ELEMENTAL COMPOSITION AND FERTILISER VALUE OF DIFFERENT TYPES OF HUMAN HAIR IN SOUTH AFRICA

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DECLARATION

I, Ntwanano Moirah Malepfane, hereby declare that:

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ABSTRACT

Salons across South African generate hair waste on a daily basis and it is disposed of at landfill sites. Human hair is an organic material that has the potential to be used as a soil amendment and nutrient source for crops, due to its high composition of nitrogen and other elements. Differences in types of hair could have effects on elemental composition, nutrient release characteristics and fertiliser value. The aim of this study was to investigate the elemental composition and the fertiliser value of African, White and Indian hair in South Africa.

The study was carried out by sampling African, White and Indian hair obtained from different salons in Pietermaritzburg, and the sampling did not distinguish age, sex, health status, geographical, livelihood of the people. The three hair types were analysed for 23 elements including nitrogen (N), phosphorus (P), sulphur (S), bases, micronutrients and heavy metals. An incubation experiment was carried out to determine the release patterns of N, P, iron (Fe), manganese (Mn), copper (Cu) and zinc (Zn) of the three hair types. The hair wastes were mixed with 100 g soil at rates equivalent to 0, 200, 400 and 800 kg N ha^{-1} and incubated for 84 days, with non-destructive sampling after 0, 14, 28, 56 and 84 days. The samples were analysed for ammonium- and nitrate-N, mineral P, and extractable Fe, Mn, Cu and Zn. A pot experiment was also set up in the greenhouse to evaluate the effect of pre-incubation time and hair type on yield parameters and uptake of nutrients by spinach (*Spinacia oleracea*).

The elements that were found to be significantly different among hair types were N, C, K, Ca, Co, Zn, Mn, Al. Indian and White hair had similar levels of N and it was higher than African hair. White hair had higher C than African and Indian hair. African hair had higher Ca than Indian hair, while White hair had similar Ca as African and Indian hair. African and Indian hair had high K than that in White hair. The Fe levels in hair were high for White>African>Indian hair respectively. African hair had high Mn, Zn and Co than the other hair types. The Al levels were high in African>Indian> White hair respectively. Incubation of the three hair types

resulted in the release of inorganic N (NH_4-N and NO_3-N), P, Cu, Zn, Mn and Fe, with mineralisation of N in the order: Indian > African > White. Leaf area, fresh weight and dry-matter of shoots and roots and N uptake of spinach increased with increase in pre-incubation time. . Effects of hair type were more evident at shorter pre-incubation time (28 days) where African hair resulted in greater dry-matter, N and S uptake, than White. Increasing pre-incubation time resulted in decline in residual soil pH, available P and exchangeable K, and had not effects on other residual soil nutrients. The minimum incubation time required for great benefit by human hair is 28 days.

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CHAPTER ONE

INTRODUCTION

1.1 Project background and justification

Large quantities of human hair waste are produced in salons as many people get their hair cut daily across the world and in South Africa. Although there is currently lack of information on the exact amount of human hair generated across South Africa, the hair waste is disposed of at landfills or incinerated, with negative effects on the environment. Incinerating hair leads to air pollution as hair has high composition of N, S and other inorganic elements (Kanwar and Paliyal, 2012; Gupta, 2014). Burning of hair leads to the release of N in the form of nitrous oxide (N_2O), S in the form sulphur dioxide ($SO_{2)}$, and carbon dioxide (CO_2), into the atmosphere. The release of these gases can contribute to increasing greenhouse gases emission. On the other hand, dumping hair at landfills tends to increase the possibilities of nitrate leaching down to ground water, after mineralisation and nitrification of the high concentration of nitrogen in the hair (Zheljazkov, 2005).

Conversely, large quantities of fertilisers are applied to improve the productivity of soils with poor fertility, particularly phosphorus (P) and nitrogen (N). However, the fertilisers are expensive and any alternatives could make a significant contribution. The use of human hair to supplement chemical fertilisers could reduce fertiliser costs, at least on a small-scale, and at the same time the practice will serve as a waste management strategy. The nutrient composition suggests that human hair could be used as soil amendment to improve soil fertility. Zheljazkov *et al.* (2008) showed that hair waste cubes contained 16.5% N, 0.01% phosphorus (P), 0.01%

potassium (K), 0.27% calcium (Ca), 0.05% magnesium (Mg), and 0.23% sulphur (S). The potential for using human hair as a soil amendment could be affected by the chemical composition of the hair, particularly N, and the rate of breakdown, which could vary among population groups.

There appears to be differences in the physical appearance of human hair among different racial groups across the world and in South Africa. It is not clear whether these physical differences could be related to chemical composition in hair. In a study carried out in Italy, Tamburo *et al.* (2015) found that elemental composition of human hair is site-specific, being determined by local environmental conditions, and that the composition may not be comparable for different places across the world. As far back as 1907, Rutherford and Hawk (1907) found differences in the percentage of N and S in different hair types from Pennsylvania, USA and concluded that the elemental composition of different hair was affected by race, gender, age and colour of hair. Japanese and Indian hair both had 15.4% N and 4.8% S and Negroid hair had 14.9% N and 4.8% S, while Caucasian hair had 15.8 % N and 5.2% S. Nicolaides and Rothman (1953) found negroid hair type to be having higher hair fat than White hair. Senofente *et al*. (2000) found significant differences in P, Cu, Se and Zn subject to age and gender, with Ca being reported to be higher in female (537 mg kg^{-1}) than males (336 mg kg^{-1}). The variation in the chemical composition among hair types could have implications on using hair as a source of nutrients for plants. In addition to environmental, racial, gender and age differences, differences in elemental composition of the human hairs can be affected by factors such as health status, livelihood and lifestyle. There is need to understand the chemical composition and release of the plant essential nutrients of the different types of human hair is to be used as soil amendments.

The main challenge of using human hair as a fertiliser material, especially as a nitrogen source, is its composition of keratin, a protein that tends to be resistant to degradation because of the cross-linking by disulphide bonds (Ignatova *et al*., 1999). Although human hair is generally known to be resistant to degradation, it has been shown to be a source of nutrients for high value crops, indicating its potential as a slow releasing fertilizer especially for N (Nustorova *et al*., 2006). When used with municipal solid waste compost, un-composted human hair has been shown to be source of nutrients for high value crops like marigold (*Calendula officinalis* (*L*.)*)*; valerian (*Valeriana officinalis* (*L*.)) and purple foxglove (*Digitalis purpurea* (L.)) (Zheljazkov *et al*., 2008). The release patterns of the nutrients from human hair needs to be clearly understood, particularly for different hair types, if they are to be used as sources of nutrients for crops.

Wastes of different hair types are produced at salons in South Africa (The Rainbow Nation), due to different racial and ethnic groups in the country, with African, White and Indian among others. There is potential to use these hair wastes as nutrient sources for plants (Zheljazkov *et al*., 2008). The effectiveness of different hair types will depend on their nutrient composition and rate of release in the soil. There is limited-to-no literature on nutrient release patterns of different human hair types. For effective management of the nutrients from human hair, it is essential to understand the nutrient release patterns. The studies by Zheljazkov (2005); Zheljazkov *et al*. (2008a, b) and Gupta and Sharma, (2014) on fertiliser value of hair, were based one hair type, which was not pre-composted and the rates of application (0, 7466.7, 14933.3 and 29866.6 kg hair/ha) were high, possibly because of the expected slow degradation of hair. Zheljazkov *et al*. (2008) suggested that time is required for the degradation of hair in order for it to release nutrients. The slow decomposition suggest that the hair could be incorporated into the soil for some time (pre-incubation), to allow for decomposition, before

plants could be grown, to benefit from the released nutrients. It is therefore essential to determine the nutrient release patterns and the minimum pre-incubation time required for greater nutritional benefits from wastes of different hair types found in South Africa.

1.2 Objectives and Hypotheses

The main objective of this study was to determine the elemental composition and the potential of using different human hair types as fertiliser. The specific objectives were to:

a) To determine the effects of hair type on elemental composition.
b) To determine the effects of human hair type and application rate on nutrient release in soil.
c) To determine the effects of pre-incubation time and hair type, as a nitrogen source, on spinach yield parameters and nutrient uptake.

The null hypotheses were:

a) Different hair types have different elemental composition.
b) Hair type affects the release of nutrients depending on the application rate.
c) The length of pre-incubation time and hair type when used as a nitrogen source affect the yield and nutrients uptake on spinach.

CHAPTER TWO

NUTRIENT COMPOSITION AND FERTILISER VALUE OF HUMAN HAIR: A REVIEW

2.1 Introduction

Human hair is made up of keratin, a protein, which is resistant to degradation because of the cross-linking by disulphide bonds (Ignatova *et al*., 1999). Different types of keratin occur and they differ in their properties depending on their function and morphology, and hence they vary in their strength. Other materials made up of keratin include horns, hooves, animal fur (including wool) and feathers (Nustorova *et al*., 2006). There are variations on the chemical composition of different keratins. Keratin material like wool and hair are differentiated by the amino acids mainly when comparing the content of cysteine (Clay *et al*., 1940). Keratin is generally resistant to degradation, but the rate differs among keratinous materials, with materials like hair degrading at a faster rate than nails (Nustorova *et al*., 2006).

Large quantities of human hair waste are being produced from salons across the world, including in South Africa, and its disposal could result in environmental pollution. Keratin materials, including human hair, could be of a valuable and as "slow-release'' fertilisers (Górecki and Gorecki, 2010). The restricting factor can be the chemical composition, which could vary with hair types.

The objective of this chapter was to review literature on previous studies done on, chemical composition, nutrient release and the potential fertiliser value from different human hair types.

2.2 Different hair types

Human hair can be an indicator of type of race one belongs to by looking at the differences in the physical appearances **(**Pruner-Bey, 1864). Human hair is usually classified based on the ethical subgroups which are African, Asian and Europeans (Buffoli *et al*., 2013). Vernall (1961) studied the size and shape of cross sections of hair from four types of men (Chinese, Western Europeans, Asiatic Indian and Negroids). From the study Chinese were found to be having the largest and most nearly round hairs; Western Europeans had the smallest hairs in cross section; the Asiatic Indian hair sections were indistinguishable in shape from those of the Western Europeans. Both are intermediate between the most nearly circular Chinese hair sections and the Negroids hair sections, which were most flattened in shape. In size of cross section, hairs of Asiatic Indians were exceeded by those of the Chinese in all respects and by Negroid hairs so far. Nicolaines and Rothman (1953) showed a difference in the fat percentage and found that Negroids had high fat percentage than whites.

2.3 Uses of human hair

Hair is a major source used to determine health status, lifestyle, sex, age and culture for forensics (Mansilla *et al*., 2011). Human hair is a major business in a place like India where its major uses includes extensions, wig, amino acids, fertilisers, ropes etc. (Jagannathan and Panchanatham, 2011). Gupta (2014) showed the various uses of human hair in different countries as wigs, fertilisers, ropes, extracting amino acids among other uses. The use of hair that is cut off and to be disposed of can be dependent on culture and ethnics (Gupta, 2014). The ethical practices of using human hair can depend on where the hair is sourced from such as hair from a superstitious person and if used as wig, they'll just prefer to burn it. Although there are

a number of uses of hair, used for centuries, mainly in Asian countries, USA and Europe, which could depend on the type, hair wastes are still disposed of at landfill sites or incinerated across the world. Keratinous materials, including hair, could be milled and baked with other wastes from animals and used as feed additives for domestic animals (Nustorova *et al*., 2006). Studies have been conducted on the best ways to utilise keratinous materials, including as fertilisers (Nustorova *et al*., 2006).

For decades different soil amendments have being used to amend soil as to improve soil fertility and crop production. Different waste products, including animal manure, sewage waste, and domestic wastes, have been used to improve soil fertility and crop production **(**Zheljazkov *et al*., 2008). Zheljazkov *et al*. (2008) showed that hair waste cubes contained 16.5% N, a high concentration when compared to other organic material like animal manure that contain about 0.2- 0.3 % N (Gupta, 2014). Whereas human hair waste is normally disposed of at landfills or through incineration, research results of Zheljazkov (2005) demonstrated that non-composted human hair waste, with an addition of municipal solid waste compost, could be used as nutrient source for crops. Human hair if used to supplement chemical fertiliser could be crucial in addressing the challenges of declining soil fertility. However, this potential is dependent on chemical composition and rate of degradations and nutrient release in soil. South Africa is a rainbow nation with different races, tribes, customs, and religions, resulting in differences in hair types and possible uses. There is a paucity of literature on the uses of human hair and the possible acceptability of human hair as a source of nutrients for plants, either for food crops or flowers, in Sub-Saharan Africa, where African hair dominates.

2.4 Chemical composition of human hair

Human hair is made up of different structures which makes it complex, and these include proteins, amino acids, lipids, elements and pigments (Robbins, 2012). Hair consists of about 65 to 95 % proteins, which are condensation polymers of many different amino acids that have different structures and molecular weights. The proteins are some of the amino acids found in hydrolyzates of human hair include glycine, alanine, valine, and the sulphur containing amino acids; cystine, methionine, cysteine, cysteic acid (Sharma *et al*., 2011). These amino acids that contain sulphur have the cross-linking disulphide bonds which make them resistant to degradation (Ignatova *et al*., 1999).

Most functional groups like hydrocarbon, hydroxyl, primary amide, and basic amino acid occur at higher frequency than disulphide bond in hair (Robbins, 2012). A strand of human hair consists of four bonds which are peptide, hydrogen, salt and disulphide and the disulphide is the second strongest bond in the hair (Daniels, 1953**).** The cross-linked disulphide bond gives the polypeptide chain great strength such that it cannot be broken by heat or water. The bond is formed when a cysteine sulphur atom on one polypeptide chain links with cysteine sulphur atom on neighbouring polypeptide chain **(**Ignatova *et al*., 1999).

The main constituent elements of human hair are carbon, hydrogen, nitrogen, oxygen and sulphur (Robbins, 2012). Other elements that are mostly found in human hair are phosphorus, potassium, calcium, magnesium, sodium, zinc, copper, manganese, iron, molybdenum, selenium, boron, aluminium, strontium, silver, mercury, arsenic, lead, antimony, titanium, tungsten and vanadium. Although heavy metals in human hair could limit its potential as a

source of nutrients, the concentrations are usually low to present any risk (Robbins, 2012). However, the elemental composition of human hair is affected by a number of factors.

2.4.1 Factors affecting elemental composition of human hair

Human hair is a complex structure that has different factors affecting it depending on the chemical composition, which differs among hair types. Human hair have been studied in different parts of the world such as in Europe, Asia and America but little or no studies have been done on the chemical composition of hair types in Sub-Saharan Africa including South Africa. The chemical composition of hair can be affected by many factors such as diet, age, gender, environmental conditions and race (Rutherford and Hawk, 1907). Huelsemann *et al.* (2009) demonstrated a study from hair of four individuals, two males and two females who were analysed for stable isotope of $\delta^{13}C$ and $\delta^{15}N$. The diet was a change from C3 to C4 plant enriched diets (corn, millet, amaranth and cane sugar) and immediately replacement of terrestrial animal products (poultry, pork and beef) by marine products in the form of tuna and cuttlefish (sepia) and resulted in a significant increase in stable isotope of $\delta^{13}C$diet of 8.5 to 9.9% and in stable isotope $\delta\ ^{15}N$diet of 1.5 to 2.2%. O'Connell and Hedges (1999) demonstrated high nitrogen isotopic values of hair keratin in oxfords residents that consumed diets of omnivores and lacto-ovo-vegetarians than vegetarians. From the literature the high intake of protein in diet leads to increase in the amount of N in hair. The differences in the cultures, customs and stable foods in the dominate races in South Africa could contribute to the elemental composition in different hair types.

Senofonte *et al.* (2000) found the elemental composition between three age groups which were: 3-6, 7-10 and 11-13 years to have been significantly different. The concentrations were

different among 3-6 and 7-10 years of Ca, Cu, P, Se and Zn; among 7-10 and 11-13 years the concentrations of As, Cu, Mo, Se and Sr; and 3-6 and 11-13 years As, Ca, Cd, Co, Mo, P and Zn were different. The other elements Al, Cr, Fe, Mg, Ni, Pb and Ti were found to have been significantly different among all the age groups. In relation to gender Clay *et al.* (1940) found high cysteine in males than females and no significant differences in N and S between males and females. The study conducted by Hrdy (1973) demonstrated the differences in diameter, medullation, scale count, kinky, curvature of hair among seven groups (Bougainville, Malaita, East Africa, Northwest European, Sioux, Ifugao and Japenese) which were sampled from different places. The African hair was found to be high in kinking than the other studied populations and the Malaitan and Bougainville were the only ones that showed some kinkiness (Hrdy, 1973).

The chemical and elemental composition of hair can be used to evaluate diseases, health conditions, and environmental exposure etc. (Puchyr *et al*., 1998). According to Buffoli *et al.* (2013) human hair is classified into African, Asian and European hair, which are the three conventional ethnic human subgroups that distinguish human hair and they differ in the macro-structure of hair such as the length, diameter, colour and cross-sectional shape among the different ethics groups. From Hrdy (1973) study on seven population variations found Mongoloid to have higher diameter than Ifugao, Sioux, Japanese, African, and European. The main cause of the differences among population was stated to have been inscribed by genetic difference and environmental exposure which has a crucial role on the chemical and physical composition of hair (Hrdy, 1973). This could be an indication of effect that a factor like environment can play on the elemental condition. The study done by Tamburo *et al.* (2015) on the coverage intervals for trace elements in human scalp in urban area of Palermo, small towns around Mt. Etan and Sardinia. No work has been done on chemical composition of human hairs

in Sub-Saharan Africa and it potential as a source of nutrients for plants. Creason (1975) stated that certain elements from hair can be a reflection of the community (Metropolitan) exposure. In the study conducted by Creason (1975) found that certain elements (Ba,Hg, Pb and V) were significant amoung age and gender due to environmental exposure such as house dust while elements like B, Cd, Cu, Fe, Li, Mn, Ag and Zn showed no significant. The enviromental exposure that differs among hair types may play a crucial role in the elemental composition of hair. The physical structure and chemical composition of hair can have effects on how a particular hair type release it nutrients as it can be affected by the compounds and biochemical variations.

Human hair studies from different places have been conducted (Gupta and Sharma, 2014; Zheljazkov *et al*., 2008; Senofonte *et al*., 2000). Gupta and Sharma (2014) conducted their study in India whereas Zheljazkov *et al.* (2008) conducted their study in America and Senofonte *et al*. (2000) conducted their study in Italy. Some parameters were higher, lower or similar among the hair types. Differences in the elemental concentrations could be due to the different digestion methods and analyses, type of hair, age, and environmental exposure among other factors (Senofonte *et al*., 2000; Puchyr *et al*., 1998). Geographic differences and the type of hair could also explain the differences in the concentrations of the elements. Human hair contains in essential macro- and micro-nutrients for plants such as N, P, K and S, which could be released to and be beneficial for improving soil fertility and plant growth and development. However, strategies need to be done to release these nutrients from hair waste.

2.5 Strategies to aid degradation and nutrient release from human hair and other keratinous materials

The breakdown of keratinous materials is very curial for their use in the agriculture, a number of strategies have been tested to enhance the process (Moreira *et al*., 2007). The release of nutrients from keratinous material can be enhanced by hydrolyses, microorganisms and under optimum conditions that can allow the breakdown of the di-sulphide bonds.

The degradation of the keratinous material such as feathers can be enhanced by thermal hydrolysis in dilute acid or base, or enzymatic digestion by specific proteases (keratinases) (Matikevičienė *et al*., 2009). In the study by Choi and Nelson (1996) N was released from feathers through hydrolysis with *Bacillus licheniformis* (Weigmann), steam hydrolysis (autoclaving), formaldehyde compared to raw feathers that were not treated. Raw feathers also released substantial N, suggesting the capability of the keratinous material, like feathers, to release constituent elements without treatment.

Choi and Nelson, (1996) showed the mineralisation of N from feathers, indicating that about 100 mg N/L of substrate could be released within 3 three weeks. The decline in nitrate-N and ammonium-N could have been due to supressed N by the microorganisms over time. Based on the chemical composition, human hair can be considered highly resistant to degradation. The keratin in human hair is resistant to degradation by proteolytic enzymes (trypsin and pepsin) because of the cross-linking by disulphide and hydrogen bonds (Ignatova *et al*., 1999). However, some microorganisms have the ability of breaking down the fibre as they use hair as source of their nutrients. The dermatophytic and non-dermatophytic microorganisms have the ability to degrade human hair (Wilson *et al*., 2007; Lal *et al*., 1999). Dermatophytes are a group

of fungi which cause infections of the skin, hair and nails; tissues that consist of keratin (Daniels, 1953) and tend to attack non-living cells. Several studies have conducted on the ability of the degradation of human hair (Sharma *et al.,* 2011; Muhsin and Hadi, 2002; Wilson *et al.,* 2007; Lal *et al.,* 1999 and Daniels, 1953).

Chrysosporium indicum colonisation was found to result the highest rate of degradation while colonisation by *Microsporum gypseum* was found to result in high amount of the product (Sharma *et al.,* 2011). Using *Chrysosporium indicum* and *Microsporum gypseum* can increase the rate of degradation if there are favourable conditions for these species. Daniels (1953) demonstrated that *Microsporum canis* (Bodin), the causative agent of animal ringworm in children and adults, is able to digest human hair keratin in vitro. It was shown by chromatographic techniques that amino acids accumulated in cultures containing human hair as sole source of nitrogen, after growth of *M. canis* for 20 days at 20 ^{o}C. The source of these amino-acids is the keratin of the hair, suggesting that the amino acids were produced by the digestion of hair keratin by the fungus. The ability of two species of *Bacillus* to degrade child's scalp hair, cow horn, hooves and human nails in vitro under static conditions was showed in a study by Lal *et al*. (1999). Child's scalp hair was found to be the most favoured keratin substrate by *Bacillus* spp. and was broken down easier when compared to other keratin materials.

Muhsin and Hadi (2002) studied four fungal species, including two dermatophytes and two saprophytes, for their degradation ability of three keratin substrates (human hair, chicken feathers and wool) liquid culture medium over three weeks of incubation. Human hair had the highest degradation rate with *Chrysosporium pannicola* and *Microsporum gypseum*. Human hair can break down fast than feathers and wool, however, more studies need to be done on the breaking down of hair at a larger scale. Keratinase activity was highest for *C. pannicola* and

M. gypseum in the culture medium with human hair and produced alkaline pH (Muhsin and Hadi, 2002). However, when applied in soil there was an opposite trend (Zheljazkov *et al.,* 2008). This could have been due to the conditions each study was conducted. In soil is like an open space where, when the hair break down there was a release of Nitrate and SO_4^- which supresses the pH and is dependent on other factors such as temperature, moisture, aeration etc. The process of human hair degradation is dependent on the dermatophytes conditions like optimum temperature, pH, moisture content etc (Wilson *et al*., 2007). *Chrysosporium indicum* colonisation was found to have the highest rate of degradation of human hair and the colonisation by *Microsporum gypseum* was found to contain high amount of protein (Sharma *et al*., 2011). Using *Chrysosporium indicum* and *Microsporum gypseum* can increase the rate of degradation if there are favourable conditions for these species. These and other dermatophyte contribute to the degradation of human hair added to soil.

Human hair can also be used as compost, where it is used with different types of organic and inorganic materials such as wood, animal manure, boxes etc (Kanwar and Paliyal, 2012). Using human hair as compost can slowly release nutrients to the soil as it degrades slowly. However, mixing hair with other compost material can increase the rate of degradation through the dermatophytic microorganisms that may be found in the composts and other wastes. the keratinolitic and dermatophitic organisms can be recovered from soil that have them (Lal *et al*., 1999; Sharma *et al*., 2011).

Using microorganisms like *M. gypseum* can increase the amount of nutrients that are released in soil (Sharma *et al*., 2011). The positive effects of using dermatophytic microorganisms are the fast release of nutrient, and the increase in the concentration of NO_3^- and NH_4^+, that are easily up taken by plants for growth and development. The negative effect of using these dermatophytic microorganisms may be pathogenic to humans and animals. Zheljazkov *et al*.

(2008) found human hair to have increased NH_4– and NO_3–N concentrations at harvest in the soil, indicating that degradation of hair occurred even without treating the hair or application of hair degrading microorganisms. It would be essential to understand nutrient release patterns of raw human hair added to soil in order to decide on the potential of the hair type as a fertiliser material.

2.6 Effects of keratinous materials in soil and plants

The high content of N in keratinous materials suggest their potential to be slow release fertiliser materials (Choi and Nelson, 1996; Górecki and Gorecki, 2010; Nustorova *et al*., 2006). The use of feathers that were hydrolysed with *Bacillus licheniformis* was shown to have the potential to be a slow release fertiliser for glasshouse plants by Choi and Nelson (1996).

Zheljazkov *et al.* (2008) conducted an experiment where using human hair as soil amendment resulted in greater concentration of Ca, S and Cu, which are essential for plant growth and development. Zheljazkov *et al.* (2008) found the addition of hair-waste to soil to increase soil inorganic N concentrations (NH_4–N and NO_3–N) at harvest and resulted in greater yield of valerian root and rhizome by five to six times when compared with the un-amended control. Soil pH was found to decrease, from 6.6 to 4.1, possibly due to mineralisation high concentrations of N and S compounds.

The high concentration of inorganic N (NH_4 and NO_3) Coupled with the decrease in pH could have significant effects on the fertility of soils and availability of nutrients, depending on the type of crop. However, results from Zheljazkov *et al*. (2008) demonstrated that un-composted

waste-wool and hair could be used as nutrient source for crops, with no negative effects on soil microbes, but could reduce soil pH at high application rates.

Adetunji *et al*., 2012 showed that the organic fertiliser was more effective on yields and productivity of cowpeas when the soil was amended with microbially hydrolysed feathers. If feathers was found to have been a good potential as an organic fertiliser, there can be a potential of human hair which is cheap and available to be a good organic fertiliser. When used as soil amendment human hair could release nutrients and other elements that may have effects on soil quality aspects such as mycorrhizae and microbial community. Mycorrhizal fungi play a key role in the nutrition, water relations, and pest resistance of crops, especially in low-input systems (Galvez *et al*., 2001).

In addition to C, N, S and P, human hair also contains significant amounts of elements Ca, Mg, Sr, B, Al, Na, K, Zn, Cu, Mn, Fe, Ag, Hg, As, Pb, Sb, Ti, W, V, Mo, I and Se. While some of the elements that can be released by degradation of human hair are essential for plant growth others, like Hg, Pb and As are not and could accumulate in the soil with continuous application of hair as a nutrient source. These effects could be affected by the hair type and release patterns of the elements when the hair is added to soil.

2.7 Different hair types in Sub-Saharan Africa and their potential as fertiliser material

Several studies done on the differences among hair types (Pruner-Bey, 1864; Clay *et al*., 1940; Nicolaides and Rothman, 1953; Rutherford and Hawk, 1907; Steggarde, 1940; Steggerda and Seibert, 1941; Hrdy, 1973; Hrdy and Baden, 1973; Buffoli *et al*., 2013; Vernall, 1961; Halat *et al*., 2009), and those on different racial groups showed differences on the chemical composition

and the morphological structure of hair types. Other causes of differences in hair chemical composition were gender, age, environmental exposure, and health status. The potential for use of hair types as a fertiliser could depend on the demands for the particular hair type for other uses. For example, Indian hair is in greater demand for use in wigs and extensions than African and White hair (Jagannathan and Panchanatham, 2011).

The Sub-Saharan African region is dominated by African people and as such the dominant hair waste type could be African hair, which would not be suitable for other uses like making wigs, because of it physical structure which is kinky and very round (Hrdy, 1973). In addition to the majority African people, South Africa also has significant proportions of Indian and White populations. The chemical composition of the hair types could differ from those from other regions of the world due to the effects of the local geographical area (Tamburo *et al*., 2015). Difference in the racial groups have differences in appearance of their hair, which could also result in differences in chemical composition (Rutherford and Hawk, 1907). The African hair appear to be kinky and curly, whereas the White and Indian hair is straight and fluffy. There could be differences in the elemental composition of the hair types in South Africa. The differences among hair types especially the elemental composition could have effects on mineralisation and the release of nutrients and other elements.

2.8 Conclusion

Even though human hair has other uses, in Asia, Europe and America, the bulk of hair waste still ends up at landfill sites and its use as fertiliser could be a viable option, but more research is required on wastes of human hair types in South Africa. While there is potential to use human hair as nutrient sources in South Africa, the elemental composition and release patterns among

hair types need to be understood. Based on the slow breakdown of raw hair, knowing the minimum pre-incubation time for breakdown and release of sufficient nutrients could have great benefits in using the different hair types as a fertiliser. The possible differences in the elemental concentration (particularly N) among different hair types can determine the amount of hair that should be applied to add a specific amount of nutrients to soils. There is a need to understand the elemental composition, release and effectiveness as sources of nutrients of the major hair types in South Africa.

CHAPTER THREE

ELEMENTAL COMPOSITION OF DIFFERENT HUMAN HAIR TYPES

3.1 Introduction

A large proportion of human hair waste produced at salons is either disposed of at landfill sites or incinerated as waste disposal strategy, with negative effects on the environment, through leaching of nitrates to groundwater or by release of sulphur dioxide and nitrous oxide gases into the atmosphere (Kanwar and Paliyal, 2012; Gupta, 2014). Hair is a tissue in the human body which has a unique character that is able to accumulate most of the elements found in the body as it is an excretory tissue and also more like a storage organ **(**Senofonte *et al*., 2000; Bowen, 1979). Human hair is made up of amino acid cysteine, the main component of keratin, the protein resistant to degradation due to the cross- linking by disulphide bonds, and as such hair contains about 5 % of sulphur (Kosanovic and Jokanovic, 2010). Since keratin is a protein, human hair contains high concentrations of N. The extent of environmental pollution, as a result of landfill disposal or incineration of human hair could depend on its chemical composition.

There is a number of research articles on research done to see the concentration of elements in human hair (Puchyr *et al*., 1998). Senofonte *et al*. (2000) analysed 19 elements to assess reference values for hair samples of young people between the age of 3 and 15 years from various urban areas of Rome. The overall analysed elements (mg/kg) were P (195), Ca (450), Mg (28.0), Zn (150), Fe (19.0), Cu (22.1), Mn (0.35), Mo (0.43), Co (0.67), Cr (0.99), Al (10.2), Se (0.77), As (0.09), Cd (0.23) , Ni (1.49), Pb (7.11), Sr (1.20), Ti (0.79), V (1.22) (Senofonte

et al., 2000). There is currently limited literature that compared the elemental composition of different hair types. Nitrogen, S and bases differ among hair type depending on the environment exposure and genetic variations. Rao *et al.* (2002) found that Fe, Mn, Zn and Cu contents in hair samples of Indian residents increased with age while the Co content decreased, whereas there were no age and gender effects on Ni, Cd and Pd. However, Halat *et al*. (2009) found no chemical or significant fine structural differences in among hair samples from different types although the results were not shown. The higher concentrations of N and S and trace elements in some hair types suggest that their degradation at landfill sites or through incineration could have greater contribution to environmental pollution than others. Application of human hair to the soil as a nutrient source could be beneficial in maintaining appropriate nutrient levels and reducing leaching losses due to uptake of the nutrients by plants. Differences among hair types in terms of plant essential nutrients could be crucial in their contribution for soil fertility and plant growth and development (Zheljazkov *et al*., 2008).

South Africa is referred to as a rainbow nation because of the different population groups, tribes and races that are in the country. The most dominant types are African, Coloureds, Indians and Europeans (White) and some salons in South Africa work with hair types from these different population groups. The potential environmental pollution and soil fertility benefits of wastes from the different hair types depend on their elemental composition. The objective of this study was to determine the effects of hair type on elemental composition of human hair from the major population groups in South Africa.

3.2 Materials and Methods

The study was conducted in Pietermaritzburg **(**29°37'33.9"S 30°24'14.0"E**)** in the KwaZulu-Natal Province of South Africa.

3.2.1 Human hair collection

Human hair waste used in this study was sampled from different hair salons in Pietermaritzburg after the approval of an ethical clearance from the University of KwaZulu-Natal's Humanities and Social Sciences Research Ethics committee. The African, European (White) and Indian hair waste cut from different salons, and was to be disposed of, was collected into plastic bags and stored. African hair was collected from salons in Scottsville and in the City Centre of Pietermaritzburg, while those for White and Indian people were collected from salons in Hayfields, Scottsville and Cascades areas of Pietermaritzburg. The salons were requested to place different hair types in separate bags without distinguishing the hair source by age, sex, livelihood, health status, environmental conditions. The hair was cut using a stainless steel scissors into small pieces of approximately 0.5 cm and samples of each hair type, from different salons, were mixed thoroughly to obtain a composite sample, before analyses for elemental composition. The hair was not washed before analysis (Assarian and Oberleas, 1977).

The samples were analysed for total carbon (C), nitrogen (N), phosphorus (P), potassium (K), sulphur (S), calcium (Ca), magnesium (Mg), sodium (Na),manganese (Mn), iron (Fe), copper (Cu), zinc (Zn), cobalt (Co), nickel (Ni), molybdenum (Mo), selenium (Se), mercury (Hg), lead (Pb), aluminium (Al), chromium (Cr), arsenic (As) and cadmium (Cd).

3.2.2 Elemental analyses

Total C and N were analysed in triplicate by a Leco TruMac CNS/ NS Carbon/Nitrogen/Sulfur Determinators (LECO Corporation, 2012). Sulphur, Ca, Mn, Fe, Cd, Mo, Si, V, Li were analysed, in duplicate, by Talbolt and Talbot Laboratories using the inductively coupled plasma-mass spectrometry (ICP-MS) (Yamashita, 2000; Puchyr *et al*., 1998) after aqua-regia (HCl/HNO_3 mixture) digestion. Potassium, Pb, Se, Zn, Cu, Cr, Ni, Hg, As, Co, Na, Mg, Al, Sb, Ba, Be, Bi, Sn and Ti were determined, in triplicate, by ICP-MS after digestion of 0.5g with 12 ml of 5:1 HNO_3:H_2O_2 acid mixture (v/v) followed by dilution to 50 ml with deionized water (Rao *et al*., 2002).

3.2.3 Energy-dispersive X-ray analyses

The hair samples were mounted on metal stubs with carbon tape in order to secure them and coated with gold using EIKO IB.3 ion coater. The coated samples were viewed with an X-Max energy-dispersive X-ray spectrometer (EDX, Oxford Instruments, Abingdon, Oxfordshire, UK).

3.2.4 Statistical analysis

The results of the elemental analyses were subjected to one-way analysis of variance (ANOVA) using GenStat 14th edition (Payne *et al*., 2011). Mean separation was done using least significant differences (LSD) at $p < 0.05$.

3.3 Results

3.3.1 Carbon, nitrogen, phosphorus and sulphur concentrations of hairs

Hair waste of Indian and White had similar levels of N, which were higher than African hair and the N content in the hairs ranged 10-13 % (Table 3.1). White hair had higher C concentration than those of Indians and Africans, which were similar, and the C content in hairs ranged 31-38 % (Table 3.1). The C:N ratios ranged 2.5-3 and were in the order: African=White >Indian. The trend of P results (ranging 230-250 mg/kg) was similar to that of total C, although there were no significant differences among the hair types. The trend of S (ranging 3-5 %) was opposite of that of the C:N ratio with the order: Indian>White>African.

Table 3.1: Concentrations of carbon, nitrogen, phosphorus and sulphur in hair types.

Elements	White	African	Indian	LSD
Carbon (%)	38.6[a]	31.0[b]	33.2[b]	2.6
Nitrogen (%)	13.9[b]	10.1[a]	13.4[b]	0.52
C:N	2.8:1[b]	3:1[b]	2.5:1[a]	0.27
Phosphorus (mg/kg)	256[a]	238[a]	239[a]	238.0
Sulphur (%)	4.4	3.5	5.0	nd[#]

Values followed by the same letter are not significant. #nd = not determined.

3.3.2 Concentrations of bases in different types of hairs

The Ca concentration in hairs ranged 1000-4000 mg/kg and African hair had higher levels than Indian hair (Table 3.2). White hair had similar levels of Ca than those of African and Indian

hairs. The three hair types did not differ in terms of Mg (ranging 120-300 mg/kg) and Na (ranging 400-900 mg/kg). However, African and Indian hair had similar K levels, which were higher than White hair. The K concentrations ranged 200-600 mg/kg.

Table 3.2: Concentrations of bases in three hair types

Element	Concentration (mg/kg)			
	White	African	Indian	LSD ($p<0.05$)
Calcium	3858[b]	4643[b]	1576[a]	2487
Magnesium	254[a]	327[a]	123[a]	226
Potassium	232[a]	647[b]	601[b]	92
Sodium	457[a]	924[a]	736[a]	590

Values followed by the same letter are not significant

3.3.3 Concentrations of micronutrients in different hair types

The Fe concentrations in the hairs ranged 318-442 mg/kg and was in the order: White > African > Indian. African hair had greater Mn, Zn and Co than White and Indian hair. The Mn concentrations in the hairs ranged 3-11 mg/kg. There were no significant differences among hair types in terms of Cu, Mo, Se and Cr (Table 3.3). The ranges of concentrations in mg/kg were 16-33 for Cu, Zn 142-256 for Zn, 13-14 for Mo, 1-2 for Se, 0.2-0.4 for Co and 1-2 for Cr.

Table 3.3: Concentration of analysed micro-nutrients of three hair types.

Micro-nutrients	Concentration (mg/kg)			
	White	African	Indian	LSD ($p<0.05$)
Iron	442.1[a]	368.5[b]	318.4[c]	15.2
Manganese	3.2[b]	11.2[a]	3.4[b]	4.4
Copper	33.1[a]	16.3[a]	20.2[a]	18.6
Zinc	184.7[a]	256.0[b]	142.6[a]	58.5
Molybdenum	13.0[a]	14.8[a]	13.7[a]	28.6
Selenium	2.1[a]	2.3[a]	1.9[a]	0.98
Cobalt	0.32[a]	0.45[b]	0.20[a]	0.15
Chromium	1.1[a]	2.5[a]	1.4[a]	1.9

Values followed by the same letter are not significant

3.3.4 Heavy metal concentrations in different types of hair

Aluminium levels in the human hairs were in the order: African > Indian > White, and ranged 142-460 mg/kg. There were no significant differences among hair types in terms of As, Cd, Pb, Hg and Ni (Table 3.4). The concentrations ranges were 0.07-0.1 mg As /kg, 0.07-0.15 mg Cd /kg, 3.1-3.4 mg Pb /kg, 2-3 mg Hg /kg, 2-3 mg Ni /kg.

Table 3.4: Heavy metal concentrations in different types of human hair

Heavy metal	Concentration (mg/kg)			
	White	African	Indian	LSD ($p<0.05$)
Aluminium	142[a]	460[b]	320[c]	120.4
Arsenic	0.07[a]	0.08[a]	0.10[a]	0.039
Cadmium	0.15[a]	0.07[a]	0.13a	0.183
Lead	3.14[a]	3.42[a]	3.26[a]	1.31
Mercury	3.68[a]	3.98[a]	2.30[a]	5.43
Nickel	2.79[a]	3.23[a]	3.69[a]	2.12

Values followed by the same letter are not significant

3.3.5 Energy-dispersive X-ray

The EDX releaved the high presence of C and O in hair (Figures 3.2, 3.4 and 3.6). The peaks of S were in the order: Indian> White> African hair, and followed the same trend as the results of the concentrations based on ICP analysis. Elements like N, S, Fe, Ca, K, Fe, Zn and Al that were significantly different among the hair types, in the concentrations, also showed peaks at EDX.

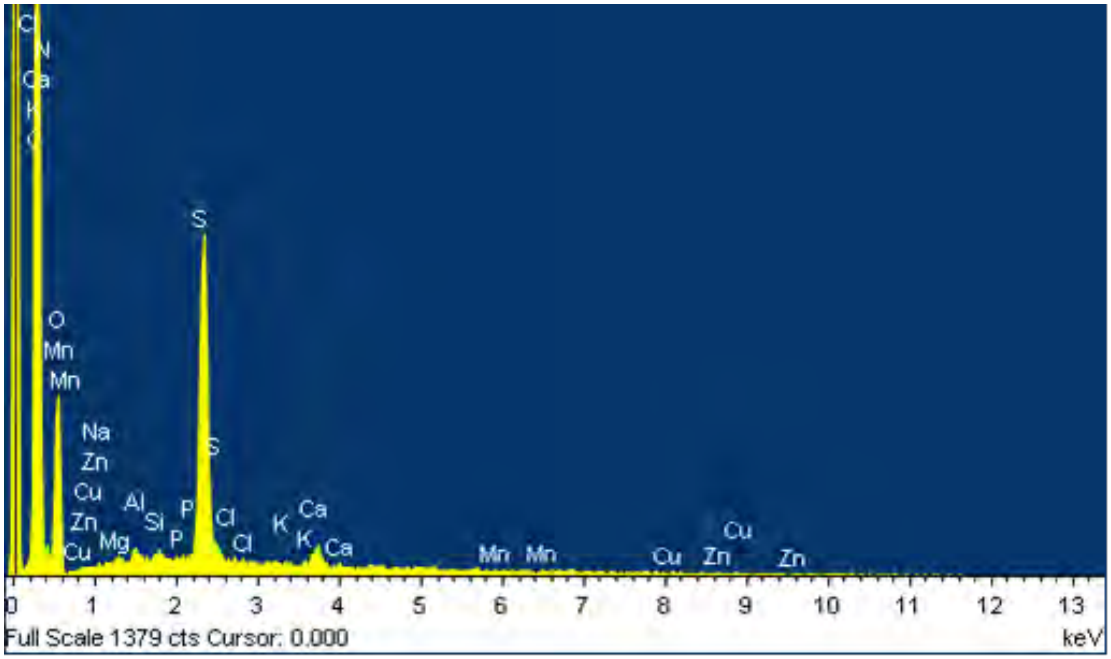

Figure 3.1: Energy dispersive x-ray (EDX) microanalysis spectra of White hair.

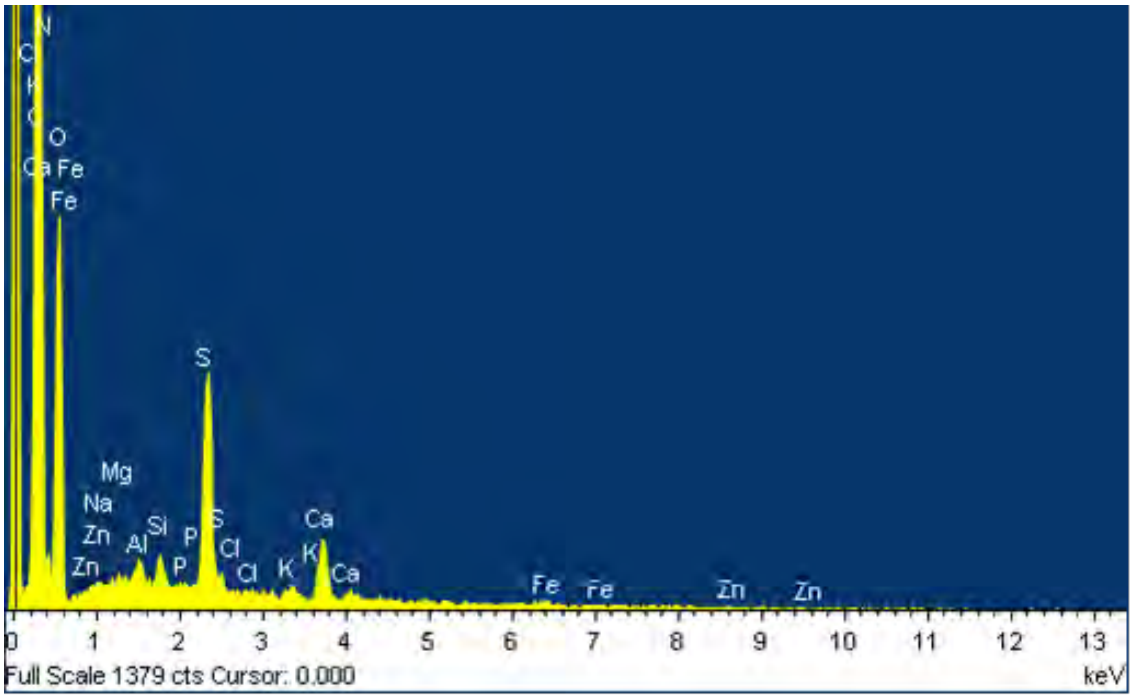

Figure 3.2: Energy dispersive x-ray (EDX) microanalysis spectra of African hair.

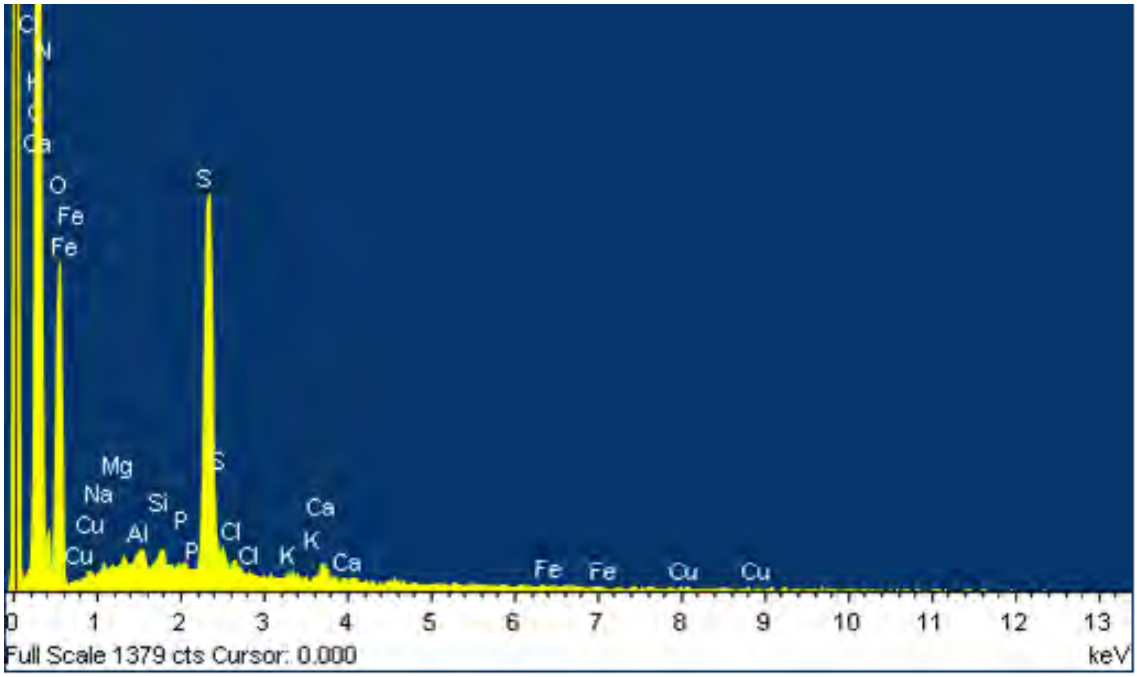

Figure 3.3: Energy dispersive x-ray (EDX) microanalysis spectra of Indian hair.

3.4 Discussion

The similar levels of N in the Indian and White hair waste could have been ascribed similarity in amino acid composition, which could be having high content of N than in African hair waste, possibly due to genetic factors (Hrdy, 1973). In addition to possible genetic variations, diet could also contribute to these differences (O'Connell and Hedges, 1999). However, Hrdy and Baden (1973) found the biochemical variation of different hair types to have been insignificant. O'Connell and Hedges (1999) showed that nitrogen isotopic values of hair keratin is dependent on the animal protein consumed in the diet, with omnivores and lacto-ovo-vegetarians having higher N than vegetarians. The N results found in this study were lower when compared with the N content found by Bowen (1979), Rutherford and Hawk (1907), Clay *et al.* (1940) and Zheljazkov *et al.* (2008). However, the N results were in the same range as those of hair wastes used by Zheljazkov *et al.* (2008) and Gupta and Sharma (2014). The lower levels could have been because composite samples of hair waste were used in this study without distinguishing

the sources by age, gender, and livelihoods, among other factors (Zheljazkov *et al.*, 2008). Since similar hair types were used in this study as in the other studies, geographical differences, which affect diets and other lifestyles, could have also contributed (Vernall, 1961; Senofonte *et al*., 1999; Tamburo *et al.*, 2015; Creason, 1975).

The C:N ratio for the African and White hair were similar to 3.2:1 from the literature (Gupta and Sharma, 2014; Zheljazkov *et al*. 2008; Rutherford and Hawk, 1907; Clay *et al.,* 1940), while the Indian hair had lower ratios. The C:N ratio results could suggest that wastes of African and white hair could degrade at a slower rate when compared to Indian hair. However, the overall degradation could depend on other factors including the chemical structures in the hair. Although there were no differences in P concentrations among the different hair-types used in this study, the concentration were higher than those in the literature (90- 200 mg/kg) (Senofonte *et al*., 1999; Gupta and Sharma, 2014; Zheljazkov *et al*., 2008), and this could be because the hair wastes were not washed before analysis.

The similarities in S in Indian and White hair could possibly have been due to the similarities in the genetic variation of these hair types. According to Robbins (2012) levels of S are affected by the level of nutrition. The lower level of S in the African hair could have been affected by the lower concentration of Cu. Although the Cu composition of the hairs was similar, African hair generally had lower levels than the other hair types. During keratinization Cu oxidises cysteine to cystine (Robbins, 2012) and this can also show from the kinkiness of African hair. The kinky hair of African hair could be due to the unusually high thiols level of cysteine, in which approximately 50% of the cysteine is oxidized to di-sulphide bonds during keratinization. Rutherford and Hawk (1907), in a study of the four racial groups (Caucasians, Indians Japanese and Negroes) found the range of S to be 4-5 % among hair types. As a result

of little or lack of literature on the different hair type on elemental composition the results of the elemental composition found in this study is compared with results from other research on elemental composition of hair of similar type. The concentration of S in White hair were in the same range as results found by Seidel *et al*. (2001).

The causes of the lower Ca and higher K in African and Indian hair than White hair were not clear but they could possibly be the effect of diet, which was not studied. Senofonte *et al*. (1999) found low concentration of Ca when compared to the concentrations found in this study. The higher concentration of Zn, Fe and Mn in African hair, than in the other hair types, could have been because of differences in diet and the hair products that are used, that may contain higher Zn concentrations than those used for the other hair types (Robbins, 2012), although these aspects were not studied. Sukumar and Subramanian (2007) found low levels of Zn in hair of urban than rural subjects, and explained the results using differences in diets. Although diets were not investigated, differences are generally known to exist among the population groups studied. The high levels of Al in African hair than in other hair types could be ascribed to Hart and Bon Voyage cooking pots, made with Aluminium, and are commonly used among African people, together with water and food they consume (Krewsk *et al.*, 2007).

The results of Pb, Cr, Ni and Cu concentrations in Indian hair type were similar to results found by Sukumar and Subramanian (2007) for the same hair type. Although environmental conditions like pollution of air, soil and water, diets and other life-styles (including smoking) could affect heavy metal composition of human hair (Robbins, 2012), there were no differences in this study. Significantly high concentrations of Ni, As, Cd and Hg were found in hair of children living in urban areas than those in rural areas of the United Arab Emirates by Hasan *et al.* (2004). The similarity of heavy metal concentrations in this study could be because

composite samples that did not distinguish gender, age, living standards and other life-styles were used.

Halat *et al.* (2009) found insignificant differences on chemical composition among hair type from different races. The S peaking on EDX peaks at the same energy at 2.3 Kev on the spectra as the results found by Zheljazkov *et al.* (2008).

3.5 Conclusions

Hair waste from the different population groups differed in elemental composition, with higher concentration of N in Indian and White, S and K were higher in Indian, and Ca, K, Mn, Zn, Co and Al in African hair, than the other hair types. The levels of the other elements occurred at similar levels among the hair types.

CHAPTER FOUR

NUTRIENT RELEASE PATTERNS FROM DIFFERENT HUMAN HAIR TYPES

4.1 Introduction

The potential of an organic waste as a source of nutrients depends on its rate of decomposition and mineralisation of the elements (Adediran *et al*., 2003). Organic sources such as animal manure, plant residue, municipal waste compost and sewage waste are used to sustain and maintain soil fertility and crop production, through the mineralisation of N, P and S among other plant essential nutrient elements. Human hair is among the organic wastes that can be used to improve soil fertility as it contains about 15 % N (Zheljazkov *et al*., 2008; Rutherford and Hawk, 1907) and about 5 % S (Rutherford and Hawk, 1907; Bowen, 1979; Robbins, 2012) among other elements. Hair waste is mainly disposed of at landfill sites, which could cause a serious environment risk through nitrate leaching into groundwater, when the waste decomposes and the N nitrifies (Gupta and Sharma, 2014; Kanwar and Paliyal, 2012).

Nitrogen is a crucial nutrient that is required by plants (Abdelmagid, 1980). Plants can only take up N in the form of NO_3-N and NH_4-N (inorganic N). Using human hair as a source of nutrient for plants can be of great benefits to soil fertility and crop production due to the high N and it can also act a slow fertiliser release as it break down slowly (Nustorova *et al*., 2006; Zheljazkov *et al*., 2008). The risk of nitrate leaching to ground water and the potential as

nutrient source could depend on the elemental composition and rate of degradation of the hair, which can both be affected by hair type.

The results in Chapter 3 indicated that African hair had lower N and S than Indian and White, higher Mn, Zn and Co than White and Indian, higher Ca and Fe than Indian hair and higher K than White hair. These variations point at possibly lower contribution of African hair to soil N and S, and higher soil Ca, K and micronutrients (Fe, Mn, Zn and Co) than at least one of the other two hair types. However, these contributions could be limited by the rate of degradation of the different hairs, which depends on the strength of keratins that is strongly determined by the number of di-sulphide and hydrogen bonds, salt linkages and other cross linkages (Nustorova *et al*., 2006). Although differences in composition of di-sulphide and hydrogen bonds, salt linkages and other cross-linkages, have not been established among White, African and Indian hair types, the rate of their degradation and nutrient release could be an indicator of the strength of the keratin and potential as a nutrient source.

A study by Gupta and Sharma (2014), on effects of un-composted hair waste as a source of nutrients for spinach, showed an increase in mineral N (NH_4-N and NO_3-N) in the soil, which indicated that the hair broke down with the effect of releasing N in available forms. However, there is a lack of literature on the release patterns of inorganic N (NH_4-N and NO_3-N) and micronutrients (Fe, Cu, Zn and Mn) from un-composted human hair and the differences among hair types. There is need to understand the nutrient release patterns of different types of human hair in order to establish their potential contribution to groundwater pollution, when added to land-fills, and to soil fertility improvement and long-term accumulation of heavy metals. The quality of most organic waste, as a nutrient source, is determined by the release of nutrients in

soil (Adediran *et al*., 2003). The objective of this study was to determine the effects of hair type and application rate on nutrient release from human hair.

4.2 Materials and Methods

The incubation study was conducted at the University of KwaZulu-Natal Pietermaritzburg campus, South Africa. The hair types used for this study were the same as those described previously in Chapter 3. The chemical properties of the hair types are also given in Chapter 3.

4.2.1 Soil

The soil used in this study was Hutton soil form (Soil Classification Working Group, 1991) collected from Baynesfield farm. The Hutton soil form was among the major agricultural soils in KwaZulu-Natal and it is well drained and aerated which is a requirement for incubation studies. Bulk soil was collected from the 0-20 cm depth after removing the surface litter, using an auger and mixed thoroughly to make composite sample. It was air dried, and sieved (2 mm) before analysis for chemical and physical characteristics. Soil pH was measure using 1 M KCl (1:2.5 soil/KCl). Phosphorus, Ca, Mg and K were determined following extraction by the AMBIC-2 method as described by Van der Merwe *et al*. (1984) and total C and N were analysed using LECO CNS analyser (LECO Corporation, 2012). Micronutrients (Fe, Cu, Mn and Zn) were extracted with DTPA method according to Lindsay and Norvell (1978).

4.2.2 Incubation study

The treatments were African, Indian and White hair applied at different nitrogen rates. The hair samples were cut approximately to 0.5 cm with a stainless steel as described in Chapter 3 and were each mixed with 100 g soil at rates equivalent to 200, 400, and 800 kg N/ha . Untreated soil was included as a control (0 kg N/ha). Each treatment was replicated three times for each sampling time. The experiment was set up with enough replication to allow for destructive sampling. The soil/hair mixtures were then placed in 500 ml plastic containers covered tightly with lids and four holes drilled just below the rim to allow air exchange. Distilled water was added to bring the samples to 100 % field capacity and then incubated in a constant temperature room at ±25°C for up to 84 days. To maintain moisture content distilled water was added regularly to 100% field capacity throughout the incubation, based on weight loss. Samples were remixed regularly during the incubation to stimulate aeration and ensure thorough contact between the soil and the hair. Samples from each treatment were destructively taken after 0, 14, 28, 56 and, 84 day of incubation and analysed for pH, inorganic nitrogen (NH_4-N and $N0_3$-N), P and micronutrients (Fe, Cu, Mn and Zn). Since keratin is generally believed to be resisted to degradation, the sampling days were chosen include increasing times of incubation and degradation of the hair and mineralisation of nutrients.

4.2.3 Analyses

The soil field capacity moisture content of -33 kPa was measured using a pressure plate. Total C and N were analysed by a TruMac CNS/ NS Carbon/Nitrogen/Sulfur Determinators. The pH was determined by weighing 10 g soil into 50 mL beakers and add 25 mL of 1 M KCl. The samples were allowed to stand for 30 min with occasional stirring using a glass rod. The pH of

the soil was measured with a Hanna pH micro-processer meter (model 211) by immersing the pH electrode into the supernatant liquid. Ammonium and nitrate N were determined by weighing 2 g fresh soil that was corrected for moisture and extracted with 20 ml of 2 M KCl. Nitrate-N was analysed using Gallery Discrete Auto-analyser and ammonium-N was determined through the colorimetric method. Ammonium was determined by making two reagents that consisted of sodium salicylate, sodium citrate, sodium tartrate and nitroprusside, sodium hydroxide and sodium hypochlorite. From the extract 0.1 ml was pipetted into a test tube and added 5 ml of reagent 1 and left for 5 minutes then added 5 ml of reagent 2 and was left for 1 hr for colour development (Anderson and Ingra, 1993). It was read at absorbance 655 nm on a Thermo Scientific UV-Vis GENESYS 20 visible spectrophotometer. Phosphorus was determined by weighing 2.5 g and extracted with 25 ml AMBIC extract as described by Van der Merwe *et al*. (1984) and was read on a Thermo Scientific UV-Vis GENESYS 20 visible spectrophotometer using the molybdenum-blue method (Murphy and Riley, 1962). Micronutrients (Fe, Cu, Mn and Zn) were extracted from 10 g of soil with 20 ml of 0.005 DTPA, adjusted pH 7.3, and were determined on the Atomic Absorption Spectrophotometer (AAS) as described by Lindsay and Norvell (1978). Net mineralised nutrients were obtained by subtracting values of the control (soil alone) from the treated soil (soil-hair mixture).

4.2.4 Statistical analysis

The results of the elemental analyses were subjected to Analysis of variance (ANOVA) using GenStat 14th edition (Payne *et al*., 2011). Mean separation was done using least significant differences (LSD) at $p \leq 0.05$.

4.3 Results

4.3.1 Soil properties

The soil used was a loam with 21.9% clay, 41.3% silt and 36.7% sand. The soil had pH 4.4, 13.7 mg available P /kg, high Ca, Mg and Fe, and low exchangeable acidity (Table 4.1).

Table 4.1: Some of the chemical characteristic of soils

Property	Value
pH (KCl)	4.4
Total C (%)	3.3
Total N (%)	0.2
Available P (mg/kg)	13.7
Exchangeable K (cmol+/kg)	0.48
Exchangeable Ca (cmol+/kg)	4.85
Exchangeable Mg (cmol+/kg)	1.75
Exchangeable acidity (cmol+/kg)	0.24
Zn (mg/kg)	6.6
Mn (mg/kg)	32.8
Cu (mg/kg)	5.6
Fe (mg/kg)	181.5

4.3.2 pH(KCl)

The pH were the same among hair types and the different rates applied from day 0 to 28 (Figure 4.1). The decline in pH occurred between days 28 and 56 and gradually decreased to day 84, except the control and soil treated with White hair at an equivalent of 200 kg N/ha. The pH significantly decreased with increase in the applied rates. Addition of Indian hair resulted in lower soil pH than White hair, while African hair resulted in similar effects to both Indian and White hair.

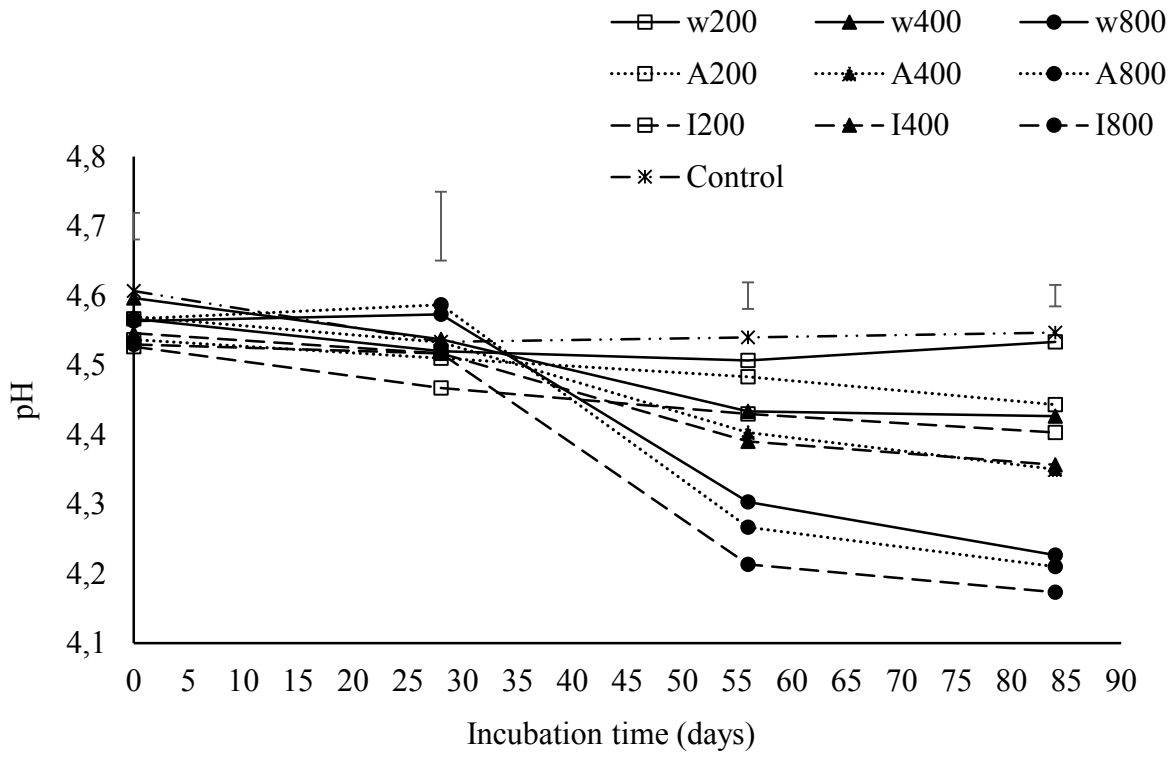

Figure 4.1: Soil pH from three hair types at different N application rates during incubation. W200-White hair at 200 kg N /ha; W400-White hair at 400 kg N/ha; W800-White hair at 800 kg N/ha; A200-African hair at 200 kg N/ha; A400-African hair at 400 kg N/ha; A800-African hair at 800 kg N/ha; I200-Indian hair at 200 kg N/ha; I400-Indian hair at 400 kg N/ha; I800-Indian hair at 800 kg N/ha and control- soil which was not treated (0 kg N/ha). Vertical error bars indicate represent LSD ($p<0.05$).

4.3.3 Ammonium-N

Hair type effects on ammonium-N concentration were only significant at day 56 where, at 800 kg N/ha, Indian hair had higher levels than White hair (Figure 4.2). At day 28, the 800 kg N/ha of all hair types released significantly higher ammonium-N than the lower rates. Indian hair continued to release significant amounts of ammonium on day 56 at all rates, whereas ammonium-N from African and White hair decreased from day 28. The ammonium-N released from the 200 and 400kg N/ha rates were low for all hair types, throughout the incubation period.

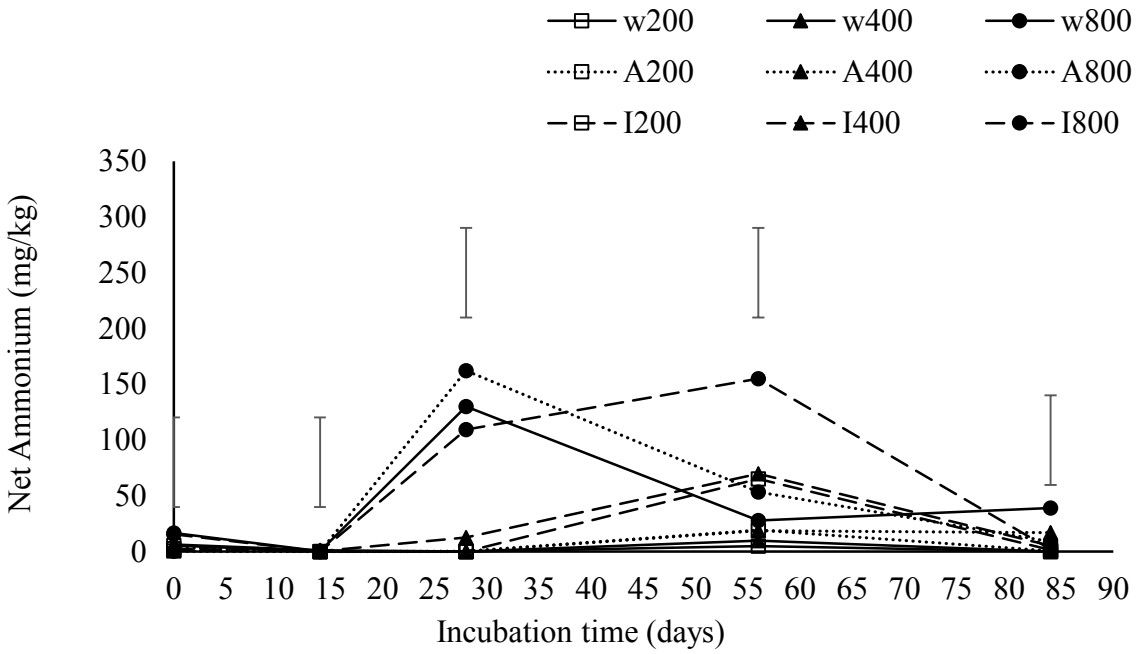

Figure 4.2: Net ammonium-N from three hair types at different N application rates during incubation. W200-White hair at 200 kg N /ha; W400-White hair at 400 kg N/ha; W800-White hair at 800 kg N/ha; A200-African hair at 200 kg N/ha; A400-African hair at 400 kg N/ha; A800-African hair at 800 kg N/ha; I200-Indian hair at 200 kg N/ha; I400-Indian hair at 400 kg N/ha; I800-Indian hair at 800 kg N/ha and control- soil which was not treated (0 kg N/ha). Vertical error bars indicate represent LSD ($p<0.05$).

4.3.4 Nitrate-N

There were no significant differences among hair types nitrate-N concentration at all the application rates within the first 28 days (Figure 4.3). After 56 and 84 days, all the hair types had similar nitrate-N for each application rate, except that at 800kg N/ha Indian hair, which had more nitrate-N than White hair after 56 days of incubation. Based on the slopes of the 800 kg N/ha, the nitrate-N release rate for Indian hair was more rapid between 28 and 56 days and slower between 56 and 84 days when compared to White hair. African hair was moderate (in between the two) throughout the incubation period. The trends were similar for lower application rates, with the 400 kg N/ha releasing nitrate-N upto 100 mg N/kg.

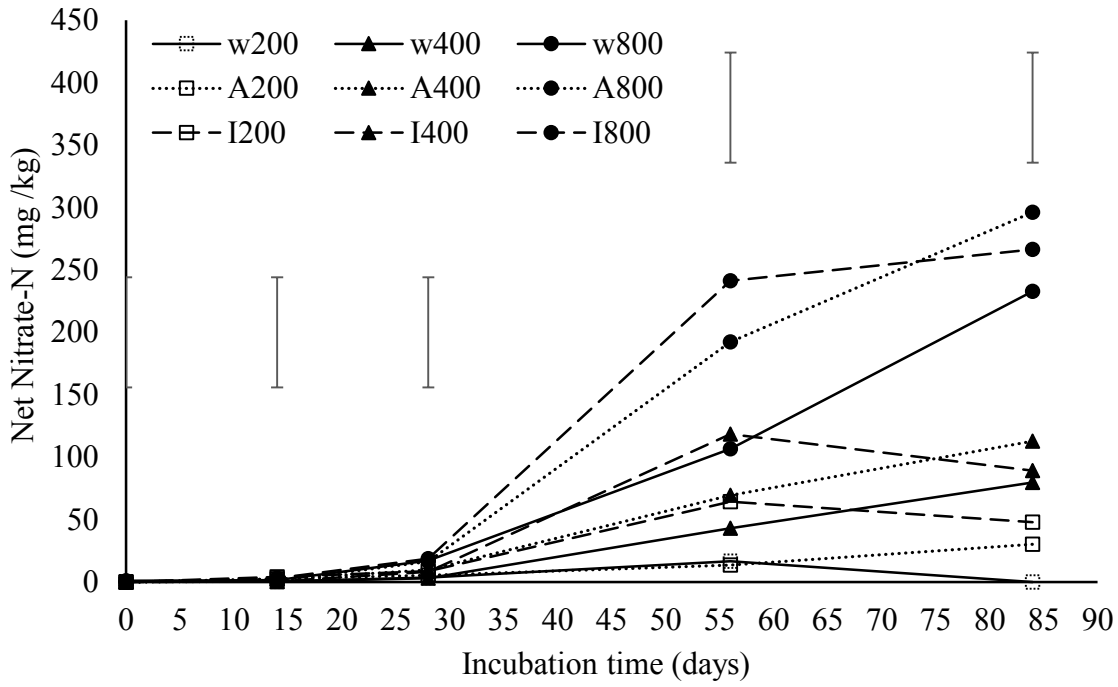

Figure 4.3: Net nitrate-N from three hair types at different N application rates during incubation. W200-White hair at 200 kg N /ha; W400-White hair at 400 kg N/ha; W800-White hair at 800 kg N/ha; A200-African hair at 200 kg N/ha; A400-African hair at 400 kg N/ha; A800-African hair at 800 kg N/ha; I200-Indian hair at 200 kg N/ha; I400-Indian hair at 400 kg N/ha; I800-Indian hair at 800 kg N/ha and control- soil which was not treated (0 kg N/ha). Vertical error bars indicate represent LSD ($p<0.05$).

4.3.5 Mineral N

There were no significant differences on mineral N concentration among hair types and among the applied rates within the first 28 days (Figure 4.4). Hair type differences occurred after 56 days of incubation where Indian hair resulted in greater mineral N than the other two at all rates and African hair treatment had more than White hair only at 800 kg N/ha. Mineral N differences among the hair types was smaller after 84 days. The 800kg N/ha rate had higher mineral N than the lower rates at 28, 56 and 84. The highest mineral N of about 390 mg/kg was for Indian hair at 800 kg N/ha after 56 days.

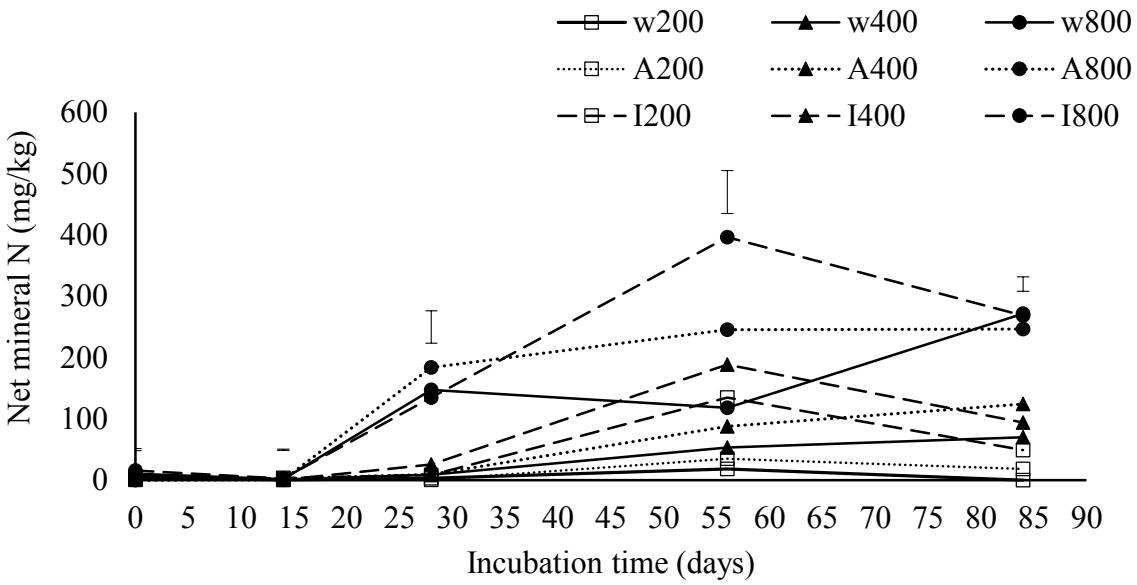

Figure 4.4: Net mineral-N from three hair types at different N application rates during incubation. W200-White hair at 200 kg N /ha; W400-White hair at 400 kg N/ha; W800-White hair at 800 kg N/ha; A200-African hair at 200 kg N/ha; A400-African hair at 400 kg N/ha; A800-African hair at 800 kg N/ha; I200-Indian hair at 200 kg N/ha; I400-Indian hair at 400 kg N/ha; I800-Indian hair at 800 kg N/ha and control- soil which was not treated (0 kg N/ha). Vertical error bars indicate represent LSD ($p<0.05$).

4.3.6 Mineral P

The African hair had more P at 800 kg N/ha on day 56 and all rates on day 84 (Figure 4.5). The other hair types were not affected by length of incubation time and application rate.

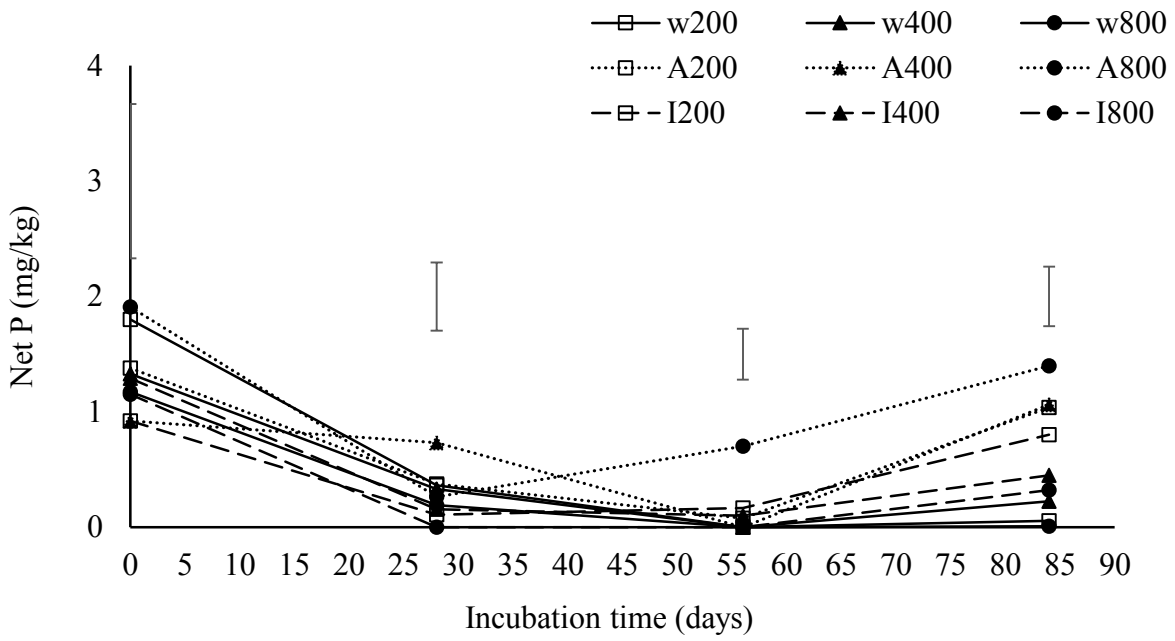

Figure 4.5: Net mineral phosphorus from three hair types at different N application rates during incubation. W200-White hair at 200 kg N /ha; W400-White hair at 400 kg N/ha; W800-White hair at 800 kg N/ha; A200-African hair at 200 kg N/ha; A400-African hair at 400 kg N/ha; A800-African hair at 800 kg N/ha; I200-Indian hair at 200 kg N/ha; I400-Indian hair at 400 kg N/ha; I800-Indian hair at 800 kg N/ha and control- soil which was not treated (0 kg N/ha). Vertical error bars indicate represent LSD ($p<0.05$).

4.3.7 Zinc and copper

There were significant interaction effects of hair types and the applied rates on extractable Zn concentration on days 28, 56 and 84 (Figure 4.6). On day 28 the 400 and 800 kg N/ha of Indian

and African hair resulted in similar concentrations of Zn, which were higher than White hair. The 800 kg N/ha rate of African hair had the most Zn throughout the incubation period. The extractable Zn decreased beyond 28 days for Indian and beyond 56 days for African and White hair. The concentration of available Cu was in the order: Indian>African>White at all rates between 14 and 56 days, with 800 kg N/ha rate having a highest concentration (Figure 4.7). The highest extractable Cu was on day 28 after which it declined.

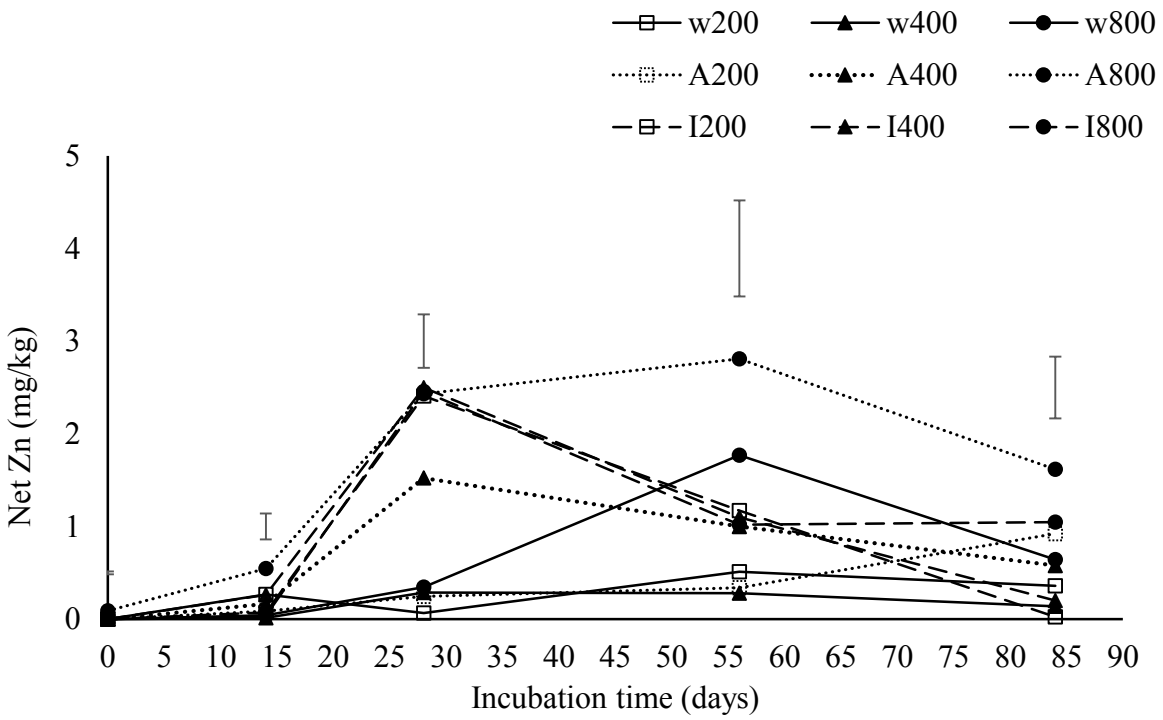

Figure 4.6: Net available zinc from three hair types at different N application rates during incubation. W200-White hair at 200 kg N /ha; W400-White hair at 400 kg N/ha; W800-White hair at 800 kg N/ha; A200-African hair at 200 kg N/ha; A400-African hair at 400 kg N/ha; A800-African hair at 800 kg N/ha; I200-Indian hair at 200 kg N/ha; I400-Indian hair at 400 kg N/ha; I800-Indian hair at 800 kg N/ha and control- soil which was not treated (0 kg N/ha). Vertical error bars indicate represent LSD ($p<0.05$).

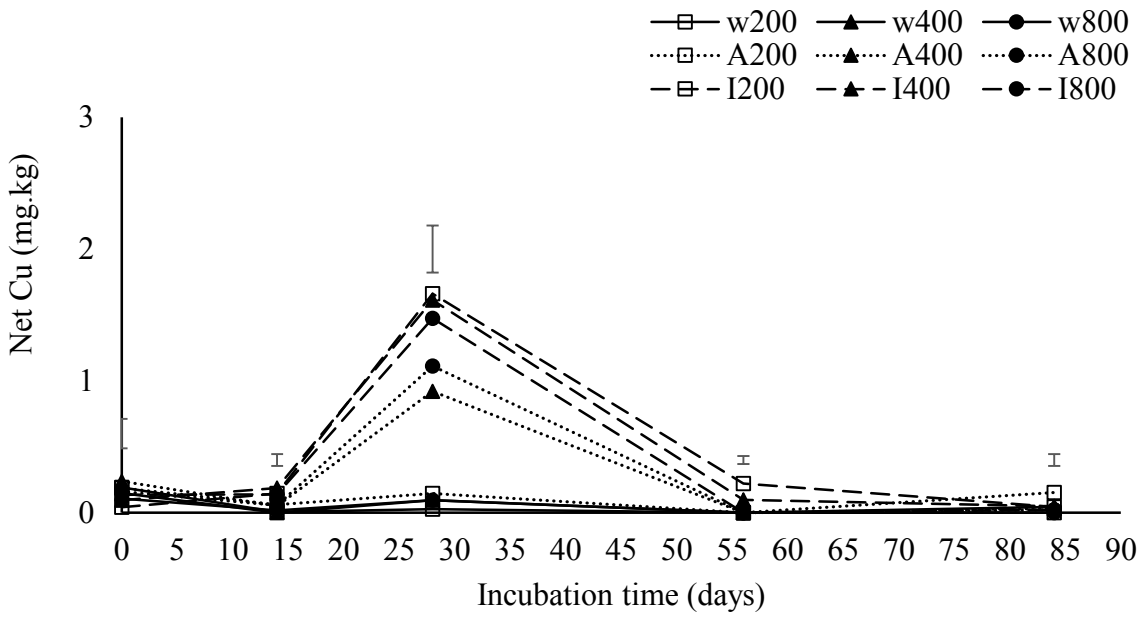

Figure 4.7: Net available copper from three hair types at different N application rates during incubation. W200-White hair at 200 kg N /ha; W400-White hair at 400 kg N/ha; W800-White hair at 800 kg N/ha; A200-African hair at 200 kg N/ha; A400-African hair at 400 kg N/ha; A800-African hair at 800 kg N/ha; I200-Indian hair at 200 kg N/ha; I400-Indian hair at 400 kg N/ha; I800-Indian hair at 800 kg N/ha and control- soil which was not treated (0 kg N/ha). Vertical error bars indicate represent LSD ($p<0.05$).

4.3.8 Manganese and iron

There were significant interaction effects of hair type and rate on the concentration of manganese among hair types at all the incubation times (Figure 4.8). The trends of concentrations of Mn were similar to those of Zn. After 28 day extractable Mn was highest, and was in the order Indian> African > White for all the different rates (Figure 4.8). At days 56 and 84 extractable Mn increased with the rate of application but the hair types did not differ.

The trends of the concentrations of Fe were similar to that of Cu. There were insignificant concentrations of Fe throughout the incubation period, except on day 28 where the 400 and 800kg N/ha rates had higher Fe than the 200 kg N/ha.

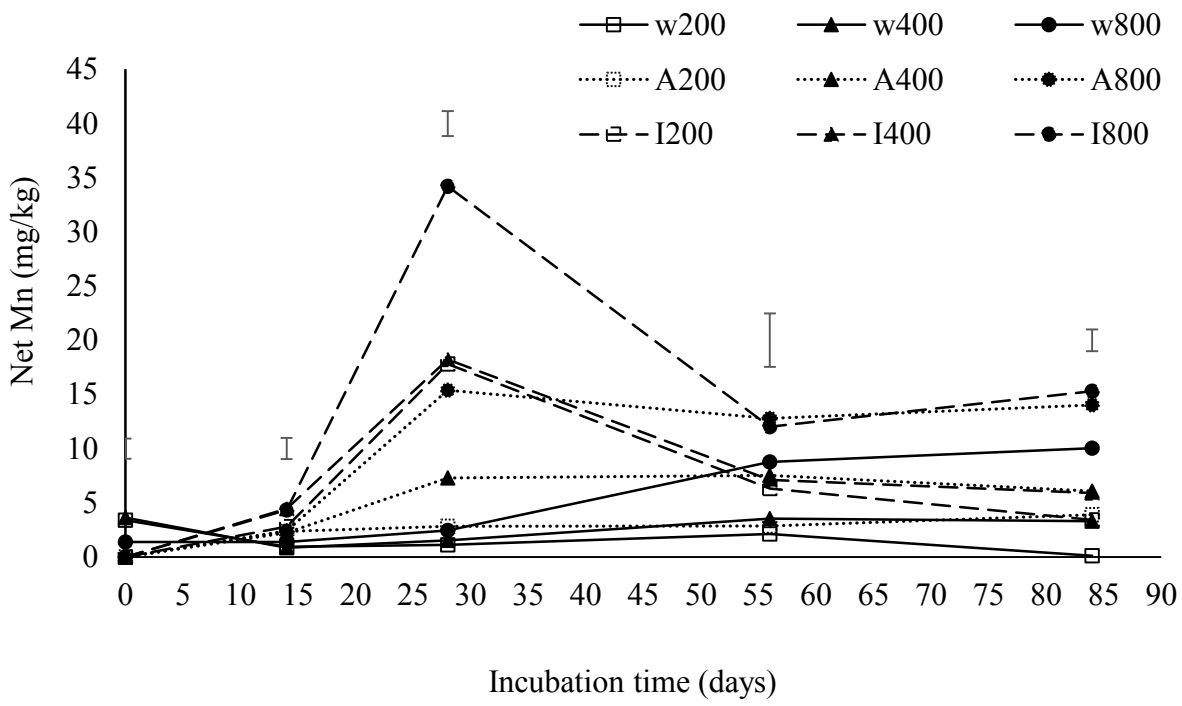

Figure 4.8: Net available manganese from three hair types at different N application rates during incubation. W200-White hair at 200 kg N /ha; W400-White hair at 400 kg N/ha; W800-White hair at 800 kg N/ha; A200-African hair at 200 kg N/ha; A400-African hair at 400 kg N/ha; A800-African hair at 800 kg N/ha; I200-Indian hair at 200 kg N/ha; I400-Indian hair at 400 kg N/ha; I800-Indian hair at 800 kg N/ha and control- soil which was not treated (0 kg N/ha). Vertical error bars indicate represent LSD ($p<0.05$).

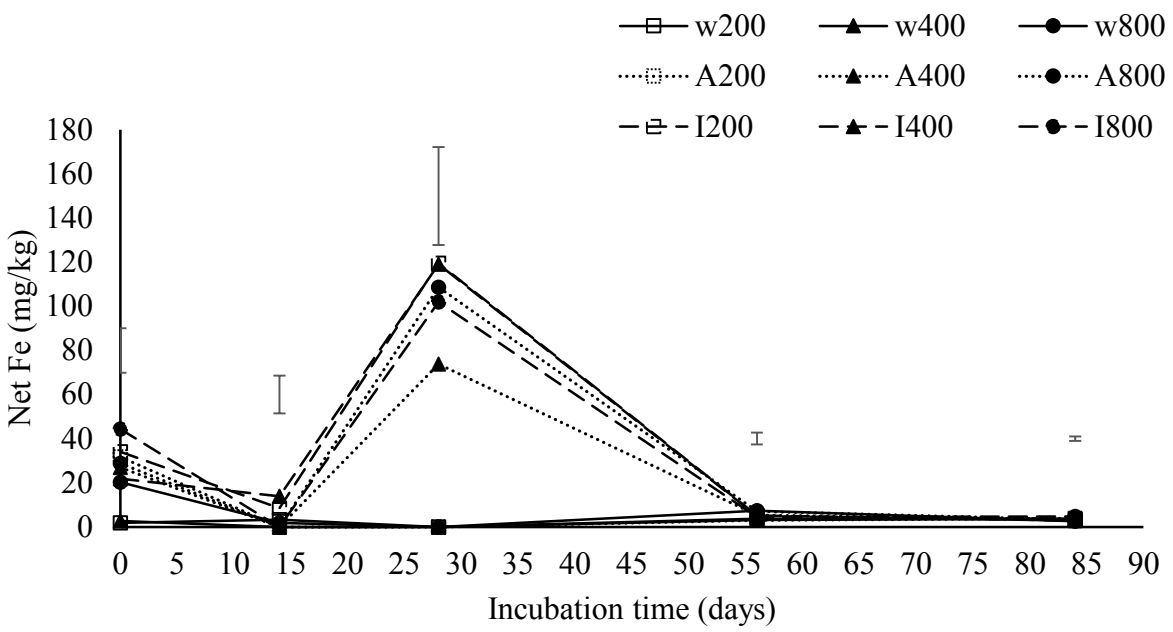

Figure 4.9: Net available iron from three hair types at different N application rates during incubation. W200-White hair at 200 kg N /ha; W400-White hair at 400 kg N/ha; W800-White hair at 800 kg N/ha; A200-African hair at 200 kg N/ha; A400-African hair at 400 kg N/ha; A800-African hair at 800 kg N/ha; I200-Indian hair at 200 kg N/ha; I400-Indian hair at 400 kg N/ha; I800-Indian hair at 800 kg N/ha and control- soil which was not treated (0 kg N/ha). Vertical error bars indicate represent LSD ($p<0.05$).

4.4 Discussion

The trends in soil pH could be explained by nitrification, with higher nitrate levels beyond 28 days of incubation, with nitrate-N in the order Indian>Africa>White while the pH was in the order Indian<Africa<White. The nitrification process releases H^+ ions which lower soil pH (Sahrawat, 2008). Oliyanka (2001) observed a decline in pH over time after applying cowdung due to nitrification and Zheljazkov *et al*. (2008) also observed a decline in pH in treatments with high N application and increased NO_3-N. The highest concentrations of ammonium-N at day 28 suggests that the different wastes from human hair decomposed and the component N

mineralised from the organic forms, as proteins and their component amino-acids (Ross, 1966), to produce the ammonium ion, within a 28 day period. The rapid increase in nitrate concentration (and mineral N) from day 28 to day 56, which coincided with decline in ammonium-N, could be explained by the nitrification of the ammonium into nitrate, with corresponding decline in pH (Ross, 1966). The high release of mineral N among hair types could be due to the high N in the hair. The incubation conditions with temperature of about 25^{o}C, field capacity moisture content, enough aeration were conducive for nitrification (Sahrawat, 2008). The more rapid release of mineral N from Indian hair than the other hair types could been ascribed by its lower C:N ratio which could have resulted in faster break down rate than the other types (Haney *et al.*, 2012). However, although African had the highest C:N ratio, it broke down faster than White hair, which suggested that, like other organic materials, the breaking down of hair may not solely depend on the C:N ratio. It could have been the compounds that make up the hair types but this was not studied in this study. The results found in this study are similar to the results found by Choi and Nelson (1996) on the keratinous material from hydrolysed feather which had high mineral N at week 5, and it decreased from 8 to 12 weeks. The high ammonium-N at day 28 was similar to what was reported by Choi and Nelson (1996).

The differences in the mineralisation rate could have been affected by the ability each hair to release of the N in the soil. After 56 days, African hair had released equivalents of 447, 161 and 32 kg $N0_3$-N/ha for the 800, 400 and 200 kg N/ha rates. The same rates had 561, 276 and 150 kg $N0_3$-N/ha, respectively, for Indian hair and 242, 100 and 39 $N0_3$-N/ha, respectively, for White hair. After 84 days African hair had released equivalents of 688, 262 and 70 $N0_3$-N/ha for the 800, 400 and the 200 kg N/ha rate, respectively, whereas Indian hair had released equivalents of 619, 207 and 111 $N0_3$-N/ha for the same rates. On the otherhand, White hair had

released equivalents of 541, 186 and 10 kg $N0_3$-N/ha, for the corresponding rates. These nitrate concentrations suggested that more than 50% of the added N was converted to nitrate-N within 56 days for African and Indian hair, and within 84 days for White, particularly for the 800 and 400 kg N/ha application rates. The N requirement for a 10 t/ha maize crop is about 220 kg N/ha (Fertiliser Society of South Africa, 2007) and as such the 400 kg N rate would release the required N, within 56 days for Indian hair and 84 days for African and White hair. However, the benefit will depend on synchrony of N availability and requirement due to the risks of losses from leaching (Bhatti and Cressser, 2015; Robertson, 1982). At that rate, Indian and African hair could result in excess N while White hair could result in less than enough, within 84 day period. However, pre-incubation will be required before planting. Using the 800 kg N/ha rate could result in increased potential for nitrate-N leaching leading to environment pollution.

Mineral P could have been affected by fixation of soil colloids due to the acidic pH of the soil and by the solubility of Zn, Cu, Fe and Mn especially on day 28 as these micronutrients were released in higher concentrations. Phosphorus is prone to fixation on the surface of Fe and Al oxides and precipitated as Fe and Al phosphates under acidic condition (Abolfazli *et al*., 2012). The reason for the slight increase on mineral P in African hair treatments after 56 (800 kg/ha) and 84 days (all rates) was not clear. While longer incubation times were required for maximum mineral N availability, incubation beyond 28 days reduced available Zn, Mn, Fe and Cu. The Zn, Mn, Fe and Cu could have precipitated with P as Zn, Mn, Fe and Cu phosphates making P not available (Lucas and Davis, 1961). The decline of pH and the concentrations of these micronutrients beyond 28 days of incubation support this view. The decline in pH from about 4.6 to about 4.3 for the 800 kg N/ha rate could have supported solubility of the metals (Fernandez and Hoeft, 2003), and supported their reaction with any available P (Lucas and Davis, 1961). The higher concentrations of Zn and Mn in the Africa and Indian hair treatments

after 28 days of incubation could be explained by the composition of these elements in these hair types compared to White. The lower concentration of Cu in White hair treatments, even though the hair has relatively higher concentration, could be explained by the slower decomposition of this hair type compared to the others as shown by mineral N results.

4.5 Conclusion

Hair type affected soil pH and nutrient release, with Indian hair resulting in lower pH, higher ammonium and nitrate-N than White hair, and micronutrient were released in the first 28 days and later declined, in the order Indian>Africa>White. At the later stages of incubation, African hair released more mineral P than the other hair types, irrespective of rates. Higher rates of hair resulted in lower pH, higher ammonium-N and micronutrients in the early stages (which later decline) and nitrate-N in the later stages of incubation.

The higher release of mineral N from Indian and African hair suggested that wastes from these hair types could be used as soil amendment to improve readily available N. The finding of this study on the N release suggested that the three hair types could be used for soil improvement.

CHAPTER FIVE

EFFECTS OF TWO HAIR TYPES AND PRE-INCUBATION TIME ON NITROGEN FERTILISER VALUE OF HUMAN HAIR

5.1 Introduction

The high rate of production of human hair waste from salons across the world could have value in soil fertility and crop improvement. While there could be some social and cultural concerns for utilising hair as a source of nutrients for food crops, it is essential to understand its potential for such an application, and, where local people do not accept hair as a fertiliser for food crops, it can be used for flowers, if the potential has been proven. Gupta and Sharma (2014), in their pot experiment, found that the addition of uncomposted hair waste to soil increased spinach yield, increased soil NH_4-N and NO_3-N, plant tissue N concentration and stimulated soil microbial biomass. They reported that the application of 29867 kg hair /ha to soil was enough to maintain a spinach crop for a minimum of 2-3 harvests without the need of the application of other fertilisers (Gupta and Sharma, 2014). The timing of placing fertilisers in soil has an effect on the optimum growth of the plant.

Microbial decomposition of organic waste and mineralisation determines the availability of N, P and S, among other nutrients (Olayinka and Adebaya, 1989). The mineralisation of N from organic materials, which is affected by many factors such as soil temperature, pH, microbial activity, moisture etc., plays a significant role in the N uptake by plants (Bhatti and Cresser, 2015; Sahrawat, 2008; Abbasi *et al*., 2007). Olayinka and Adebaya, (1989) showed that incubation of cow dung for a period of four weeks before planting enhanced the growth and

dry-matter yields of maize and the length of pre-incubation increased the uptake of N, and P but did not affect K, Ca, Mg and Na uptake.

The results of the incubation study in Chapter 4 showed that Indian and African hair degraded faster than White hair and that a 28 day incubation period results in significant mineralisation of N from human hair. In order to test the potential of such pre-incubation on the value of human hair as a source of nutrients, a fast-growing crop, which is resistant to disease and pests is preferred. Spinach is a leafy annual crop grown for its leaves and it is one of the most important crops that are grown worldwide mostly in China, USA, Indonesia (Welbaum, 2015). In South Africa the crop is grown mainly in KwaZulu-Natal, but other provinces produce it as well. Spinach can grow in wide range of soils but can do best in fertile sandy loam with pH range of 6.0 to 7.0. Spinach need to be well fertilised, especially with N, by growers to enhance its leafiness and rapid growth that occur during the short time between emergence and harvest (Welbaum, 2015).

The objective of this study was to determine the effects of hair type and pre-incubation period on dry-matter yield, N uptake, other tissue elemental composition and residual soil nutrient composition.

5.2 Materials and Methods

The study was conducted as a pot trial under glasshouse conditions at the University of Kwa-Zulu Natal Pietermaritzburg campus located 29°37'33.9"S 30°24'14.0"E in the Kwa-Zulu-Natal Province of South Africa. The hair types used for this study were African and White as that described previously in Chapter 3. The chemical properties of the hair types were also

given in Chapter 3. The soil used for this study was the same as that described previously in Chapter 4. The chemical properties of the soil are also given in Chapter 4.

5.2.1 Pot trial

The soil used in this study was a Hutton soil form collected from Baynesfield farm (described in Chapter 4). Experimental pots were prepared by adding 2.2 kg of soil to each pot along with the different hair types. African and White hair wastes were applied at 400 kg N/ha rate. This experiment was done using the hair only as a source of N not P and K. As such, all the pots (including the control) were treated with P and K added to achieve recommended rates for spinach for the soil form used. Phosphorus was applied as NaH_2PO_4 to achieve an equivalent of 178 kg P/ha and K to 192 kg K/ha using KCl. The amended soils were pre-incubated for 0, 28, 56 and 84 days before planting spinach seedlings as done by Olayinka and Adebaya (1989) with sawdust. The pre-incubation was done in such a way that the planting of spinach in all treatments was done at the same time. Each hair type was triplicated for each pre-incubation time. The experiment had a positive and negative control which were not pre-incubated. The positive control was treated with 46% urea at 200 kg N/ha, instead of human hair, while the negative control was not treated with N. Both the positive and negative controls were amended with the same K and P as for the hair treatments.

Two seedlings (56 days old), of the Ford Hook cultivar of giant spinach (*Spinacia oleracea*), bought from Sunshine Seedling Services, were planted in each pot. The pots were arranged in randomised complete block design in the glass house using the random number table, for randomisation in each block. The plants were allowed to grow for 6 weeks, with irrigation

using tap water to correct for water loss due to evaporation (at least once a day). The glasshouse temperature was 23 ^{o}C during the day and 20 ^{o}C at night.

After six weeks, the leaf area (LA) was calculated by measuring the length and width of a leaf from each pot using a 30 cm ruler. The leaf width was measured from one end of the leaf to the other end (in the middle position of the leaf length) and dividing that sum by the number of plants measured for each treatment according to Msibi *et al.* (2014). The number of leaves were counted per pot per plant before harvest. At harvest, shoots (upper part of the plant) were cut approximately 1 cm above the soil surface and the soil emptied from the pots to separate the roots. Both the shoots and roots were washed with tap water, to remove soil particles, and spread on towel paper to remove excess water, before they were weighed for fresh weight and then oven dried at ± 70 ^{o}C for 72 h. After oven drying the samples were weighed for dry matter and ground using a pestle and mortar to pass through a 1 mm sieve. The ground shoots and roots were analysed for total C and N. The shoots ground samples was then digested using a microwave digester and analysed for S, P, Ca, Mg, K, Cu, Zn, Fe and Mn. The residual soil was air-dried, ground with a mortar and pestle to pass through a 2 mm sieve and analysed soil pH, available P, exchangeable K, Ca, Mg and extractable Zn, Cu, Mn and Fe.

5.2.2 Analyses

The pH was determined by weighing 10 g soil into 50 mL beakers and add 25 mL of 1 M KCl. The samples were allowed to stand for 30 min with occasional stirring using a glass rod. The pH of the soil was measured with a Hanna pH micro-processer meter (model 211) by immersing the pH electrode into the supernatant liquid. The ground shoot samples were weighted (0.25g) and 10 ml of concentrated HNO_3 and HCl (3:1) were added (Gupta and

Sharma, 2014). The samples were pre-digested for 30 minutes and then digested using a MARS CEM 5 microwave for an hour. The digests were then analysed for P, S, Ca, Mg, K, Cu, Zn, Fe and Mn using the Varian 720-ES ICP-OES (inductively coupled plasma- optical emission spectrometer). Residual soil P, Ca, Mg and K were determined following extraction by the AMBIC-2 method as described by Van der Merwe *et al*. (1984) and micronutrients (Fe, Cu, Mn and Zn) were extracted with DTPA method according to Lindsay and Norvell (1978). The Ca, Mg, K, Fe, Cu, Mn and Zn concentrations were determined using the Varian AA 280-Fast Sequential Atomic Absorption Spectrophotometer (FS-AAS) while P was read on a Thermo Scientific UV-Vis GENESYS 20 visible spectrophotometer using the molybdenum-blue method (Murphy and Riley, 1962).

5.2.3 Statistical analysis

All data were analysed statistically by subjecting them to analysis of variance (ANOVA) using GenStat 14th edition. Least significant differences (LSD) (at $p < 0.05$) were used to separate the treatment means.

5.3 Results

5.3.1 Leaf area and number of leaves

There was an increase in LA with pre-incubation time for the plants grown with hair as a source of N, except that for African hair, pre-incubation for 28 days resulted in higher leaf area than for 56 days (Table 5.1). The negative control had similar LA as the hair treatments (both African and White) that were not pre-incubated, whereas hair treatments that were pre-

incubated for 56 days had similar LA with the positive control, and the 84 day pre-incubation resulted in higher levels. The number of leaves in all the treatments were the same except for White hair pre-incubated for 56 days, which had higher number of leaves (Table 5.1).

Table 5.1: LA and Number of leaves.

Treatment	Pre-incubation days	LA (cm^2)	Number of leaves $plant^{-1}$
Positive control		90	9.5
Negative control		60	8.2
African hair	0	55	9.3
	28	121	9.2
	56	100	9.2
	84	127	9.5
White hair	0	52	8.8
	28	94	9.3
	56	96	11.5
	84	119	9.5
LSD (P <0.05)		19.5	1.9

5.3.2 Shoot and root fresh-weight and dry-matter

There were no significant differences among hair types on fresh-weight for shoots and roots (Figure 5.1a and 5.2a). Shoots fresh-weight increased with increase in pre-incubation time for both hair types except that African hair pre-incubated for 28 days had greater weight than for 56 day pre-incubation (Figure 5.1a). The negative control had similar shoot fresh weight with

those of the hair treatments that were not pre-incubated whereas the positive control had similar weight as the hair treatments pre-incubated for 28 (except for African hair which was higher) and 56 days. Root fresh-weights followed the same trend as that of shoots, in terms of relationships among hair types and rates and relative to the controls. There were significant interaction effects of hair type and pre-incubation time on shoot (Figure 5.1b) and root (Figure 5.2b) dry-matter. The results of shoot and root dry-matter followed the same trend as the fresh-weights.

Visual observations of the spinach, at six weeks after planting (Appendix 2 and 3), showed that growth increased with pre-incubation time. In the treatments with hair that were not pre-incubated, the plants had similar growth as the negative control and yellowish as though it had less nitrogen. At day 28 it looked more nourished and had a bigger biomass than day 56 and 84 even though day 84 had bigger leaves from the observation.

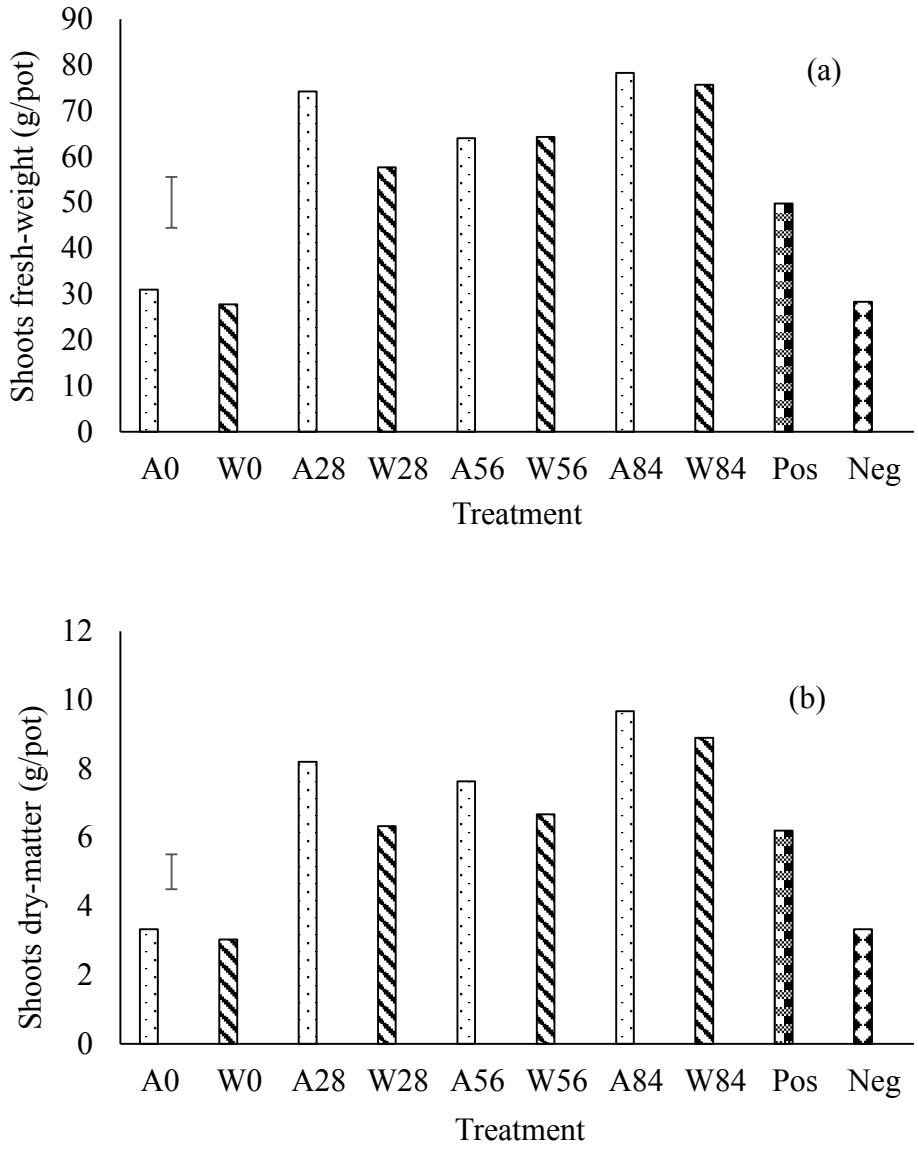

Figure 5.1: Shoots fresh-weight (a) and dry-matter (b) as affected by hair type and pre-incubation. A0- African hair at 0 pre-incubation day, W0- White hair at 0 pre-incubation day, A28- African hair pre-incubated for 28 days, W28- White hair pre-incubated for 28 days, A56- African hair pre-incubated for 56 days, W56- White hair pre-incubated for 56 days, A84- African hair pre-incubated for 84 days, W84- White hair pre-incubated for 84 days, Pos- Positive control, Neg-Negative control. Vertical bars indicate error bars of LSD ($p<0.05$).

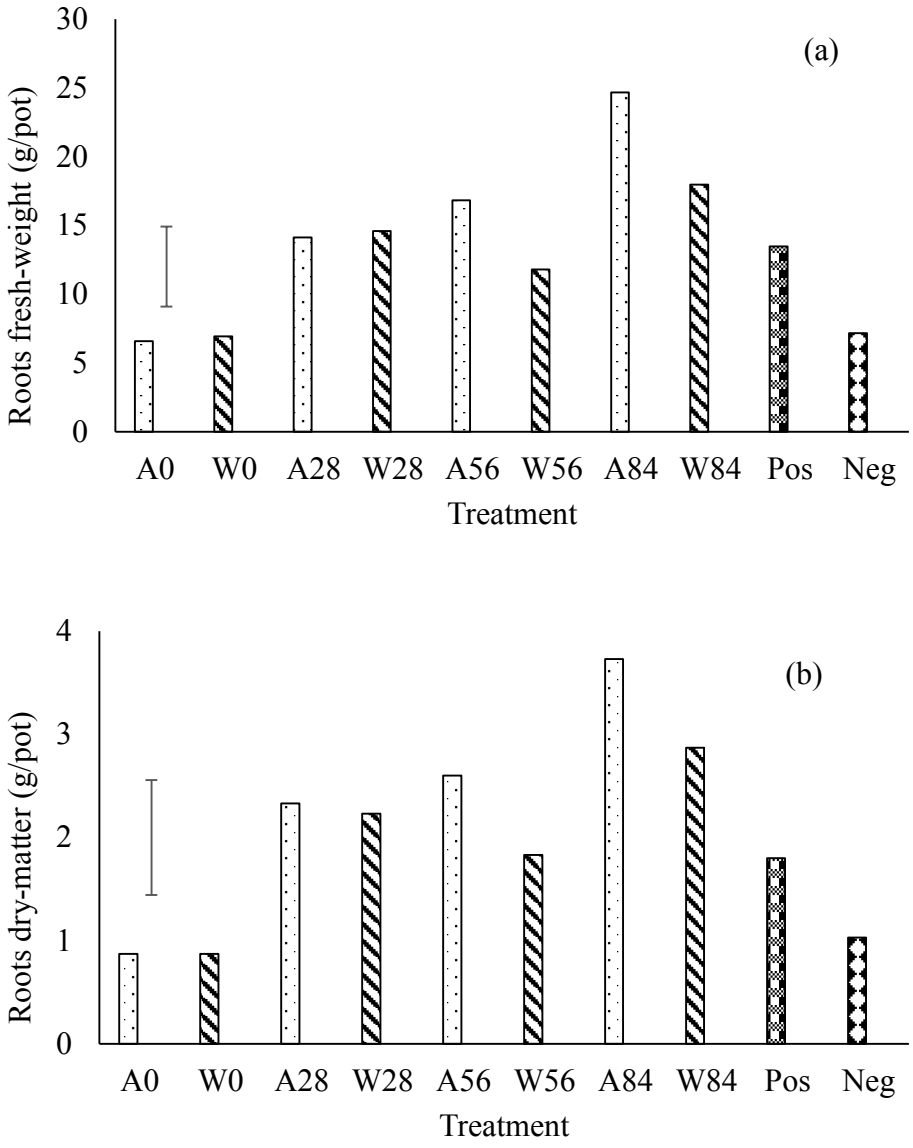

Figure 5.2: Roots fresh-weight (a) and dry-matter (b) as affected by hair type and pre-incubation. A0- African hair at 0 pre-incubation day, W0- White hair at 0 pre-incubation day, A28- African hair pre-incubated for 28 days, W28- White hair pre-incubated for 28 days, A56- African hair pre-incubated for 56 days, W56- White hair pre-incubated for 56 days, A84- African hair pre-incubated for 84 days, W84- White hair pre-incubated for 84 days, Pos- Positive control, Neg-Negative control. Vertical bars indicate error bars of LSD ($p<0.05$).

5.3.3 Concentration and uptake of N and S in spinach shoots

There were no significant differences between hair types at each pre-incubation time on shoot N content and uptake (Table 5.2). Pre-incubated hair treatments had similar tissue N content (except African hair pre-incubated for 28 days), which was higher than the negative control. The highest tissue N was in hair treatments that were pre-incubated for 28 days. Shoot N uptake was lower in the negative control and hair treatments that were not pre-incubated. All pre-incubated hair treatments had greater N uptake than the positive control. The N uptake in pre-incubated hair treatments was in the order 28>84>56 for African hair and 84>28>56 for White. The highest N uptake was in the African hair treatment that was pre-incubated for 28 days.

There were no significant differences between hair types on shoot S concentration for all pre-incubation periods (Table 5.2). For the pre-incubated treatments, tissue S concentration declined with period of pre-incubation, with the hair pre-incubated for 84 days having lower S content than for 28 days (similar to controls). Pre-incubation of hair resulted in increase in S uptake for both hair types, with the highest uptake being after 28 days of pre-incubation, where African hair resulted in the higher uptake than White. Shoot S uptake in the controls was similar to hair treatments that were not pre-incubated (day 0) for both hair types.

Table 5.2: Concentration and uptake of N and S by spinach shoots fertilised with African and White hair after different pre-incubation times.

Treatment	Pre-incubation days	Tissue N	N uptake	Tissue S	S uptake
		%	g/pot	%	g/pot
Control	Positive	1.8	0.1	0.27	0.02
	Negative	1.5	0.05	0.30	0.01
African hair	0	1.7	0.06	0.35	0.02
	28	2.4	2.0	0.44	0.09
	56	1.8	1.4	0.33	0.05
	84	1.9	1.8	0.27	0.05
White hair	0	1.7	0.05	0.44	0.02
	28	2.2	1.4	0.44	0.07
	56	1.9	1.3	0.31	0.04
	84	2.1	1.8	0.29	0.05
LSD		0.3	0.03	0.11	0.02

5.3.4 Concentration and uptake of phosphorus and potassium in spinach

Tissue P concentration in the hair treatments decreased with increase in pre-incubation time (opposite to dry-matter yield results) with the highest concentrations in hair treatments that were not pre-incubated (similar to the negative control) and lowest in those treatments that were pre-incubated for 84 days (Table 5.3). The uptake of P was similar among all treatments irrespective of hair type of pre-incubation period (Table 5.3). The highest tissue K concentration was in the negative control and the hair treatments that were not pre-incubated

(Table 5.3). The hair treatments that were pre-incubated for 56 days had higher tissue K than those pre-incubated for 28 and 84 days and the positive control. The K uptake was higher in the pre-incubated hair treatments than the controls and hair treatments that were not pre-incubated, except the White hair treatment pre-incubated for 28 days, which was similar to the positive control (Table 5.3).

Table 5.3: Concentration and uptake of P and K by spinach shoots fertilised with African and White hair after different pre-incubation times.

Treatment	Pre-incubation days	Tissue P	P uptake	Tissue K	K uptake
		%	x10^{-3} g/pot	%	g/pot
Control	Positive	0.35	22	6.28	0.38
	Negative	0.64	21	7.88	0.26
African hair	0	0.65	22	8.30	0.28
	28	0.27	23	6.46	0.53
	56	0.23	17	7.34	0.56
	84	0.17	16	5.82	0.56
White hair	0	0.87	27	8.54	0.26
	28	0.36	22	7.07	0.45
	56	0.24	16	7.66	0.51
	84	0.20	18	6.11	0.54
LSD ($p<0.05$)*		0.18	7.8	0.79	0.072
LSD ($p<0.05$)**		0.10	8.9	0.32	0.077

*Treatments including controls; ** pre-incubated hair treatments only

5.3.5 Concentration and uptake of calcium and magnesium in spinach

Tissue Ca concentration in hair treatments was not affected by pre-incubation and hair types, except the African hair pre-incubated for 28 days, which had lower Ca than when pre-incubated for 56 and 84 days (Table 5.4). Calcium uptake increased with pre-incubation time and, when pre-incubated for 84 days, African hair had higher uptake (Table 5.4). Both controls had similar Ca uptake with hair treatments pre-incubated for up to 28 days. African hair treatments pre-incubated for 56 and 84 days resulted in greater tissue Mg than all other treatments (Table 5.4). Magnesium uptake had the same trend as Ca.

Table 5.4: Concentration and uptake of Ca and Mg by spinach shoots fertilised with African and White hair after different pre-incubation times.

Treatment	Pre-incubation days	Tissue Ca	Ca uptake	Tissue Mg	Mg uptake
		%	g/pot	%	g/pot
Control	Positive	1.38	0.084	1.39	0.085
	Negative	1.57	0.054	1.22	0.041
African hair	0	1.62	0.055	1.31	0.044
	28	1.39	0.11	1.42	0.12
	56	2.17	0.17	1.81	0.14
	84	2.30	0.22	1.89	0.18
White hair	0	1.56	0.047	1.42	0.043
	28	1.26	0.080	1.24	0.078
	56	1.75	0.12	1.51	0.100
	84	1.81	0.16	1.54	0.14

LSD ($p<0.05$)*	0.72	0.053	0.39	0.030
LSD ($p<0.05$)**	0.37	0.059	0.19	0.032

*Treatments including controls; ** pre-incubated hair treatments only

5.3.6 Concentration and uptake of iron and manganese in spinach

There were no differences in tissue Fe for both hair types except White hair pre-incubated for 56 days, which had higher levels. Pre-incubated African hair treatments (28, 56 and 84) and White hair pre-incubated for 56 days had higher Fe uptake than all other treatments (Table 5.5). Tissue Mn decreased with pre-incubation time for both hair types. Pre-incubated hair treatments had higher Mn uptake than the positive control, which had higher uptake than hair treatments without pre-incubation and negative control (Table 5.5). African hair resulted in greater Mn uptake than White hair, for the 56-day pre-incubation.

Table 5.5: Concentration and uptake of Fe and Mn by spinach shoots fertilised with African and White hair after different pre-incubation times.

Treatment	Pre-incubation days	Tissue Fe	Fe uptake	Tissue Mn	Mn uptake
		%	x 10^{-4}g/pot	%	x 10^{-4}g/pot
Control	Positive	277	17	756	46
	Negative	371	12	747	31
African hair	0	389	13	1027	34
	28	332	27	833	68
	56	340	26	942	72
	84	348	33	747	72
White hair	0	380	11	1327	41
	28	303	19	918	58
	56	561	38	792	52
	84	239	21	741	66
LSD ($p<0.05$)*		211	16	170	11
LSD ($p<0.05$)**		120	18	87	12

*Treatments including controls; ** pre-incubated hair treatments only

5.3.7 Concentration and uptake of copper and zinc in spinach

There were no differences in Zn concentrations among all the hair treatments except the White hair pre-incubated for 84 days, with lower Zn, which was similar to both controls (Table 5.6). All pre-incubated hair treatments had similar Zn uptake (irrespective of time), which was higher than both controls and hair treatments without pre-incubation. Tissue Cu decreased with

increase with pre-incubation time, with White hair pre-incubated for <56 days having higher levels than corresponding African hair treatments. The trend of Cu uptake was similar to that of Zn.

Table 5.6: Concentration and uptake of Cu and Zn by spinach shoots fertilised with African and White hair after different pre-incubation times.

Treatment	Pre-incubation days	Tissue Cu	Cu uptake	Tissue Zn	Zn uptake
		mg/kg	x 10^{-5}g/pot	mg/kg	x 10^{-4}g/pot
Control	Positive	20	12	192	12
	Negative	23	8	231	8
African hair	0	26	9	281	9
	28	23	19	296	24
	56	23	17	292	22
	84	21	21	274	27
White hair	0	29	9	323	10
	28	26	17	278	18
	56	24	16	272	18
	84	20	17	232	20
LSD ($p<0.05$)*		4	3.9	65	5.7
LSD ($p<0.05$)**		2	4.4	36	6.4

*Treatments including controls; ** pre-incubated hair treatments only

5.3.8 Residual soil nutrient concentrations

Hair strands were observable in residual soil from hair treatments that were not pre-incubated and none could be observed in the other pre-incubation times. Soil pH decreased with increase in pre-incubation time. Residual soil K concentration decreased with increase in pre-incubation time, with pre-incubated African hair treatments having no measurable residual levels. There were no significant differences among the different hair treatments on residual soil P, Ca, Mg, Fe, Mn, Zn and Cu concentrations (Table 5.7 and 5.8). All hair treatments irrespective of pre-incubation time had similar soil Zn level, whereas all the treatments had similar soil Fe, Mn and Cu levels, except for White hair treatment pre-incubated for 56 days, which had higher Fe and Mn levels. There was no significant difference on White hair on the concentration of Ca, significance at day 0 and 84 pre-incubation time. There was no significant difference among the treatments on soil Mg except the negative control and African hair treatment that was not pre-incubated that had higher Mg levels than all other treatments.

Table 5.7: Residual soil macronutrients properties after harvest of spinach fertilised with African and White hair after different pre-incubation times.

Treatment	Pre-incubation time	pH (KCl)	P	Ca	Mg	K
			mg/kg			
Control	Positive	4.3	7.2	791	183	53
	Negative	4.4	7.5	792	187	130
African hair	0	4.5	6.6	767	186	155
	28	4.3	6.4	719	181	0.0
	56	4.2	6.2	726	179	0.0
	84	4.1	6.0	680	177	0.0
White hair	0	4.4	7.1	761	185	155
	28	4.4	6.2	775	185	79
	56	4.3	6.2	718	183	45
	84	4.2	6.0	743	181	0.0
LSD (P <0.05)		0.07	0.7	50.3	6.1	20.2

Table 5.8: Residual soil micronutrients properties after harvest of spinach fertilised with African and White hair after different pre-incubation times.

Treatment	Pre-incubation time	Zn	Mn	Fe	Cu
		mg/kg			
Control	Positive	8.5	48	63	1.7
	Negative	9.2	46	65	1.7
African hair	0	7.9	46	63	1.6
	28	7.8	46	64	1.5
	56	6.9	47	64	1.3
	84	7.7	45	64	1.6
White hair	0	7.6	46	63	1.7
	28	8.2	43	63	1.5
	56	8.1	51	66	1.6
	84	8.0	44	64	1.6
LSD ($p<0.05$)		1.3	2.8	1.4	0.7

5.4 Discussion

The increase in LA, fresh and dry-matter of both shoots and roots with increase in pre-incubation time could be explained by N-uptake results which were higher with greater pre-incubation time. Higher yield for yellow poppy were obtained by Zheljazkov *et al*. (2008) after planting them in pots that were treated with hair waste that were planted with lettuce and wormwood which were basically pre-incubated for 214 days. Gupta and Sharma (2014) observed increased yield of spinach with addition of uncomposted hair.

The absence of hair strands in residual soil of pre-incubated hair treatments, whereas strands occurred in those without pre-incubation, indicated that the added hair broke down with pre-incubation, possibly releasing nutrients. The practical significance of the results is that when human hair is to be used as a source of nutrients, it needs to be pre-incubated at least for 28 days (the shortest period tested). The greater dry-matter could have been a result of the release of N and S over time leading to appreciable amounts available for plant uptake. Wang *et al*. (2009) observed an increase in NO_3-N resulted in higher biomass yield. The dry-matter results were supported by those of N and S uptake which followed the same trend, suggesting that greater uptake of N and S stimulated growth and accumulation of dry-matter. Olayinka and Adebayo (1989) found that pre-incubation of cow dung for 4 weeks (28 days) before planting enhanced the growth and dry-matter yields of maize. The decline in pH of the residual soil supported the view that nitrification, and possibly S mineralisation and oxidation to sulphate-S occurred and released of H^+ (Aulakh *et al*., 2002, Wainwright *et al*., 1986).

Mineralisation of N, with more NO_3 being released, could have made the pH of the soil to decrease (Zheljazkov *et al*., 2008). Sanchez-Monedero *et al*. (2001) observed a decline in pH during organic waste composting due to nitrification. The decrease in soil pH with the addition of hair waste as result of mineralisation and oxidation of N and S has been reported by Zheljazkov *et al.* (2008a, b). The higher LA, fresh and drymatter of shoots and roots in African than White hair treatement for the 28 day pre-incubation time could be a result of greater nitrate-N in African hair treatment, possibly as a result of more rapid degradation. Pre-incubation for 56 and 84 days meant that extra incubation allowed White hair to release similar amounts of nitrate-N, and possibly sulphate-S, than shorter periods where African hair could have had a more rapid rate of degradation and mineralisation. The decline in tissue N and S

with increase in pre-incubation could be a dilution effect as a result of greater dry-matter accumulation.

Even though P was supplemented, to reach the recommended rate, tissue P and P uptake were not affected by addition of hair (whether African or White) nor with increase in pre-incubation time. Increasing pre-incubation time could have resulted in P mineralisation which was also associated with decline in pH, and the low soil pH (<pH 5), which declined with pre-incubation time, could have reduced availability of P (Lucas and Davis, 1961). The low pH could have resulted in formation of Al, Fe, Mn phosphates (precipitation of P), limiting P uptake (Lucas and Davis, 1961). This view was supported by the declined in residual soil available P as pH declined with pre-incubation time (Holford, 1997). Ahmadil *et al*. (2010) found a decrease in P with incresae in N application in spinach.

In addition to responses to N and S uptake, the dry-matter yield could have also been a result of greater availability of Ca, Mg and K with incubation time. The similarity in trends between dry-matter and uptake of Ca, Mg, K, Fe and Mn suggested a response to availability of these elements (Malakouti, 2008; Kayode and Agboola, 1985). The higher uptake of Ca, Mg, Fe, Mn in pre-incubated African hair than White hair treatment could be explained by the higher composition of these elements in African hair, which when degrades will release them, and make them available for uptake. The higher Cu and Zn in pre-incubated hair treatments than the controls and hair that was not pre-incubated indicated that the dry-matter was also a response to the availability of these micronutrients (Gupta and Sharma, 2014).

The decrease of residual nutrients in the soil, with pre-incubation time, could indicate that the vigorous growth of plants resulted in uptake of these nutrients and exhausted them. This effect was more evident for K where no residual soil K could be measured in the longer pre-incubation treatments, suggesting that the plants grow vigorously in response to higher N levels and

exhausted all the K supplied by the soil and added as fertiliser. Zheljazkov *et al.* (2008) found high amounts of residual nutrients in the control than in hair treatments. The longer the pre-incubation resulted in greater dry-matter increase nutrients uptake and exhausting them from the soil. The length of pre-incubation time did not have effect on Ca, Mg and Na which was in agreement with Olayinka and Adebaya (1989). The addition of hair did increased the concentration of Ca when compared with the positive and negative control indicating that Ca was released from the decomposition of hair.

5.5 Conclusion

Pre-incubation of human hair resulted in greater leaf area, fresh weight and dry-matter of spinach and uptake of macro- and micronutrients. Effects of hair type were more evident at shorter pre-incubation time (28 days) where African hair resulted in greater dry-matter, N and S uptake, than White. Increasing pre-incubation time resulted in decline in residual soil pH, available P and exchangeable K, and had not effects on other residual soil nutrients.

CHAPTER SIX

GENERAL DISCUSSIONS, CONCLUSION AND RECOMMENDATIONS

6.1 General discussion

Significant amount of human hair waste is generated by salons daily people shave their hair and is typically disposed of at landfills. The disposal of the hair is serious problem as hair is a rich source of important nutrients such as N, macronutrients and micronutrients. The high quantities of nutrients make hair waste material a valuable resource, if used as fertilizer, particularly if conditions are conducive for its decomposition. Hair when applied to the soil could release nutrients that could have an effect on soil qualities such as chemical and physical properties and these effects could differ with hair type. The differences in hair types can be affected by various factors such as gender, age, health status etc. The potential for the different types of hair, as fertilizer, is determined by the mineralisation of element in the hair, which could be affected by the hair type, application rate and the length of incubation time. The main objective of this study was to evaluate the elemental composition, nutrient release and the potential for using different human hair types as fertiliser.

The higher N and S concentrations and lower C:N ratio in the Indian and White hair waste were not clearly understood but could have been ascribed to genetic factors and diet which possibly affected amino-acid (Hrdy, 1973; O'Connell and Hedges, 1999; Robbins, 2012). Whereas Hrdy and Baden (1973) found insignificant biochemical variation among different hair types,

O'Connell and Hedges (1999) showed that nitrogen composition of hair keratin was dependent on the animal protein consumed in the diet, with omnivores and lacto-ovo-vegetarians having higher N than vegetarians. The lower N and S concentrations, and higher C:N, in African hair suggested that this hair type could have be inferior and could degrade slower than Indian and White hair. However, the concentration of nitrate- and mineral N in soil during the incubation study was in the order Indian>African>White, suggesting that other factors than N concentration and C:N could be involved. While the more rapid release of mineral N from Indian hair than the other hair types could been ascribed to its lower C: N ratio, African hair broke down faster than White hair, even though it had the highest C:N ratio, which suggested that, like other organic materials, the breaking down of hair may not solely depend on the C:N ratio. A study conducted by Adediran *et al*. (2003) found that the N mineralization rate was positively correlated with total N and lignin content and negatively related to the C:N ratio, cellulose, polyphenol and polyphenol: total N. The lignin content, cellulose, polyphenol and proteins composition of the hair types were not studied. Hair is made up of protein and lipids, which consist of fatty acids, gylcerides, esters and hydrocarbons, which differ depending on hair type, age, gender etc. (Bogdanov *et al*., 2006). Increasing the rate of application resulted in greater mineral N with a rate equivalent to 400kgN/ha releasing about 200kg nitrate-N/ha depending on hair type, and as such this could be the most appropriate rate to supply enough N for spinach and maize for high yields. Higher rates of hair application will result in heavy leaching losses and possible pollution of groundwater. Bhatti and Cresser (2015) observed high leaching of nitrate in ground water due changes in seasons from soils under grass.

The rapid mineralisation of N, and possibly S, and nitrification explained the lower pH in soils incubated with Indian and African hair in the incubation study and this was also observed in the residual soil from the pot trial. Even though the soil used was acidic, which could suppress

nitrification, the results of nitrate-N indicated that the process was not suppressed in this study. Zheljazkov *et al.* (2008) observed lower pH with increase in hair waste application due to the breaking down of N and S compounds. The greater nitrate- and mineral-N in African than White hair, based on the incubation study, could explain the higher LA, fresh weight, dry-matter and N and S uptake of spinach in African hair treatment pre-incubated for 28 days before planting could be done. The lack of differences between hair types when pre-incubated for longer than 28 days could be because similar mineral N was available to the plant because long enough time was allowed for White hair to mineralise to the same level as African hair. The 6 week period was extra incubation period, such that those pre-incubated for 0, 28, 56 and 84 days had overall incubation periods of 42 (greater than 28 but less than 56 days), 70, 98 and 126 days. The increase in the incubation time could have been the reason of greater dry-matter for treatments pre-incubated for 28 days which had higher S uptake. This can also be related to the decline in pH from the incubation study in chapter four, making S to be more available for uptake. The results of the incubation study showed that higher nitrate- and mineral-N in African hair than White hair occurred after 56 days of incubation but there were no differences after 84 days of incubation (Zheljazkov *et al*., 2009). Whereas greater mineralisation resulted in greater availability, uptake of N and S with incubation time, with higher levels for African hair after 28 days of pre-incubation, the resultant decline in pH could have negative effects on availability of P and micronutrients (Zheljazkov *et al*., 2008a;b).

The lack of differences in P concentrations among the different hair types in both the incubation study and the pot trial suggested that the addition of hair to soils may not result in differences in the levels of P released in the soil. However, the faster degradation of Indian and African hair could result in greater mineralisation of the P at least in the short-term. However, the available P released could be affected by the decline in pH as a result of nitrification (Lucas

and Davis, 1961). The decline in pH in the incubation study coincided with the period of decline of P availability, possibly as a result of fixation to sesquioxides and precipitation of Al, Fe, Cu, Mn and Zn phosphates (Fernandez and Hoeft, 2003). This could also resulted in a decrease in P uptake with increase in pre-incubation time. This view was supported by the availability of these metal elements, in the incubation experiment, where the highest concentration occurred after 28 days of incubation and declined significantly thereafter, to low levels. Such declines in available micronutrients suggested that availability and uptake of these elements by plants could be limited. Although the incubation study suggested that availability of Fe, Mn, Cu and Zn declined with incubation time longer than 28 days, uptake of these elements by spinach increased with pre-incubation time, which suggested that spinach has a mechanism of making these elements available. A possible mechanism could be the release of organic acids like oxalate in the rhizosphere **(**Sánchez-Rodríguez *et al*., 2014). The organic acids can increase the availability of micronutrients in soil. These findings were supported by the results of the residual soil micronutrient composition, which were similar for all the treatments. The use of human hair as a source of N should be used with lime to neutralise the acidity (Zheljazkov *et al*., 2008).

The higher Ca, K, Fe, Mn in African hair than White hair, possibly be an effect of diet, suggested that degradation of African hair would release more of these elements. Although the incubation study did not monitor the bases, the results of the pot trial showed that there are able to mineralize and be taken up by plants and the uptake was affected by the composition of the hair.

The similarity of heavy metals in the different hair types suggested that the addition of hair to soils may need result in differences in the levels of metals released in the soil. However, the

faster degradation of Indian and African hair could result in greater accumulation of the metals at least in the short-term. However, this aspect was not studied during the incubation and pot trials.

6.2 Conclusion

The elemental composition was different among hair types in South Africa. Differences in hair type affected nutrient release in soil, especially at higher application rates. Pre-incubation of human hair, irrespective of type, increases spinach yield and nutrient uptake.

6.3 Recommendations

While this work showed potential for using human hair as a fertilizer, its impact will depend on the quantities of hair waste available. Research is required on the amount of the different types of human hair waste generated in South Africa. More research needs to be done on the chemical composition of different hair types, with special focus on effects of the factors such as gender, age, lifestyles, diets, social and economic endowment etc. White hair release nutrients in a slow rate than the other hair types, so it is recommended that a research be done on the biochemical variations that make up the hair types. Effects of co-application of human hair and lime, on P availability in soil need to be studied.

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APPENDICES

Appendix 1: Heavy metal concentrations in different types of human hair

Heavy metal	Concentration (mg/kg)		
	White	African	Indian
Arsenic	0.0682	0.0761	0.1028
Barium	8.71	16.72	11.03
Bismuth	0.23	0.04	3.38
Lithium	0.58	0.52	0.24
Silicon	434	1090	480
Strontium	9.27	12.87	10.03
Tin	3.18	3.23	2.65
Titanium	2.19	8.96	6.99
Vanadium	0.21	1.29	0.49
Silver	2.59	0.99	1.19

Appendix 2: Picture showing response of spinach leaf matter after different pre-incubation times with African hair.

Appendix 3: Picture showing response of spinach leaf matter after different pre-incubation times with White hair