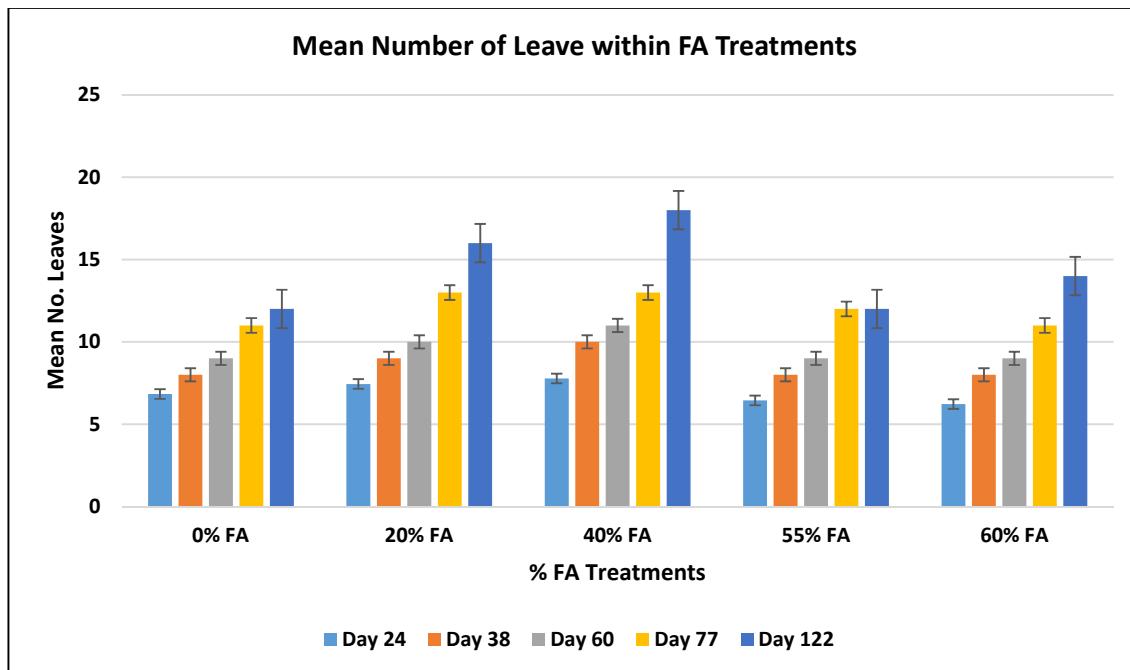


Figure 11: Mean No. of Leaves with Treatments across Time

The analysis of graphs in Figure 12 indicates that the development of the leaves was linear, within all the FA treatments, across time, with slight deviations from the trendline. There were similarities in the development of the leaves from the plants grown in the 0 % and 55 % FA treatments, with the day 77 leave count above the trend line and those at days 24, 38, 60 and 122 slightly on the trendline. The mean number of the leaves from the plants grown in the 20 % and 60 % FA treatments also displayed similar trends, with those counted at day 60 being below the trend and those at day 122 slightly above. The leaves counted in the 40 % FA treatment plants were different, those on days 24 and 122 treatment being above the trendline and 40 % and 55 % being below.

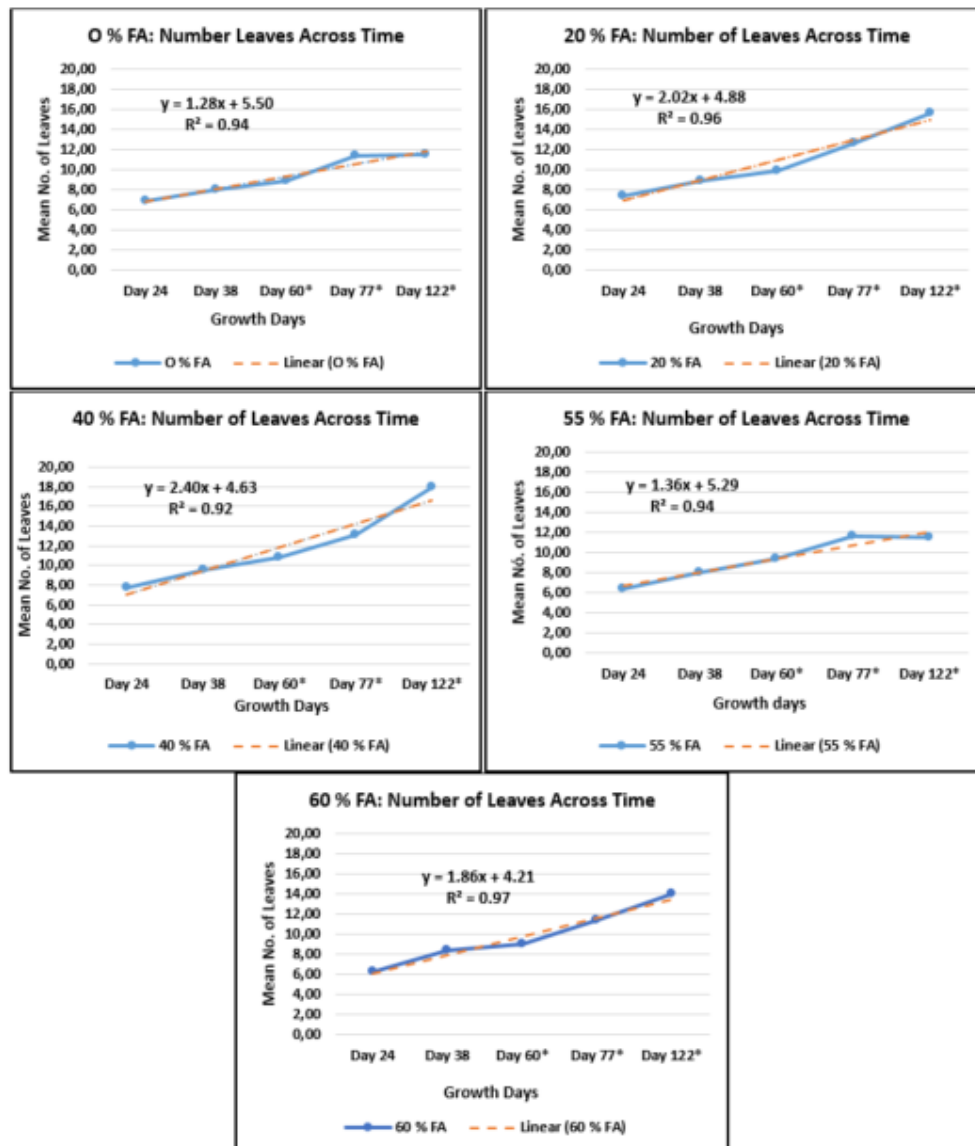


Figure 12: Trendline Analysis - Mean No. of Leaves with Treatments across Time

a) 0 % FA Treatment

The ANOVA indicated that there was a significant difference ($p < 0.05$: F value of 20.90 and a P value of 1.13×10^{-07}) between the mean no. of leaves of the 0 % FA treatment plants across time (days 24, 38, 60, 77 and 122). The mean no. of leaves increased from 7 ± 0.25 to 8 ± 0.63 , 9 ± 0.40 , 11 ± 1.32 and 12 ± 1.87 at days 24, 38, 60, 77 and 122, respectively, which is a normal indicator of growth. The number of leaves at day 24 seem to correlate well with the expected no. of leaves at vegetative stage V5, which normally commences at around day 20. The days 38 and 60 mean no. of leaves are lower than the expected literature no. of leaves at V8 (eight leaves) and V12 (twelve leaves), which normally develop at days 30 and 60, respectively. Similarly, the number of leaves at days 77 and 122 falls short of the typical 20 leaves at the

reproductive stage, which commences at around day 70 (O'Keeffe 2009).

The Q test indicated that 70 % of the comparisons of the no. of leaves across time were significantly different ($p < 0.05$). The remaining 30 % indicated that there was no significant difference ($p > 0.05$) between days 24 and 38, days 38 and 60 and days 77 and 122 with P values of 0.39, 0.64 and 1, respectively.

b) 20 % FA Treatment

The ANOVA indicated that there was a significant difference ($p < 0.05$) between the mean no. of leaves of the 20 % FA treatment plants across time, with an F value of 43.28 and a P value of 6.89×10^{-11} . The mean no. of leaves increased from 7 ± 0.81 to 9 ± 0.53 , 10 ± 0.44 , 13 ± 0.87 and 16 ± 2.34 at days 24, 38, 60, 77 and 122, respectively. In this case the day 24 and 38 no. of leaves are closely correlated to the literature values at V5 and V8. The number of leaves at days 60, 77 and 122 falls short of the typical V16 for day 60 and the 20 leaves at the reproductive stage, which commences at around day 70 (O'Keeffe 2009).

The Q test indicated that 80 % of the comparisons of the no. of leaves across time were significantly different ($p < 0.05$). The remaining 20 % indicated that there was no significant difference ($p > 0.05$) between days 24 and 38, and days 38 and 60, with P values of 0.23 and 0.62, respectively.

c) 40 % FA Treatment

The ANOVA indicated that there was a significant difference ($p < 0.05$: F value of 32.71 and a P value of 1.34×10^{-09}) between the mean no. of leaves of the 40 % FA treatment plants across time, with an. The mean no. of leaves increased from 8 ± 1.13 to 10 ± 1.41 , 11 ± 0.96 , 13 ± 1.46 and 18 ± 2.83 at day 24, 38, 60, 77 and 122, respectively. The scenario is similar to the preceding one for the 20 % FA treatment, with regard to the literature trends.

The Q test indicated that 70 % of the comparisons of the no. of leaves across time were significantly different ($p < 0.05$). The remaining 30 % indicated that there was no significant difference ($p > 0.05$) between days 24 and 38, days 38 and 60, and days 60 and 77, with P values of 0.42, 0.65 and 0.18, respectively.

d) 55 % FA Treatment

The ANOVA indicated that there was a significant difference in the mean no. of leaves of the 55 % FA treatment plants across time, with an F value of 29.45 and a P value of 3.93×10^{-09} . The

mean no. of leaves the 55 % treatment displays the similar lag that was observed in the stem length analyses for similar FA treatment. The mean no. of leaves increased from 6 ± 0.40 to 8 ± 0.61 , 9 ± 0.67 , 12 ± 0.74 and 12 ± 1.87 at days 24, 38, 60, 77 and 122. The no. of leaves at day 24 is the only one that correlates well with the literature, which suggests 5 leaves at V5, which commences at around day 20. The remaining treatments fall short of the no. of the typical 16 leaves at the suggested day 60 V16 stage and the 20 leaves at the reproductive stages, which commences at around day 70 (O'Keeffe 2009).

The Q test indicated that 80 % of the comparisons of the no. of leaves across time were significantly different ($p < 0.05$). The remaining 20 % indicated that there was no significant difference ($p > 0.05$) between days 38 and 60, as well as days 77 and 122, with P values of 0.21 and 1, respectively.

e) 60 % FA Treatment

The ANOVA indicated that there was a significant difference in the mean no. of leaves of the 60 % FA treatment plants across time, with an F value of 40.05 and a P value of 1.59×10^{-10} . The mean no. of leaves increased from 6 ± 0.69 , 8 ± 0.67 , 9 ± 0.42 , 11 ± 1.54 and 14 ± 1.79 at days 24, 38, 60, 77 and 122, respectively. This outcome is similar to the 55 % FA treatment, when compared to the suggested literature values.

The Q test indicated that 90 % of the comparisons of the no. of leaves across time were significantly different ($p < 0.05$). The remaining 10 % indicated that there was no significant difference ($p > 0.05$) between days 38 and 60, with a P values of 0.85.

4.1.3 Effects of FA on the length of the roots

The analyses of the average root lengths were done on data collected at days 60, 77 and 122, when the plants for the three sets were sequentially harvested. Figure 13 below illustrates mean root lengths, which displays a similar growth pattern to the stem length, particularly for data collected on days 60 and 122.

The analysis of graphs in Figure 14 indicates dissimilarities between the trends of the root length within time (days 60, 77 and 122) across all the FA treatments. The root lengths at day 60 were above the trendline for plants grown in 40 % and 55 % FA treatments; and lower for 0 % and 60 % FA treatments. On the contrary, the day 77 data revealed 20 % treatment plants as being above the trendlines compared to those from the 0 % FA treatment, while 40 %, 55 % and 60 % plants were slightly on the trendline. The data at day 122 indicated that plants that were grown

in the 20 % and 40 % FA treatment generally had longer lengths (above the trendline), whilst those grown in the 0 %, 55 % and 60 % FA treatment were shorter.

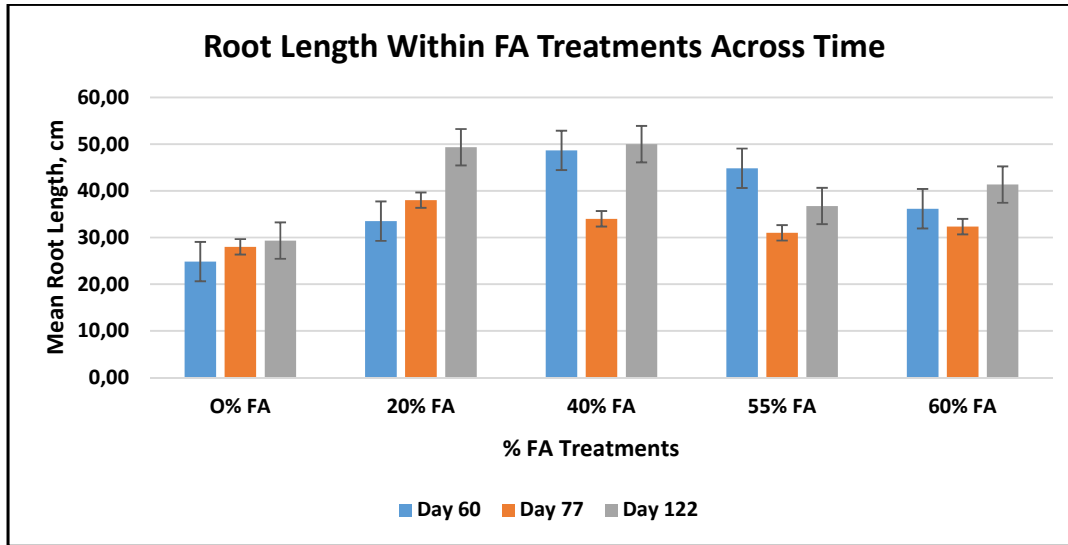


Figure 13: Mean Root Length within FA Treatments Across Time

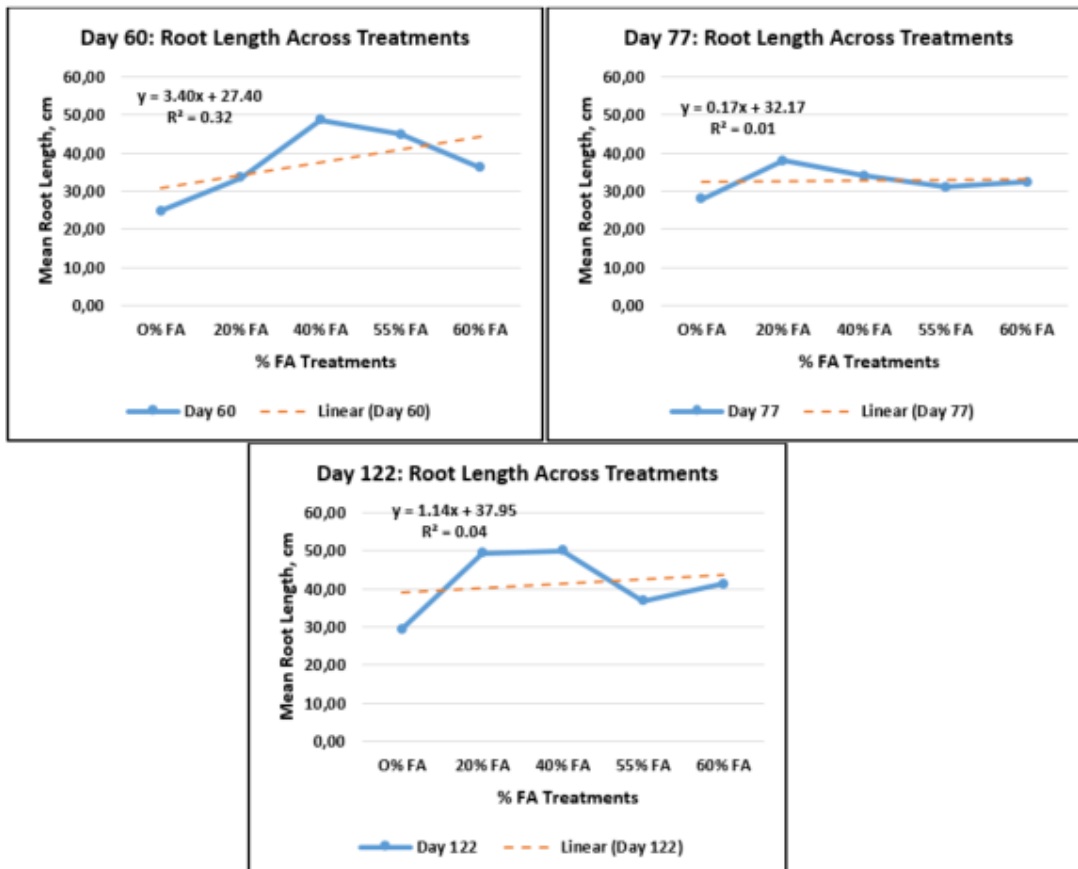


Figure 14: Trendline Analysis _ Mean Root Length within Time across FA Treatments

a) Day 60 – Root Lengths across treatments

The ANOVA indicated that there was a significant difference ($p < 0.05$: F value = 8.91 and P value = 0.00013) between the mean lengths of the roots of the plants, which were harvested at day 60, across the FA treatments. The longest mean root length was 48.67 ± 11.04 cm for the plants that grew in the 40 % FA treatment, followed by 44.83 ± 7.73 cm and 36.17 ± 6.34 cm for 55 % and 60 % FA treatments, respectively. The shortest roots were 24.83 ± 6.52 and 33.5 ± 5.99 cm for plants grown in 0 % and 20 % FA treatments.

The Q-Test also reveals the significant difference between the root lengths of plants grown in the 0 % FA treatment and those grown in both 40 % and 55 %; those grown in 20 % FA treatment compared to 40 %. There was no significant difference between the lengths of the roots per plant grown in the 0 % FA treatment and those in both 20 % and 55 %; 20 % compared to 55 % and 60 %; 40 % compared to both 55 % and 60 %; and 55 % compared to 60 %.

b) Day 77 – Root Lengths across treatments

The ANOVA conducted on the data collected from plants that were harvested on day 77 indicated that there was no significant difference ($P > 0.05$: F value = 0.41 and P value = 0.79) between the mean lengths of the roots across the different treatments in this set. The longest mean root length was 38.00 ± 1.73 cm for the plants that grew in the 20 % FA treatment, followed by 34.00 ± 7.94 , 32.33 ± 16.20 , 31.00 ± 0.41 and 28.00 ± 1.73 cm for 40 %, 60 %, 55 % and 0 % FA treatments, respectively.

This observation is also supported by the Q test, which also yielded results that indicate that there was no significant difference ($p > 0.05$) between the FA treatments.

c) Day 122 – Root Lengths across treatments

The ANOVA indicated that there was a significant difference ($p < 0.05$: F value = 2.89 and P-value of 0.0411), between the mean root lengths of the plants grown in the different FA treatments. The longest mean root length was 50.00 ± 11.61 cm for the plants that grew in the 40 % FA treatment, followed by 49.33 ± 8.31 , 41.33 ± 8.36 , 36.75 ± 18.93 and 29.33 ± 9.77 cm for 20 %, 60 %, 55 % and 0 % FA treatments, respectively.

On the contrary, the Q test yield indicated that there was no significant difference ($p > 0.05$) between the FA treatments.

In general, the statistical analysis suggests that the increments of FA treatment did not have a significant effect on the length of the roots, across the treatments. A second observation in this analysis is that the mean root lengths of the plants that were harvested at days 60 and 122 across all treatments were almost similar and higher compared to those at day 77. This suggests that at day 60, the roots were already getting pot bound, resulting in their density increasing with the limited size of the planting pots.

4.1.4 Effects of FA on the mass of the total plant biomass

Figure 15 below illustrates the mean masses of the biomass (below plus above ground) for the plants which were harvested on days 60, 77 and 122. There was not much difference between the mean masses of the plants, which were measured at days 60 and 77. The plants that grew in 20 %, 40 % and 60 % FA treatments had higher masses at day 122, compared to those in 0 % and 55 % FA treatments.

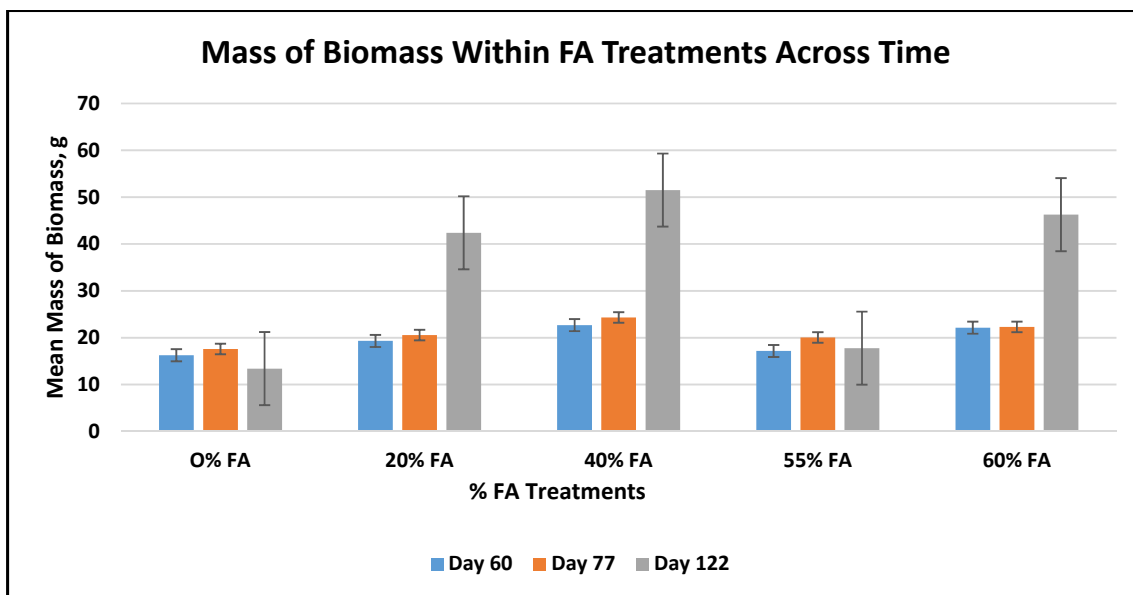


Figure 15: Mean Mass of Plant Biomass within FA Treatments across Time

The analysis of the trendlines in Figure 16 indicated a similar close to linear pattern in the measurements done on the plants, which were harvested on days 60 and 77, compared to those harvested at day 122. The masses of the plant biomass from the day 122 harvest relatively deviated significantly from the trendline. It is also evident from these trends that the plants which were grown in the 40 % FA treatment generally had higher masses, whereas those grown in the 55 % FA treatment were lower.

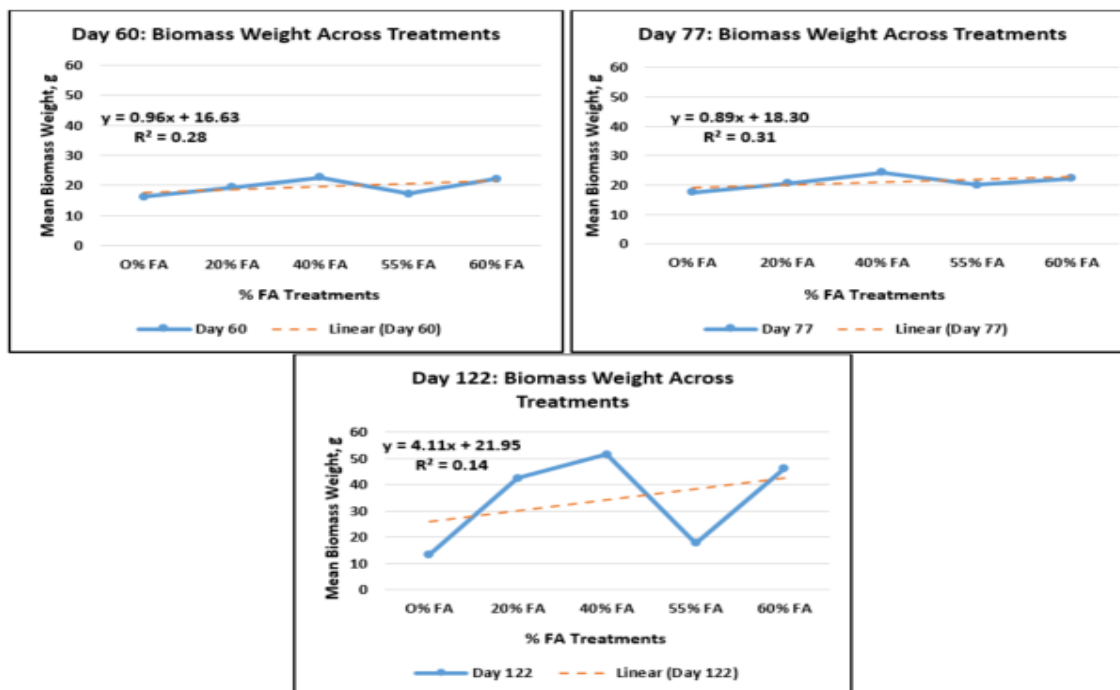


Figure 16: Mean Mass of Plant Biomass within Time across FA Treatments

a) Day 60 –Plant Biomass across treatments

The ANOVA performed on the mean dry biomass of plants that were harvested on day 60 of growth indicated that there is a significant difference ($p < 0.05$; F value = 19.44; P-value = 2.22×10^{-7}) between the masses of the plants grown in the different FA treatments. The highest masses were 22.70 ± 1.61 and 22.15 ± 1.25 g for plants grown in 40 % and 60 % FA treatments, respectively. This was followed by 19.31 ± 1.26 , 17.17 ± 1.74 and 16.26 ± 2.01 g for 20 %, 55 % and 0 % FA treatments respectively.

The Q test, also suggest that there was significant difference ($p < 0.05$) between the mean mass of the dry biomass of the plants grown in the 0 % FA treatment and 20 %, 40 %, and 60 % FA treatments. There was also a significant difference when comparing the dry biomass grown in 20 % compared to both 40 % and 60 %; 40 % compared to 55 %; and 55 % compared to 60 %. There was no significant difference between the values for plants grown in 0 % compared to those grown in 55 %; plants grown in 20 % FA compared to those in 55 %; and those grown in 40 % compared to those grown in 60 % FA treatment.

These variations in the mean biomass across the treatments indicated that FA had an effect on the growth and development of the maize plants. It was also evident that the plants, which grew in the 40 % FA treatment had more mass compared to those in other treatments, followed by those in 60 % and 20 %.

b) Day 77 – Plant Biomass across treatments

The ANOVA indicated that there is a significant difference ($p < 0.05$: F value of 7.28 and P value of 0.00087) between the masses of the plants grown in the different FA treatments. The highest masses were 24.33 ± 1.15 and 22.32 ± 2.57 g for plants grown in 40 % and 60 % FA treatments, respectively. This was followed by 20.58 ± 1.26 , 20.05 ± 2.05 and 17.59 ± 2.47 g for 20 %, 55 % and 0 % FA treatments respectively.

The Q-Test performed on the total biomass of each plant yielded P-values higher than 0,05, which suggests that there was no significant difference across the treatments. This implies that the concentration of FA did not affect the total biomass in this set of harvested maize plants.

c) Day 122 – Plant Biomass across treatments

The ANOVA performed on the dry biomass of plants that were harvested on day 122 of growth indicated that there is a significant difference ($P < 0.05$: F value = 4.35 and a P-value of 0,00761) between the masses of the plants grown in the different FA treatments. The highest masses were 51.52 ± 12.54 , 46.27 ± 40.44 (one outlier) and 42.40 ± 14.01 g for plants grown in 40 % 60 % and 20 % FA treatments, respectively. This was followed by 17.78 ± 16.34 , and 13.42 ± 7.44 g for 55 % and 0 % FA treatments respectively.

The P-values from Q-Test, also suggested that there was significant difference between the dry biomass of the plants grown in the 0 % FA treatment and those grown in 40 % FA treatments. There was significant difference when comparing the dry biomass grown in 40 % compared to those grown in 55 % FA treatment. There was no significant difference between the readings for plants grown in 0 % compared to those grown in 20 %, 55 % and 60 %; plants grown in 20 % FA compared to those in 40 %, 55 % and 60 %; those grown in 40 % compared to those grown in 60 %; and those grown in 55 % compared to 60 % FA treatment.

In conclusion, although there are some similarities in the growth patterns compared to the stem length, the overall statistical analysis suggests that the different FA treatments did not significantly influence the mass of the plants at each harvest.

4.2 Laboratory Chemical Analysis

4.2.1 Soil Properties

The laboratory results for the soil analyses that was conducted by the Agricultural Research Council (ARC), as part of this study, indicated that the pH (H_2O) of the soil increased from 5.64

for 0 % FA treatment to 7.56 at 60% FA treatment. The pH measured for the original soil, which was not treated with FA had a pH of 5.22.

Table 6: Original Soil Properties

Parameter	Analysis Method Used	Value
pH	pH (H ₂ O)	5.22
CEC	Ammonium Acetate Digestion	27.06 cmol kg ⁻¹
Extractable cations, Ca	Ammonium Acetate Digestion	3164.94 mg kg ⁻¹
Extractable cations, K	Ammonium Acetate Digestion	1969.28 mg kg ⁻¹
Extractable cations, Mg	Ammonium Acetate Digestion	2219.20 mg kg ⁻¹
Extractable cations, Na	Ammonium Acetate Digestion	116.90 mg kg ⁻¹
Total P	Dry Combustion	1425.22 mg kg ⁻¹
Total N	Dry Combustion	3724 mg kg ⁻¹
Total C	Walkley-Black/Dry Combustion	41650 mg kg ⁻¹
Ba	ICP-MS	70.64 mg kg ⁻¹
Cr	ICP-MS	378.03 mg kg ⁻¹
Mn	ICP-MS	1043.28 mg kg ⁻¹
Mo	ICP-MS	1.19 mg kg ⁻¹

The CEC analysis, including extractable cations of Ca, K, Mg and Na, yielded CEC values that decreased from 27.06 cmol kg⁻¹ in the untreated original soil at day zero to 18.091 cmol kg⁻¹ in the 0 % FA treatment after 122 days of plant growth before increasing again to 21.38 cmol kg⁻¹ with 20 % FA treatment. This was followed by declines to 16.33, 12.02 and 11.59 cmol kg⁻¹ with 40 %, 55 % and 60 % FA treatments, respectively. These figures represent the total CEC for extractable cations, such as Ca²⁺, Mg²⁺, K⁺ and Na⁺, which are present in the soil, noting that they have different molecular weights; and they are the main cation exchange elements (Basu *et al.*, 2008).

Figure 17 below indicates a relationship between pH and CEC, as well as the extractable cations Ca, Mg, K and Na, which indicates that pH increases with Ca, Mg and Na concentrations, whereas the K concentration and CEC decreased.

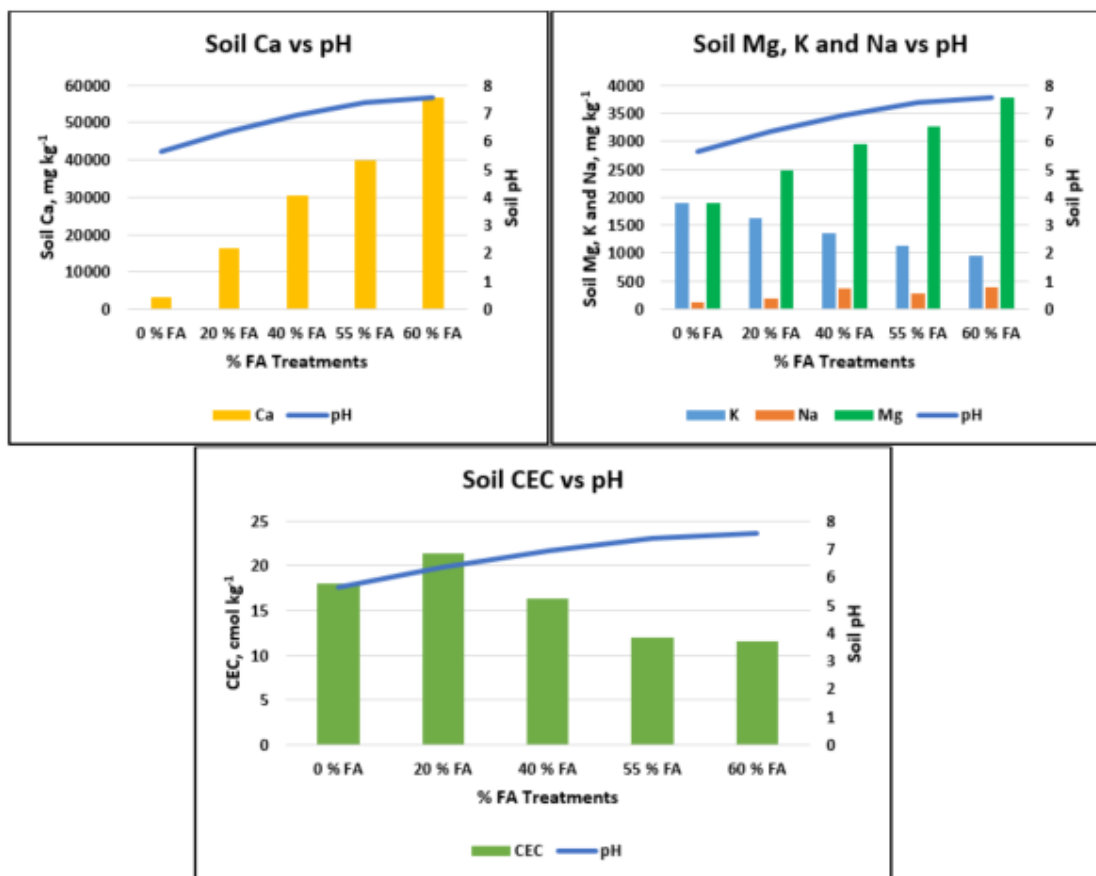


Figure 17: Soil CEC, Ca, K, Mg and Na; vs pH across treatments

The Ca concentration slightly declined from 3164 mg kg⁻¹ at day zero, in the original (unused) soil collected from the farm, to 3082 mg kg⁻¹ in 0 % FA treatment after day 122 of maize plant growth before increasing with the FA treatment to 16455, 30423, 39901 and 56686 mg kg⁻¹ in 20 %, 40 %, 55 % and 60 % FA treatments, respectively.

The concentration of Mg and P (see Figure 18) display opposite patterns, when compared to each other across the FA treatments. The concentration of Mg decreased from 2219 mg kg⁻¹ in the untreated original soil to 1895 mg kg⁻¹ in 0 % FA treatment after 122 days of plant growth. The FA treated soils contained slightly high amounts of P, which were 2482, 2955 and 3259 mg kg⁻¹ in 20 %, 40 %, 55 % and 60 % FA treatments, respectively at day 122. The content of P decreased from 1425 mg kg⁻¹ in the original soil at day zero to 1405 mg kg⁻¹ in 0% FA after 122 days of plant growth. The concentrations of P in the treated soils were 1931, 1381, 1327 and 1261 mg kg⁻¹, in 20 %, 40 %, 55 % and 60 % FA treatments, respectively after day 122.

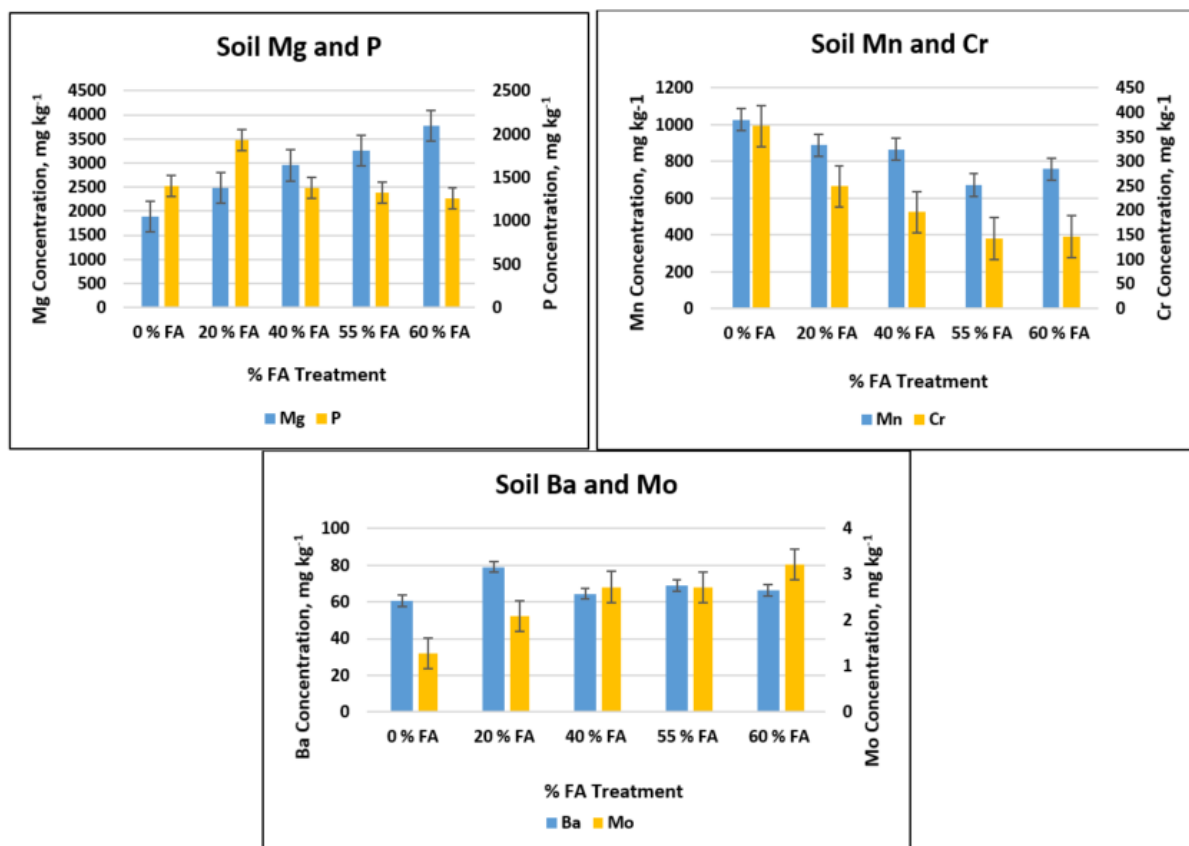


Figure 18: Soil Concentrations of Mg, P, Mn, Cr, Ba and Mo, across FA Treatments

Figure 18 also illustrates the concentrations of the HMs Mn, Cr, Ba and Mo in the soil, across all five FA treatments. The amount of Mn declined from 1043 mg kg⁻¹, in the untreated original soil, to 1027 mg kg⁻¹, after 122 days of plant growth; and the FA treated soils contained 889, 866, 671 and 758 mg kg⁻¹ of Mn in the 0 %, 20 %, 40 %, 55 % and 60 % FA treatments, respectively after 122 days of plant growth. Whereas, the Cr concentration declined from 378 mg kg⁻¹, in the original soil, to 372 mg kg⁻¹ in the 0 % FA treatment after 122 days. The concentrations of Cr were 249, 197, 142 and 146 mg kg⁻¹ in the 20 %, 40 %, 55 % and 60 % FA treatments, respectively.

The Ba and Mo concentrations were lower compared to Mn and Cr and ranged from 60 to 80 mg kg⁻¹ for Ba and 1 to 3.5 mg kg⁻¹ for Mo. The amount of Ba declined from 71 mg kg⁻¹, in the original soil, to 61 mg kg⁻¹ in the 0 % FA treatment after 122 days of plant growth. These Ba concentrations fluctuated in the FA treated soils, and they were 79, 64, 69 and 66 mg kg⁻¹ in the 20 %, 40 %, 55 % and 60 % FA treatment, respectively. The Mo concentration slightly increased from 1.20 mg kg⁻¹ in the original soil to 1.28 mg kg⁻¹ in the 0 % FA treatment after 122 days. These concentrations of Mo were slightly higher in the FA treated soils, which contained 2.09, 2.73, 2.72 and 3.22 mg kg⁻¹ in the 20 %, 40 %, 55 % and 60 % FA treatments, respectively, after 122 days of plant growth.

4.2.2 Plant Nutrient Analysis

a) Above and Below Ground C and N

Figure 19 below illustrates concentrations, which were determined from total combustion, of both C and N in the below ground and above ground biomass, as well as the amount of C and N that was accumulated per plant, across treatments at day 122. These analyses were based on 45 plant samples, consisting of three below ground and three matching above ground replicate samples for each of the five FA treatments. The analysis indicates that concentrations of both C and N in the below ground biomass were lower than those in the above ground plant biomasses, across all the FA treatments.

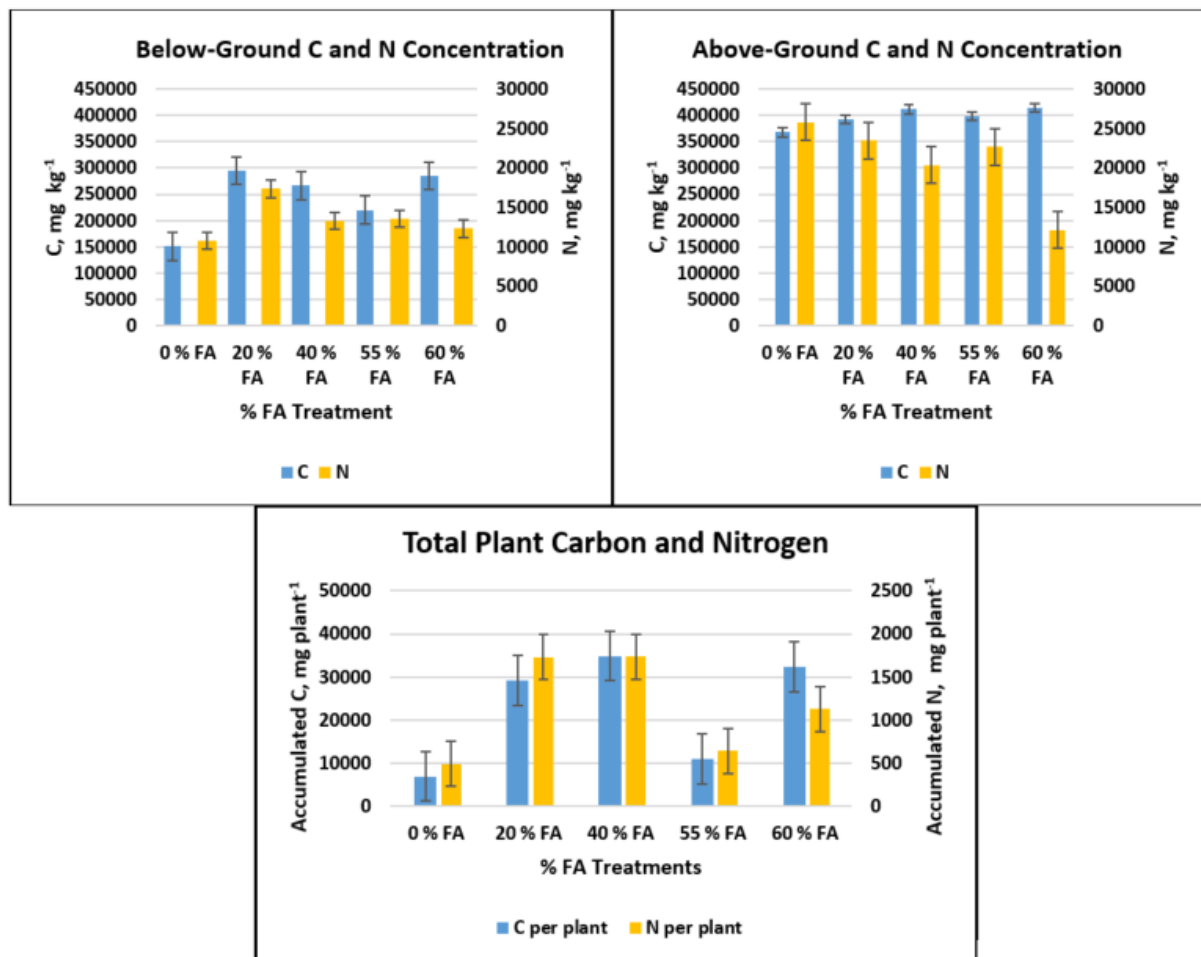


Figure 19: Below and Above Ground Biomass C and N across FA Treatments

The accumulated C and N per plant (see Figure 19) was calculated using the total concentrations (below and above ground) and the mean masses across the FA treatments, for the plants that were harvested at day 122. The comparison between the accumulated C and N per plants varied across the treatments, with the plants that were grown in 0 % and 55 % FA having lower

accumulation, compared to those in 20 %, 40 % and 60 % FA treatments. The estimated total C that accumulated was 6958.63 and 11006.23 mg plant⁻¹ in the 0 % and 55 % FA treatments, respectively; and higher at 29143.48, 32333.11 and 34915.67 mg plant⁻¹ in the 20 %, 60 % and 40 % FA treatments, respectively. The total accumulated N was 490.96 and 643.85 mg plant⁻¹ in the 0 % and 55 % FA treatments, respectively; and 1131.61, 1730.15 and 1735.89 mg plant⁻¹ in the 60 %, 20 % and 40 % FA treatments, respectively. These trend are similar to those of the mean plant biomass, across treatments in Figure 15.

Below Ground Biomass

The ANOVA indicated that there was no significant difference ($p>0.05$) in both the below ground (roots) total C and N across the FA treatments, and this was confirmed by the Q test ($p>0.05$) between the various treatments. The below ground biomass mean concentrations of C were 150959 ± 82157 ; 294861 ± 33311 ; 266171 ± 84017 ; 220044 ± 65507 and 284946 ± 116874 mg kg⁻¹ in the plants from the 0 %, 20 %, 40 %, 55 % and 60 % FA treatments, respectively. Whereas the concentrations of N were 10771 ± 3114 , 17345 ± 1093 , 13302 ± 4554 , 13555 ± 3685 and 12308 ± 5348 mg kg⁻¹ in plants from the 0 %, 20 %, 40 %, 55 % and 60 % FA treatments, respectively.

Above Ground Biomass

This ANOVA indicated that there were significant differences ($p<0.05$) in the total C (P value = 0.016; F value = 5.18) and total N (P value = 0.046; F value = 3.58) concentrations in the above ground biomasses between all the five FA treatments. The above-ground biomass mean concentrations of C were 367697 ± 4043 ; 392485 ± 17906 ; 411518 ± 8080 ; 398935 ± 4023 and 414148 ± 24214 mg kg⁻¹ in the plants from the 0 %, 20 %, 40 %, 55 % and 60 % FA treatments, respectively. Whereas the concentrations of N were 25822 ± 1123 , 23460 ± 1401 , 20391 ± 7435 , 22654 ± 1835 and 12159 ± 7349 mg kg⁻¹ in plants from the 0 %, 20 %, 40 %, 55 % and 60 % FA treatments, respectively.

The Q-tests only indicated significant differences ($p<0.05$) for total C in 0 % compared with 40 % and 60 % FA treatments; and a significant difference ($p<0.05$) for total N only for 0 % vs 60 % FA treatments.

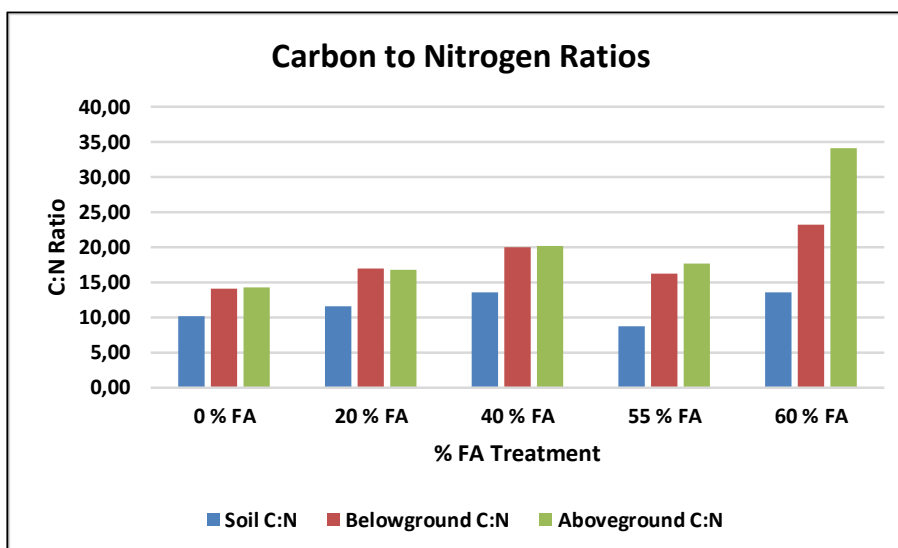


Figure 20: C to N Ratios as Indicators of C and N Supply Balance

The variation of the C:N ratios across all treatments for all these samples is illustrated in Figure 20. The C:N ratios for the soil increased from 10.10 in 0 % FA treatment to 11.58 and 13.61 for both 20 % and 40 % FA treatments, before dropping to 8.67 for 55 % FA and increasing again to 13.61 in 60 % FA treatment. A similar trend is displayed by both the below and above ground C:N ratios increasing between 0 % and 40 % FA treatments before dropping in 55 % FA soils and increasing in 60 % FA treatments. The C:N ratio for the untreated soil that was not used in the experiment was 11.18. The mean C:N ratio of 11.46 (SD = 1.96) falls within the range of C:N ratios in the literature.

b) Below and Above Ground Ca, Mg and P

The analysis of the graphs in Figures 21 and 22 indicates that the Ca concentration in the below ground biomass, across the treatments, is higher with a range of 4200 to 9600 mg kg⁻¹, compared to the above ground Ca concentrations, which ranged between 4600 and 6000 mg kg⁻¹. Both Mg and P above ground concentrations were, however, higher in the above ground biomass compared to below ground. The above ground Mg concentration ranges between 4500 and 5700 mg kg⁻¹ as the Ca, whereas its below ground concentration was lower with a range between 2730 to 4300 mg kg⁻¹, across the FA treatments.

The accumulated amounts of Ca, Ma and P per plants, in Figure 21, were also determined using the product between the mean masses of the plants harvested at day 122 and total concentrations of each of the three elements, across the FA treatments. The accumulated Ca in the 0 %, 20 %, 40 %, 55 % and 60 % FA treatment plants were estimated to be 126.90, 568.39, 773, 22, 265.05 and 658.47 mg plant⁻¹, respectively. The Ma accumulated was 111. 81, 337.53,

425.96, 177.68 and 658.47 mg plant⁻¹; and P was 64.80, 209.15, 274.94, 84.48 and 193.11 mg plant⁻¹, all in 0 %, 20 %, 40 %, 55 % and 60 % FA treatments, respectively.

Similar to the C and N accumulation in the preceding section, the plants in 0 % and 55 % FA treatments had the lowest accumulation, compared to those in the 20 %, 40 % and 60 % FA treatments. These patterns were also influenced mainly by the means masses of the plants per treatment.

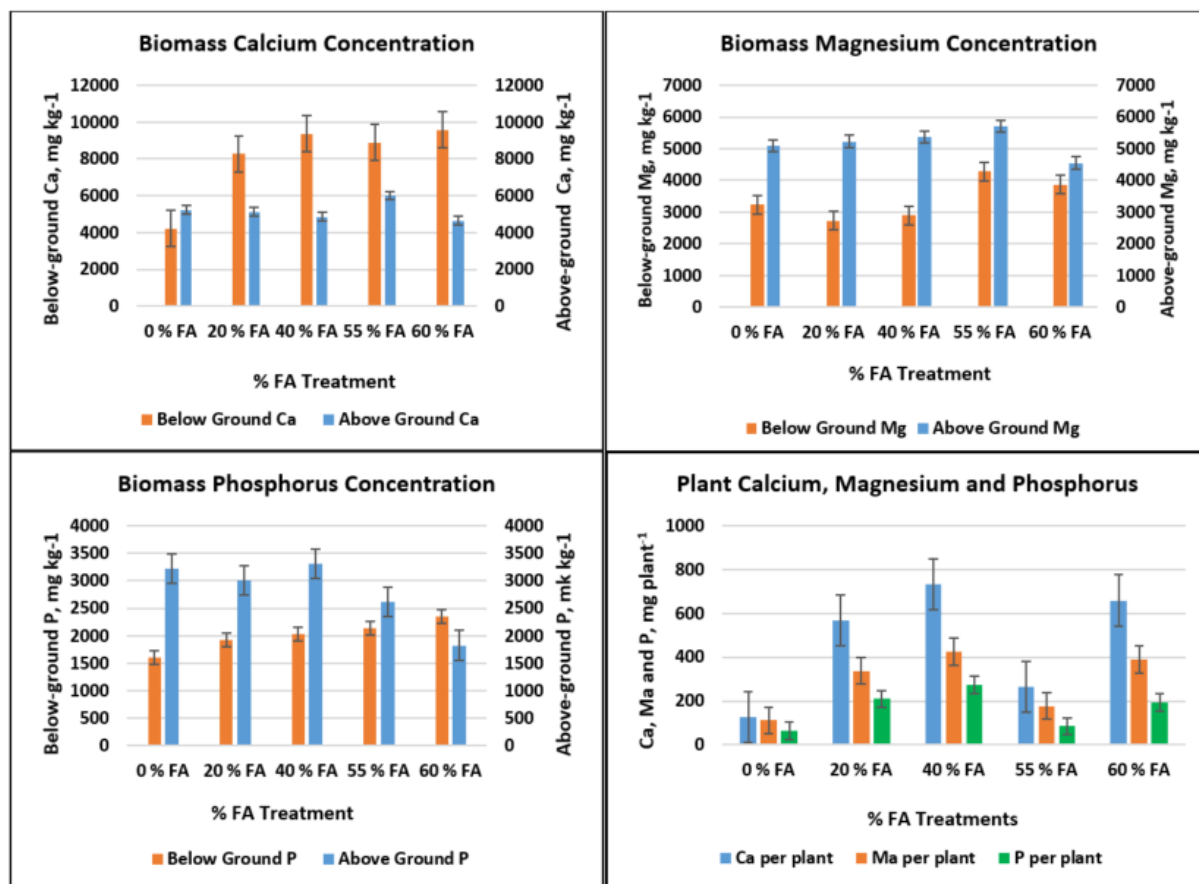


Figure 21: Above and Below Ground Concentrations of Ca, Mg and P

ANOVA - Ca Concentration

The ANOVA indicated that there was a significant difference ($p < 0.05$: P value = 0.014 and F value = 5.72) between the below ground concentrations of Ca, across the FA treatments. The corresponding Q test only indicated a significant difference ($p < 0.05$) between the below ground concentration in 0 % FA treatment compared to 20 %, 40 %, 55 % and 60 % treatments. Both the ANOVA and Q test indicated that there was not significant difference ($p > 0.05$) between the Ca concentration in the above ground biomass.

After day 122 of maize growth, the below ground biomass Ca concentration, was 4220 mg kg⁻¹ in 0 % FA treatment followed by 8271 mg kg⁻¹ in 20 % and 9378 mg kg⁻¹ in 40 %, before declining to 8896 mg kg⁻¹ in 55 % and rising again to 9586 mg kg⁻¹ in 60 % FA treatment. Whereas the above ground biomass Ca concentration decreased from 5239 mg kg⁻¹ in the plants that grew in 0 % FA treatment to 5134 and 4853 mg kg⁻¹ in 20 % and 40 % FA treatment plants, before increasing to 6010 mg kg⁻¹ in 55 % and decreased thereafter to 4652 mg kg⁻¹ in 60 % FA treatment plants.

ANOVA - Mg Concentration

The ANOVA indicated that there was a significant difference ($p < 0.05$: P value = 0.016 and F value = 5.14) between the concentrations of Mg in below ground biomass, across the FA treatments; and the Q test only indicated there were significant differences ($p > 0.05$) when comparing the concentrations in 20 % and 40 % FA treatments against those in 55 % FA treatment. Both the ANOVA and Q test indicated that there was no significant difference ($p > 0.05$) between the Mg concentration in the above ground biomass.

The Mg concentration in the below ground biomass decreased from 3233 mg kg⁻¹ in the below ground biomass of 0 % FA treatment plants to 2735 mg kg⁻¹ in 20 % FA plants, before increasing to 2894 and 4288 mg kg⁻¹ in 40 % and 55 % FA treatment, respectively, then decreased to 3862 mg kg⁻¹ in 60 % FA treatment below ground biomass. In the above ground biomass, the Mg concentrations increased from 5101 mg kg⁻¹ in 0 % FA treatment to 5226, 5374 and 5705 mg kg⁻¹ in 20 %, 40 % and 55 % FA treatments, respectively, before decreasing to 4551 mg kg⁻¹ in 60 % FA treatment.

ANOVA - P Concentration

Both the ANOVA and the Q test indicated that there was no significant difference ($p > 0.05$) between the concentrations of P in the below ground biomass, across FA treatments. On the contrary, the ANOVA indicate a significant difference ($p < 0.05$: P value = 0.0017 and F value = 9.87) between the concentrations of P in the above ground biomass; similarly, the Q test indicated the same when comparing P concentrations in above ground biomass of plants grown in 60 % FA against those grown in 0 %, 20 % and 40 FA treatments.

The concentration of P in the below ground biomass increased across the FA treatments, from 1609 mg kg⁻¹ in the 0 % FA treatment to 1929, 2039, 2134 and 2349 mg kg⁻¹, in 0 %, 20 %, 40 %, 55 % and 60 % FA treatments, respectively. The above ground biomass P concentrations, were higher and displayed a different pattern, with the concentration decreasing from 3221 mg

kg⁻¹ in 0 % FA treatment to 3004 mg kg⁻¹ in 20 %, then increasing to 3307 mg kg⁻¹ in 40 % before decreasing to 2617 and 1826 mg kg⁻¹ in 55 % and 60 % FA treatments.

4.2.3 Heavy Metal Accumulation

Figure 22 below indicates the comparison between below and above ground mean concentrations of Mn, Cr, Ba and Mo, which were determined from the maize plants that were harvested after 122 days of growth.

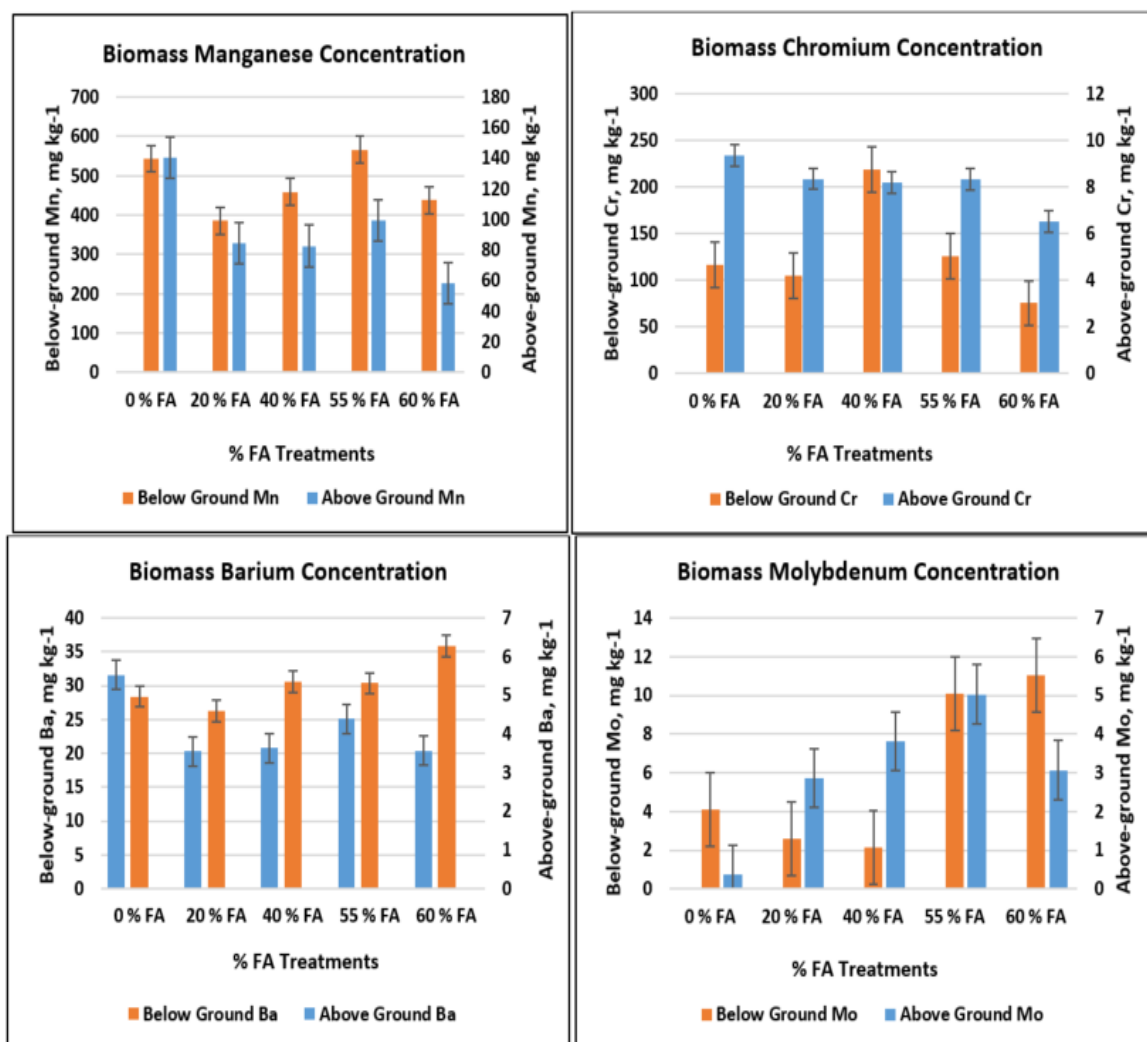


Figure 22: Below and Above Ground HM Concentrations

a) ANOVA – Ba and Cr Concentrations

The ANOVA and the Q test for both Ba and Cr indicated that there are no significant differences ($p > 0.05$) between their respective concentrations in the below ground biomass, across all the FA treatments. Similar results were obtained for both the Ba and Cr concentrations in above ground biomass.

The mean concentration of Ba in the below ground biomass decreased from 28.40 mg kg⁻¹ in 0 % FA treatment to 26.27 mg kg⁻¹ in 20 % FA, before increasing to 30.60 mg kg⁻¹ in 40 % FA treatment. This was followed by another decline to 30.33 mg kg⁻¹ in 55 % FA treatment and an increase to 35.80 mg kg⁻¹ in 60 % FA treatment. In the above ground biomass, the concentrations across the FA treatments also assumed a similar pattern, whereby the mean concentration declined from 5.53 mg kg⁻¹ in 0 %FA treatment to 3.55 mg kg⁻¹ in 20 % FA. This was followed by increments to 3.63 and 4.38 mg kg⁻¹ in 40 % and 55 % FA treatments, respectively, before declining to 3.56 mg kg⁻¹ in 60 % FA treatment.

Similarly, the mean Cr concentration in the below ground biomass decreased from 116.20 mg kg⁻¹ in 0 % FA treatment to 104.70 mg kg⁻¹ in 20 % FA, before increasing to 219.10 mg kg⁻¹ in 40 % FA treatment. This was followed by another decline to 125.60 and 75.33 mg kg⁻¹ in 55 % and 60 % FA treatments, respectively. The Cr concentration in the above ground biomass decreased from 9.36 mg kg⁻¹ in 0 % FA treatment to 8.35 and 8.20 mg kg⁻¹ in 20 % and 40 % FA treatments, respectively, before increasing to 8.34 mg kg⁻¹ in 55 % FA and then dropping to 6.53 mg kg⁻¹ in 60 % FA treatment.

b) ANOVA – Mn Concentration

The ANOVA indicated that there was no significant difference ($p > 0.05$: P value = 0.64 and F value = 0.66) between the Mn concentrations in the below ground biomass, across all the FA treatments. These results were contrary to the ANOVA on the above ground concentration of Mn, which indicated that there was a significant difference ($p < 0.05$: P value = 0.006 and F value = 7.14), between the FA treatments.

The Q test that there was no significant difference ($p > 0.05$) between below ground Mn concentrations across all FA treatments. On the contrary, the Q test only indicated a significant difference ($p < 0.05$) when comparing the above ground Mn concentration in the 0 % FA treatment plants against 20 %, 40 % and 60 % FA treatments.

The mean Mn concentration in the below ground biomass decreased from 543.77 ± 145.98 mg kg⁻¹ in 0 % FA treatment to 385.17 ± 41.88 mg kg⁻¹ in 20 % FA, before increasing to 458.90 ± 12.40 and 566.10 ± 173.61 mg kg⁻¹ in 40 % and 55 % FA treatments, respectively. The concentration in the 60 % FA treatment below ground biomass was 437.37 ± 276.69 mg kg⁻¹.

The above ground Mn concentration decreased from 140 ± 19.57 mg kg⁻¹ in 0 % FA treatment to 84.47 ± 25.00 and 82.70 ± 20.61 mg kg⁻¹ in 20 % and 40 % FA treatments, respectively;

before increasing to $99.10 \pm 10.80 \text{ mg kg}^{-1}$ in 55 % FA and declining to $58.37 \pm 19.10 \text{ mg kg}^{-1}$ in 60 % FA treatment.

c) ANOVA – Mo Concentration

The ANOVA indicated that there was a significant difference ($p > 0.05$: P value = 0.02 and F value = 4.83) between the Mo concentrations in the below ground biomass, across all the FA treatments. The Q test only indicated a significant difference ($p < 0.05$: P value = 0.053) when comparing the concentrations in 40 % FA treatment against those in 60 % FA. The ANOVA of the Mo concentration in the above ground biomass also indicated that there is a significant difference ($p < 0.05$: P value = 0.002 and F value = 9.14) between the FA treatments. The Q test only indicated a significant difference when comparing the 0 % FA treatment concentration against 20 % (P value = 0.05), 40 % (P value = 0.01), 55 % (P value = 0.001) and 60 % (P value = 0.04) FA treatments.

The mean Mo concentration in the below ground biomass decreased from 4.11 mg kg^{-1} in 0 % FA treatment to 2.57 and 2.14 mg kg^{-1} in 20 % and 40 % FA, respectively, before increasing to 10.09 and 11.02 mg kg^{-1} in 55 % and 60 % FA treatments, respectively. The above ground concentration increased from 0.37 mg kg^{-1} in 0 % FA treatment to 2.86, 3.81 and 5.02 mg kg^{-1} in 20 % 40 % and 55 % FA treatments, respectively; before decreasing to 3.06 mg kg^{-1} in 60 % FA treatment.

In general, the analyses indicate that the Mn, Cr and Ba concentrations in the below-ground biomass are higher than in the above ground biomass, across all the FA treatments. The same is observed for Mo, except for the 20 % and 40 % FA treatments, in which the above ground Mo concentration are higher. All these concentrations are below those in the Kusile Waste Assessment report, which concluded that the Kusile FA was low risk, in terms of the legislation. Both the Mn and Cr concentrations were mostly reported to be below 1000 mg kg^{-1} , across the treatments, except to Mn in the 0 % FA treatment, which was 1027 mg kg^{-1} ; whereas the concentrations of Ba and Mo were 351 and 24.1 mg kg^{-1} , respectively (see Table 3).

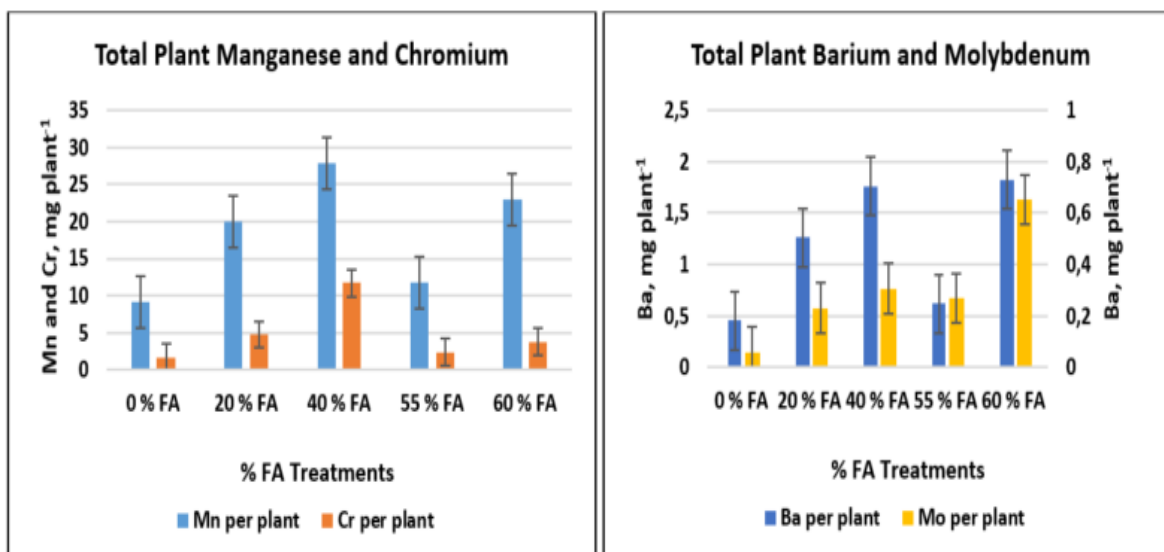


Figure 23: HM Accumulation per Plant, mg

Figure 23 indicates the accumulated HMs (Mn, Cr, Ba and Mo) per plant, which were determined using the same method use for C, N, Ca, Ma and P in the preceding sections. The accumulation of Mn per plant was the highest, with 9.18, 19.91, 27.90, 11.83 and 22.93 mg, across all the FA treatments (0 %, 20 %, 40 %, 55 % and 60 %); similarly, Cr per plant was 1.68, 4.97, 11.71, 2.38 and 3.79 mg; and Ba per plant was 0.46, 1.26, 1.76, 0.62 and 1.82 mg. The accumulation of Mo was the least of all four HMs with 0.06, 0.23, 0.31, 0.27 and 0.65 mg, in 0 %, 20 %, 40 %, 55 % and 60 % FA treatments, respectively.

The pattern of the accumulated HMs across all the FA treatments is similar to those of the C, N, Ca, Ma and P, as indicated in Figures 19 and 21. All of these also seem to be influenced by the total mass of the day 122 plant biomass, which is indicated in Figure 15. The accumulation of all these four HMs (Mn, Cr, Ba and Mo) per plant increased with the % FA treatments, as clearly evidenced in the trend from 0 % to 40 % FA treatments. This is while, Figure 18 indicated that both the Mn and Cr concentrations in the soil decreased across the FA treatments. The Mo concentrations in the soil increased, whereas Ba remained slightly constant.

In general, the maize plants seem to be partially capable of reducing the concentrations of Mn and Cr in the soil, more than they do with those of Ba and Mo. This observation will assist in the understanding whether maize plants are hyper-accumulators of HMs and whether they can be used for ameliorating HM contaminated soils.

5. DISCUSSION

The purpose of this study was to establish whether the application of FA to the soil would have any effect on the growth and yield of maize plants. In order to achieve this, the first objective was to evaluate the effects of FA on the soil pH and CEC, and plant nutrition. The second objective was to evaluate the FA effect on plant growth, with emphasis on stem lengths, number of leaves, root length and the mass of the dry biomass. The third and last objective being to evaluate whether maize plants can partially decontaminate the soil, through phytoremediation. The discussion will thus focus on whether the results, which were analysed in the preceding section satisfied these objectives.

5.1 Effect of FA on the Soil pH, CEC and the Plant Nutrition.

a) Soil pH, Ca concentration and CEC

The key aspects in plant growth and development are the quality of the soil and its attributes, the pH and CEC. Generally, the pH of untreated soils has a positive correlation with its CEC, meaning soils with lower pH (acidic) would have low CEC, which will increase with pH. There are several traits that affect the pH of the soil, such as H^+ and Al^{3+} cations which makes the soil acidic (low pH); OH^- and Ca^{2+} , which tends to make the soil less acidic or alkaline (moderate to high pH).

The concentration of Ca in this study increased across all the five FA treatments and the same was observed with the soil pH. This scenario is supported by studies that suggest that FA increases the alkalinity of soil due to its Ca content (Ayanda *et al.*, 2018; Basu *et al.*, 2009; Chandrakar *et al.*, 2018). Similarly, the soil pH also increased from 5.22 to 7.56 across the FA treatments and over the 122 days of growth. These results are corroborated by published studies, included by Basu *et al.* (2008), Srivastava *et al.* (2016), Panda and Biswal (2018).

These measured pH values are within the recommended pH range of 5.5 to 8 for most crops, including maize plants; and are closer to the pH range of 5.6 to 6.4 for Gauteng and Mpumalanga Provinces (Courtman *et al.*, 2012). The increase in the pH across all the FA treatments did not negatively affect the development and growth of the maize plants.

The expectation is that the increase in the concentration of Ca, which increased with higher FA treatments, would have the same effect. The analysis of the CEC values across the FA treatments (see Figure 17) indicated that the CEC values ranged between 11.59 and 21.36 $cmol\ kg^{-1}$, after 122 days of plant growth, were within the range of 15 to 25 $mg\ kg^{-1}$ for fertile soils

(Rayment, 2016). These were, however, lower than the CEC of 27.06 cmol kg⁻¹ that was measured in the original soil, which contained less Ca.

The CEC values across the FA treatments did not increase with pH and Ca beyond the 20 % FA treatment, where it had the highest value of 21.36 cmol kg⁻¹, before declining to 16.33 12.02 and 11.59 cmol kg⁻¹ in 40 %, 55 % and 60 % FA treatments, respectively. This decline in the soil CEC across the FA treatments is attributed to the negative correlation between the two parameters (Akbulut and Arasan 2010; Adari and Prasad (017). Rajpoot, Kumar and Kumar, (2018); Tulabandula and Jayanth, (2021), also established that there was a negative correlation between FA and CEC.

Both the pH and CEC also tended to stabilise between 55 % and 60 % FA treatment; whereas the soil Ca concentration increased further from about 3100 to 56000 mg kg⁻¹, across the FA treatments. The total Ca that was absorbed by the above-ground biomass, was lower at 5239, 5134, 4854, 6010 and 4652 mg kg⁻¹ for the plants that were grown in 0 %, 20 %, 40 %, 55 % and 60 % FA treatments, respectively. These figures are slightly closer to the estimated concentration of 5000 mg kg⁻¹ required for the growth of the above-ground biomass to full maturity, Marschner (2012) (see Table 1).

In conclusion, these observations suggest that the pH, Ca concentration, and CEC did not negatively affect the growth of the maize plants, across all the FA treatments.

b) Plant Nutrients

The waste assessment for the Kusile CFPP's FA indicate that its composition has no organic carbon and nitrogen. The finding of this assessment is corroborated by studies conducted by Ayanda *et al.* (2018); Basu *et al.* (2009); Chandrakar *et al.* (2018) etc., who also agree that FA generally contains all the elements that are available in the soil, but has very low C and N content.

The ratio of C to N is often used as an indicator of the balanced supply for plant development and growth (Dietzel *et al.*, 2017; Ordonez *et al.*, 2020). In this study, this ratio varied across all the FA treatments, and they were estimated based on the laboratory soil analysis to be 10.10, 11.58, 13.61, 8.67 and 13.61 for 0 %, 20 %, 40 %, 55 % and 60 % FA treatments, respectively. Similarly, these ratios were 14.01, 17.00, 20.01, 16.23 and 23.15 for the below-ground biomass; and 14.24, 16.73, 20.18, 17.61 and 34.06 in the above-ground biomass.

Dietzel *et al* (2017) pointed out that the C:N in the roots increased with depth, with C:N values ranging between 11 and 12 at soil depths of 0 to 60 cm; and ratio of 19 at soil depths between 60 and 100 cm, before the experiment and this was due to increased C in deeper depths. These values also increased overtime during the experiment, but the root C did not vary much (Dietzel *et al.* 2017). Ordonez *et al.* (2020) reported a mean C:N ratio of 30.40 after three years of repeated cultivation, with root samples collected from up to the soil depth of 210 cm, but this was lower than values reported by Dietzel *et al.* (2017) over a six year study period.

The C:N values for this experiment are within the reported literature values. Ordonez *et al.* (2020) and Dietzel *et al.* (2017), also established that there was a positive correlation between the root C:N ratios and the root to shoot (stem and leaves) (R:S ratio) biomass ratios. According to Amos and Walters (as cited in Hauck *et al.* 2015), various studies stated that the RS differs at the different maize phenological stages. The RS values of 2:3 (67 %), 1:3 (33 %) and 1:5 (20 %) were suggested for emergence stage (day 6), flowering (day 60 plus) and physiological maturity (days 118 to 125), respectively Magaia *et al.* (2016).

According to Marschner (2012), in order to achieve adequate growth to full maturity, the dry biomass of each maize plant requires about 15000, 2000, 5000 and 2000 mg kg⁻¹ of N, P, Ca and Mg, respectively each, as indicated in Table 1. The mean concentrations of these elements, which were determined in the dry above-ground biomass of the maize plants that were harvested at day 122, were mostly higher than the those suggested in literature and the variations are indicated in Table 7 below. These are presented in percentages indicating how they differ from values in cited studies.

Table 7: Above-Ground - Variation of Day 122 Concentrations vs Literature Values

	N	P	Ca	Mg
0 % FA	72.15 %	61.05 %	4.77 %	155.05 %
20 % FA	56.40 %	50.18 %	2.68 %	161.30 %
40 % FA	35.94 %	65.37 %	-2.93 %	168.70 %
55 % FA	51.03 %	30.83 %	20.19 %	185.23 %
60 % FA	-18,94 %	-8.68 %	-6.97 %	127.53 %

The bioavailability of elements in the soil solution and their interaction influence their relative absorption by plant roots and their translocation to the various parts of the above-ground biomass. The sufficient absorption of elements, deficit or over-supply has an influence on plant growth and development. Marschner (2012) states that excessive uptake of nutrients can be advantageous because these excess nutrients can act as essential reserves, which can be utilised later when the soil nutrient concentrations are low.

In other instances, the availability of other elements has an impact on the absorption of their counterparts, e.g. the absorption of Mg is negatively affected when there is availability of cations like, Ca and K, amongst others (Marschner, 1995; Gunes *et al.*, 2002, as cited by El-Dissoky *et al.*, 2017). This negative effect does not occur, though, when the concentration of the extractible Mg is higher than that of K (Schulte, 2004, as cited by El-Dossoky *et al.*, 2017); and the ratio of Ca to Mg are also indicators of whether there is a nutrient imbalance (El-Dossoky, 2017). Marschner (2012) also pointed out that maize plants tend to have selective uptake of nutrients and in some instance competition amongst some elements, which have the same valency and size of ions.

5.2 The Impact of Fly Ash on the Growth Plants

The phenological growth stages of maize plants were introduced earlier in the literature section of the report, namely, vegetative phase, which takes about 60 days, followed by the reproductive ('R') phase for another 100 days (Zhao *et al.*, 2011; O'Keeffe, 2009). Each of these phases are characterised, amongst others, by the no. of leaves developed and stem length after certain number of days after the emergence of the maize plant from the soil to full maturity at about 130 days (see Figure 1) (Zhao *et al.*, 2011; O'Keeffe, 2009). The above-ground traits such as the stem length, number of leaves, biomass accumulation, grain yield, stability/anchoring etc. depends on the ability of the roots to absorb nutrients from the soil, thus making the root system an essential part of any plant (Hauck *et al.*, 2015; Magaia *et al.*, 2015).

a) Stem Length

The stem length data, which was collected from all the maize plants, across the FA treatments, on days 24, 38, 60, 77 and 122 after emergence from the soil, showed that plants grew during the experiment. It was also observed that the plants that grew in 0 % FA treatment were mostly shorter than those in the soil that was treated with FA. This was confirmed by the ANOVA, which indicated a significant difference ($p < 0.05$) between the stem lengths, within time across the FA treatments; and within treatments across time.

This proves that FA does have a positive impact on the growth of maize plants. If the contrary was true, the plants in 0 % FA treatment would not have been shorter than their counterparts, which were grown in the FA treated soils. There is, however, evidence of stunted growth, when comparing the measured stem lengths at a particular stage against the literature, as indicated in Table 8. which could be caused by several factors such as nutrient and water deficiency. Another factor that can result in stunted growth is the under development of the root system.

These mean stem lengths across all the treatments at day 77 were shorter than those estimated for field maize, which at this stage has commenced with the transition into the reproductive stage (R). At this stage, stem lengths normally start to stabilise at 270 cm until final harvesting (O’Keeffe 2009). This stunted growth could be attributed to several issues, such as confined growth with the planting pots, the spacing of the plant in the greenhouse, amongst other differences between pot culture and field grown plants.

b) No. of Leaves

The development of the leaves over the number of days of growth also displayed an increase as expected, as indicated in Figure 9. There was also an indication of significant difference ($p < 0.05$) through ANOVA, within a time period across treatments and also within each treatment across time. This positive outcome is, however, overshadowed by the number of leaves across the treatments being lower than the literature figures for all the number of days of the maize plants’ growth.

These variances in the number of leaves could be caused by several factors, such as leaves, particularly the older lower ones, withering and falling off during the growth of the plants. Some of the leaves can succumb to diseases caused by the deficiency of nutrients or the plants having absorbed too much HMs (O’Keeffe, 2009) and heat stress (Abirdew *et al.*, 2018; Udom & Kamalu, 2019; Zhao & Nan, 2007).

c) Root length

The statistical analysis suggests that the increments of FA treatment did not result in any significant effect on the length of the roots across the treatments. A second observation in this analysis is that the mean root lengths of the plants that were harvested at days 60 and 122 across all treatments were almost similar and higher compared to those at day 77, which may provide evidence that the plants were becoming pot bound.

The root system is essential for the absorption of nutrients from the soil and their subsequent translocation to the above-ground parts of the maize plants for growth and development. According to du Plessis (2003), an unrestricted root system a maize plant can grow to a minimum of 1.5 m and 2 m sideways and downwards respectively. It is then clear that growth of roots in this experiment was also influenced by “mechanical impedance and water stress” (Magaia *et al.*, 2015), which are bound to occur in the 20 litre planting bags after several months of growth. Roots are essential for the absorption of plant nutrients from the soil and their subsequent

transfer and the growth of the above-ground maize plant structures (Hauck *et al.*, 2015; Marschner, 2012; Magaia *et al.*, 2019).

d) Mean mass of total plant biomass

The total biomass of the maize plants at full maturity is dependent on several aspects, in particular the full and healthy development of the root system (O'Keeffe, 2009; Song, Jin and He, 2019). There was more than sufficient supply of the essential nutrients to ensure adequate development and growth of the maize plants.

The lack of sufficient water, also affect plant development, most importantly after sowing the seed, since they can result in poor or reduced germination (O'Keeffe, 2009; Song, Jin and He, 2019). The lack of water or its insufficient supply can negatively affect plant photosynthesis processes, resulting in irreversible damages such as, reduced leaf area index (LAI), chlorophyll content, reproduction and biomass at later growth stages. The plants were watered adequately during this experiment which rules out water supply as a potential aspect that could affect the development and growth of the maize plants, and subsequently the mass of the biomass.

The plant biomass can also be affected by the presence of HMs in excess, because this tends to affect the optimal development and growth of the above-ground parts of the plant in many ways, which includes stunted growth, leaf health, yield etc (O'Keeffe, 2009). There was evidence of stunted growth of the stems in the experiment, which could affect the mass of the plant biomass.

5.3 The Ability of Maize Plants to Partially Decontaminate the Soil through the Phytoextraction of Heavy Metals (HMs)

This section focuses on two matters, namely, the concentration of the HMs across the FA treatments after 122 days of plant growth; and the bioavailability of HMs that were absorbed by the plants across the FA treatments, in order to determine if the plant can really decontaminate soils.

a) The Concentration of the HMs in the Soil, across the FA treatments

Manganese (Mn)

The availability of Mn, as an essential nutrient that catalyses the plant metabolism for antioxidants, is required in small amounts for efficient plant development and growth (Alejandro *et al.*, 2020; Nozulaidi *et al.*, 2016). Both the deficiency and over supply of Mn have negative

impacts on the plants, as discussed in the literature review section of this report. The concentrations of Mn in the soil ranged between 670 to 1030 mg kg⁻¹ across the FA treatments, which is within acceptable range of the legal TCT values in Table 3.

Chromium (Cr)

The non-essential HMs, such as Cr, can be toxic to plants through their increased availability in the soil and accumulation in the plants. The photosynthesis processes, transpiration of plants, stem lengths, number of leaves, leaf index, fresh and dry biomass and respiration can be affected by high amounts of Cr (Anjum *et al.*, 2016). The measured concentrations in this study ranged between 140 to 380 mg kg⁻¹, across the FA treatments, which was very low compared to the legal TCT values in Table 3.

Barium (Ba)

There are no current studies or evidence that Ba affects plant development and growth, (Nogueira *et al.*, 2010; Abreu *et al.*, 2012) and its concentrations in natural soils ranges from 100 to 3000 mg Kg⁻¹ (Nogueira *et al.*, 2010). The concentrations of Ba measured in the soil samples after 122 days of plant growth ranged lower between 60 to 80 mg kg⁻¹ and are also below the legal TCT values in Table 3.

Molybdenum

Molybdenum (Mo) is one of few essential microelements that are present in agricultural soils in average ranges between 1 to 2 mg Kg⁻¹, and is required for plant development and growth; and its availability in soils is determined by factors such as, the concentrations of iron oxides, water content and organic compounds in the soil colloids (Bodi *et al.*, 2015). The concentrations of Mo in this study ranged between 1.20 and 3.25 mg kg⁻¹ across the FA treatments and according to the figures in Table 1, a maize plant requires about 0.1 mg kg⁻¹ of Mo for efficient growth.

It has been confirmed that the presence of Mo in plants does not have any negative effects on the development and growth of maize and it is involved in essential N₂ metabolism processes, such as N₂ fixation and nitrate reduction (Bodi *et al.*, 2015; Rana *et al.*, 2020). Apart from N metabolism processes, Mo is an essential catalyst in reactions of several enzymes, such as phytohormones (Rana *et al.*, 2020). Modi *et al.* (2015), also states that insufficient availability of Mo results in reduced photosynthesis, low sugar and vitamin C content, while it physically manifests itself through rolled up plant leaf edges; narrow edges in young leaves, as well as spots; while older leaves may experience chlorosis, necrosis and stunted growth.

In conclusion, concentrations of all these HMs were within the legal TCT ranges in Table 3. This

is an indication that this FA can be used for commercial purposes, including agriculture, instead of it being left to stockpile next to CFPPs.

b) The Accumulation of HMs in the Plant Above Ground Biomass

There is a clear difference between the Mn, Cr, Ba and Mo concentrations (see Figure 18) in the soil compared to those which were measured in the below and above-ground dry biomass (see Figure 22). Figure 18 indicates that the concentrations of Mn and Cr in the soil decreased across the FA treatments; whereas that of Ba remained fairly constant and Ba increased across the FA treatments. The concentrations of Mn, Cr and Ba remained mostly higher in the soil, across all the FA treatments, compared to those absorbed by the above ground biomass; whereas the Mo was higher in the plants than in the soil. Similarly, there was high concentrations of the HMs in the below ground biomass compared to the above biomass.

It is clear from above that the plants tended to absorb Mo from the soil as opposed to Mn, Cr and Ba, thus meaning that the plants can bioaccumulate Mo in their above ground biomass more than they do with Mn, Cr and Ba. The bioaccumulation of these HMs in the plants is characterised by the bioaccumulation factor (BAF), which is a ratio of the concentration of the HMs in the plant above ground biomass to that in the soil (Aladesanmi *et al.*, 2019), and the BAFs are indicated in Table 8, across all the FA treatments.

Table 8: Bioavailability factors of Ba, Cr, Mn and Mo

BA Factor	Ba	Cr	Mn	Mo
0 % FA	0.091	0.025	0.136	0.292
20 % FA	0.045	0.034	0.095	1.370
40 % FA	0.056	0.042	0.096	1.399
55 % FA	0.063	0.059	0.148	1.852
60 % FA	0.054	0.045	0.077	0.952

NB: B/A Factor = Plant HMs Concentration/Soil HMs Concentration

The BAFs that are below 0.50 indicate that the plants absorbed less 50 % of the bioavailable element in the soil, such as Ba, Cr and Mn, across all the FA treatments; and Mo in the 0 % FA treatment (Aladesanmi *et al.*, 2019). The higher BA factors indicates that the plant can absorb and hyper-accumulate the particular HM, such Mo. This could also assist in determining the ability of maize plants in the phytoextraction of HMs from contaminated soils (Aladesanmi *et al.*, 2019; Wuana and Okieimen 2010).

It is important to note that at the same time, the soil concentrations of Mn and Cr decreased across the FA treatment, whereas those of Ba were constant and Mo increased. This suggests

that the below ground biomass retained the Mn and Cr, compared to Mo, which was translocated mostly to the above ground biomass. This could mean that, maize plants can phytoextract Mn and CR, but they are immobilised in the below ground biomass, compared to Mo.

There other factors that influence the uptake of these HMs, such as the presence of other elements, the competition amongst each other, soil properties, the chemical form of the HM, such as chelates, climate, water sources etc. (Aledesanmi *et al.*, 2019). Coupled with this is the selective absorption of these HMs by the maize plants compared to other nutrients (Marschner 2012).

It is important to note that even though maize plants can be accumulators of HMs (Wuana and Okieimen 2010), these HMs can have negative impact of the development and growth of these plants. The excessive presence of these HMs can negatively affect the plant physiology, and also be present in food, resulting in health risks (Aledesanmi *et al.*, 2019).

6. CONCLUSION

The pH of the soil increased to acceptable levels for plant growth, across the FA treatments due to the presence of Ca in the FA. This is despite the decrease in the CEC beyond the 20 % FA treatment, which is attributed to the structure and texture of the FA. There was evidence that the FA treatment enhanced the development of the plants, particularly the plants that were grown in the 40 % FA treatment, compared to those which were grown in the 0 % FA treatment. Based on the bioaccumulation factors, the above ground biomass of the maize plants also displayed a high affinity for Mo followed by Mn, Cr and Ba. The maize plant can phytoextract Mn and Cr, but it retains them in the roots, with limited translocation to the above ground biomass.

The general conclusion is that the application of FA in the soil did not negatively affect the development and growth of the maize plants, mainly up to the 40 % FA treatment.

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