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Nitrogen, phosphorus and potassium availability as influenced by humate and fulvate soil amendment

by

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DECLARATION

I, Auges Gatabazi declare that the dissertation, which I hereby submit for the degree of MSc (Agric) Agronomy at the University of Pretoria, is my work and has not previously been submitted by me for a degree at this or any other tertiary institution.

Signature___________________________________

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ABSTRACT

Citrus fruit in South Africa is produced mainly for the export market where it competes with other countries such as Spain, Turkey, USA and Egypt. South Africa is the third largest exporter of citrus after Spain and Turkey. Therefore, quality and shelf life play an important role in maintaining the competiveness of South African produced citrus. Plant nutrients and especially the macro nutrients such as nitrogen (N), phosphorus (P) and potassium (K) play an important role in ensuring yield, quality, and shelf life. However, the efficiency of applied fertiliser is less than 50% for N, less than 10% for P and about 40% for K due to the leaching. Thus, by using humate and fulvate amendments the N leaching from soils can be reduced. The objectives of this study were to determine the effects of humate on: (1) The culturable soil community and microbial activity in a sandy clay and a sandy clay loam soil; (2) the reduction in N, P and K losses; (3) the uptake of N, P and K in potted citrus and (4) the cation exchange capacity of soils.

Four experiments were conducted: Experiments on the viable microbial population and dehydrogenase activity were done in a microbiology laboratory, leaching column studies were done in a soil physics laboratory and pot trials were conducted in a glass house at the experimental farm of University of Pretoria.

Sandy clay and sandy clay loam soils were supplemented with 220-50-80 kg ha^{-1} which represent 100% of the recommended N, P and K application rate and 165-37.5-60 kg ha^{-1}, which represents 75% of the recommended N, P and K application rate. The soils were further amended with humate low ash and humate high ash or with fulvate at a rate of 200 kg ha^{-1}. Controls included soils without any amendments and with 100% and 75% of the N, P and K recommendation. Experiments on microbial population and dehydrogenase activity were done in triplicate and leaching column and pot trials had four replications.

Quantification of heterotrophic bacteria and fungi in both soils indicated, after four weeks, an increase in bacterial and fungal counts for soils treated with humates and a fulvate compared to soils with no humic acids. Results from leaching column experiments indicated a decrease in N leaching when humates and fulvate were added to the soils, while inconsistent results were found for P and K leaching in both soils. Pot trials indicated that humates and fulvate reduced N and P leaching, while N, P and K uptake were higher for the soils with humate or fulvate. The study indicates that humates and a fulvate increased the cation exchange capacity of both soils.

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ABREVIATIONS

ATP – Adenosine 5’-triphosphate

C – Carbon

Ca – Calcium

CEC – Cation exchange capacity

COOH – Carboxyl

CFU – Colony forming unit

CGA – Citrus growers association

CO_2 – Carbon dioxide

CRBD – Completely randomized block design

Cu – Copper

Da = $1.6605402\ 10^{-24}$ g

EC – Electrical conductivity

Fe – Iron

Ha – High ash

ICP – Inductively Coupled Plasma

K – Potassium

KH_2PO_4 – Potassium dehydrogen phosphate

KNO_3 – Potassium nitrate

La – Low ash

Mg – Magnesium

Mn – Manganese

N - Nitrogen

NO_2^- – Nitrite

NO_3^- – Nitrate

OH^- – Hydroxyl

P – Phosphorus

PDA – Potato dextrose agar

pH – The pH of a solution is a negative algorithm to the base ten of the hydrogen ion activity in the solution ($pH = - \log_{10}{}^{a}H$)

S – Sulphur

TSA – Trypticase soy agar

TTC – Tryiphenyl tetrazolium chloride

Zn – Zinc

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CHAPTER 1

GENERAL INTRODUCTION

1.1 Problem statement

South Africa is a major producer of fruit in the Southern Hemisphere and competes on the international market with other production countries such as Spain, Turkey, USA and Egypt (Figure 1.1). Citrus is the number one fruit export product for SA followed by pome and grapes, is exported to nearly 70 countries ranking South Africa as the third largest exporter of citrus. In South Africa citrus is produced on approximately 60 000 ha by more than 1 400 farmers, that provide jobs for more than 100 000 people (Fresh Produce Exporters Forum, 2007).

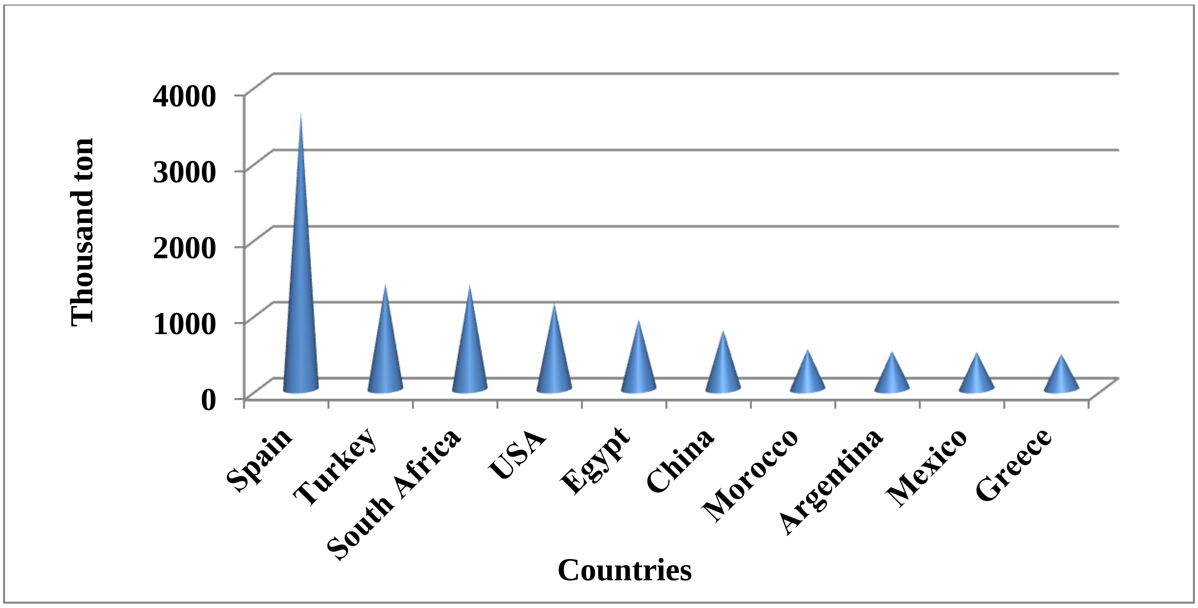

Figure 1.1 Ranking of the world fresh citrus export for the 2010-2011 season (Citrus Growers Association of Southern Africa, 2012)

Citrus is a perennial crop with a production period of 3 to 50 years. Fertilisation begins in the nursery, where small trees are grown for two years before being transplanted at commercial farms. Optimum fertilisation (good fertiliser management) also aims to ensure profitability. There is no point in producing citrus if it is not profitable for the farmer. Citrus trees need to be fertilised to ensure optimum growth, yield and fruit quality such as fruit size, skin thickness, and texture, acid and total soluble solids (TSS) and juice content (Fertiliser Handbook, 2007). Leaf and soil analyses are used to determine the correct amount of nutrients to be applied (Alva *et al.*, 2001, Institute for Tropical and Subtropical Crops, 1996).

Nutrients not taken-up by the crop can be leached (Alva *et al.*, 2001) if over irrigation or fertilisation or high rainfall events occur, as was found for a silt soil where a significant amount of the phosphorus (P) applied leached directly after an irrigation event (Toor *et al.*, 2005). Avnimelech & Raveh (1976) reported for a clay loam soil, nitrogen (N) losses of approximately 50 kg NO_3-N ha^{-1} a^{-1} (47% of the applied N) due to leaching. In sandy soils (< 5% clay), potassium (K) losses are higher than in clay soils (Askegaard *et al.*, 2003) with annual K-leaching reaching a maximum of 30 kg ha^{-1} (Olesen & Vester, 1995). The application of N-fertiliser may result in a significant increase in N-leaching, which may reduce ground water quality, especially in sandy soils (Paramasivan & Alva, 1997). Depending on the soil type and drainage, annual N leaching in citrus orchard may range between 20 and 160 kg ha^{-1} with N application rates of 60 to 520 kg ha^{-1} (Ramos *et al.*, 2002)

Humic substances are organic components produced from the decomposition of plant and animal remains. They are divided into three groups: Humic acids, fulvic acids and humins. Humic and fulvic acids are alkaline-soluble, while humin substances are insoluble in diluted acid or alkali solutions (Pettit, 2004; Eladia *et al.*, 2005).

The influence of humic acids on nutrient availability in other crops is well documented. For example, in lettuce the N-content of the leaves and the availability of soil P increased with humic acids applications (Cimrin & Yilmaz, 2005), while fruit quality and yield of watermelons also improved with the addition of humic acids, although cultivar played a role in the response to the humic acid application (Salman *et al.*, 2005). In grapevines the inorganic N applied could be reduced with 50% when humic acids were added, with an increase in yield and a decrease in the NO_3 and NO_2 content of the berry juice (Eman *et al*., 2008). Shaaban *et al*. (2009) reported that when the amount of N, P and K-fertilisers for a crop in a silt clay soil was reduced by 50% the yield increases. According to Ebtisam *et al*. (2012) and Sharif *et al.* (2002) the benefits of humic acids were due to improving of soil properties such as: water holding capacity, soil aggregate formation, EC, pH, and increase in microorganism activity.

Similar benefits are expected for citrus, however, little information exists on the use of humates and fulvate to reduce N, P and K losses in citrus orchards. Therefore, the aim of this study is to determine the influence of humates and fulvate on soil microbial activity, leaching and uptake of N, P and K in potted citrus.

1.2 Hypotheses and objectives

From literature it is clear that humic acids and fulvates have a beneficial effect on the availability of applied N, P and K. It is envisaged that N, P and K-fertiliser availability can be improved. Therefore, the research was formulated with the hypotheses that humates and fulvate addition to N, P and K-fertilisers could:

1. Increase the heterotrophic microbial community and microbial activity in sandy clay and sandy clay loam soils.
2. Reduce N, P and K losses.
3. Increase the uptake of N, P and K in potted citrus.
4. Decrease the fertiliser application due to the decrease in N, P and K-leaching.
5. Increase cation exchange capacity of the soils.

The objectives of the study were to determine the influence of humates and fulvates on:

1. The culturable soil community and microbial activity in a sandy clay and a sandy clay loam soil.
2. The reduction in leaching of N, P and K.
3. The uptake of N, P and K in potted citrus.
4. Cation exchange capacity of soils.

1.3 Format of dissertation

This dissertation is divided into six chapters. Chapter 1 provides the general introduction and contains the problem statement, hypotheses and objectives of the dissertation.

Chapter 2 provides the reader with a literature review of the origin of humic substances (humic acid, fulvic acid, humate and humin), the influence of humic substances on physical, chemical and biological properties in the soil and plant response to humic application.

Chapter 3 illuminates the effect of humates and fulvate on the microbial soil community by looking at:

1. Culturable heterotrophic bacteria and fungi and
2. Microbial enzyme activity (Dehydrogenase activity) under laboratory conditions.

Chapter 4 relates the effect of humates and fulvate on the leaching of N, P and K under laboratory conditions.

Chapter 5 reflects on the effect of humates and fulvate on the leaching of N, P and K and on the uptake of N, P and K in pot trials.

Then Chapter 6 presents a general conclusion by providing a summary of the study and answering the research hypotheses.

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CHAPTER 2

LITERATURE REVIEW

2.1 Origin of humic substances

Humic substances are a mixture of natural organic materials that remain after the decomposition of animals and plants (Hopkins & Stark, 2003) and are found in soils, compost, sewage, water, marine peat bogs, lake sediments and brown coal lignite (Stevenson, 1982). The different pathways for the formation of soil humic substances from plant residues are illustrated in Figure 2.1.

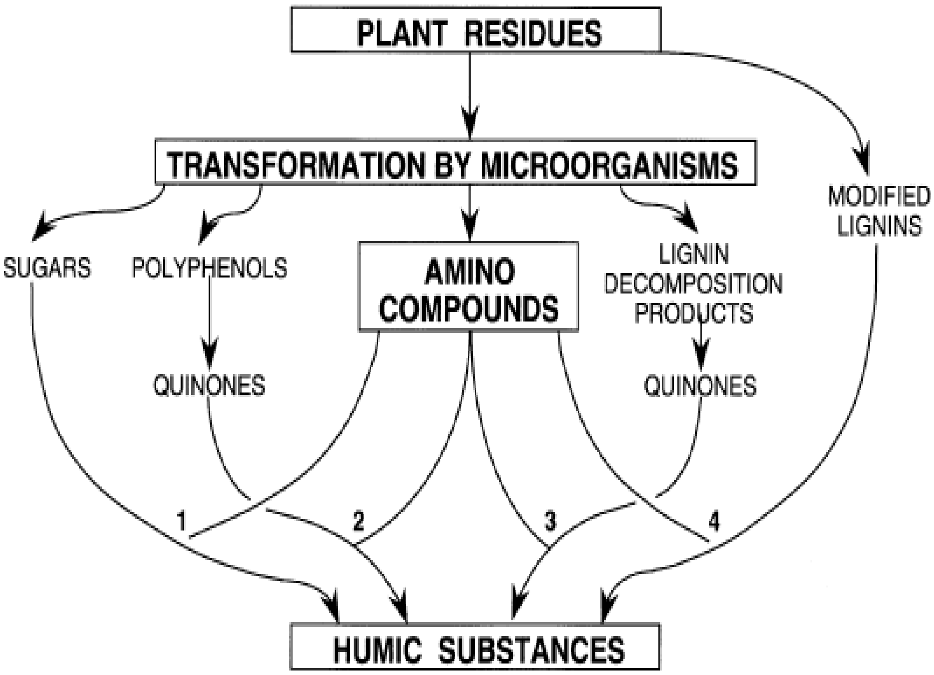

Figure 2.1 Different pathways for the formation of soil humic substances from plant residues (Stevenson, 1982)

Plant residues are broken down by microorganisms to form amino and other organic compounds such as sugars (pathway 1), polyphenols and different lignin compounds are further broken down to quinones (pathways 2 and 3) and modified lignins (pathway 4). These products may then react with amino compounds to form humic substances (Stevenson, 1982). The humic substances consist of three main groups: humic acids, fulvic acids and humins (Hopkins & Stark, 2003), which are discussed below.

2.2 Humic acids, fulvic acids, humates and humins

2.2.1 Humic acids

Humic acids consist of a mixture of weak carbon chains and carbon rings that are water soluble at a pH greater than 2, and are believed to be complex macro-molecules composed of linked aromatic groups and complexes of amino acids, peptides, amino sugars and aliphatic compounds (Figure 2.2) (Selim *et al.*, 2009).

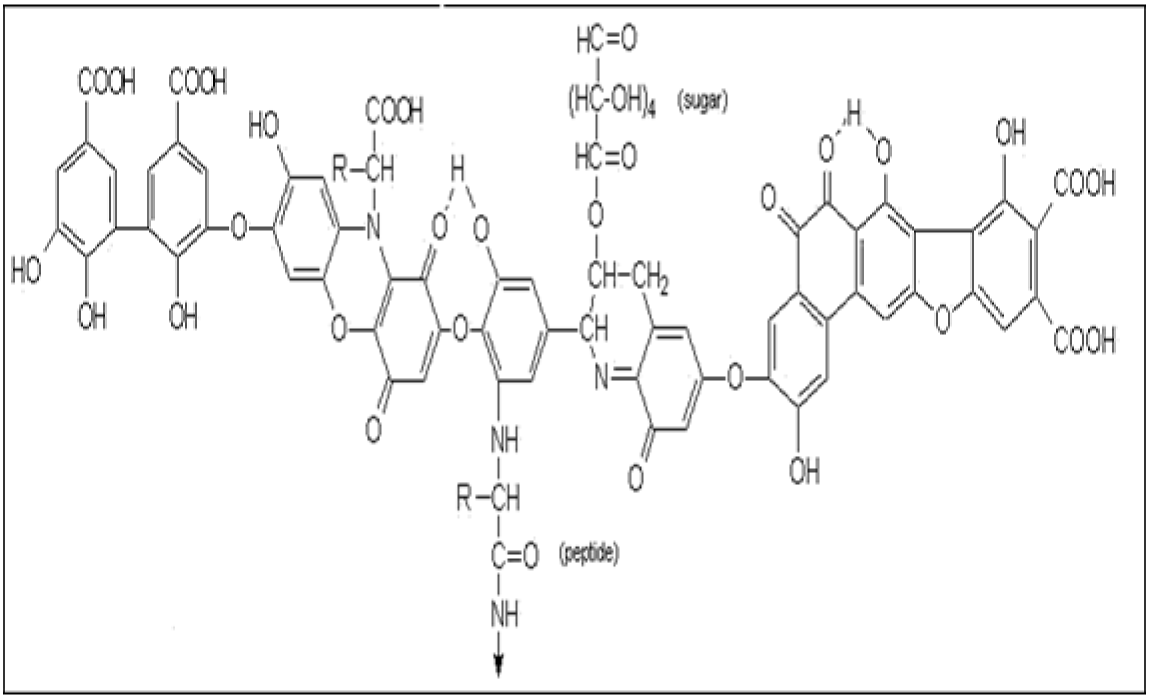

Figure 2.2 The basic structure of humic substances (Stevenson, 1982)

The chemical composition and structure of humic substances is determined by the process of decomposition of plant and animal tissues. The molecular size, compositions, weight and position of functional groups varies depending on the age and source of the material. Various carboxyl (COOH) groups are bounded to aromatic rings with phenolics (OH) and sugars to form long complex polymer chains of humic substances (Figure 2.2). Chemical analysis of humic acids, from various sources, shows a high content of C and O and depending on the source may also contain different concentrations of Na, Ca, K, Mg, Mn, Al, S, P, Zn, Fe, N, and Cu (Rupiasih & Vidyasagar, 2009). Although humic and fulvic acids have been studied for more than 200 years the actual structure and properties are still elusive (Jeffrey *et al.*, 1996). The main differences between humic and fulvic acids are that fulvic acid has a lower molecular weight than humic acid and contains more carbohydrate and carboxylic groups (Giannouli *et al.*, 2009). The molecular weight of humic acid ranges from 3 000 to 1 000 000 Da (1 Da = 1.6605402 10^{-24} g) while the molecular weight of fulvic acid ranges from 500 to 5 000 Da (Stevenson, 1982).

2.2.2 Fulvic acids

Fulvic acids are compounds with aromatic organic acids and weak aliphatic chains that are soluble under low and high pH conditions. The molecular structure of fulvic acids resembles that of humic acids and humins. However, the oxygen content of fulvic acids is double than that of humic acids and form part of the hydroxyl (-OH) and carboxyl (-COOH) groups, which increases the chelating properties of fulvic acid compounds (Pettit, 2004).

2.2.3 Humates

Humates are produced by treating humic or fulvic acids with NaOH or KOH. The alkali is a reagent used to extract organic matter such as humic acids and fulvic acids and helps with the isolation of a considerable fraction of organic matter (Bremner & Harad, 1958). Humates manufactured from brown coal contain a large number of phenolic and carboxylic groups that serve as a carbon source in the soil for microorganisms (Imbufe *et al.*, 2004). The humates increase biological activity and improves chemical reactions in soils by binding other nutrients (Shujrah *et al.*, 2010).

2.2.4 Humins

Humins are the fraction of humic substances that is insoluble in a water solution at both a low and high pH. Humins are slow to decompose and have a wide range of molecular weight that ranges between 100000-10000000 Da (Pettit, 2004; Jeffrey *et al.*, 1996).

2.3 Influence of humic and fulvic acids on the physical, chemical and biological properties of soil

2.3.1 Influence of humic and fulvic acids on the physical properties of soil

The large surface area and charge associated with humic and fulvic acids increases the cohesive forces causing fine soil particles and clay to bind to each other to form macro and micro-aggregates that leads to an increase in the water holding capacity of the soil (Ebtisam *et al.*, 2012). For example, Sharif *et al.* (2002) and Piccolo *et al*. (1996) reported that when humic acids were applied at a rate of 50-100 mg kg^{-1}, the aggregate stability improved. Piccolo & Mbagwu (1990) reported that humic acid, fulvic acid and humin serve as a carbon and energy source for microorganisms and the functional groups of COOH, OH and phenolic groups play a role in improvement of soil structure. It was also reported that humic acids may improve the soil physical properties due to an increase in the organic content of the soils

(Selim *et al*., 2009). The effect of humic acids seems to be associated with chelating nutrients that influence physical properties of the soil (Hishamo & Sherif, 2007).

2.3.2 Influence of humic and fulvic acids on the chemical properties of soil

Humic and fulvic acids containing N may serve as a slow release N-fertiliser when applied at high quantities to the soil (Nisar & Mir, 1989). Jeffrey *et al.* (1996) also found that humic and fulvic acids form soluble complexes with cations in the soil that result in the long distances migration of these cations. On the other hand when humic acids were applied with N, P and K-fertiliser through drip irrigation, the leaching of N and K was reduced but P availability increased (Selim *et al*., 2009).

2.3.3 Influence of humic and fulvic acids on the biological properties of soil

The applications of humic acids increase and stimulate microbial growth in the soil (Sharif *et al*., 2002; Selim *et al*., 2010) and Piccolo *et al*. (1992) found that the carboxyl groups of humic acids serve as a carbon source that increase the biological growth. Visser (1985) reported that when humic acids are applied at a rate of 30 mg l^{-1} the heterotrophic and autotrophic microbial activity increases due to improved cell membrane permeability (Valdrighi *et al*., 1996).

2.4 Plant response to humic and fulvic acid applications

2.4.1 Nutrient uptake

In gerbera, the N, P, K, Ca and Mg uptake increased with humic acid application (Nikbakht *et al*., 2008), while Verlinden *et al*. (2009) studied the effect of humic substances on nutrient uptake in grass, maize, potato and spinach crops and found that N, P and Mg-content increased significantly. Sharif *et al.* (2002) reported that the potential effect of humic acids on nutrient uptake and cation exchange capacity are related to the chemical and biological content of the products.

Humic acid sprayed on the leaves of irrigated wheat resulted in the increase of carbohydrates which affect biological yield (Shaaban *et al*., 2009). When humic acids were sprayed at a concentration of 1g l^{-1} on gerbera plants, the macronutrients (N, P, K and Mg) and micronutrients (Fe and Zn) of the leaves increased (Nikbakht *et al.*, 2008).

Khaled & Fawy (2011) studied the effect of different concentrations of humic acids on nutrient content, plant growth and soil properties under saline conditions. They found that soil applied humus improved the N-uptake of maize while humic acid application enhances P, K Mg, Ca, Zn and Cu uptake. Salman *et al*. (2005) reported similar trends on fruit yield and quality of watermelon where humic acids increased the N, P and K-content of the leaves. Turgay *et al. (*2011) reported that humic substances stimulate micronutrient status, plant growth and grain yield in a bread wheat cropping system over two experimental seasons.

2.4.2 Root growth

It was found that maize root development significantly improves with the application of humic acids (Sharif *et al*., 2002) and they also found that the shoot biomass in maize increased when humic acid was applied at a rate of 50 to 300 kg ha^{-1}. They concluded that the improvement was due to the increase in soil microbial population, microbial activity, water holding capacity, nutrient availability and increase in the cation exchange capacity of the soil.

Nikbakht *et al*. (2008) reported an increase in fresh and dry weight and root growth due to the presence of hormone-like cytokines, gibberellins and indole acetic acids in humic acids. Sara *et al.* (2010) also showed that the use of humic and fulvic acids affect root architecture due to auxin-like hormones in humic substances.

2.4.3 Plant growth and yield

A correlation between plant growth and the amount of humic acids applied exist, because humic acids act as a chelating agent for nutrients that increase their availability (Tahir *et al.*, 2011). When humic acids were applied in pot trials at the rate of 60 mg kg^{-1}, plant growth and shoot weight of wheat plants increased (Tahir *et al.*, 2011). The same tendencies was reported for maize in a pot trial where, the shoot weight increased significantly ($p<0.005$) when humic acid was applied at the rate of 50 mg kg^{-1} (Sharif *et al.*, 2002). When humic acids extracted from leonardite, shoot growth in wheat increased with an increase in K, Mg, Ca, Fe and B-content of the plant (Katkat *et al*., 2009). In another study done by Silvia *et al.* (2004) higher nitrate content in maize leaves was reported when treated with humic substances compared to untreated plants.

A study done by El-Bassiony *et al.* (2010) showed that when humic acids were applied to snap beans, the number of leaves, branches, fresh and dry weight of the whole plant increased. They also reported an improvement in the green pod yield and quality, measured as pod length, weight, pod chlorophyll content, fibre, total protein and N, P and K-content. In another study Selim *et al*. (2009) reported that humic substances increase tubers size, yield, quality and starch content of potato cultivated in a sandy soil.

The applications of humic acids have shown to increase crop yield under different cultivation practices, the increasing was due to the influence of carboxylic and phenolic components, associated with humic acids (Kalaichelvi *et al.*, 2006). Salman *et al.* (2005) concluded that the fruit yield of a watermelon crop increased with the application of humic acids. Humic acids applied at a rate of 200 mg l^{-1}, increased the Fe and Zn-content of chlorophyll in melon and soybean plants (Chen *et al.*, 2004). Prakash *et al.* (2011) reported that the application of potassium humate increased the total biomass, protein, ash, moisture content and fibre in mushrooms (*Pleurotus florida*). Another experiment was done on the potato plants with humic acid applied at a rate of 120 kg ha^{-1} and it was found that tuber production, chlorophyll, nitrate, starch, ascorbic acid and protein content increased (Selim *et al.*, 2009).

2.5 Conclusions

Humic acids consist of a mixture of weak carbon chains and rings and are water soluble at a pH greater than 2 (Selim *et al.*, 2009), and serve as a catalyst for microorganism activity and stimulation of microbial growth in the soil (Sharif *et al.*, 2002). Humic acid increases soil fertility and crop production and plants show a more active metabolism and improve respiration activities which are attributed to the carboxyl and hydroxyl group of humic acids (Petronio *et al.*, 1982; Rajpar *et al.*, 2011). Humic acids improve macro and micro-nutrient uptake and plant growth (Nikbakht *et al.*, 2008).

Fulvic acids are water-soluble at low and high pH-conditions, are smaller molecules with double the amount of oxygen atoms and twice the CEC of humic acids due to the high number of carboxyl groups (Pettit, 2004).

Humates and fulvates obtained by alkaline extractions from brown coal have beneficial effects such as the increase in biological activity and improves the physical and chemical properties of soils due to the higher phenolic and carboxylic groups (Hishamo & Sharif, 2007; Shujrah *et al.*, 2010).

Humins are fractions of humic substances with low solubility at all pH levels and the macro-organic humins increases cation exchange and soil fertility (Jeffrey *et al*., 1996).

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CHAPTER 3

THE EFFECT OF HUMATES AND FULVATE ON THE VIABLE MICROBIAL POPULATION

3.1 Introduction

Microorganisms are involved in processes such as N fixation, the solubilisation of P and trace elements, and stabilising soil aggregates (Tinker, 1984; Tisdall, 1994; Gyaneshwar *et al.*, 2002). Several studies were performed to evaluate the effect of humic and fulvic acids on soil microbial communities and results varied according to the source and structure of the acids (Vaughan & Malcolm, 1985; Valdrighi *et al*., 1996). Sharif *et al*. (2002) performed laboratory incubation studies to determine the effect of humic acids on soil biological properties. He reported that the application of 0.5 and 1.0 kg ha^{-1} humic acid increased the bacterial populations of the soil by 355-476% and the fungal populations by 610-716%, due to the establishment of a favourable biochemical environment. Furthermore, Valdrighi *et al*. (1996) studied the effect of compost derived humic acids on plant biomass and soil microbial populations and found that humic acids increased the vegetative growth of chicory and enhanced bacterial populations in soil.

Enzymes, produced by microorganisms, plant roots and soil animals, play a crucial role in biochemical nutrient cycling in soil. For example, the biological oxidation of organic compounds is mainly a dehydrogenation process catalysed by dehydrogenase enzymes (Weaver *et al.*, 1994). Soil dehydrogenase activity represents a group of intracellular enzymes occurring in living soil microbes and can be used as an indicator of poor or good quality soil (Garcia *et al*., 1997). Since the group of enzymes are not active as an extracellular enzyme in soil, it is considered a good indicator of overall microbial activity (Alef & Nannipieri, 1995). Several researchers found that these enzymes respond to changes in soil quality related to anthropogenic activities (Rao *et al.*, 2003) such as soil pollution with heavy metals (Hinojosa *et al.*, 2004) and/or herbicides (Wingfield *et al.*, 1977).

Lizarazo *et al.* (2005) used dehydrogenase activity in conjunction with alkaline phosphatase activity to evaluate the effect of three commercially available humic amendments. They found that fulvic acids, with a high Kjedahl-N content, resulted in constant high enzyme activities while humus lignite resulted in the highest increase in dehydrogenase activity while humus peat showed no effect. They concluded that the materials (various humic substances), although extensively utilized and recommended for the enhancement of plant and microbial growth, all perform in a different way.

The effect of humic amendments on the microbial populations in soil can therefore not only be predicted based on their humic and/or fulvic acid content but also on their structural characteristics, which dependents on their origin. The aim of this study is to determine the effect of two humates (low and high ash content) and a fulvate on the bacterial and fungal numbers in two soil types as well as to determine its effect on the soil microbial activity based on the dehydrogenase enzymes.

3.2 Materials and methods

3.2.1 Soil sampling and analysis

Two soil samples, (sandy clay and sandy clay loam) were collected from the upper part of the soil profiles (0-20 cm) at the Hatfield Experimental Farm of the University of Pretoria. The soil samples were air dried, milled and sieved through a 2 mm sieve before conducting physical and chemical analyses according to the methods described by the Standard of Soil Science South Africa (SSSSA) use by the Soil Science Laboratory of the University of Pretoria. Soil texture was determined with a hydrometer method and soil EC and pH were determined from a 1:2.5 soil:water suspension. Ca, Mg, K and Na were extracted with ammonium acetate and concentrations determined using Inductively Coupled Plasma Atomic Emission Spectroscopy (ICP-OES). The phosphate content of the soils was determined using Bray I method and concentration determined with ICP-OES. NO_3^- and NH_4^+ were extracted with KCl and analysed with the Kjeldahl method (Non-Affiliated Soil Analysis Work Committee, 1990). Results from the soil analysis are shown in Table 3.1.

Table 3.1 Physical and chemical properties of selected soils

	Water content	**Sand**	**Silt**	**Clay**	**pH**	**EC**	**Elemental analysis**						
			(%)				**K***	**Ca***	**Mg***	**Na***	**P****	**NO_3^{-***}**	**NH_4^{+***}**
						(mSm^{-1})				**mg kg^{-1}**			
Sandy clay	11.7	58	6	36	5	19	25	104	18	9	78	28	8
Sandy clay loam	1.3	78	2	20	6	14	67	501	163	5	25	16	5

* NH_4OAc extractable cations
** Bray-I
*** Kjeldahl method

3.2.2 Chemical analysis of selected humates and fulvate

Two commercially available humates differentiated by their ash content and one fulvate (highly soluble) were used in this study. Nitrogen, C and H concentration were determined by dry oxidation with a Carlo-Erba instrument. Samples were digested with nitric-perchloric acid and K, Ca, P, Mg, Mn, Zn, Cu concentrations determined with ICP-AES. pH were determined using a 1:5 and EC from a 1:10 soil:water suspension. Moisture and ash content were determined by drying samples at 70°C for 24 hours and the difference in weight before and after drying was regarded as water loss. Ash content was determined by heating a known weight of the samples at 600°C for at least one hour. Results from the humates and fulvate analysis are shown in Table 3.2.

Table 3.2 Moisture content and chemical properties of humates and fulvate

	Moisture	**Ash**	**pH**	**EC**	**Elemental analysis**								**Elemental analysis**					
					N*	**C***	**H***	**S****	**K****	**Ca****	**Na****	**Fe****	**P****	**Cl^{-}****	**Mg****	**Mn****	**Zn****	**Cu****
	(%)			**(mS m^{-1})**	**(%)**								**(mg kg^{-1})**					
Humate (La)	7.79	14.28	9.8	2390	0.6	34	3.07	10	12.3	1.25	3.77	0.4	147	975	1535	69.6	15.7	5.9
Humate, (Ha)	5.83	63.69	10.7	729	0.12	40	1.62	7.8	0.1	5.54	0.1	0.1	113	1685	1167	206	24.9	1.4
Fulvate	5.75	32.24	4.9	346	0.28	17	5.16	0.8	0.2	0.02	3.24	0.6	297	857	346.5	21.1	55.1	60

* Dry oxidation
** Nitric-perchloric acid digested and determined on ICP-OES

La= Low ash

Ha= High ash

Table 3.3 Loading of elements

Loading elements	**C**		**H**		**K**		**Ca**		**Na**		**Mg**	
	kg ha^{-1}	mg kg^{-1}	kg ha^{-1}	mg kg^{-1}	kg ha^{-1}	mg kg^{-1}	kg ha^{-1}	mg kg^{-1}	kg ha^{-1}	mg kg^{-}	kg ha^{-1}	mg kg^{-1}
Humate (La)	68	17.00	6.14	1.53	24.6	6.15	2.50	0.62	7.54	1.88	30.7	7.67
Humate (Ha)	80	20.00	3.24	0.81	0.2	0.05	11.08	2.77	0.20	0.05	23.34	5.83
Fulvate	34	8.50	10.32	2.58	0.4	0.10	0.04	0.01	6.48	1.62	6.93	1.73

3.2.3 Experimental design

All experiments were conducted at the microbiology laboratory of the Department of Plant Production and Soil Science (University of Pretoria). Sandy clay and sandy clay loam soil were supplemented with two different fertiliser application rates 220-50-80 kg ha^{-1} which represents 100% of the recommended N, P and K-application rate for a commercial 13 year old citrus orchard and 165-37.5-60 kg ha^{-1}, which represents of 75% of the recommended N, P and K-application rate. Nitrogen, P and K were applied in the form of ammonium nitrate (NH_4NO_3), potassium dihydrogen phosphate (KH_2PO_4) and potassium nitrate (KNO_3), respectively.

Fertilised soil was further amended with either humate low ash (La), humate high ash (Ha) or fulvate at an application rate of 200 kg ha^{-1}. Controls included soil without humate or fulvate and soil without fertiliser and humate or fulvate: All experiments were done in triplicate and a summary of the treatments used is shown in Table 3.4.

Table 3.4 Summary of the treatments used

No	Treatments	Treatment description	N, Pand K (kg ha^{-1})	Humate and Fulvate (kg ha^{-1})
1	Control 0	Soil without fertiliser and humate or fulvate	0-0-0	0
2	Control 75	Soil + N, P and K recommended 75%	165-37.5-60	0
3	Control 100	Soil + N, P and K recommended 100%	220-50-80	0
4	Humate (La) 75	Soil + N, P and K recommended 75% + humate (La)	165-37.5-60	200
5	Humate (La) 100	Soil + N, P and K recommended 100% + humate (La)	220-50-80	200
6	Fulvate 75	Soil + N, P and K recommended 75% + fulvate	165-37.5-60	200
7	Fulvate 100	Soil + N, P and K recommended 100% + fulvate	220-50-80	200
8	Humate (Ha) 75	Soil + N, P and K recommended 75% + humate (Ha)	165-37.5-60	200
9	Humate (Ha) 100	Soil + N, P and K recommended 100% + humate (Ha)	220-50-80	200

3.2.4 Microbial analysis

3.2.4.1 Quantification of heterotrophic bacteria and fungi

Bacterial and fungal populations in all treatments and controls were enumerated at each sampling interval (fortnightly for one month). Serial dilutions, up to 10^{-6} in sterile Ringers solution (quarter strength) were used for enumeration of microbial populations by plate counts. Total bacteria and fungi were counted using the spread plate technique. One gram of the sampled soil was placed in sterilised container with 9 ml Ringers solution. The bottles were shaken for 20 min at 230 rpm in order to remove microbial cells from the soil particles. One hundred microliters of the soil dilutions (10^{-1} to 10^{-6}) were spread in triplicate onto Tryptone Soy agar (tenth strength) and Potato Dextrose agar (full strength) for the enumeration of bacteria and fungi, respectively. The plates were incubated at 25°C and bacteria and fungi enumerated after 2 and 3 days, respectively.

3.2.4.2 Dehydrogenase activity

Dehydrogenase activity was determined according to the method described by Alef & Nannipieri (1995). Five gram of field-moist soil was placed in 50 ml Greiner tubes and incubated with 2 ml of 3% 2, 3, 5 triphenyl tetrazolium chloride (TTC) for 24h at 30°C. After incubation, 10 ml of acetone was added and the suspension was homogenized with agitation for 2h (once every 30 minutes) and then centrifuged at 4000 rpm for 5 min. Reactive products were measured at 546 nm (red colour) using a spectrophotometer. A sample without soil containing 2 ml buffer instead of TTC, was used as a control. Dehydrogenase activity was calculated as follows:

$$\text{Dehydrogenase activity} = \frac{TPF\frac{(\mu g)}{ml}\times 45}{dwt\times 5} \qquad (2)$$

Where, *TPF* is standard solution, *dwt* is the dry weight of one gram moist soil, 5 is the weight of moist soil used (g) and 45 is the volume of solution added to the soil sample in the assay (Alef & Nannipieri, 1995).

3.2.5 Statistical analysis

The data was analysed using analysis of variance and the means of the results were compared using least significant difference (LSD) with the software Statistical Analyses System (SAS) version 9.2.

3.3 Results and Discussion

3.3.1 Heterotrophic bacteria in sandy clay and sandy clay loam soils

Heterotrophic bacterial numbers in sandy clay loam and sandy clay soils were higher when amended with either humate or fulvate as compared to bacterial numbers in control soils (Figures 3.1 and 3.2). Several studies have shown a similar effect of humic acids on microbial growth (Visser, 1985; Valdrighi *et al.*, 1996; Tikhonov *et al.*, 2010). This beneficial effect of humic substances on microorganisms can be indirect through its high cationic exchange capacity, providing essential cations like chelated Fe (Burk *et al.*, 1932; Toledo *et al.*, 1980) and therefore aiding microbial growth. Humic substances can also affect microbial growth through direct methods such as 1) supplying nutrients which can serve as an energy source and building blocks (Filip & Bielek, 2002; Vallini *et al.*, 1997; Tikhonov *et al.*, 2010; Charest *et al.*, 2005) and by 2) improving membrane permeability to nutrient uptake (Visser, 1985; Valdrighi *et al.*, 1996). The addition of higher concentrations of N, P and K (100%) on the bacterial numbers in control soils and soil treated with humate and fulvate increase after 2 weeks depending on treatments (Figure 3.1). Higher bacterial numbers in sandy clay loam were observed when humates (low and high ash) were applied compared to fulvate application. In contrast, fulvate resulted in a rapid increase in bacterial numbers present in sandy clay soil. The effect of soil and its ability to buffer changes should however not be discarded. Overall, bacterial numbers were higher in the sandy clay soil for all treatments possibly due to higher PO_4 and NO_3 concentrations, higher moisture content as well as higher clay content to buffer chemical changes. In most cases the initial increase in bacterial numbers, when soil was amended with either humates or fulvate was rapid, followed by either a slower increase or decrease after a 2 week incubation period (Figure 3.2). This trend could be ascribed to the availability of nutrients from the humate or fulvate during the first two weeks followed by the depletion of the readily available sources and more stable to microbial attack.

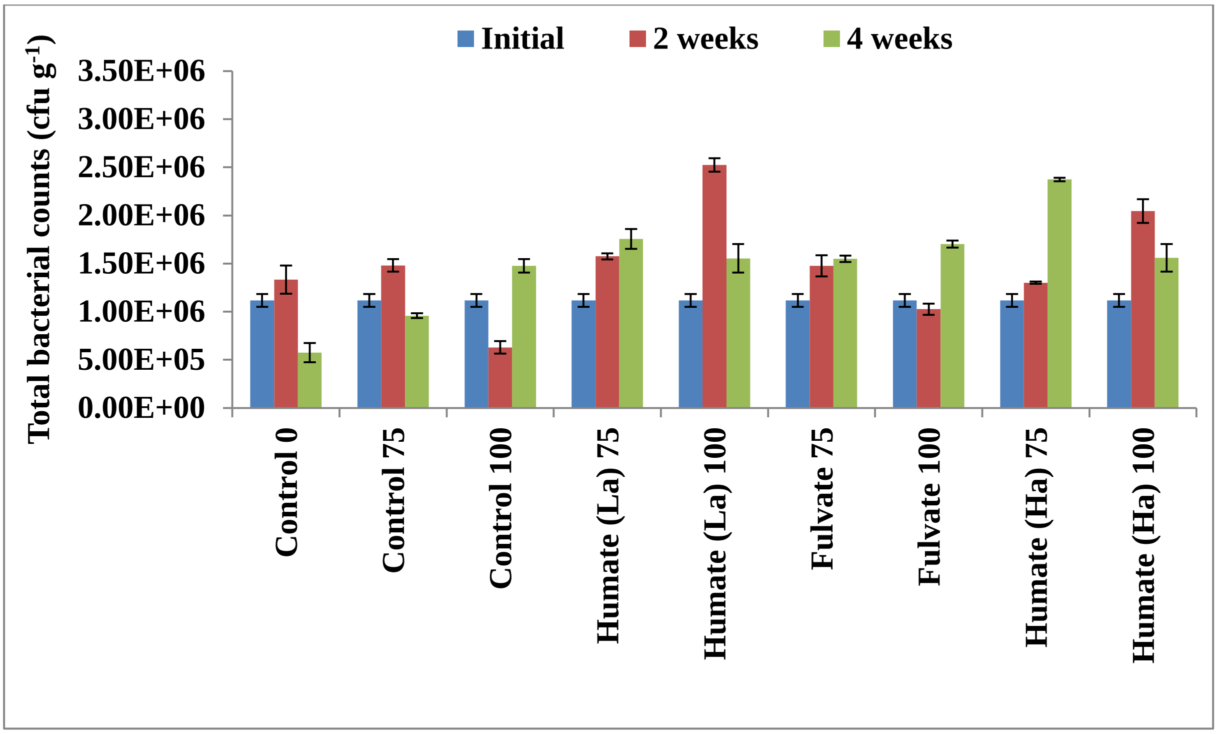

Figure 3.1 Culturable heterotrophic bacteria (cfu g^{-1}) in a sandy clay loam.

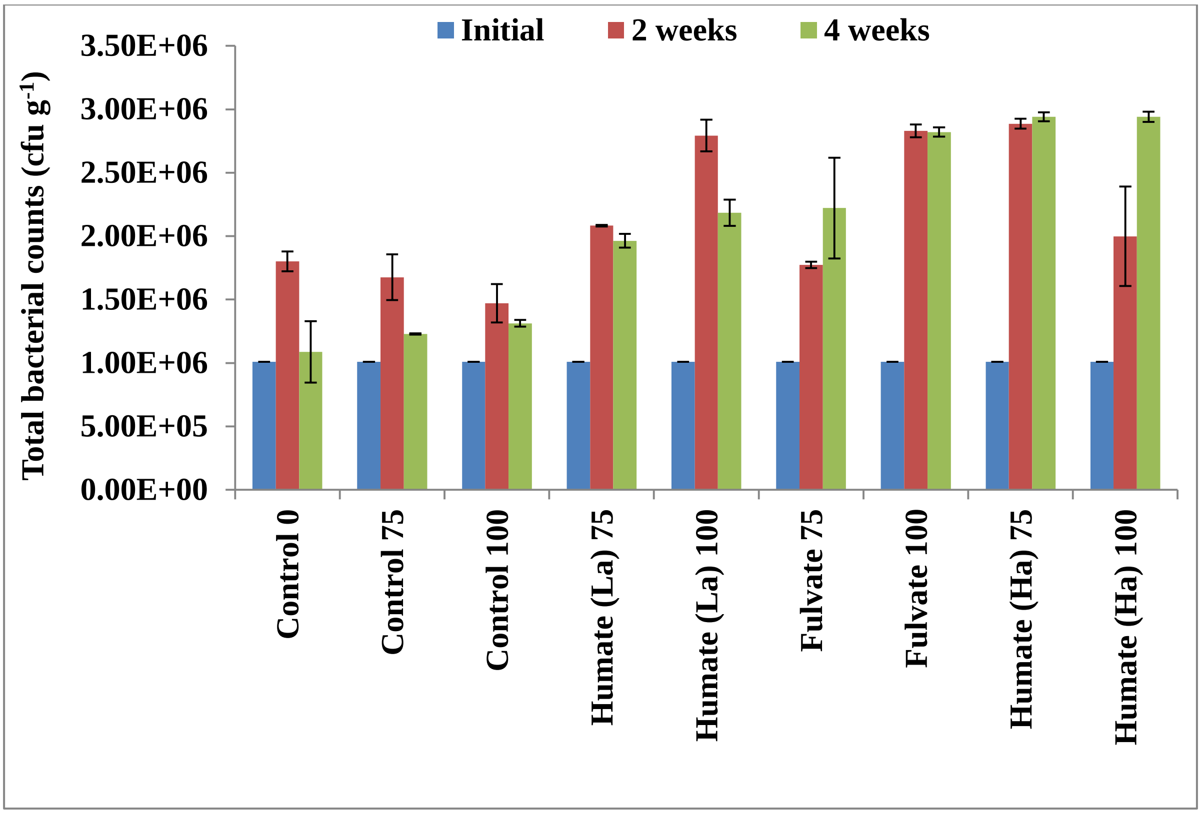

Figure 3.2 Culturable heterotrophic bacteria (cfu g^{-1}) in a sandy clay soil.

3.3.2 Heterotrophic fungi in sandy clay loam and sandy clay soils

The highest fungal counts in a sandy clay loam soil (Figure 3.3) were observed when amended with humate (La and Ha) under 100% N, P and K levels, while fulvate and humate (Ha) in the presence of 100% N, P and K levels performed the best when applied to sandy clay soil (Figure 3.4). The addition of 100% N, P and K to a sandy clay loam soil resulted in higher fungal counts for the control and humate/fulvate treated soil after 4 weeks than the controls. In a sandy clay soil, amendment with higher concentrations of N, P and K had a less prominent effect, only showing noticeable higher fungal counts for soil treated with fulvate (Figure 3.4). Fungal numbers in both soils were at its maximum for all control and humate/fulvate treated soil amended with N, P and K after 4 weeks. Similarly to bacterial populations, fungal numbers were lower in sandy clay loam amended with fulvate as compared to fungal numbers in sandy clay soil amended with fulvate. The molecular weight of fulvic acids are lower than that of humic acids and can therefore have a greater effect on the growth and activities of microorganisms (Charest *et al.*, 2005). Overall, the addition of humates or fulvate to both soils showed a higher increase in the fungal population as compared to control soils. These increases are believed to be due to the nutrient content of humates and fulvates, supplying the fungi with an energy source and building blocks (Filip & Bielek, 2002; Vallini *et al.*, 1993; Tikhonov *et al.*, 2010; Charest *et al.*, 2005). Similarly, fungal counts were shown to increase beyond 4 weeks when soil was amended with different potassium humate products in a study done by Van Tonder (2008). Results published by several other researchers (Dackman *et al.*, 1987, Manici *et al.*, 2003 and Albertsen *et al.*, 2005) demonstrated that application of organic substances resulted in an increase in fungal numbers.

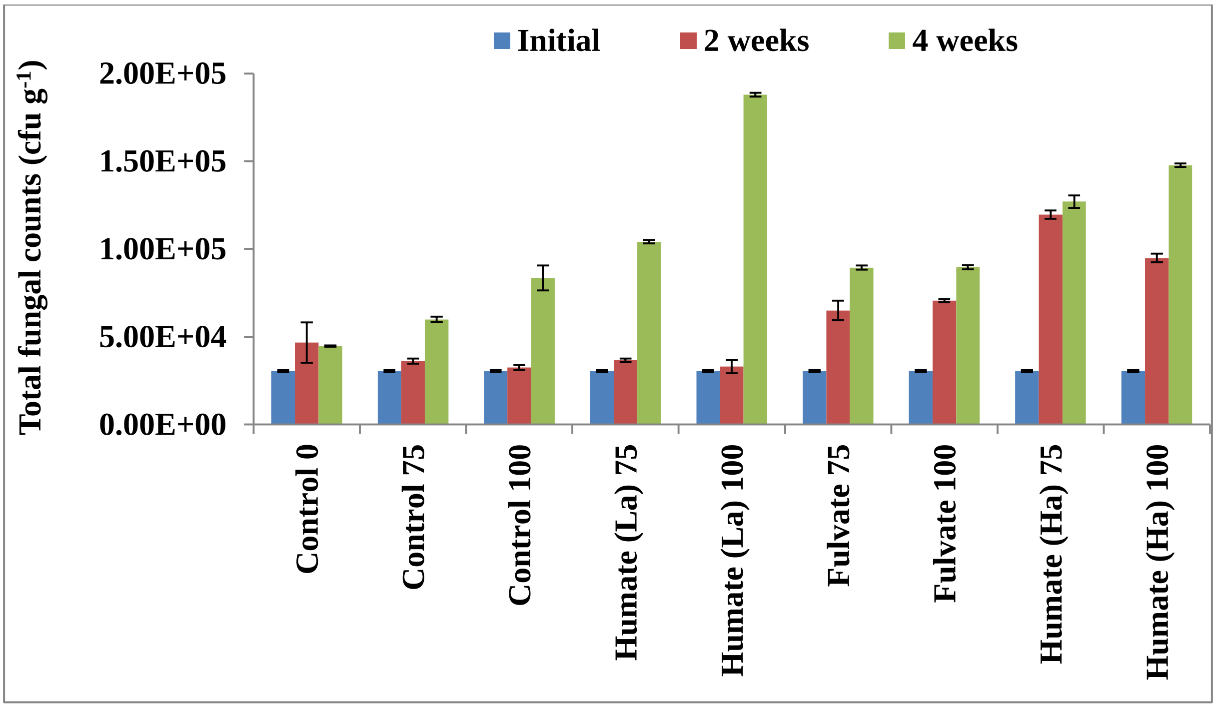

Figure 3.3 Culturable heterotrophic fungi (cfu g^{-1}) in a sandy clay loam soil.

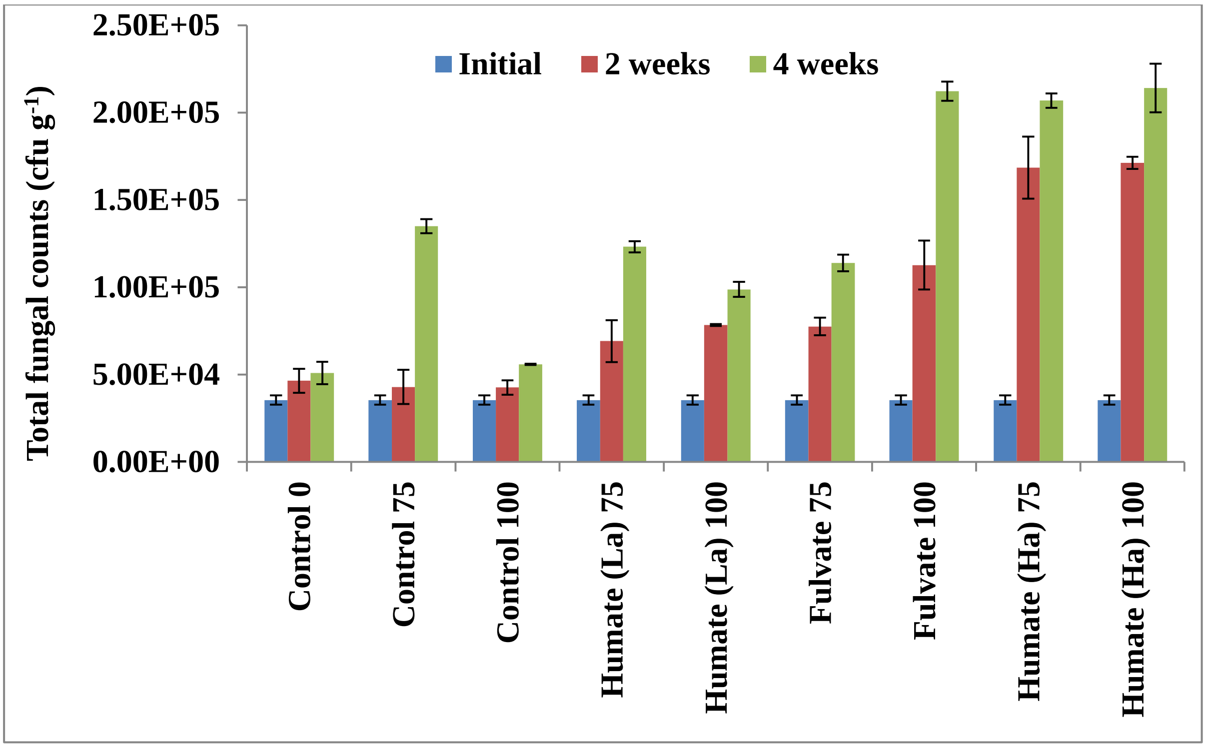

Figure 3.4 Culturable heterotrophic fungi (cfu g^{-1}) in a sandy clay soil.

3.3.3 Dehydrogenase activity in sandy clay loam and sandy clay soils

Sandy clay loam soil amended with humate (La) and 100% N, P and K showed the highest dehydrogenase activity after 4 weeks (Figure 3.5). Among the control samples, the highest microbial activity was seen when soil was amended with 100% N, P and K. For all the sandy clay loam soil treatments and controls microbial activity decreased during the first two weeks after amendments followed by an increase in activity after 2 weeks which could be ascribed to the community adapting to its new environment. This is plausible as sandy soils have a lower ability to buffer chemical changes as compared to clay soils. Fulvate treated sandy clay loam soils showed the lowest increase in dehydrogenase activity as compared to soils treated with humate (low and high ash). Moreover, the carbon content of fulvic acid used in this study was lower than that of the humic acids (Table 3.2), thus supplying less available carbon for microbial biomass production. Studies done by Lizarazo *et al.* (2005) showed an increase in dehydrogenase activity after an aridisol was supplemented with a fulvic acid and a humus lignite (containing mainly humic acids). Dehydrogenase activity increased in all controls and treated sandy clay soils after the initial sampling (Figure 3.6). This increase could be due to the higher moisture content of this soil which could increase microbial activity and chemical reactivity. The addition of humates/fulvate resulted sometimes in a slightly higher microbial activity as compared to control soils. Moreover, the addition N, P and K also resulted in a slight increase in the microbial activity in some cases.

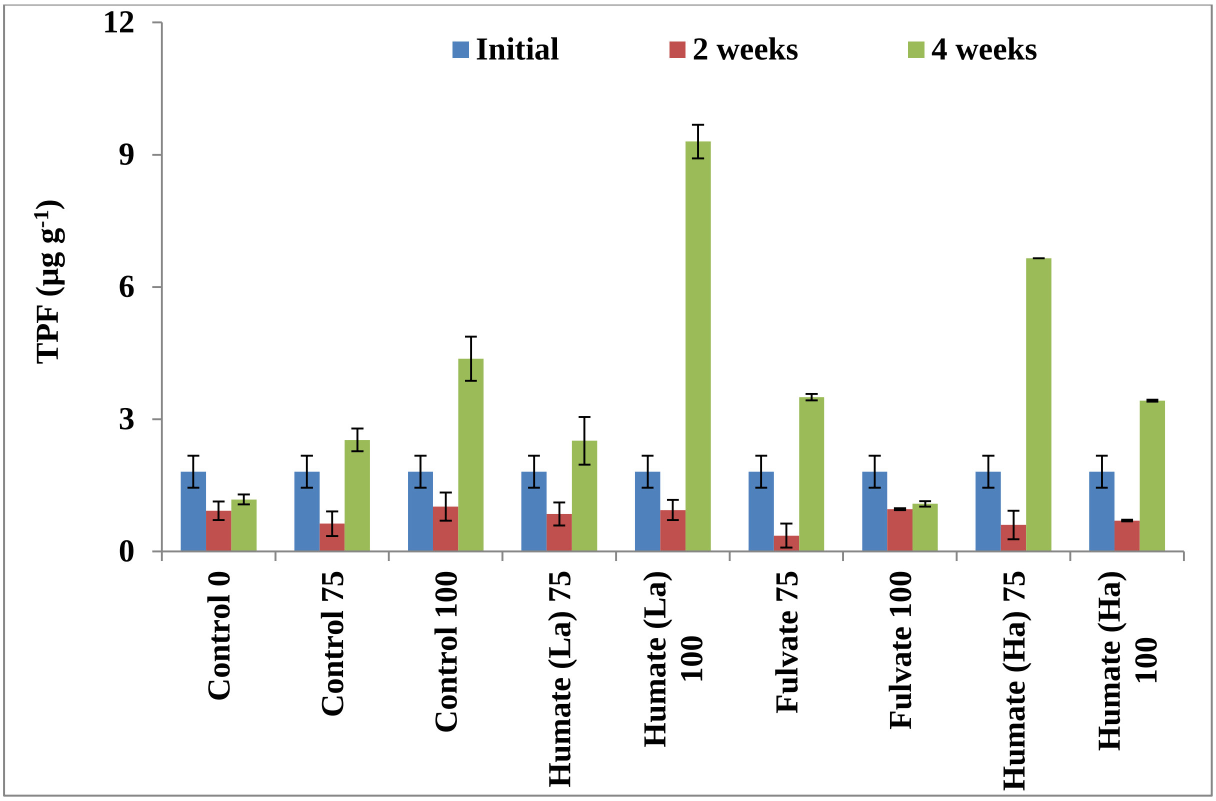

Figure 3.5 Dehydrogenase activity as measured by TPF (µg g^{-1}) in sandy clay loam soil.

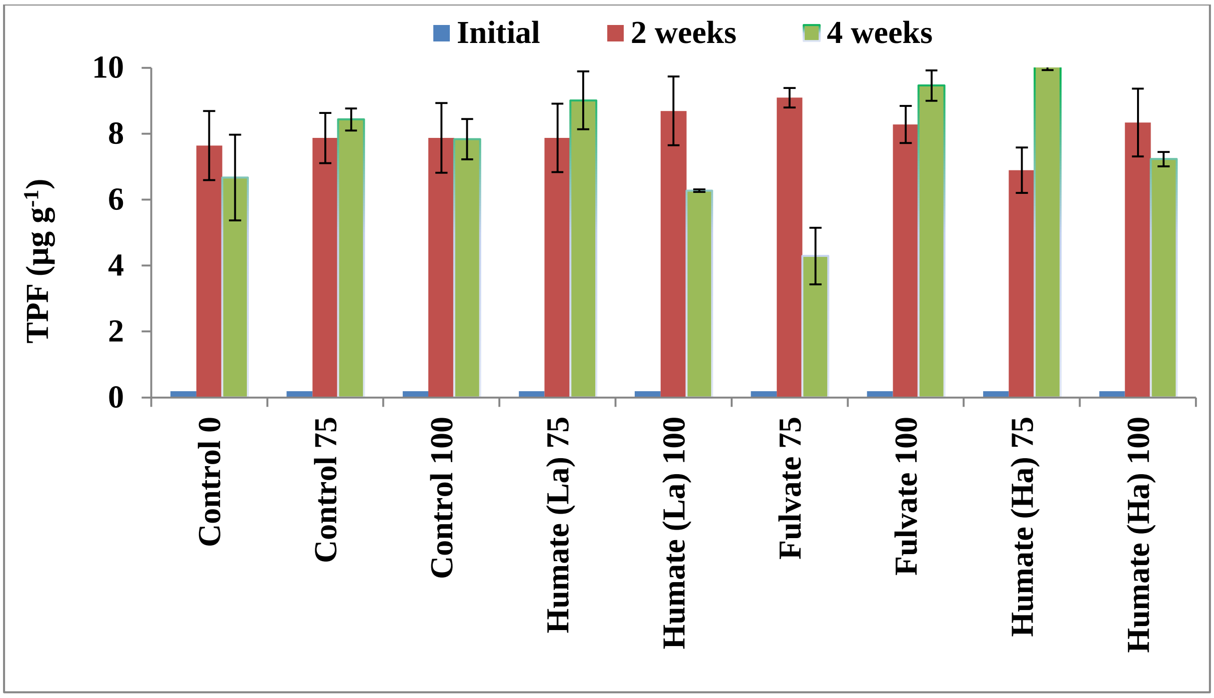

Figure 3.6 Dehydrogenase activity as measured by TPF (µg g^{-1}) in sandy clay soil.

3.4 Conclusion

The overall effect of the two humates (low and high ash) and the fulvate used in this study on the bacterial and fungal numbers as well as on the microbial activity in a sandy clay loam and sandy clay soil was of a positive nature. The amendment of sandy clay soil with humates and a fulvate showed a higher increase in bacterial and fungal numbers than in sandy clay loam soil. In sandy clay loam soils the bacterial and fungal numbers were lower when fulvate was added as with humate application. Furthermore, fulvate appeared to have a more pronounced effect on bacterial and fungal populations when added to sandy clay soil. This is believed to be due to the higher clay content and moisture percentage of the sandy clay soil, buffering changes in soil and allowing for chemical reactions to take place due to its higher water holding capacity. In most cases the addition of N, P and K at both concentrations (75 and 100%) resulted in an increase in bacterial and fungal numbers, with the 100% application showing overall highest numbers for most treatments. The extent of humates and fulvates on microbial populations seems to be highly dependent on the type of soil which it is applied to and the chemical composition and structure of these compounds.

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CHAPTER 4

NITROGEN, PHOSPHORUS AND POTASSIUM LEACHING AS INFLUENCED BY HUMATES AND FULVATE

4.1 Introduction

Nitrogen, P and K are essential macro nutrients for all plants (Hopkins & Ellsworth, 2005). Baligar *et al*. (2001) found that the efficiency of N, P and K-fertilisers applied to soil is less than half for N, less than 10% for P, and approximately 40% for K. Ledgard *et al*. (1996) reported for a silt loam soil that received 990 mm yr^{-1} rain, that the N leaching was 18 kg ha^{-1} when 220 kg ha^{-1} yr^{-1} and 31 kg ha^{-1} yr^{-1} when 360 kg ha^{-1} N was applied to soil. Phosphorus losses from agriculture areas to surface water resources have been significant (Kleinman *et al.*, 2002), while K leaching was estimated to be 0.6 mg l^{-1} at 1 m depth (Askegaard & Eriksen, 2000).

Humic acid is a promising natural resource that also can be manufactured commercially to be utilised as an alternative to increase crop production and to reduce fertiliser application (Sharif *et al*., 2002; Selim *et al*., 2009). The N content of lettuce and soil P availability increased with humic acid application (Mesut & Yilmaz, 2005), and the fruit quality and yield of watermelons also increased with the addition of humic acid (Salman *et al*., 2005). For other crops it was also reported that with the addition of humic acid the soil N, P and K application can be reduced (Shaaban *et al*., 2009). For irrigated wheat, it was found that soil fertilisers could be reduced to 75% of the recommended application (Shaaban *et al*., 2009). For tomatoes the fertilisers could be reduced by 25% (Abdel –Mawgoud *et al*., 2007) and for grapevines the N application was reduced by 50% without compromising yield or quality of the crop (Eman *et al*., 2008).

In a study on the influence of humic acid on the growth of maize plants it was found that humic acid applied to the soil at rates more than 100 kg ha^{-1} did not have any significant effect on maize yield (Sharif *et al.,* 2002). However, Jones *et al* (2007) reported that humic acid increases yield and nutrient availability at higher rates (72–145 kg ha^{-1}).

The aim of this study was to determine the effect of humates and fulvate on the leaching of N, P and K in two types of soils under controlled conditions.

4.2 Materials and methods

4.2.1 Experimental layout

This experiment was conducted at the soil physics laboratory of the Department of Plant Production and Soil Science at the University of Pretoria. Soil samples were collected in October 2011 from the Experimental Farm of the University of Pretoria and were analysed for selected chemical and physical properties (Table 3.1).

The leaching experiment was conducted in a laboratory using columns consisting of Plexiglas (0.1 m diameter and 0.3 m high). Each column was fitted with five filters of four different sizes that ranged from 5 µm to 2 mm (Figure 4.1). The leaching studies were conducted on two types of soil (sandy clay loam and sandy clay) and consist of nine treatments (Figure 4.2) and four replications.

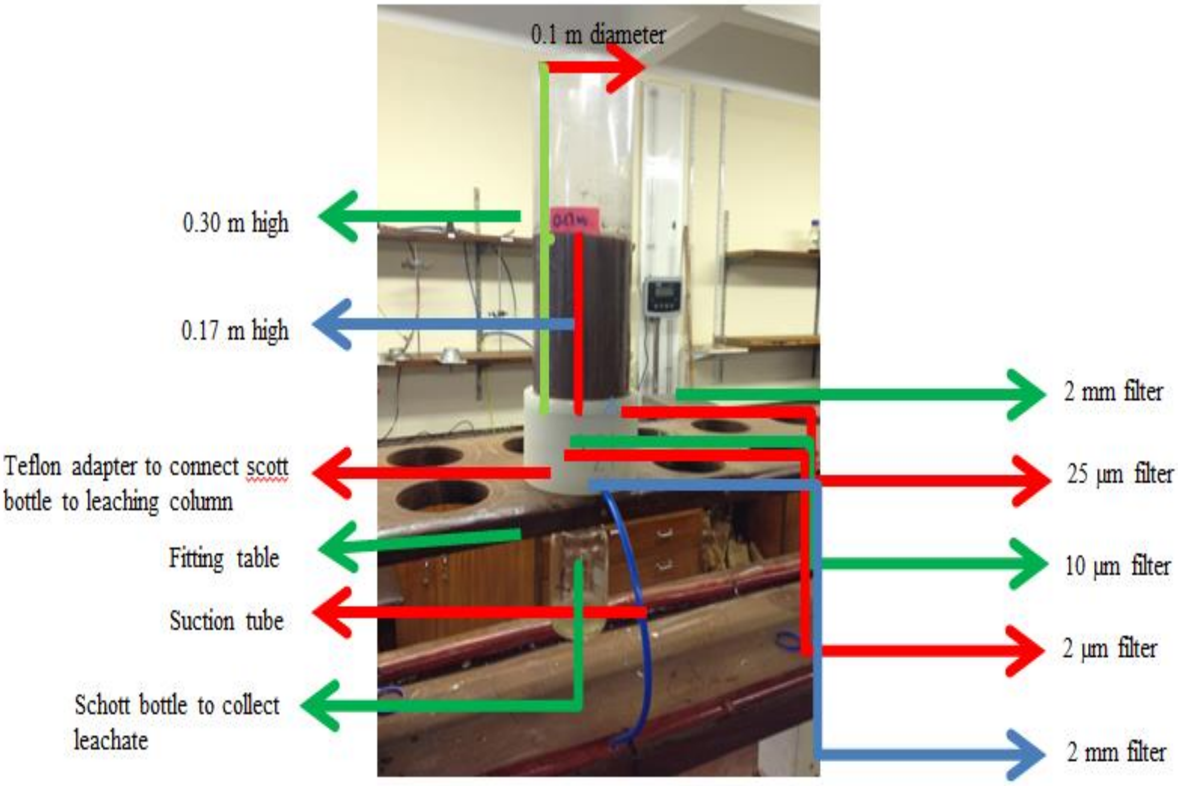

Figure 4.1 Leaching column

The leaching columns were arranged in a completely randomised block design (CRBD) on laboratory benches. The filter material were inserted and arranged as shown in Figure 4.1 and the suction tubes and Schott bottles were then connected to the columns. The soils were mixed prior to filling the leaching columns with 50 mg kg^{-1} (equivalent to approximately 200 kg ha^{-1}) humates or fulvate. Nitrogen, P and K were then added to the soils at two concentration levels, 100% (220-50-80) and 75% (165-37.5-60) of the fertiliser recommendation for citrus and thoroughly mixed prior to filling the leaching columns (Fertiliser Handbook of South Africa, 2007). The different columns were filled with different soils to a height of 0.17 m at a bulk density of approximately 1498 kg m^{-3}. Soils in the leaching columns were left for 14 days to react with N, P, and K fertilisers and the humates and fulvates.

Figure 4.2 Example of leaching columns used

4.2.2 Application of leaching water

The volume of water applied to each column was calculated from bulk density, porosity and the pore space of the soils.

$$\rho = \frac{m}{v} = \frac{2\ kg}{0.001335\ m^{-3}} = 1498\ kg\ m^{-3} \tag{1}$$

Where: ρ is bulk density m is mass and v is volume

$$\Phi = 1 - \frac{\rho Bulk}{\rho Particle} = 1 - \frac{1498\ kg\ m^{-3}}{2650\ kg\ m^{-3}} = 0.435 \tag{2}$$

Where: Φ is porosity, $\rho Bulk$ is bulk density and $\rho Particle$ is particle density

$$\rho\varsigma = \Phi * v = 0.435 * 1335\ cm^3 = 580.7\ cm^3 = 580.7\ cm^3 \approx 580.7\ ml \tag{3}$$

Where: ρς is pore space, Φ is porosity and ν is volume.

The amount of water applied to each column was assumed to be equal to pore space and was calculated using eq.3. The soils in the leaching columns were subjected to three times of wetting and drying cycles after 30 days. After wetting, the leachate was collected from the different treatments and filtered with a Whatman no 2 filter to remove turbidity and analysed.

4.2.3 pH, EC and N, P and K analyses of the leachate and soils

4.2.3.1 pH and EC of the leachate

The pH and EC of the leachate were measured according to the methods described in the Handbook of Standard Soil Testing Methods for Advisory Purposes (Non-Affiliated Work Committee, 1990).

4.2.3.2 Determination of NH_4^+ and NO_3^- concentration of the leachate

For NH_4^+ determination, 15 ml of a 50 % (v/v) NaOH solution and to 25 ml boric acid was added to the total leachate collected from each treatment and distilled for 6 minutes. The distillate was titrated with 0.01 M HCl and NH_4^+ concentration was calculated. For the determination of NO_3^- concentration, a spatula tip of Devarda Alloy was added to the distilled samples and left until the solution was completely reduced. The solution was then redistilled for 6 minutes with 25 ml of boric acid. The distillate was titrated with 0.01 M HCl and the titrated amount of NO_3^- was calculated. All procedures were done according to the Handbook of Standard Soil testing Methods for Advisory Purposes (Non-Affiliated Soil Analysis Work Committee, 1990).

4.2.3.3. Determination of P and K concentration of the leachate

Phosphorus and K concentration were determined from 15 ml of the filtered leachate with axially viewed Inductively Coupled Plasma Atomic Emission Spectrometer (ICP-AES) according to the procedure described by the Handbook of Standard Soil Testing Methods for Advisory Purposes (Non-Affiliated Soil Analysis Work Committee, 1990).

Nitrogen, P and K, concentrations were calculated using the following formula:

$$\frac{ppm * \frac{leachate\,(g)}{1000}}{2} \qquad (4)$$

Where: mg kg $^{-1}$ (ppm) is mg per kilogram solution as measured by ICP-AES, *leachate (g)* is the mass of leachate per treatment, 1000 is to convert g to kg and 2 is the mass of soil in kg used in the column.

4.2.3.4 Determination of N, P and K in the soils

NO_3^- and NH_4^+ were extracted from the soils with KCl and determined with the Kjeldahl method. Phosphorus was extracted with the Bray I method and K with ammonium acetate and then the concentration was determined with ICP-OES (Non-Affiliated Soil Analysis Work committee, 1990).

4.2.4 Statistical analysis

The data were analysed using analysis of variance and the means of the results were compared using least significant difference (LSD) with the Statistical Analyses System (SAS) version 9.2.

4.3 Results and discussion

4.3.1 pH measurements of the leachate

There were significant differences ($p<0.01$) between pH-values of the leachate of the different treatments. For both soils the pH of the leachate was higher when humate or fulvate were added in the soil as compared to the controls (Table 4.1).

There was a significant difference ($p<0.01$) in the initial pH of sandy clay measured at 15 days. The pH of the leachate of the humate (Ha) combined with N, P and K fertilisers treatments were significantly higher compared to the fulvate and humate (La) fertiliser combinations or fertilisers alone. During the second cycle of leaching, humate (La and Ha) combined with 100% or 75 % N, P and K-fertilisers and fulvate combined with 100% N, P and K fertilisers resulted in a higher pH compared to the 75 % N, P and K-fertiliser treatment and the control. For the third leaching cycle the pH of the leachate of the humate (Ha) combined with 100% N, P and K-fertilisers were in general higher compared to the other treatments, although not significantly.

In a sandy clay loam, the treatment consisting of humate (La) and 100% N, P and K-fertilisers, the fulvate combined with fertilisers 100% N, P and K-fertilises and the treatment of humate (Ha) combined with fertilisers were significantly ($p<0.01$) higher than the control. For the second leachate (after 30 days of the mixture) the pH increased for the humate and fulvate (Ha and La) combined with 100% N, P and K. Then after 60 days (leachate from third cycle) the pH increased significantly for the treatments consisting of humate (La) 100, fulvate 100 and humate (Ha) 75 and 100 compared to the controls.

These results are in accordance with the study of Shujrah *et al.* (2010) that reported that humates increase the pH of acidic soils after 60 days of incubation. Imbufe *et al.* (2004) also reported that humates increase the pH buffering of acidic soil.

Table 4.1 pH of the leachate collected form sandy clay and sandy clay soils

	Treatments	**Sandy clay**			**Sandy clay loam**		
		15 days	**30 days**	**60 days**	**15 days**	**30 days**	**60 days**
1	Control 0	4.8^{c}	5.0^{d}	5.4^{ab}	$4.8d^{e}$	5.1^{bcd}	4.8^{d}
2	Control 75	5.4^{b}	5.3^{cd}	5.7^{ab}	4.7^{e}	5.3^{abc}	4.9^{cd}
3	Control 100	5.4^{b}	5.7^{ab}	5.3^{b}	5.1^{cd}	5.4^{abc}	5.1^{bc}
4	Humate (La) 75	5.4^{b}	5.7^{ab}	5.8^{ab}	4.7^{e}	4.7^{d}	5.0^{bcd}
5	Humate (La) 100	5.3^{b}	5.7^{ab}	5.7^{ab}	5.4^{bc}	5.7^{a}	5.7^{a}
6	Fulvate 75	5.3^{b}	5.5^{bc}	5.7^{ab}	5.4^{bc}	4.9^{cd}	5.0^{cd}
7	Fulvate 100	5.4^{b}	5.6^{ab}	5.7^{ab}	5.5^{b}	5.6^{ab}	5.3^{b}
8	Humate (Ha) 75	5.9^{a}	5.7^{ab}	5.8^{ab}	5.9^{a}	4.9^{cd}	5.6^{a}
9	Humate (Ha) 100	5.9^{a}	6.0^{a}	6.0^{a}	5.3^{bc}	5.9^{a}	5.7^{a}
	LSD	**0.36**	**0.35**	**0.36**	**0.36**	**0.55**	**0.26**

Values in each column followed by the same letter were not significantly different $p < 0.01$.

4.3.2 EC measurements of the leachate

The results from the EC measurements of the leachate of a sandy clay and a sandy clay loam soil mixed with humates or fulvate and different concentrations of fertilisers are presented in Table 4.2. From these results it is clear that the EC of the leachate increase with increase in fertiliser concentration for all treatments. The EC measurements done of the leachate of the soils containing fertilisers with humate or fulvate combined with 100% or 75 % N, P and K after 15 days were higher or equal to the corresponding controls except for humate (La) 75 which were lower than control 75 and 100. The data shows that the EC decreases with each leaching cycle in both soils. Electrical conductivity of the leachate of the sandy clay soil was higher than for the sandy clay loam for all each cycle and treatment. The increase in EC is mainly explained by humates and fulvate that plays an important role in chelating cations in the soils that may increases their mobility. Shujrah *et al.* (2010) reported that potassium humate increased the EC after 30 days of incubation with an acid soil.

Table 4.2 EC of leachate collected from sandy clay and sandy clay loam soils (mS m^{-1})

	Treatments	**Sandy clay**			**Sandy clay loam**		
		15 days	**30 days**	**60 days**	**15 days**	**30 days**	**60 days**
1	Control, 0	45.9^{d}	12.6^{d}	7.9^{d}	16.7^{e}	3.4^{e}	4.6^{e}
2	Control 75	98.3^{c}	34.4^{c}	16.0^{6c}	69.2^{d}	21.3^{d}	14.9^{d}
3	Control 100	101.4^{bc}	42.4^{abc}	24.1^{b}	105.0^{bc}	27.6^{b}	17.6^{bc}
4	Humate (La) 75	88.4^{c}	45.4^{ab}	25.8^{ab}	88.6^{cd}	27.9^{b}	15.3^{d}
5	Humate (La) 100	126.4^{b}	48.3^{a}	27.5^{a}	105.2^{bc}	25.9^{bc}	22.4^{a}
6	Fulvate 75	101.3^{bc}	37.2^{bc}	18.0^{c}	89.5^{cd}	32.6^{a}	17.3^{bc}
7	Fulvate 100	112.8^{bc}	48.1^{a}	23.0^{b}	105.0^{bc}	32.0^{a}	17.3^{bc}
8	Humate (Ha) 75	105.0^{bc}	46.5^{ab}	18.7^{c}	122.6^{ab}	25.2^{bc}	16.3^{cd}
9	Humate (Ha) 100	162.9^{a}	44.0^{ab}	24.3^{b}	144.5^{a}	24.1^{cd}	19.2^{b}
	LSD	**25.87**	**9.36**	**2.93**	**25.54**	**3.19**	**1.93**

Values in each column followed by the same letters were not significantly difference $p<0.01$.

4.3.3 N concentration of the leachate

Nitrogen concentration of the leachates of the soils mixed with humate, fulvate and fertilisers are presented in Figure 4.3. These results indicate that humates and fulvate application have a significant ($p<0.01$) effect on reducing N leaching. For sandy clay soil the N concentration of the leachate of control 0 was 9.7 mg kg^{-1} (37.9 kg ha^{-1}), for control 75 it was 10.56 mg kg^{-1} (42.2 kg ha^{-1}) and for control 100 it was 11.6 mg kg^{-1} (46.7 kg ha^{-1}). Whereas N concentration for humates and fulvate combined with fertilisers varied between 2.3 mg kg^{-1}(9.1 kg ha^{-1}) and 6.3 mg kg^{-1} (25.1 kg ha^{-1}) (Figure 4.3).

The results for the sandy clay loam are presented in Figure 4.3. The N concentration of the leachate of for control 0 was 5.75 mg kg^{-1} (22. 99 kg ha^{-1}), for control 75 it was 14.50 mg kg^{-1} (57.98 kg ha^{-1}) and for control 100 it was 16.88 mg kg^{-1} (67.50 kg ha^{-1}). The N concentration of the leachate from the humates and fulvate treatments varied between 1.90 mg kg^{-1} (7.59 kg ha^{-1}) for the lowest N and 5.08 mg kg^{-1} (20.31 kg ha^{-1}) for the highest N. Humates and fulvate mixed with N, P and K fertilisers manifested a significant influence ($p<0.01$) on reducing N leaching compared to the controls. Shaaban *et al*. (2009) reported that the applications of humic acids reduce the leaching of N fertiliser in a silty clay soil and Ortega & Fernandez (2007) also reported that humic and fulvic reduce N due to high stimulation of microbial growth. On the other hand Avnimelech & Raveh (1976) reported that half of the N leached when fertilisers were applied.

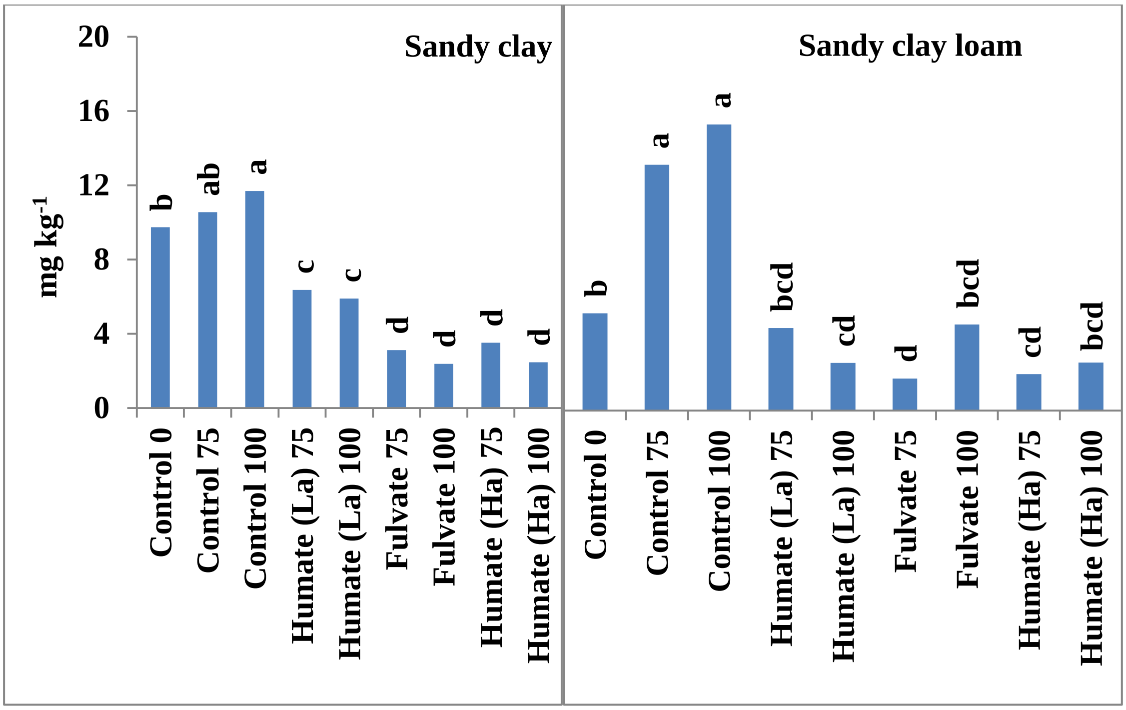

Figure 4.3 Nitrogen leached (mg kg^{-1}) from sandy clay and sandy clay loam soils.

4.3.3.1 N mass balance for sandy clay and clay loam soil

The N balance of the sandy clay and sandy clay loam soil was calculated from the following formula:

$$N\ mass\ balance = N\ input\ (Initial + fertiliser) - N\ output\ (Leachate + retained)\ (5)$$

Where N_{intial} is the in situ N from the mineral and organic complexes of the soils used, $N_{fertiliser}$ is N added from fertiliser, $N_{leachate}$ is N from the leaching water collected and $N_{retained}$ is N from soil analysed at the end of the trial. On average the N mass balance error for sandy clay soil was 5.2% (Table 4.3), while for sandy clay loam it was 16.6% (Table 4.4). This may be due to the N mass balance components such as atmospheric losses, nitrification and denitrification and the mineralisation of N from the humates and fulvates which were not considered in calculating the mass balance error.

In general for both soils, the percentage of the applied N that was leached varied between 2.7-8.3% for humates and fulvate and between 12.9-27.5% for the control treatments. The leaching reduction from humates and fulvate treatments was approximately 300% compared to the controls. Therefore it is can be concluded that humates and fulvate were beneficial in reducing N leaching from the soils, and it is reasonable to expect that this will translate to increased availability to crops.

Table 4.3 N mass balance for the sandy clay soil.

No	Treatments	mg kg^{-1}								
		Initial	Applied	Total applied	Leached	% of total N leached	Retained	Total leached + Retained	*Mass balance error	*% error
1	Control 0	35.4	0	35.4	9.74	27.51	24.66	34.4	1	2.8
2	Control 75	35.4	41.25	76.65	10.56	13.78	66.26	76.86	-0.17	0.2
3	Control 100	35.4	55.01	90.41	11.69	12.93	73.70	85.39	5.02	5.5
4	Humate (La) 75	35.4	41.25	76.65	6.37	8.31	64.14	70.51	6.14	8.0
5	Humate (La) 100	35.4	55.01	90.41	5.90	6.53	86.60	92.50	-2.09	2.3
6	Fulvate 75	35.4	41.25	76.65	3.12	4.07	86.16	89.28	-12.63	16.4
7	Fulvate 100	35.4	55.01	90.41	2.39	2.64	88.76	91.15	-0.74	0.8
8	Humate (Ha) 75	35.4	41.25	76.65	3.52	4.59	80.70	84.22	-7.57	9.8
9	Humate (Ha) 100	35.4	55.01	90.41	2.48	2.74	88.60	91.08	-0.67	0.7

* Error was calculated by Total applied – Total leached + Retained, * Mass balance error divided by Total leached + Retained x 100.

Table 4.4 N mass balance for the sandy clay loam soil.

No	Treatments	mg kg^{-1}								
		Initial	Applied	Total applied	Leached	% of leached compare to applied	Retained	Total leached + Retained	*Mass balance error	%
1	Control 0	20.9	0	20.90	5.75	27.51	15.83	21.58	-0.68	3.2
2	Control 75	20.9	41.25	62.15	16.88	27.16	39.06	55.94	6.21	9.9
3	Control 100	20.9	55.01	75.91	14.50	19.10	40.51	55.01	20.90	27.5
4	Humate (La) 75	20.9	41.25	62.15	2.82	4.53	75.78	78.60	-16.45	26.4
5	Humate (La) 100	20.9	55.01	75.91	4.88	6.42	52.60	57.48	18.43	24.2
6	Fulvate 75	20.9	41.25	62.15	5.08	8.17	63.25	68.33	-6.18	9.9
7	Fulvate 100	20.9	55.01	75.91	1.90	2.50	62.71	64.61	11.30	14.8
8	Humate (Ha) 75	20.9	41.25	62.15	2.82	4.57	71.16	74.00	-11.85	19.0
9	Humate (Ha) 100	20.9	55.01	75.91	2.15	2.83	62.75	64.90	11.01	14.5

* Error was calculated by Total applied – Total leached + Retained

4.3.4 P concentration of the leachate

The P concentration of the soils mixed with humate, fulvate and fertilisers are presented in Figure 4.4 for sandy clay and sandy clay loam. These results indicate that there is a significant trend for sandy clay and for sandy clay loam ($p<0.05$). The highest significant P leaching for the sandy clay soil was found for the fulvate 100 treatment. High P leaching was also recorded for humate (La) 100. The lowest P leaching was for the humate (Ha) 100 and control 0 treatments. For sandy clay loam, the results indicated the highest P leaching was for humate (La) 75, whereas the lowest P leaching was found for the control 0.

In general, the results showed that P leaching was the highest for the humate and fulvate combined with fertilisers treatments. Even though P leaching varied between 0.01 and 0.89 mg kg^{-1} across the difference soil types, the range of variation is low. These results are supported by the research conducted by Zhang (2008) who investigated, the effect of soil properties on P subsurface migration in sandy soils using column leaching, from which he found that P loss by leaching is low when Ca concentration in the soil solution is high. It is also well known that P does not easily leach in the soil due to various factors such as Ca (For the sandy clay the Ca content was 104 mg kg^{-1} and for sandy clay loam it was 501 mg kg^{-1}) and Fe and this could be the main reason why P leaching was lower for all treatments.

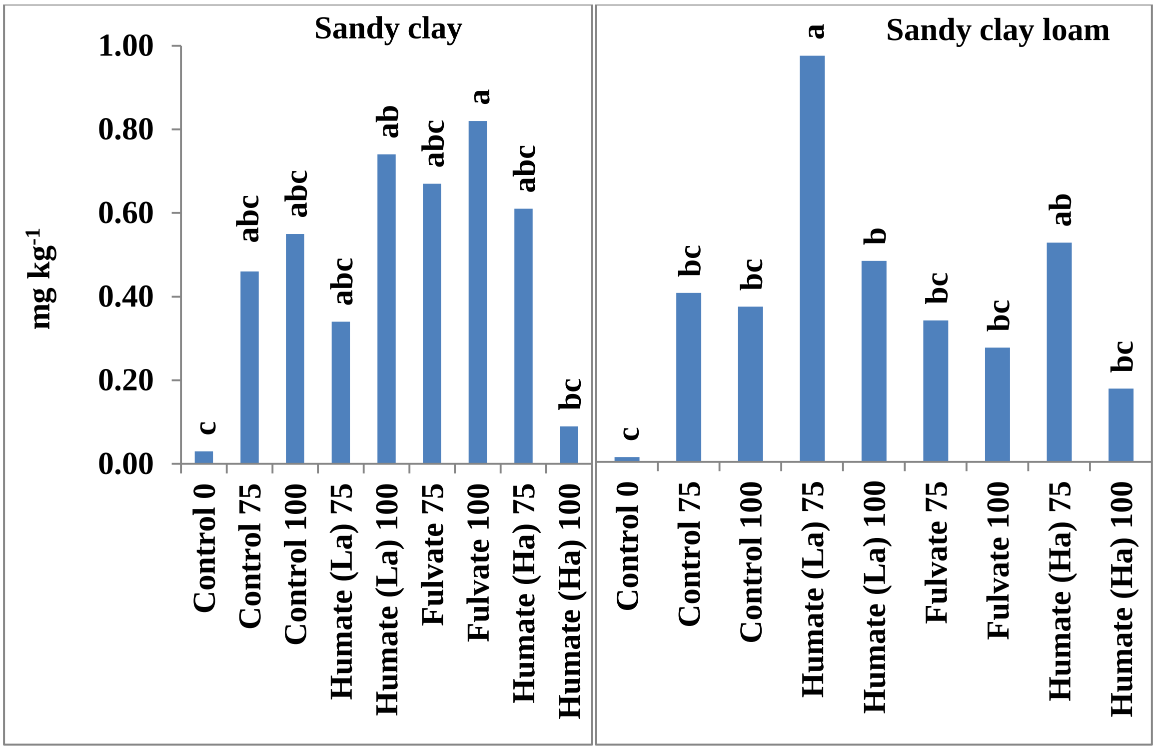

Figure 4.4 Phosphorus leached (mg kg^{-1}) from sandy clay and sandy clay loam soils.

4.3.5 K concentration of the leachate

The K concentration in the leachates of the different soils mixed with humate, fulvate and fertilisers are presented in Figure 4.5 for sandy clay and for sandy clay loam soils. The data shows that there is a significant ($p<0.01$) difference between the treatments in both soils. For sandy clay soil, the leaching of K from control 0 was 2.88 mg kg^{-1} (11.51 kg ha^{-1}) while for fertilisers and humates or fulvate combined with fertilisers varied between 6.75 mg kg^{-1} (26.99 kg ha^{-1}) and 9.14 mg kg^{-1} (36.55 kg ha^{-1}). For sandy clay loam, K leaching for control 0 was 1.15 mg kg^{-1} (4.59 kg kg^{-1}), for control 75 it was 6.81 mg kg^{-1} (27.23 kg ha^{-1}) and for control 100 it was 7.60 mg kg^{-1} (30.39 kg ha^{-1}). These results indicated that K leaching was high for humate (La) combined with fertiliser and for control 100 compared to the rest of the treatments although not significantly. The maximum leaching of K was for humate (La) 100.

In general, humates and fulvate did not decrease K leaching in both soils. Research done by Kolohchi & Jalali (2007) on the effect K leaching in sandy soil found that a high concentration of Ca increases K in the soil solution. Table 3.2 shows that Ca concentration is high in the sandy clay and sandy clay loam used, thus this could be the reason why K leaching was manifested with the treatments treated with fertilisers and humates and fulvate combined with fertilisers.

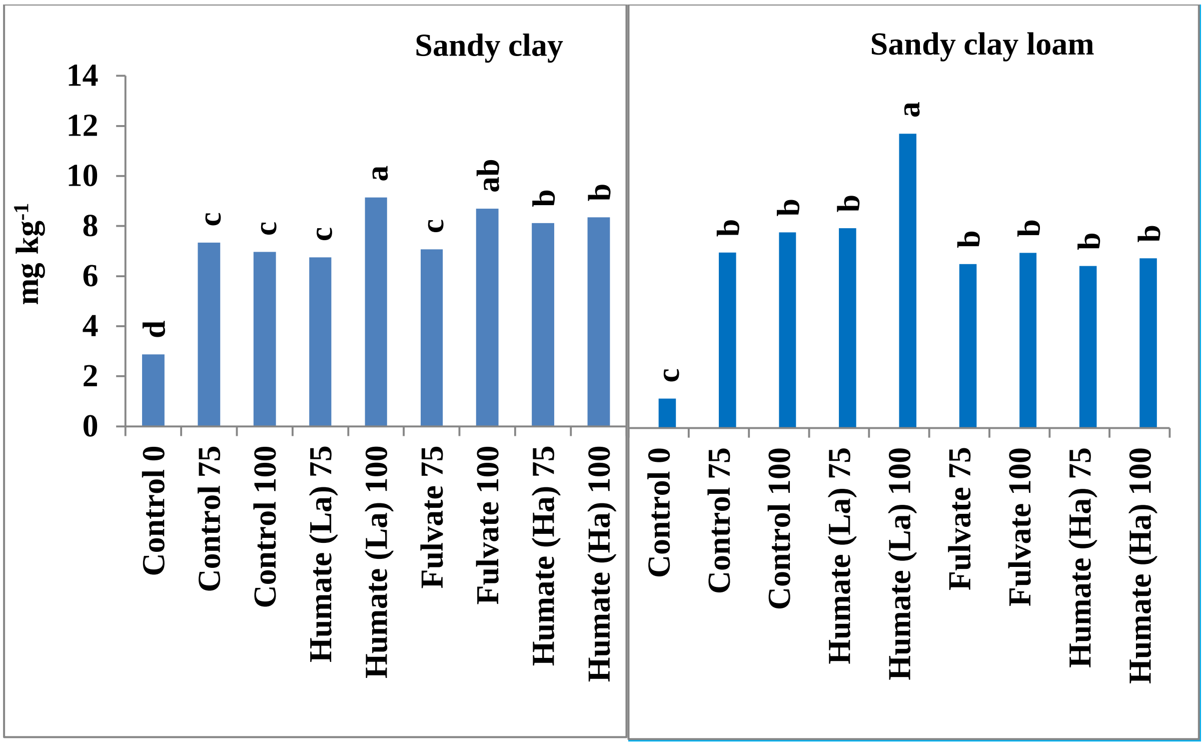

Figure 4.5 Potassium leached (mg kg^{-1}) from sandy clay and sandy clay loam soils.

4.4 Conclusions

These results showed that humates and fulvates mixed with fertilisers increase the pH and EC of the leachate of both soils. The addition of humate or fulvate to soils mixed with fertilisers showed a high significance ($p<0.01$) in decreasing the N concentration of the leachate of both soil types. Humic acids play an important role in the soil and increases nutrient availability and also increase chemical and biological properties of the soils by adding macronutrients. It was reported that humic acids increase carbon content and water holding capacity of the soils that reduces nutrient leaching (Hussein & Hassan, 2012).

Inconsistent results were found for P and K in both soil types and treatments due to the high concentration of P and K in the humates and fulvate. Therefore, humate and fulvate did no reduce P and K. The interaction between humic substances and P increases soil fertility at various soil layers (Selim *et al.*, 2010). The research done on the effect of the application of humic substances on quality and nutrition of potato tubers showed that the application of humic substances to the soil increases soil nutrient content (Ahmed, 2012).

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CHAPTER 5

INFLUENCE OF HUMATES AND FULVATE ON N, P AND K LEACHING AND UPTAKE IN POTTED CITRUS

5.1 Introduction

Citrus is grown on a wide variety of soil types and needs N, P and K to ensure optimum yield and quality (Fertiliser Handbook OF South Africa, 2007). Nitrogen is essential for synthesis of plant chlorophyll, proteins and enzymes. Phosphorus for phospho-proteins, phospho-lipids, ATP, ADP formation and root growth and K increases translocation and synthesis of proteins and stimulates enzyme activity (El-Bassiony *et al*., 2010).

Nitrate leaching and runoff into rivers and estuarine ecosystems are responsible for algal blooms and eutrophication that pose a public health risk (Beman *et al.*, 2005). The primary source of N pollution comes from fertiliser application, which is expected to triple by 2050 (Tilman *et al.*, 2001). Nitrate leaching from arable and horticultural land was found to be approximately half the N applied (Goulding, 2000) and Cuttle & Scholefield (1995) found that N leaching is influenced by climate and the soils physical, chemical and biological properties. Therefore, humates and fulvates potentially can limit this. Phosphorous losses by surface runoff from arable soils cause freshwater eutrophication, while the amount of K leached depends on rainfall and soil types (Alfaro *et al*., 2004).

The uses of organic soil amendments such as humic acids to increase crop production on a sustainable basis have become imperative because of the high cost of chemical fertilisers (Sharif *et al.*, 2010). Humic acids are commercially available as soluble salts in the form of humates and fulvates. They serve as source of trace elements and a easily metabolisable carbon source. Humate and fulvate can contribute to structure formation and in turn can increase soil aeration and promoting soil microbial activities. Humic substances contain long chains of hydrocarbon, fatty acids and esters (Hayes & Clapp, 2001). As a result, leaching of NO_3-N and K is reduced (Sharif *et al.*, 2002). Humic acids significantly increase the macro and micro-nutrient content of plant leaves (Petronio *et al.*, 1982; Nikbakht *et al.*, 2008; Pettit, 2004). Eman *et al*. (2008) found that the use of humic acids can reduce mineral N fertiliser application and soil and water pollution. Humic substances play an important role in reducing nutrients losses, degradation and leaching of cations by acting as a chelate. Many researchers recorded that humic acids form chelates with cations and the beneficial influences of humic acids seem to be supplementary to its cation-chelating ability by improving the physical, chemical and biological properties of the soil (Hishamo & Mohammad., 2007). Rajpar *et al.* (2011) reported that humic acid increases soil amendment and crop production even in unfertile soils. The addition of humic substances improves the structural and water retention properties of degraded soils. Humic substances have many hydrophilic and hydrophobic functional groups. Hydrophilic soils play the role of storing soil moisture and complexation of polyvalent cations in soil surfaces, while hydrophobic soils reduces soil slaking by preventing water loss (Mbagwa, 2003; Piccolo *et al.*, 1996).

In most studies involving humic substances or humates the shoot and root yield increased at low concentrations of 50 and 100 mg kg^{-1} (Sharif *et al*., 2002). It was also found that humate application increases seedling growth, plant growth, yield and marketable fruit compared to a control (Bray, 1976).

This chapter assesses the effect of humates and fulvate soil amendment on N, P and K leaching and uptake in potted citrus.

5.2 Materials and methods

5.2.1 Soil selection and analyses

A potted trial was conducted from November 2011 until April 2012 in a glass house at the Hatfield Experiment Farm of University of Pretoria (25° 45’S 28° 16’E).

Two soils of different textural classes (sandy clay soil and sandy clay loam soil) were used. The sandy clay soil sample was collected from the top 0.20 m Hutton soil profile at the Hatfield Experimental Farm. While the sandy loam clay soil was collected from the top 0.20 m soil profile at Tarlton, Krugersdorp (28° 02’S 39° 33’E). The soil samples were air-dried and sieved with a 2 mm-sieve. Physical and chemical analyses were performed on the soil samples at the beginning of the experiments using the methods of the Non-Affiliated Soil Analysis Work Committee (1990). $NH_4^+$and NO_3^- were extracted using 1M of KCl and analysed with the Kjeldahl method. The concentration of Bray-1 extractable P was determined using Inductively Coupled Plasma Atomic Emission Spectrometer (ICP-AES) and soluble and exchangeable Ca, K, Mg and Na were determined with 1M NH_4OAc (Non-Affiliated Soil Analysis Work Committee, 1990). Cations in solution were also determined by means of ICP-AES. pH was measured to determine any pH changes in the soil with humate and fulvate application. Chemical and physical properties of the soil are given in Table 5.1.

Table 5.1 Physical and chemical properties of selected soils

	Moisture (%)	Sand (%)	Silt (%)	Clay (%)	pH	EC mS m^{-1}	Elemental analysis mg kg^{-1}						
							K*	**Ca***	**Mg***	**Na***	**P****	**NO_3^-*****	**NH_4^+*****
Sandy clay	11.7	58	6	36	5.9	19	106	602	170	23.6	1.8	5.26	3.66
Sandy clay loam	1.0	78	4	10	4.4	56	38	182	32	16.6	0.41	15.1	5.50

* NH_4OAc extractable cations
** Bray-1
*** Kjeldahl method

5.2.2 Experiment layout

The pots were laid out in a completely randomized block design (CRBD) with five treatments and four replicates. The treatments consist of: 1) control 0, containing neither fertiliser nor humates and fulvate; 2) control 75, which represents of 75% of the recommended N, P and K-application rate; 3) humate (La) 75, which represents 75% of the recommended N, P and K-application rate with humate low ash (200 kg ha^{-1}); 4) fulvate 75 which represents 75% of the recommended N, P and K-application rate with fulvate (200 kg ha^{-1}); and 5) humate (Ha) 75, which represents 75% of the recommended N, P and K-application rate with humate high ash (200 kg ha^{-1}). Details of the treatments are given in Table 5.2. The 75% N, P and K-fertiliser application rates are equivalent to 165, 37 and 60 kg ha^{-1} of N, P and K respectively. Humates and fulvate were mixed with the soil at a rate of 200 kg ha^{-1}. Details of the fertiliser and chemical and physical properties of humates and fulvate used are described in Chapter 3 (section 3.2).

Small ‘Delta’ Valencia citrus trees were planted in 10 litre pots and left for one month to acclimatise. During this period, each pot was irrigated to field capacity with 3.4 L of distilled water every two days. The quantity of water irrigated was increased to 3.9 L when leaching was performed. The leachate was collected and analysed for N, P and K. At the end of the trial, leaf, bark and root samples were also analysed to determine its concentration for N, P and K.

To determine the influence of humate and fulvate on the CEC of the soil an experiment was done with a complete randomized block design with four treatments and four replicates.

Table 5.2 Details of the treatments for the pot trial

No	**Treatment**	**N, P and K (kg ha^{-1})**	**Humate and fulvate (kg ha^{-1})**
1	Control 0	0	0
2	Control 75	165-37.5-60	0
3	Humate (La) 75	165-37.5-60	200
4	Fulvate 75	165-37.5-60	200
5	Humate (Ha) 75	165-37.5-60	200

La= Low ash Ha= High ash

5.2.3 pH, EC and N, P and K determination of the leachate, soil and plant

5.2.3.1 pH and EC determination

The methodology used is described in Chapter 4, section 4.2.3.1.

5.2.3.2 Leachate analysis

The leachate collected was filtered with Whatman no 2 filter paper to remove soil particles. Ammonium (NH_4^+) and nitrate (NO_3^-) concentration were determined with the Kjeldahl method within 24 hours of sample collection, 15 ml of the filtered solution was used to determine P and K. All procedures were done according to the Handbook of Standard Soil Testing Methods for Advisory Purposes (Non-Affiliated Soil Analysis Work Committee, 1990).

5.2.3.2 Soil analysis

NH_4^+, NO_3^-, K and P were analysed according to the standard procedures of the Soil Science Department of the University of Pretoria as described in Chapter 4.2.4.4 of ALASA (1998).

5.2.3.3 Plant analysis

The leaf, bark and root of potted citrus tree were sampled after 5 months. Four samples were taken from each treatment and washed with distilled water to remove foreign material. Samples were oven-dried for two to three days at 50^{o}C until constant mass. The samples were milled and analysed according to the procedures described by ALASA (1998).

Nitrogen concentration was determined with an auto-analyser after H_2SO_4-digestion. Phosphorus and K were determined with ICP-AES after nitric acid and perchloric acid were used to digest the plant material.

5.2.3.4 Statistical analysis

The data of the leachate, soil and plant material were analysed using analysis of variance (ANOVA) and the means of the results were compared using least significant difference (LSD) with the statistical analyses system (SAS) version 9.2.

5.3 Results and discussion

5.3.1 pH of the leachate of treatments

The pH of the leachate from both soils slightly increased for the humate treatments compared to the fulvate and control treatments (Table 5.3 and 5.4).

These results correlate with those found by Shujrah *et al*. (2010) on the impact of potassium humate on selected chemical properties of an acidic soil. They found that 100 kg ha^{-1} of K-humate increased the pH of the soil compared to their control treatments.

Table 5.3 pH of the leachate collected from the sandy clay soil

Sandy clay		**Leachate 1**	**Leachate 2**	**Leachate 3**
Treatments				
1	Control 0	6.0^{c}	5.2^{c}	5.6^{c}
2	Control 75	6.1^{c}	6.2^{ab}	6.0^{a}
3	Humate (La) 75	6.6^{a}	6.5^{a}	5.9^{b}
4	Fulvate 75	6.1^{c}	6.0^{b}	5.8^{b}
5	Humate (Ha) 75	6.3^{ab}	6.2^{ab}	6.1^{a}
	LSD	**0.12**	**0.31**	**0.08**

Table 5.4 pH of the leachate collected from the sandy clay loam soil

Sandy clay loam		**Leachate 1**	**Leachate 2**	**Leachate 3**
Treatments				
1	Control 0	5.5^{c}	5.3^{e}	5.3^{c}
2	Control 75	6.0^{b}	6.2^{b}	5.7^{b}
3	Humate (La) 75	5.9^{b}	5.9^{c}	5.8^{ab}
4	Fulvate 75	5.5^{c}	5.6^{d}	4.8^{d}
5	Humate (Ha) 75	6.3^{a}	6.5^{a}	5.9^{a}
	LSD	**0.13**	**0.13**	**0.14**

Values in each column with the same letter were not significantly different $p<0.01$.

5.3.2 EC of the leachate of treatments

The electrical conductivity of the leachate of the soils (sandy clay and sandy clay loam) amended with humate and fulvate was higher than the leachate of the soils without the humate and fulvate (Table 5.5 and 5.6). These results are similar to those found by Imbufe *et al*. (2004), who found that potassium humate increased the electrical conductivity of acidic vineyard soils. In general, for both soils and treatments, the results showed that, EC decreases with leaching cycles.

Table 5.5 EC leachate of sandy clay soil (mS m^{-1})

Sandy clay **Treatments**		**Leachate 1**	**Leachate 2**	**Leachate 3**
1	Control 0	21.6e	20e	19.7e
2	Control 75	33.2d	29.6d	30.2d
3	Humate (La) 75	82.7a	66.3b	54.4b
4	Fulvate 75	78b	70.5a	68.5a
5	Humate (Ha) 75	38.4c	55.4c	45.2c
	LSD	**0.49**	**1.13**	**0.43**

Values in each column with the same letter were not significantly different p<0.01.

Table 5.6 EC leachate of sandy clay loam soil (mS m^{-1})

Sandy clay loam **Treatments**		**Leachate 1**	**Leachate 2**	**Leachate 3**
1	Control 0	31.0d	27.4e	25.2d
2	Control 75	33.6d	31.5d	30c
3	Humate (La) 75	65.2b	45.1c	43.1b
4	Fulvate 75	78.3a	71.2a	63a
5	Humate (Ha) 75	52.7c	48.3b	44.9b
	LSD	**3.02**	**0.72**	**2.83**

Values in each column with the same letter were not significantly different p<0.01.

5.3.3 N concentration of leachate

For both soil types the application of humate and fulvate treatments significantly reduced N leaching compared to the controls (p<0.01). For the sandy clay soil the N leaching for the soils without humate and fulvate ranged between 4.7 mg kg^{-1}(18.5 kg ha^{-1}) and 13.4 mg kg^{-1} (53.5 kg ha^{-1}). For the humates and fulvate treatments the values were between 2.4 mg kg^{-1} (9.5 kg ha^{-1}) and 6.2 mg kg^{-1} (24.7kg ha^{-1}). For sandy clay loam, the leaching of N varied between 2.7 mg kg^{-1} (10.7 kg ha^{-1}) and 7.9 mg kg^{-1} (31.5 kg ha^{-1}) for the controls, while for N leached from humates and fulvate treatments, the values varied between 1.3 mg kg^{-1} (5.1 kg ha^{-1}) to 5.3 mg kg^{-1} (21.1 kg ha^{-1}) (Figure 5.1). These results are in accordance with Selim *et al*. (2012) who reported that the application of 120 kg ha^{-1}of humic substances reduced the leaching of nutrients in irrigated potatoes in a sandy soil and Stevenson (1994) also showed results where humic acids reduced N leaching due to microbial and chemical reactions.

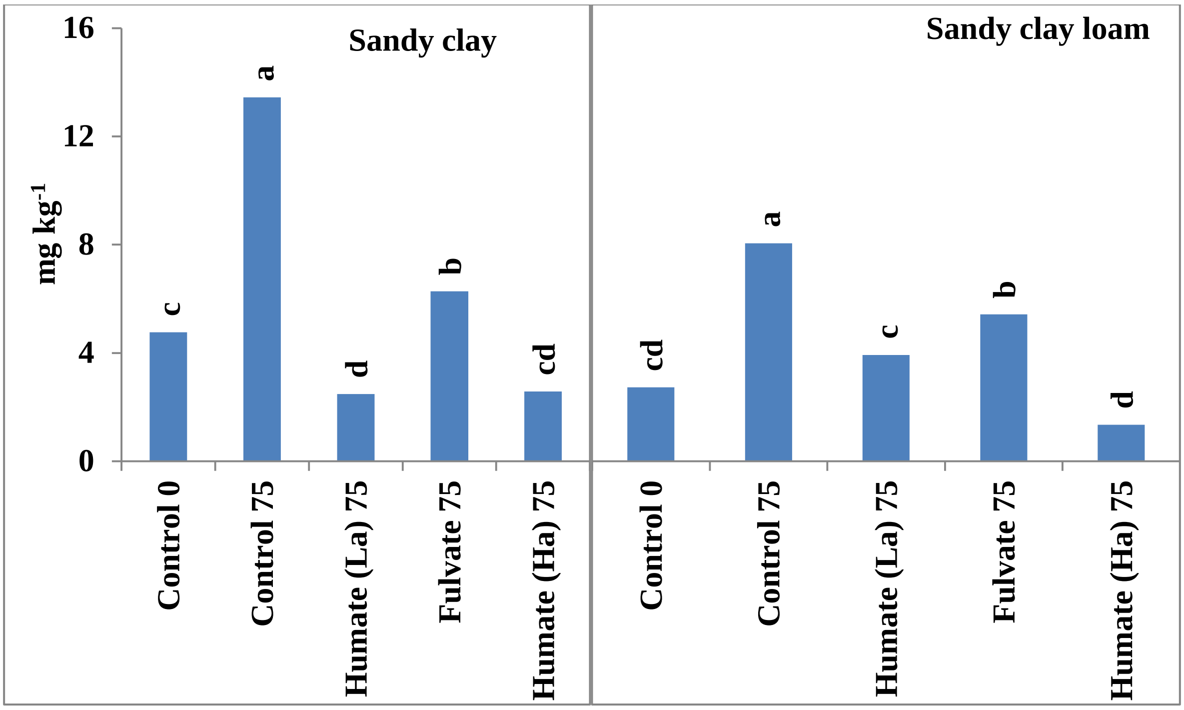

Figure 5.1 Nitrogen leached from sandy clay and sandy clay loam soils.

5.3.4 P concentration of leachate

Humates and fulvate treatments significantly reduced P leaching ($p<0.01$) in both soils (Figure 5.2). The amount of P leached for controls (0 and 75) were 0.018 mg kg^{-1} (0.71kg ha^{-1}) for the sandy clay soil. For the sandy clay loam soil, the amount of P leached, for control 0 and control 75, varied between 0.017 mg kg^{-1} (0.067 kg ha^{-1}), and 0.035 mg kg^{-1} (0.119 kg ha^{-1}). For the humate and fulvate treated soils the P concentration in the leachate was between 0.006 mg kg^{-1} (0.02 kg ha^{-1}) and 0.009 mg kg^{-1} (0.04 kg ha^{-1}). For the sandy clay soil the P concentration in the leachate was between 0.004 mg kg^{-1}(0.01 kg ha^{-1}) and 0.006 mg kg^{-1} (0.02 kg ha^{-1}). In both soils, the results showed that the application of humate and fulvate reduced P leaching, probably due to the P uptake by the roots, which is in accordance with a report from Shaaban *et al.* (2009) that application of humic acids considerably decreased P concentration in the leaching water of irrigated wheat.

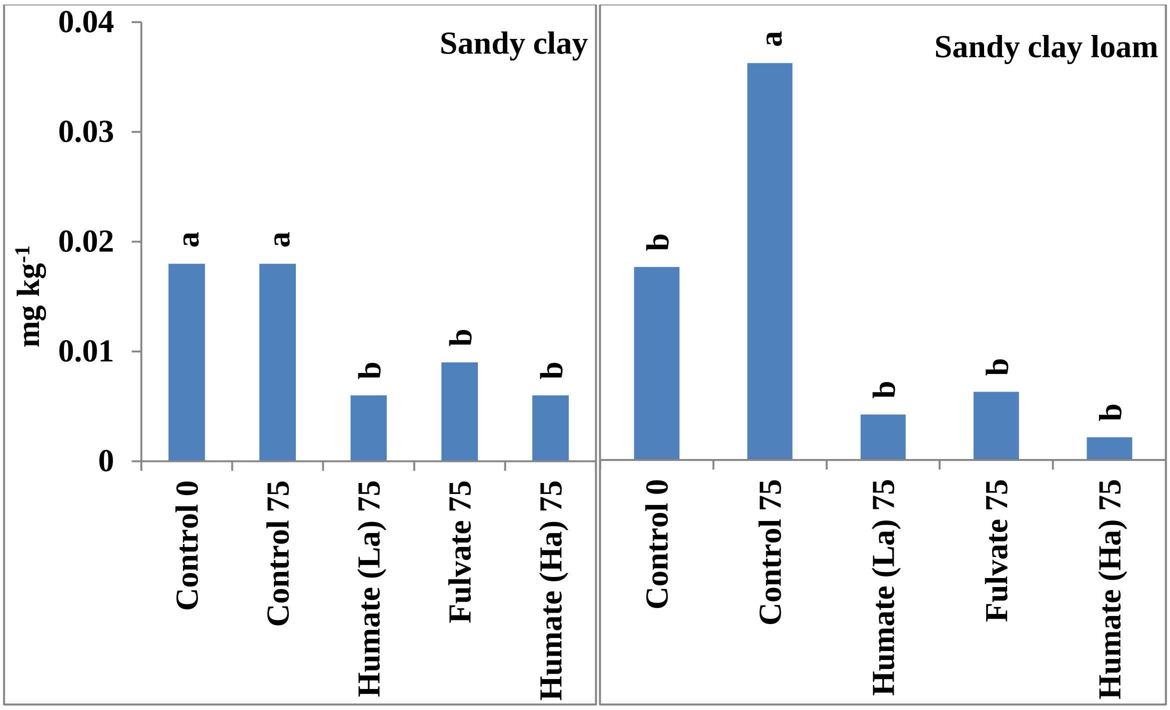

Figure 5.2 Phosphorus leached from sandy clay and sandy clay loam soils.

5.3.5 K concentration of leachate

In Figure 5.3 the K concentration for the leachate for sandy clay soil and sandy clay loam are given. Humate (La) 75 for sandy clay soil and treatment humate (Ha) 75 for sandy clay loam showed a decrease in K leaching. This can either be an artefact or there must be some substantial evidence that the chemical composition differs and therefore reduce the leaching of K. The high K leaching could be due to the addition of K through the humates and fulvate treatments (Table 3.2).

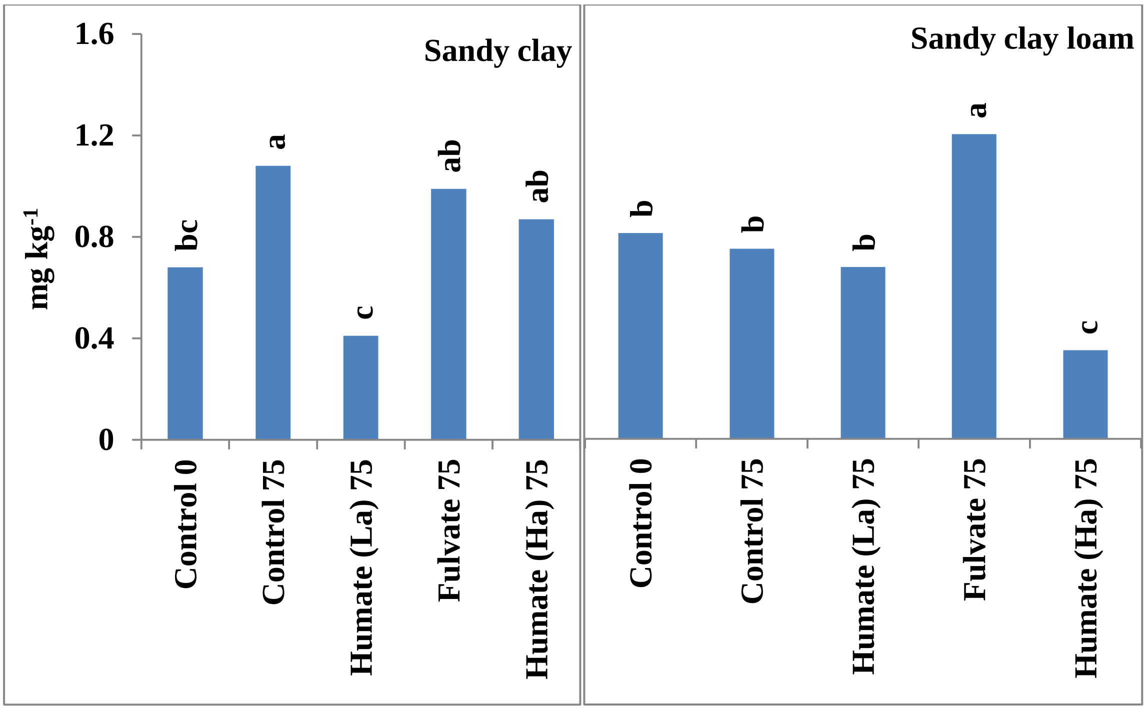

Figure 5.3 Potassium leached from sandy clay and sandy clay loam soils.

5.3.6 Plant analyses

5.3.6.1 Nitrogen concentration

Results for the N content of the leaf, bark and root of 'Delta' Valencia planted in a sandy clay soil are presented in Table 5.7 and in the sandy clay loam in Table 5.8. The results from plant analysis for the 'Delta' Valencia planted in sandy clay soil indicated that except for the fulvate treatment no significant differences in the N concentration of the leaves were found. The fulvate treatment also resulted in the highest N content in the bark, while humate (Ha) treated plants has the lowest N in bark. There were no significant differences in the root N content among the treatments (Table 5.7). Similar results were found by Silvia *et al.* (2004) where N content in roots was not significantly affected by humic substances

In sandy clay loam soil the humate (Ha) treatment resulted in a significantly higher leaf N content than for the humate (La) and control 0 treatment, but was not significant different from that of the fulvate and control 75 treatments. The N content in the bark was significantly higher for humate (La) treatment, while control 0 was significantly lower. The N in the bark for the humate (Ha) and fulvate treatments were not significantly different from the control 75. Fulvate and humate treatments resulted in significantly higher root N than for the controls.

Mesut & Yilmaz (2005) found that humic acids increased N uptake by lettuce and improves nutrient availability. Hishamo & Mohammad (2007) also reported improved N uptake by a maize crop in sandy clay loam due to the complexion of nutrients of humic acids. While Kalaichelvi *et al*. (2006) found increased N uptake in tomato and wheat due to humic acid amendments. Nikbakht *et al*. (2008) found that, humic acids significantly increased N in the leaves of maize and wheat.

Table 5.7 N content of 'Delta' Valencia planted in a sandy clay soil

Sandy clay		**Leaf**	**Bark**	**Root**
Treatments		**g kg^{-1}**	**g kg^{-1}**	**g kg^{-1}**
1	Control 0	13.6^{b}	10.3^{bc}	7.1^{a}
2	Control 75	16.5^{ab}	11.1^{ab}	7.8^{a}
3	Humate (La) 75	19.5^{ab}	11.3^{ab}	9.1^{a}
4	Fulvate 75	23.4^{a}	13.1^{a}	7.8^{a}
5	Humate (Ha) 75	21.2^{ab}	8.0^{c}	7.1^{a}
	LSD	**8.36**	**2.30**	**4.31**

Table 5.8 N content of 'Delta' Valencia planted in a clay loam soil

Sandy clay loam		**Leaf**	**Bark**	**Root**
Treatments		**g kg^{-1}**	**g kg^{-1}**	**g kg^{-1}**
1	Control 0	13.9^{b}	6.2^{c}	5.7^{b}
2	Control 75	18.4^{ab}	7.3^{b}	6.0^{b}
3	Humate (La) 75	16.4^{b}	13.7^{a}	8.9^{a}
4	Fulvate 75	18.1^{ab}	9.1^{b}	11.2^{a}
5	Humate (Ha) 75	24.7^{a}	9.2^{b}	9.1^{a}
	LSD	**7.12**	**2.88**	**2.76**

Values in each column with the same letter were not significantly different $p<0.05$.

5.3.6.2 Phosphorus concentration

In the sandy clay soil, the humate and fulvate treatments had no significant effect on the P content of the leaves, bark and root of the citrus plants (Table 5.9). Similar results were reported by (Eman *et al*., 2008) for grapevine who found that humic acids combined with fertilisers in a sandy soil did not significantly increase the P uptake. P concentration in the leaves and bark was slightly higher than roots for both soils (Table 5.9 and 5.10). In the sandy clay loam soil the humate (Ha) treatment significantly increased the P content of the leaves compared to the other treatments. Plants treated with humate (La) had significantly higher P concentration in the bark than in control 75 (Table 5.10). However, there were no significant differences among the other treatments. Application of humic acids increased P content in the roots compared to the control treatments. The fulvate treatment resulted in the highest P content in the roots although it was not significantly higher than the humate (Ha) treatment. Similar results were found for snap-bean (El-Bassiony *et al.*, 2010), maize (Eyheraguibel *et al.*, 2008) and gerbera plants (Nikbakht *et al.*, 2008).

Table 5.9 P content of ‘Delta’ Valencia planted in a sandy clay soil

Sandy clay loam		**Leaf**	**Bark**	**Root**
Treatments		**g kg^{-1}**	**g kg^{-1}**	**g kg^{-1}**
1	Control 0	0.866^{a}	0.378^{a}	0.0065^{a}
2	Control 75	0.697^{a}	3.259^{a}	0.007^{a}
3	Humate (La) 75	1.259^{a}	0.388^{a}	0.0073^{a}
4	Fulvate 75	0.942^{a}	0.377^{a}	0.0065^{a}
5	Humate (Ha) 75	1.009^{a}	0.438^{a}	0.0066^{a}
	LSD	**3.42**	**0.16**	**0.0026**

Table 5.10 P content ‘Delta’ Valencia planted in a sandy clay loam soil

Sandy clay loam		**Leaf**	**Bark**	**Root**
Treatments		**g kg^{-1}**	**g kg^{-1}**	**g kg^{-1}**
1	Control 0	0.616^{b}	0.432^{ab}	0.003^{c}
2	Control 75	0.767^{b}	0.257^{b}	0.004^{bc}
3	Humate (La) 75	0.954^{b}	0.513^{a}	0.006^{b}
4	Fulvate 75	0.739^{b}	0.326^{ab}	0.008^{a}
5	Humate (Ha) 75	1.411^{a}	0.380^{ab}	0.006^{b}
	LSD	**0.443**	**0.226**	**0.002**

Values in each column with the same letter were not significantly different $p<0.05$.

5.3.6.3 Potassium concentration

In general, there was no significant difference in the K content of the leaves and bark between the different treatments of ‘Delta’ Valencia planted in a sandy clay soil (Table 5.11). One exception was the significant difference in leaf K content between humate (La) and humate (Ha) treated plants. Futhermore, K in the bark was higher with the humate (Ha) treatment than with the fulvate and control 75 treatments. Humates and fulvate showed no significant effects on K in the root.

In Table 5.12 results for K concentration in leaves, bark and root of “Delta’ Valencia planted in sandy clay loam soil are presented. Unlike the K content in bark, the K contents in the leaves and roots were significantly affected by humate and fulvate treatments compared to control 0. Leaf K was higher with humate treatments than for control 0, which had the lowest leaf K. Hence, the K content of the fulvate treated leaves were not were no significantly different from the controls.

Table 5.11 K content of ‘Delta’ Valencia planted in a sandy clay soil

Sandy clay loam		**Leaf**	**Bark**	**Root**
Treatments		**g kg^{-1}**	**g kg^{-1}**	**g kg^{-1}**
1	Control 0	11.4^{ab}	4.6^{ab}	7.7^{a}
2	Control 75	8.8^{ab}	4.1^{b}	8.8^{a}
3	Humate (La) 75	13.8^{a}	4.7^{ab}	8.6^{a}
4	Fulvate 75	9.2^{ab}	4.5^{b}	8.8^{a}
5	Humate (Ha) 75	7.1^{b}	6.9^{a}	7.6^{a}
	LSD	**6.39**	**2.35**	**0.91**

Table 5.12 K content of ‘Delta’ Valencia planted in a sandy clay loam soil

Sandy clay loam		**Leaf**	**Bark**	**Root**
Treatments		**g kg^{-1}**	**g kg^{-1}**	**g kg^{-1}**
1	Control 0	8.3^{c}	6.2^{a}	4.2^{c}
2	Control 75	10.2^{abc}	2.8^{a}	5.9^{bc}
3	Humate (La) 75	13.4^{a}	3.7^{a}	7.8^{ab}
4	Fulvate 75	8.7^{bc}	5.1^{a}	10^{a}
5	Humate (Ha) 75	11.6^{ab}	6.1^{a}	8.1^{ab}
	LSD	**3.15**	**3.89**	**2.34**

Values in each column with the same letter were not significantly different $p<0.05$.

5.3.7 Influence of humate and fulvate on CEC

Humate and fulvate treatments resulted in higher CEC in both soils compared to the control (Figures 5.4). Similar results were reported by Shujrah *et al*. (2010) using K-humate and Sharif *et al.* (2002) reported that the potential effects of humic acids on cation exchange capacity are related to the chemical and biological content of the products. Humic acids, from various sources contains high amounts of C and O and depending on the source may also contain different concentrations of Na, Ca, K, Mg (Rupiasih & Vidyasagar, 2009). Chapter 3, section 3.3.1 and 3.3.2 showed that humate and fulvate increases bacteria and fungi growth that could be the main reason for the increasing of the CEC of the soils, due to the increase in organic matter when the microorganisms die. However, the reason for the increase in the CEC of the soil is unclear and further research into this phenomenon needs to be conducted.

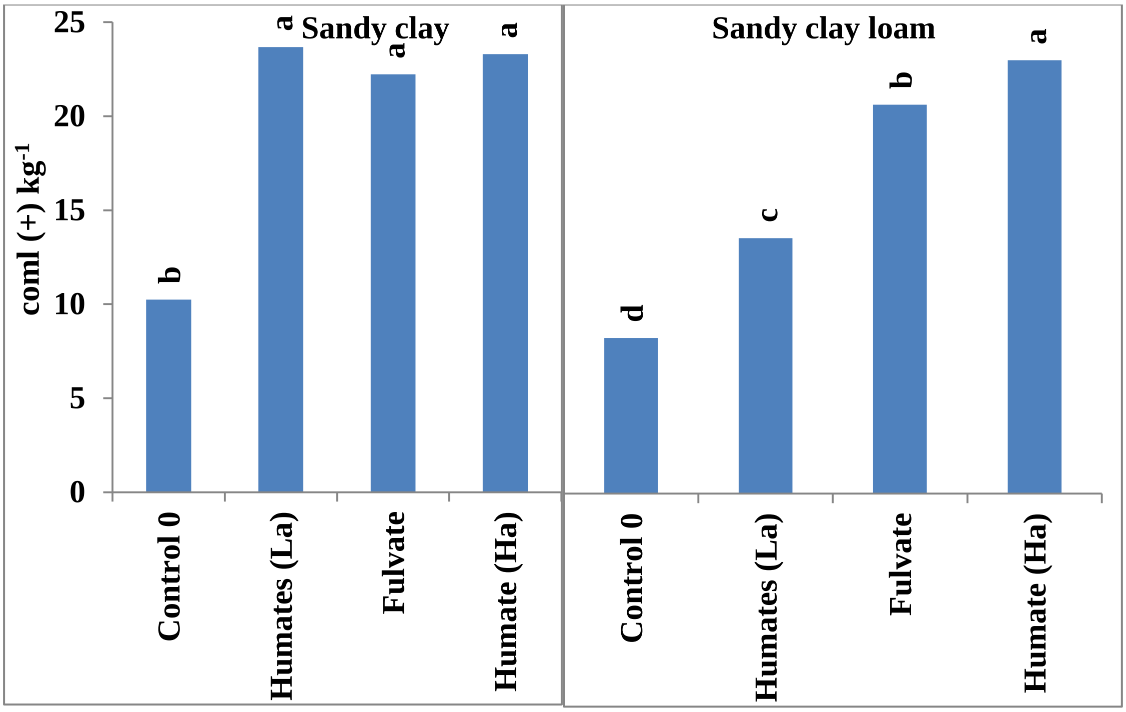

Figure 5.4 CEC from sandy clay and sandy clay loam soil.

5.4 Conclusions

The results of this study showed that humate and fulvate treatments significantly increased the pH and EC of the soils and generally reduced the leaching of N and P for both soils (sandy clay and sandy clay loam). However, no significant effect was observed for K.

Humate and fulvate treatments in the sandy clay soil affected N and K contents of citrus leaves and bark but not roots. P uptake was not significantly affected by the different treatments. For the sandy clay loam soil, N and P in leaves, bark and root were significantly affected by the different treatments. However, humate and fulvate treatments affected K content of the leaves and roots but not the bark. Similar results of N, P and K uptake were previously observed in sugar cane (Hishamo & Mohammad, 2007) maize, potato, spinach (Verlinden *et al.*, 2009) and barley (Ayuso et *al*., 1996).

Thus, humates and fulvate combined with N, P and K increased the nutrient availability, CEC and uptake of nutrients (N, P, K) and reduced the N, P, leaching from both soils.

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CHAPTER 6

SYNTHESIS AND CONCLUSIONS

6.1 Synthesis

The results of this study demonstrate that humates and fulvate combined with applied N, P and K fertilisers increases the heterotrophic bacterial and fungal populations of both soils. Total bacterial counts in the sandy clay and sandy clay loam soil, after two weeks of incubation increased when humates and a fulvate combined with applied N, P and K fertilsers where mixed with the soil. After four weeks, the bacterial counts were the highest in the soils treated with humates and fulvate and N, P and K compared to the soils containing no humates and fulvate. Treatments of humates and fulvate combined with N, P and K resulted in higher fungal counts than the treatments without humates and fulvates. After four weeks, fungal counts were still growing with the treatment with humate and fulvate. It was also found that that humates and fulvate increases dehydrogenase (microbial activity) in the sandy clay and sandy clay loam soils compared to the controls.

Results from an experiment with leaching columns showed that, the pH and EC leachate of soils amendment with humates, fulvates and N, P and K increased. It was also found in this experiment that humates and fulvate combined with N, P and K amendment reduced the leaching of N in both soil types. Inconsistent results were found for K and P and the different soil types and treatments.

The results from the pot trials clearly show that humates and fulvates combined with N, P and K fertilisers increase the pH and EC in the leachate of both soils. Humates and fulvate combined with N, P and K amendment significantly reduced N and P leaching in both soils, but did not reduce K leaching.

In general, N, P and K content of the leaf, bark and root increased when humate and fulvate combined with N, P and K were added to the soil. However, humate and fulvate combined with N, P and K did not increase the root N, P and K content in sandy clay soil.

Humates and fulvates combined with N, P and K amendment increase CEC in both the sandy clay and sandy clay loam. The increase in CEC maybe due to the indirect result of microorganisms that increases the organic matter when they die (Chapter 3, section 3.3.1 and 3.3.2). The study indicates that the use of humates and fulvate combined with N, P and K amendment is beneficial for nutrient availability in both soils due to increasing of microbial population and activity in the soils. Therefore more experiments are needed to confirm the sustainability of humates and fulvate under field conditions.

6.2 Recommendations

The experiments were done in the laboratory (columns laboratory, microbial laboratory) and in a pot trial under controlled conditions. This need to be scaled-up to field conditions to validate the findings of the study. Field trials in orchards need to be done on the effect of humate and fulvate combined with N, P and K fertilisers on:

- Nutrient leaching and uptake in citrus orchards under irrigation conditions.
- Influence of different soils.
- Agriculture economics study to quantify the impact this has on the profitability of citrus production and also the influence on export quality citrus.

APPENDIX

I. Summary of ANOVA tables (N, P and K-Leaching)

Table 1: Summary of ANOVA table on the leaching of Nitrogen sandy clay soil (Tukey's Studentized Range)

Dependent Variable	**R-Square**	**Coeff Var**	**Root MSE**	**Mean**	
	0.92	19.99	1.23	6.19	
Source	**DF**	**Type III**	**Mean Square**	**F Values**	**Pr>F**
TRT	8	427.12	53.34	34.83	<0.01
REP	3	3.45	1.15	0.75	0.53

TRT= Treatment, REP= Repetition, DF= Degree of freedom

Table 2: Summary of ANOVA table on the leaching of Nitrogen sandy clay loam soil (Tukey's Studentized Range)

Dependent Variable	**R-Square**	**Coeff Var**	**Root MSE**	**Mean**	
	0.90	32.42	2.04	6.31	
Source	**DF**	**Type III**	**Mean Square**	**F Values**	**Pr>F**
TRT	8	973.70	121.71	29.05	<0.01
REP	3	30.45	10.15	2.42	0.09

TRT= Treatment, REP= Repetition, DF= Degree of freedom**,**

Table 3: Summary of ANOVA table on the leaching of Phosphorus clay soil (Tukey's Studentized Range)

Dependent Variable	R-Square	Coeff Var	Root MSE	Mean	
	0.36	99.74	0.49	0.49	
Source	**DF**	**Type III**	**Mean Square**	**F Values**	**Pr>F**
TRT	8	2.48	0.31	1.29	0.05
REP	3	0.88	0.23	1.22	0.32

TRT= Treatment, REP= Repetition, DF= Degree of freedom**,**

Table 4: Summary of ANOVA table on the leaching of Phosphorus sandy clay loam soil (Tukey's Studentized Range)

Dependent Variable	**R-Square**	**Coeff Var**	**Root MSE**	**Mean**	
	0.50	75.94	0.28	0.38	
Source	**DF**	**Type III**	**Mean Square**	**F Values**	**Pr>F**
TRT	8	1.94	0.24	2.96	0.05
REP	3	0.08	0.02	0.35	0.78

TRT= Treatment, REP= Repetition, DF= Degree of freedom

Table 5: Summary of ANOVA table on the leaching of Potassium sandy clay soil (Tukey's Studentized Range)

Dependent Variable	**R-Square**	**Coeff Var**	**Root MSE**	**Mean**	
	0.94	6.91	0.50	7.27	
Source	**DF**	**Type III**	**Mean Square**	**F Values**	**Pr>F**
TRT	8	108.08	13.51	53.35	<0.01
REP	3	0.34	0.11	0.45	0.72

TRT= Treatment, REP= Repetition, DF= Degree of freedom

Table 6: Summary of ANOVA table on the leaching of Potassium sandy clay loam soil (Tukey's Studentized Range)

Dependent Variable	**R-Square**	**Coeff Var**	**Root MSE**	**Mean**	
	0.89	17.40	1.18	6.78	
Source	**DF**	**Type III**	**Mean Square**	**F Values**	**Pr>F**
TRT	8	221.41	27.68	19.92	<0.001
REP	3	34.47	11.48	8.27	0.006

TRT= Treatment, REP= Repetition, DF= Degree of freedom

II. Summary of ANOVA tables Pot trial N, P and K-Leaching

Table 7: Summary of ANOVA table on the leaching of Nitrogen sandy clay soil (Tukey's Studentized Range)

Dependent Variable	**R-Square**	**Coeff Var**	**Root MSE**	**Mean**	
	0.92	23.93	1.46	6.13	
Source	**DF**	**Type III**	**Mean Square**	**F Values**	**Pr>F**
TRT	4	329.38	82.34	38.15	<0.01
REP	3	4.51	1.50	0.70	0.57

TRT= Treatment, REP= Repetition, DF= Degree of freedom

Table 8: Summary of ANOVA table on the leaching of Nitrogen sandy clay soil loam (Tukey's Studentized Range)

Dependent Variable	**R-Square**	**Coeff Var**	**Root MSE**	**Mean**
	0.89	22.74	0.93	4.11

Source	**DF**	**Type III**	**Mean Square**	**F Values**	**Pr>F**
TRT	4	83.05	20.76	23.64	<0.01
REP	3	2.57	0.85	0.98	0.43

TRT= Treatment, REP= Repetition, DF= Degree of freedom

Table 9: Summary of ANOVA table on the leaching of Phosphorus sandy clay soil (Tukey's Studentized Range)

Dependent Variable	**R-Square**	**Coeff Var**	**Root MSE**	**Mean**	
	0.72	32.07	0.0041	0.0130	
Source	**DF**	**Type III**	**Mean Square**	**F Values**	**Pr>F**
TRT	4	0.01	0.02	17.11	<0.01
REP	3	0.02	0.00	5.14	0.05

TRT= Treatment, REP= Repetition, DF= Degree of freedom

Table 10: Summary of ANOVA table on the leaching of Phosphorus sandy clay soil loam (Tukey's Studentized Range)

Dependent Variable	**R-Square**	**Coeff Var**	**Root MSE**	**Mean**	
	0.7224	75.2086	0.0108	0.0144	
Source	**DF**	**Type III**	**Mean Square**	**F Values**	**Pr>F**
TRT	4	0.0030	0.0007	6.40	0.05
REP	3	0.0006	0.0002	1.88	0.18

TRT= Treatment, REP= Repetition, DF= Degree of freedom

Table 11: Summary of ANOVA table on the leaching of Potassium sandy clay soil (Tukey's Studentized Range)

Dependent Variable	**R-Square**	**Coeff Var**	**Root MSE**	**Mean**	
	0.65	27.95	0.23	0.82	
Source	**DF**	**Type III**	**Mean Square**	**F Values**	**Pr>F**
TRT	4	1.15	0.28	5.44	0.05
REP	3	0.04	0.01	0.25	0.85

TRT= Treatment, REP= Repetition, DF= Degree of freedom

Table 12: Summary of ANOVA table on the leaching of Potassium sandy clay soil loam (Tukey's Studentized Range)

Dependent Variable	**R-Square**	**Coeff Var**	**Root MSE**	**Mean**	
	0.80	23.09	0.17	0.74	
Source	**DF**	**Type III**	**Mean Square**	**F Values**	**Pr>F**
TRT	4	1.38	0.34	11.56	0.05
REP	3	0.09	0.03	1.08	0.39

TRT= Treatment, REP= Repetition, DF= Degree of freedom

III. Summary of ANOVA tables Cations Exchange Capacity of sandy clay and sandy clay soil

Table 13: Summary of ANOVA table on the CEC sandy clay (Tukey’s Studentized Range)

Dependent Variable	**R-Square**	**Coeff Var**	**Root MSE**	**Mean**	
	0.97	7.47	1.17	15.75	
Source	**DF**	**Type III**	**Mean Square**	**F Values**	**Pr>F**
TRT	3	504.32	168.10	121.21	<0.01
REP	3	1.91	0.63	0.46	0.71

TRT= Treatment, REP= Repetition, DF= Degree of freedom

Table 14: Summary of ANOVA table on the CEC sandy clay loam (Tukey's Studentized Range)

Dependent Variable	R-Square	Coeff Var	Root MSE	Mean	
	0.89	13.06	2.59	19.86	
Source	**DF**	**Type III**	**Mean Square**	**F Values**	**Pr>F**
TRT	3	498.37	166.12	24.67	<0.01
REP	3	26.94	8.98	1.33	0.32

TRT= Treatment, REP= Repetition, DF= Degree of freedom