TITANIUM COMPOUNDS AND PLANT GROWTH

BY

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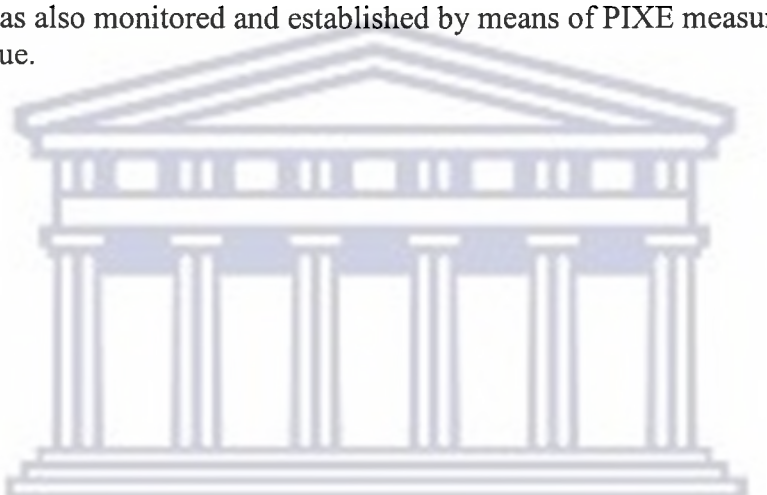
A THESIS IN THE DEPARTMENT OF CHEMISTRY
UNIVERSITY OF THE WESTERN CAPE

PRESENTED IN PARTIAL FULFILLMENT OF THE REQUIREMENTS OF THE MASTER'S DEGREE (MSc) IN CHEMISTRY

ABSTRACT

The effect of titanium compounds on the growth of tomato plants, *Lycopersicum esculentum* (cultivar Floridade), was studied under greenhouse conditions. Aqueous suspensions (TiO_2) and solutions (complexes of titanium (IV) with L(+)-ascorbic acid and oxalic acid) were applied as foliar sprays and soil treatments. A new compound of titanium (IV) with L(+)-ascorbic acid was prepared and characterised. Some titanium compounds appeared to enhance some growth parameters in some instances, but titanium did not consistently result in improved growth or increased nitrogen concentration within the plants. Titanium uptake was also monitored and established by means of PIXE measurements on plant tissue.



ACKNOWLEDGEMENTS

I would like to pass my special thanks to my supervisors, Dr L M Raitt and Mr J Aalbers, for their help and advice during the project, especially concerning the botanical aspects and to Dr DL Key for advice on chemical aspects.

Special thanks are also due to Dr Przybilowicz (husband and wife team) for their expertise in the use of the microprobe at the National Accelerator Centre and their help in the mounting of PIXE samples, and Mr Hansforth for his assistance in the analysis of samples by ICP.

Last but not least I would like to thank my parents and also Mr M Selepe for their support without which this work would still be incomplete .

CONTENTS

CHAPTER 1

The Chemistry of Vitamin C and its complexes with metal ions

1. Introduction

L (+) ascorbic acid, also known as vitamin C ($C_6H_8O_6$), is widely known for its role in the cure and prevention of scurvy. It is found in a wide variety of plants and animals. It is essential to man, though its biochemical role is only poorly understood. Recently more attention has been given to the biochemistry of this molecule, especially since the findings of Hollis *et al.* (1985) on its antitumor properties, in conjunction with chemotherapy in neuroblastoma, malignant melanoma and on the anticancer properties of some organometallic titanium and tin ascorbate complexes. Vitamin C is chemically the simplest of the vitamins and for this reason was among the first to be isolated, characterized and purified, and to have its structure determined. It was isolated by Szent - Gyory (1928) from adrenal cortex of cattle and later from orange juice and cabbage water. Herbet determined its chemical structure in 1933.

1.1.1 Structure and properties of vitamin C and its metal complexes

The crystal structure of vitamin C has been determined by means of x-ray crystallography and neutron diffraction by Hvoslef (1968) as shown in figure 1.1

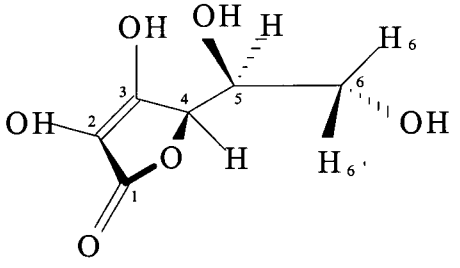
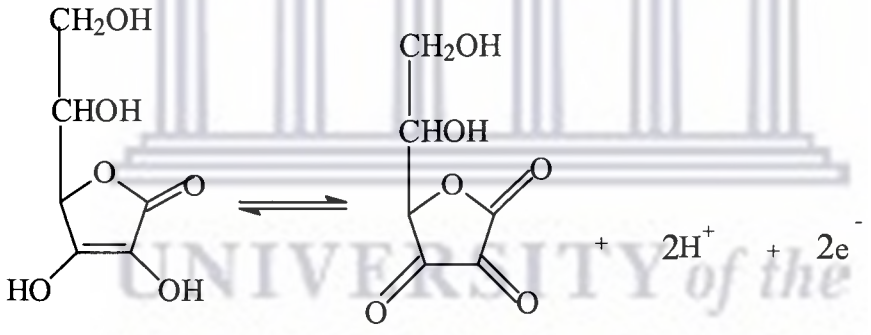


Figure 1.1: The X-ray crystal structure of vitamin C

The structure of L (+)-ascorbic acid has been characterised by various physical methods. The UV spectrum of L (+)-ascorbic acid in aqueous solution at pH 2.0 reveals a λ_{max} at 243 nm which undergoes a shift to 265 nm at pH 7.0 as a result of ionisation of the C_3-OH proton (Davies *et al.* 1991). The spectra obtained are consistent with $\pi \rightarrow \pi^*$ excitation of a conjugated C-C bond in a five-membered lactone ring (Davies *et al.* 1991). The IR spectrum analysis of L(+)-ascorbic acid shows ν(O-H) stretch bands between 4000-2000 cm^{-1} that is due to the enolic OH groups on C_2 and C_3. There is a strong absorption at 1754 cm^{-1} attributed to ν(C=O) stretching vibration, also an intense doublet at 1675 $cm^{-1}$and 1660 cm^{-1}arising from ν(C = C) stretching vibrations (Benetis *et al.* (1981) and Davies *et al.* (1991)). The band at 1460 cm^{-1} is attributed to CH_2 scissoring vibration. The finger-print region contains at 1320 cm^{-1}ν(O-H) deformation of C_2 -OH, 1275 cm^{-1} ν(C_2-O) stretch band, 1140 cm^{-1}ν(C_5-O) band and 1025/990 cm^{-1} (lactone ring deformation). The ^1H NMR spectrum of L (+)-ascorbic acid was carefully studied in D_2O, the four OH protons are exchanged and do not appear as separate signals. The remaining four (H_6, $H_{6'}$, H_5, and H_4) comprise an ABMX system, the two protons on C_6 being non-equivalent because of the chirality of C_5 (Davies *et al.* 1991).

L(+)-ascorbic acid, because of its ene-diol structure, has a highly complex chemistry. It is known to form both mono- and di-anion species, depending on the pH of the aqueous solution. In aqueous solution it exhibits a strong reducing action and is oxidized readily to produce dehydroascorbic acid (Hvoslef 1969, Seib & Tolbert 1982 ; Basch *et al.* 1986), this property being much less evident in non-aqueous media. The mono-anion forms at pH 4-5 with deprotonation of O_3-H (HA^-), while the di-anion form at pH 11-12 with deprotonation of O_2-H(HA^{-2}) (Hvoslef 1969). L (+)-ascorbic acid is also susceptible to degradation under anaerobic conditions, giving rise to furfural and carbon dioxide. In aqueous solution the oxidation of L (+)-ascorbic acid to dehydroascorbic acid involves a two electron redox process, as shown below.

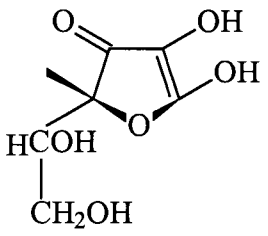


Despite being a simple molecule, its ene-diol structure provides it with a highly complex chemistry. It has extensive redox chemistry involving comparatively stable radical intermediates. L (+)-ascorbic acid is synthesized from the hexose D (+)- glucose. This remains the most important synthetic method yet devised for vitamin C, and with some modification, still provides the foundation for the modern method for its commercial manufacture. L (+)-ascorbic acid is known to form both mono- and di-anion species due

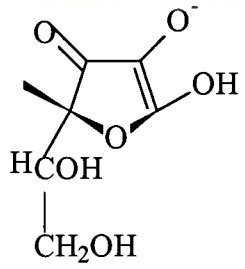
to the ionisation of acidic protons on the C_2- and C_3- OH groups, depending on the pH of the solution. In aqueous solution it exhibits a strong reducing action and is readily oxidized to produce the dehydro-acid. The reducing properties are due to the grouping - C (OH) – C=O (OH) with two conjugated double bonds. Cruez (1981) and Davies *et al* (1991) indicated that the oxidation and reduction reaction of L (+)- ascorbic acid and its redox behavior are complicated by the simultaneous transfer of protons. Furthermore L (+)-ascorbic acid, its free radical and dehydroascorbic acid all have acid-base properties. The properties are summarized in table 1.1. The structures of the various redox products are shown in figure 1.2. In addition to this the formation of a stable radical HA^- which is itself a strong acid with a pKa of 0.45 has been observed.

Figure 1.2 : Structure of (i) L(+)-ascorbic acid H_2A, (ii) ascorbate ion (HA^-), (iii) protonated radical HA., (iv) ascorbate radical A^- and (v & vi) dehydroascorbic (ref. 6)

(i) H_2A

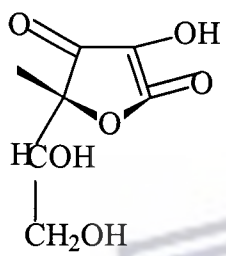


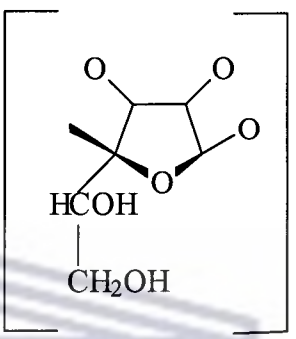
(ii) HA^-



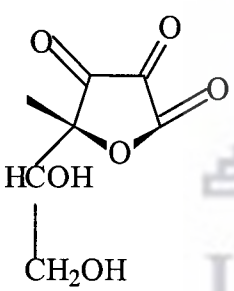
(iv) A^-

(iii) $HA^{\cdot}$





(v) A



(vi) dehydroascorbic acid

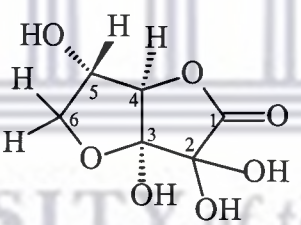


Table 1.1 . Dissociation constants for L(+)-ascorbic acid (H_2A) , ascorbate radical ($HA^{\cdot}$) and dehydroascorbic acid

Equilibrium	pKa
$H_2A \rightarrow H^+ + HA^-$	$pK_1 = 4.0$
$HA^- \rightarrow H^+ + A^{2-}$	$pK_2 = 11.3$
$HA^{\cdot} \rightarrow H^+ + A^-$	$pK = 0.45$
$A \rightarrow H^+ + A^-$(conjugate base of A)	$pK = 8$

L(+)-ascorbic acid is a moderately weak acid in the loss of the first hydrogen ion and a very weak acid in the loss of the second. The radical $HA^{\cdot}$ is a strong acid, comparable with mineral acids. All these species have their own redox behavior.

							E°/V
A	+	$2H^+$	+	$2e^-$	$\Leftrightarrow$	H_2A	0.40
A	+	H^+	+	$2e^-$	$\Leftrightarrow$	HA^-	0.28
A	+	$2e^-$			$\Leftrightarrow$	A^{2-}	-0.05
A	+	e^-			$\Leftrightarrow$	A^-	0.16
A	+	e^-			$\Leftrightarrow$	A^{2-}	0.05
$HA^{\cdot}$	+	e^-			$\Leftrightarrow$	HA^-	0.70

Hence the acid-base properties and redox changes may all play a part in the interaction of ascorbic acid species with metal ions. It has been known for many years that L(+)-ascorbic acid is easily oxidized by dioxygen and catalyzed by the presence of copper (II) ions (Creutz 1981; Davies *et al.* (1991). There have been many investigations of the

reactions of L(+)-ascorbic acid under these conditions. Metal ions capable of undergoing redox reactions are thought to catalyze the auto-oxidation of intermediate metal-ascorbate – dioxygen complexes. These oxidations are important in the loss of vitamin C in drinks and food .

1.2. Complexes formed by L(+)-ascorbic acid

The interaction of L (+)-ascorbic acid with transition metal ions are known to be of considerable importance in many biological systems (Seib & Tolbert 1982), because of several potential donor sites on L(+)-ascorbic acid for the complex formation with metal ions. It is generally found that the reactions exhibit pH dependencies which can be related to the acid dissociation steps of L(+)-ascorbic acid. Martell (1982) studied and speculated on the co-ordination of ascorbic acid with metal ions. He concluded that ascorbic acid is a weak bidentate ligand, which coordinates metal ions to form monoprotonated chelates at low and intermediate pH and deprotonates chelates at high pH. Chelate formation in aqueous solution is weak. The acid anions can potentially use most of its donor oxygen atoms to form three-dimensional polymeric structures. This can occur through O_5, O_6 chelation , O_3 , O_5, O_6 chelation , O_3, O_5, O_6 chelation , O_1, O_2 chelation and O_2, O_3 chelation as well as unidentately bonded ascorbate anions. There have not been many reports of the isolation of solid complexes of L(+)-ascorbic acid.

Jabs *et al.* (1990) has described the isolation of some crystalline complexes of a variety of transition metal ions such as TiO^{2+} , Cr^{3+} , Mn^{2+} , Co^{2+} , Ni^{2+} and Zn^{2+}. The complexes were prepared by reacting metal ion sulfate with L(+)-ascorbic acid in the presence of barium trioxide metal ions in the absence of oxygen, to give a reaction of the type given below. The substances were all precipitated from solution with organic solvents and had poor elemental analysis results.

$$NiSO_4 . 7H_2O + Ba(OH)_2 + 2H_2A \quad \Leftrightarrow \quad Ni(HA)_2. 4H_2O + BaSO_4 \quad + \quad 5H_2O$$

Jabs *et al.* (1990) also investigated the reactions of titanium (IV) compounds with mono-alkali and di-alkali metal salts of L(+)-ascorbic acid and 5,6-O- isopropylidene L(+)-ascorbic acid respectively in organic solvents such as ethanol and tetrahydrofuran. They proposed the ene-diolate group to be the co-ordination site for both of the di-anions. Martinez & Uribe (1982) isolated a deep blue iron(III) complex of the ascorbate system. They concluded that the complex has the formula $[(HA)_2Fe)]^+$. In acid solution this color appears as a transient blue when solutions of L(+)-ascorbic acid and Fe^{3+} are mixed and before the reduction of iron(III) to iron(II) occur (Lawrence & Ellis 1972). Furthermore, Martinez & Uribe (1982) concluded from chemical studies and from mossbauer spectrum that iron is present as iron(III) , in the following structure.

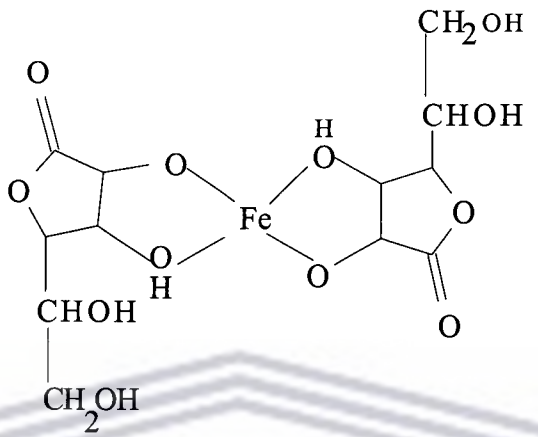


Figure 1.3: Suggested structure for the iron(III)-ascorbic acid complex

The only X-ray crystal structure determined to date is of a complex of ascorbic acid of the type cis-[Pt(RNH_2)$_2$-ascorbate] , where (R= cyclohexane), (Hollis *et al*. 1985). As expected the ascorbate acts as a bidentate ligand, with a surprising feature of the co-ordination, (expected to be through (O_2, O_3), but occurs through the C_2 and O_5. NMR studies show that C_2-O5 binding of the ascorbate di-anion complexes. This mode of co-ordination should be considered in existing and future studies of metal ascorbate interaction. The cis-diamineplatinum ascorbate chelates are prepared from corresponding diaminediaque complex , cis-[Pt(RNH_2)$_2$-(H_2O)$_2$][NO_3]$_2$ (where R= cyclohexane) by reaction with sodium ascorbate in aqueous solution under nitrogen.

Basch *et al.* (1986) discussed the possible electronic structure of the related complex cis-diamine (ascorbato) platinum (II). In calculating the ligand binding energy they used the molecule cis-Pt $(NH_3)_2CH_3OH(OCH_3)$ as a model based on valence electron self-consistent field theory. They concluded that there is a strong Pt-C bond to the ascorbate moiety, which exhibits a large trans influence. The calculated value is 800 $kJmol^{-1}$ more than 160 $kJmol^{-1}$ greater than the Pt-O bond energy. There has been speculation about other complexes of L (+)-ascorbic acid, but none of the speculation indicated the structure determined for the platinum complex by Hollis *et al.* (1985) and Davies *et al.* (1991) .

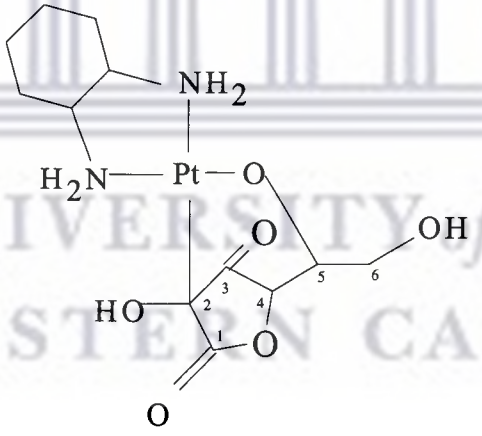


Figure 1.4: The cis-diamine platinum (II) complex of ascorbic acid

Kriss (1988) suggested that dipositive cations complex from Ca^{2+}, VO^{2+}, Mn^{2+} to Zn^{2+} and Cd^{2+} are monodentate. However, a ^{13}CNMR by Nordenskioeld *et al.* (1981) using spin-lattice relaxation times, concluded that for metal ions Co^{2+}, Fe^{2+} and Mn^{2+} at

pH 8.5, the ascorbate -metal ion complexes are formed by chelation with O_2 and O_3 of the ascorbate ion. Similar techniques were applied to the Ni^{2+} ions in the presence of ascorbic acid, with formation of NiO metal species at a high pH of 8.5. It is reasoned that the complex possesses a pseudo-octahedral symmetry from the electronic spectrum, while ^{13}C relaxation data showed that the ascorbate is chelated to the metal through O_2 and O_3. The proposed structure is given below.

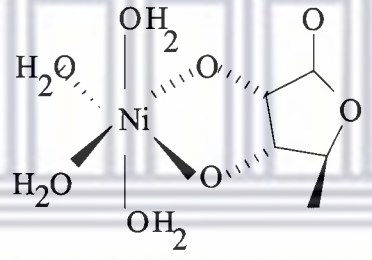


Figure 1.5: Structure of the nickel (II) complex of L(+)-ascorbic acid

A recent development has been the preparation of the amorphous, blue complex (ascorbato) pentamine ruthenium (III) as the trifluoromethane sulphonate salt, prepared from reaction of ascorbic acid in alkaline solution with $[(NH_3)_5RuCl]Cl_2$, followed by precipitation using sodium tetrafluoromethane sulphonate. A similar complex was made from the related tetramethyl reductive acid as shown in Figure 1.6 and 3-O-methylascorbate figure 1.7.

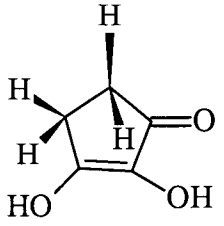


Figure 1.6: Tetramethyl reductic acid

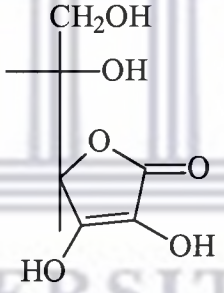


Figure1.7: 3-O-methylascorbic acid

The complexes were thoroughly studied using ESR spectroscopy, cyclic volt-ammetry and NMR spectroscopy . Other complexes that have been prepared, investigated and characterized using IR and thermal stability are the Lanthanide's from cerium (III) to lutecium (III), yttrium and lanthanum (Davies 1984; Bram *et al.*1988).

1.3. **Oxidation of ascorbic acid by metal ions**

The redox reaction of transition metal ions with L (+)-ascorbic acid is of fundamental importance in determining the co-ordination of ascorbic acid with metal ions. Creutz (1981) examined the complexity of such reactions thoroughly. The reactions of metal complexes with ascorbic acid as with other reducing agents can be classified into three categories.

(i) Outer-sphere electron transfer.

(ii) Inner-sphere electron transfer, occurring subsequent to the substitution of a ligand for ascorbate.

(iii) A bridging mechanism in which ascorbate is bound as a ligand of the complex before the electron transfer.

While L(+)-ascorbic acid reacts mainly through an outer-sphere mechanism, inner sphere electron transfer and its oxidation by metal complexes has been reported by Hvoslef (1968) and Davies *et al.* (1991). The role of transition metals in the catalysis of redox reactions of L(+)-ascorbic acid frequently involves a step in which the transition metal ion is itself reduced. These reactions usually involve the formation of an intermediate followed by electron transfer. The transfer of an electron from the reducing agent to the metal normally occurs without any bond being formed between the reductant and the oxidant, i.e. there is no metal ligand bond formation that occurs. In aqueous solution

many transition metal ions are reduced by L(+)-ascorbic acid. A good example would be that of Co (III) ions in water. The high positive charge of the cobalt causes the solution to be acidic *via* hydrolysis with the formation of hydroxo species at higher pH values. A similar reaction has been demonstrated for other aqua ions such as aquamanganese (III). Many other metal ions in high oxidation states have been shown to oxidize L(+)- ascorbic acid .

1.4 **Preparation and characterization of titanium complexes of vitamin C**

Although preparation of some titanium complexes of vitamin C has been described by Jabs *et al.* (1990), the compounds were impure and poorly characterized.

1.4.1 **Introduction**

Titanium ascorbate complexes have been used as foliar sprays on plants to enhance plant growth Pais (1983). Titanium in plants is said to promote the synthesis of chlorophyll (Dumon & Ernst 1988). It was also found that titanium complexes of L(+)-ascorbic acid appear to promote some photosynthetic process (Tajmir-Riahi 1991). It is also believed to have an auxin effect (Martinez & Uribe 1992). Its application can increase the crop yield on average by 10-20 % and also improve the quality of the product Pais (1983). It

is also known that titanium dioxide is able to promote photocatalytic processes (Pais 1983; Dumon & Ernst (1988) and for example the photolysis of water; this is discussed in Chapter 2. It is possible that the foliar spray of the titanium ascorbic acid system hydrolyses to produce $TiO_2.nH_2O$. TiO_2 can act as a photocatalyst to fix atmospheric nitrogen:

$$N_2 + 3H_2O \rightarrow 2NH_3 + 3/2\ O_2$$

1. 5 Preparation of titanium ascorbate complexes

In a cold, hydrolyzed but clear solution of $TiCl_4$ in water, two equivalents (19.27 g, 0.109 mol) L(+)-ascorbic acid were dissolved , after which 4.5 equivalents of sodium hydroxide were carefully added to bring the pH to 1.6. The dark orange solid was precipitated with ethanol and analysed, the reaction is show below.

$$TiCl_4 + 2HA + 4NaOH \rightarrow TiO(HA)_2.2H_2O + 4NaCl + H_2O$$

1.6 Results and Discussion

1.6.1 Elemental analysis

Compound 1 : **Titanium Ascorbate $TiO(HA)_2.2H_2O$**

Elements	Calculated	Found 1	Found 2
C	29.79	32.01	32.01
H	4.00	4.09	4.09
Ti	10.64	10.60	10.60

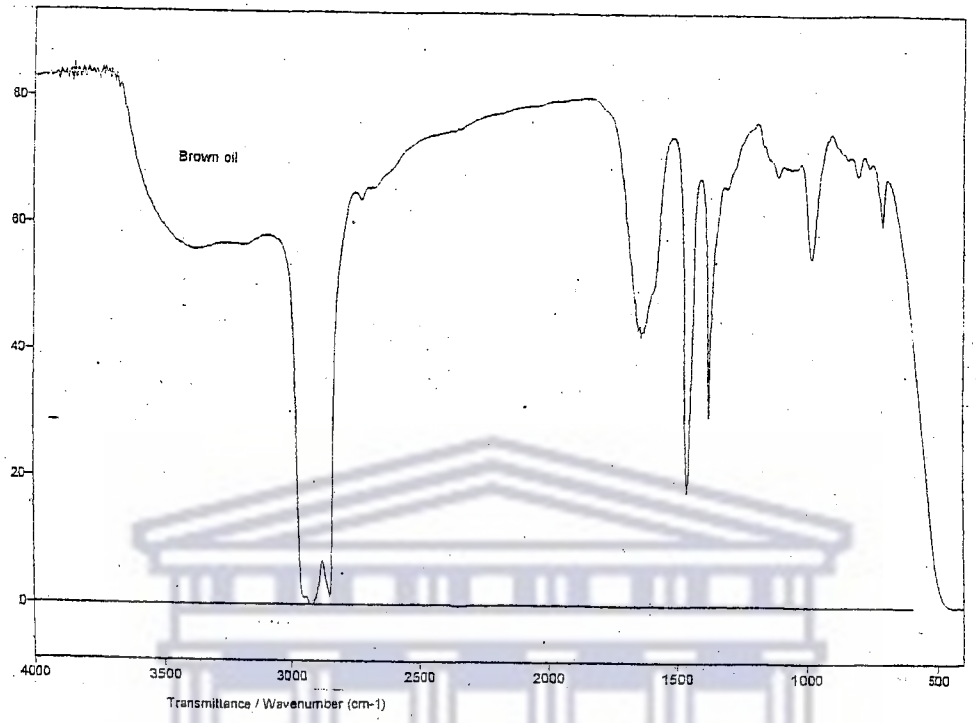


Figure 1.1 The Spectrum of L(+)-Ascorbic acid

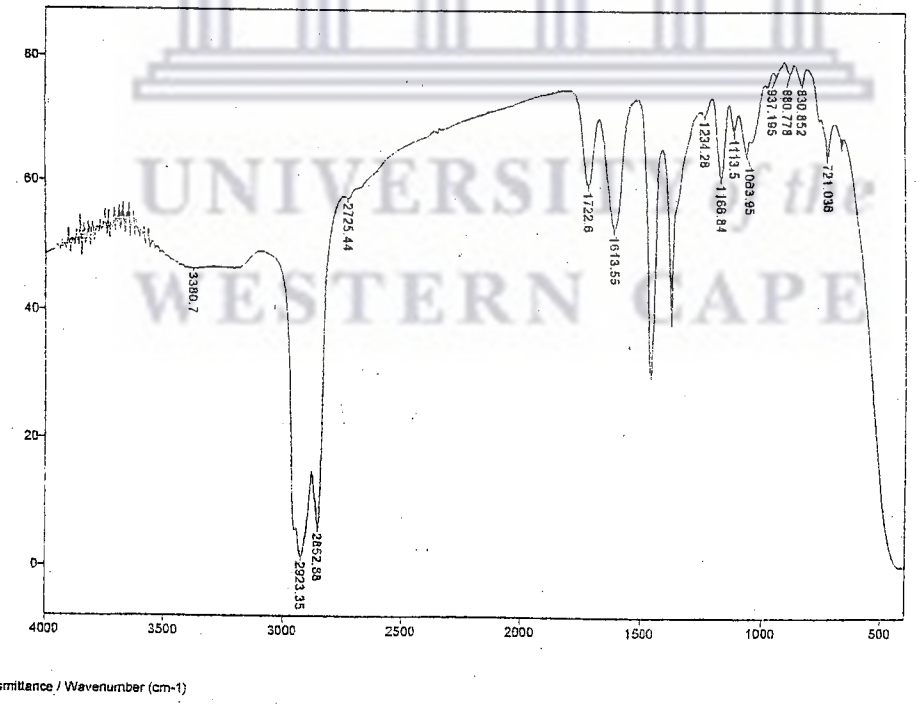


Figure 1.2 The Spectrum of $TiO(HA)_2.2H_2O$

In the area of 2600-3000 cm^{-1} the bands are classified as the stretching vibrations of aliphatic OH-groups of side chains with intermolecular H-bonding (see figure 1.1). From Evtushenko *et al.* (1977) the bands in the area 2750-2600 cm^{-1} of the spectrum of the free $Hasc^-$ anion (figure 1.1) are classified as the stretching vibrations of OH-groups with intramolecular H-bonding. Evtushenko *et al.* (1977) was unable to locate these bands in the spectra of Zn^{2+} and vanadyl ascorbate and concluded that the intramolecular H-bonding only occurs in the free anion and is broken through its interaction with metal ions. The classification of these bands as OH- stretching vibration seems to be correct. Similar results have been found in NaHA, and $Ca(HA)_2.2H_2O$ where additional intermolecular H-bonding was established (Tajmir-Riahi 1991; Tajmir-Riahi & Bohai 1992).

They are also present in the spectra of Ti-ascorbate ($TiO(HA)_2.2H_2O$ see figure 1.2) in which the corresponding bands at 2923 and 2852 cm^{-1} are found. In the spectrum of the free acid L-H_2A the strong band at 1752 cm^{-1} was classified as the CO- stretching vibration and the very strong bands at 1665 cm^{-1}, to the C=C stretching vibration (figure 1.1). Yatsimirskii (1980) suggested to classification of both bands at 1752 cm^{-1} and 1665 cm^{-1} to the CO groups that are involved in intramolecular H-bonding. The bands can be seen very clearly in the spectra of Na- and K-ascorbate at 1600 cm^{-1} and 1590 cm^{-1} as a doublet (Tajmir-Riahi 1991 ; Tajmir-Riahi & Bohai 1992).

Yatsimirskii (1980) argued that the doublet at 1665 cm^{-1} cannot be classified as the C=C vibrations because it can be seen in the spectrum of $TiO(HA)_2.2H_2O$ without any shift relative to that in L- H_2A, whereas it is missing in the $Ti(OH)_2(HA)_2$ (Benetis *et al.* 1981). The band at 1613 cm^{-1} in Figure 1.2 corresponds to the band of KHA and NaHA at 1592 cm^{-1}. The spectrum of $TiO(HA)_2.2H_2O$ shows a shoulder band at 1722 cm^{-1} .

In conclusion, all bands between 1760 and 1630 cm^{-1} correspond to the CO-group of the lactone ring that is involved in different binding situations. The doublet at 1665 cm^{-1} corresponds to the CO-stretching vibration that are involved in intermolecular H-bonding. The band at 1355 cm^{-1} in the spectrum of $TiO(HA)_2.2H_2O$ corresponds to the stretching vibration of C=C double bonds (see Figure 1.2 above).

1.8 REFERENCES

Basch, H., Krauss, M. & Stevens, W.J. 1986. Inorg. Chem. 25: 4777.

Bram, D.M., Dell, S.D., Kumar, R., Clarke, M.J., Rodriguez, V., Sherban, M. & Charkoudian, J. 1988. J. Am. Chem. Soc. 110: 1498-1506.

Benetis, N., Holmen, K., Kowalewski, J., Nordenskioeld, L. & Wahlberg, O. 1981. Acta. Chim. Scand. A35: 513.

Creutz, C. 1981. Inorg. Chem. 20: 4449.

Davies, M.B., Partridge, D.A. & Austin, J. 1991. Vitamin C: Its Chemistry and Biochemistry. Royal Society of Chemistry, London. 3, 124.

Davies, M.B. 1984, Inorg. Chim. Acta. 92: 141.

Dumon, J.C. & Ernst, W.H.O. 1988. J. Plant. Physiol. 133: 203-209.

Evtushenko, N.P., Yatsimirskii, K.B., Kriss, E.E . & Kurbatova, J. 1977. Russ. J. Inorg. Chem. 22(6): 839.

Hollis, L.S., Amundson, A.R. & Stern, E.W. 1985. J. Am. Chem. Soc. 107: 274.

Hvoslef, J. 1968. Acta Cryst Sect. B. 24: 1431.

Hvoslef, J. 1969. Acta . Cryst Sect. A. 35: 513.

Jabs, W., Gaube, W., Fehl, C. & Lukowski. R.1990. Inorg. Chim. Acta. 175: 273-276.

Kriss, E.E.. 1988. Russ J. Inorg. Chem. 25: 1362.

Lawrence, G.S. & Ellis, K.J. 1972. J. Chem. Soc., Dalton Trans: 1667.

Martinez, P. & Uribe, D. 1982. Z .Naturforsch. B: Anorg. Chem .37B: 1446.

Martell, A.E. 1982. In Ascorbic Acid: Its Chemistry, Metabolism and Uses. Advances in Chemistry Series (Edited by P.A. Seib and B. M. Tolbert. American Chemical Society, Washington, DC.). Ch.7.

Nordenskiold, L., Kowalewski, J. & Benetis, N. 1981. Inorg. Chim. Acta. 56: L21-L22.

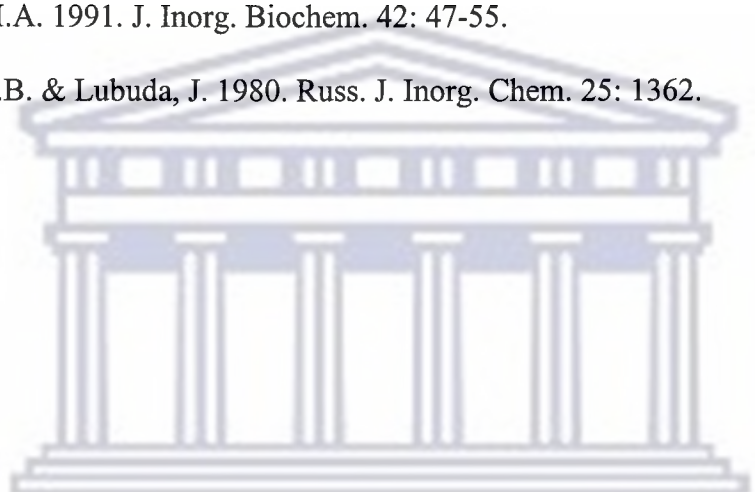
Pais, I. 1983. J. Plant. Nutr. 6 (1): 3-131.

Seib, P.A. & Tolbert, B.M. 1982. Ascorbic Acid: Its Chemistry, Metabolism and Uses. Adv. Chem. Ser. Amer. Chem. Soc. Washington, DC: 53.

Tajmir-Riahi, H.A. & Bohai, D.M. 1992. J. Inorg. Biochem. 45: 73-84.

Tajmir-Riahi, H.A. 1991. J. Inorg. Biochem. 42: 47-55.

Yatsimirskii, K.B. & Lubuda, J. 1980. Russ. J. Inorg. Chem. 25: 1362.



CHAPTER 2

Titanium in Plants

2.1 INTRODUCTION

For almost half a century after the classical research with water cultures of Sachs & Knop (1860), it was generally accepted that for most plant species, ten elements were essential for healthy plant growth. These elements were carbon, hydrogen, oxygen, nitrogen, phosphorus, sulphur, potassium, calcium, magnesium and iron. For a few plants it was suggested that some additional element might be necessary for plant growth (Stiles 1958). It was not until 1930 when G.Bertrand came with the first definite statement that other elements might be necessary for normal plant growth. Since 1930 it has been known that plant life needs some other elements in much lower concentration, between 0.001 to 0.1 percent. These latter elements are known as trace elements or micronutrient elements. Till 1960 the only known trace elements in plants were iron, boron, manganese, copper, zinc, and molybdenum. Arnon & Stout (1939) were the first to qualify the principal of essentiality, which states that;

(i) Lack of these elements cause incompletion of the plant life cycle

(ii) These elements must be part of the essential plant composition or metabolites.

(iii) If they are (not) essential for plant life, deficiency or disease occurs which can be reversed by the application of these elements.

Recently, progress on the impact of trace elements on plant metabolism has been stimulated by the extensive need of managing and storing industrial wastes (Salomons & Forstner 1988). One of these elements is titanium. Titanium is the tenth most

abundant element in the earth's crust, and is present in many ores. The primary ore being ilmenite, and then rutile and anatase. It is generally distributed as the oxide in surface soils. Its distribution in soil profiles has been well documented by Swaine (1955), Wells (1960) and Mitchell (1964). Regardless of its abundance, its increasing importance in metallurgy and the vast amount of titanium found in certain industrial waste (Sierp 1967; Whitehead 1984), there is no thorough synthesis of its occurrence, accumulation, physiological role, and hence biochemical cycling, in biota (Stiles 1958 ; Dumon & Ernst 1988). There is no evidence to show the essentiallity of titanium for the growth of higher plants, nor is there evidence that the element can be toxic to them (Pratt 1966). The absence of toxicity of titanium may be related to its insolubility in the pH range 4 - 8, where plants grow well if given the essential elements. In recent reviews on trace elements, titanium has been excluded (Woolhouse 1982 , Lauchli & Bieleski 1983; Fiedler & Rosler ,1987).

2.1.1 **Occurrence of titanium in soils**

Titanium has been regarded as being immobile in soils. Its distribution in the lithosphere has been estimated to be less than 1mg.kg^{-1} and in general there is little indication of downward movement following mineral weathering. During weathering titanium minerals such as geikeilite ($MgTiO_3$), ilmenite ($FeTiO_3$), Perovskite ($CaTiO_3$) and titinite or spliene ($CaTiSiO_5$), as well as anatase and rutile (TiO_2) may become available to

plants. Joffe & Pugh (1934) and Karim (1953) demonstrated titanium oxide accumulation in the A_2 horizons of podsolized soils. The authors suggested the use of titanium distribution as a significant index for the classification of soils. Tamura *et al.* (1955) and Silverman & Muroz (1971) reported up to 25 percent titanium oxide in lateritic Hawaiian soils. Mineralogical analysis showed that titanium oxide is present as the mineral anatase. In Hungarian soils they (Tamura *et al.* 1955; Silverman & Muroz 1971) reported high titanium levels with a fairly uniform distribution. The titanium present in these soils is fairly unavailable to plants. When given to plants there seem to be no response, probably because these salts are transformed very rapidly into insoluble forms.

In tropical regions where soils are characterised by intense weathering over long periods, good evidence exist for the mobility of titanium. The residual accumulation of TiO_2 can be observed in the topsoil with steep concentration gradients from as high as 1.08 percent in topsoil to 0.36 percent in the hard bed rock of a hornblende granulite in India (Satyanarayana & Thomas 1962). In southern American topsoil it was reported that the titanium concentration is 1.0 percent to 2.76 percent in certain ignious rocks (Hardy & Follet-Smit 1931). In some soils of volcanic origin in Hawaii, the titanium concentration amounts to 2 percent. Pellet & Fribourg (1905) found 2 percent titanium oxide in the Egyptian soil and 0.47 per cent in a French soil, while Geilmann (1920) reported as much as 1 percent of titanium oxide in soils Hawaii, but most of the samples

had from 0.3 to 0.6 percent. Lee (1943) found 0.5 percent titanium oxide as the usual value for pedocals, but reported that red earth soils had 1 to 3 percent, but differed from red soils from basalt, which had up to 6.15 percent titanium oxide. These findings were different from Chernozem soils which had 0.45 to 0.53 percent (Kaminskaya 1941), with podsols having a wider range of titanium content. In soils directly above titanium enriched ores, the concentration of titanium can increase up to 10 percent. Even in Scottish soils, which are comparatively young and little weathered there are indications suggesting the inertness of titanium. In young soils, under temperate climate, the concentration of titanium remains low in soils over limestone, marl and in loamy sand, but increases to 6.3 per cent in loes-like and alluvial clays (Tonkonozhenko & Khlyupina 1974). In addition to the effect of soil acidity, the soil organic compounds can influence the availability of titanium to plants as with other chemical elements (Table 2.1). McBride (1981), Van der Werff (1981) and Dumon & Ernst, (1988) demonstrated the solubility of titanium in three acidic soils.

Table 2.1: The solubility of titanium in three acidic soils

Soil Type	pH H_2O	total concentration (mg. Kg^{-1} dry wt.)	Ti extractable by demineralised water mol dm^{-3}	1molar acetic acid mol dm^{-3}	1molar oxalic acid mol dm^{-3}
Peat[1]	2.7	2037	0.0	0.0	123
Brown earth[1]	3.0	1025	0.0	0.0	77
Sand[2]	6.8	72	4.4×10^{-9}	1.72	n.d

1. after Nautsch-Laufer (1974).
2. Ram *et al* (1983), n.d = not determined

Swaine and Mitchell (1960) reported acetic acid extractable titanium of up to 3.5 $mg.kg^{-1}$ in the A horizons of podsols developed on granitic and felsitic lava. They interpreted these relatively high levels as possible evidence of mobilisation and impending translocation of titanium. Summary reports as to how titanium is accumulated by plants are very sketchy. It is however clear that soil titanium concentrations vary widely, but can be as high as 10 percent. Titanium forms 25 percent by mass of TiO_2. In South Africa, along the Natal Coastline, about 80 percent of titanium is extracted from titanium dioxide, and used mainly in the manufacturing of paints, gas turbines, etc.

2.1.2 **Titanium in aquatic organisms**

The titanium concentration in sea water has been found to be very low at 0.02 $\mu g\ dm^{-3}$ (Goldberg 1965). This is probably due to the low solubility of titanium during rock weathering and soil formation. Whitehead (1984) reported higher values of 50-100 times the above. Living cells are able to take up elements from solution against a concentration gradient. This is well demonstrated by marine organisms, many of which contain trace elements at concentrations as high as 10^6 times their level in sea water. Bowen (1966) mentioned that the concentration of trace elements by marine organisms shows the following pattern:

(i) the orders of affinity for marine organisms is 4+ and 3+ elements >

2+ transition metals >+2 Group IIA metals > 1+ Group I metals.

(ii) the order of affinity of plankton and brown algae for 4+ and 3+ groups and for 2+ transition metals is different .

For example: Plankton Fe > Al >Ti > Cr , Si > Ga

Zn >Pb >Cu> Mn >Co> Ni >Cd

Brown algae Fe > Ca >Cr > Ga > Ti > Al >Si

Pb > Mn > Zn > Cd >Co > Ni

In phytoplankton the titanium concentration varied between 6 and 137 mg kg^{-1} dry matter. This seems to be governed by selectivity. In diatoms the titanium concentration was 1254 mg kg^{-1} dry wt silicates, being strongly bound in the silicate structure (Riley & Chester 1971). Titanium concentrations in other marine plants, like multicellular algae, varied according to species. The range was from less than 6 mg Ti kg^{-1} dry mass in *Laminaria* and *Ulva* spp (Dumon & Ernst 1988), increasing with a greater range in *Spirulina* spp. In other algae for example, as in *Fucus* spp, the titanium level was 90 mg Ti kg^{-1} dry mass (Dumon 1975). A similar value of 91 mg kg^{-1} of titanium was observed in *Zostera marina*, growing in a submersed saltmarsh (Dumon 1975). Many of the figures given were determined on unwashed algae, so that surface adsorption could not be distinguished from uptake by the algae, which severely reduces the biological relevance of the bio-concentration factor.

Major elements in ocean water are usually stable or almost so, considerable variation in their concentration ratios may be found in the marine environment under some atypical conditions. In river water the composition is largely controlled by the nature of the rocks with which the water has been in contact. Readily hydrolysable compounds, such as titanium compounds, are not appreciable enriched near continental sediments.

Summary

Usually the lower organisms concentrate trace elements more readily than higher ones. The power to concentrate a particular element or groups of elements is often an attribute of a particular family or species. Concentrations may reach up to 90 $mg.kg^{-1}$ dry mass.

2.1.3 **Titanium in land plants**

Many authors studied the presence of titanium in plants. They investigated titanium concentrations in all systematic groups of terrestrial plants. These include bryophytes, fungi, lichens, angiosperms and fossil plant remnants (Dumon 1975). A number of plant organs have been found to contain TiO_2. In potatoes the titanium content is about 0.34 $mg.kg^{-1}$ (of titanium) dry mass. Titanium as a non-essential microelement has a wide range of actions, which are not confined to a particular plant species. In order to analyze the content of titanium in plant species, plants must be washed to avoid surface contamination. Takala & Olkkonen (1985) studied the titanium content of lichens in

Finland. They concluded that the titanium concentration correlated highly significantly with that of sulphur from wet and dry deposition. They found that the titanium content of lichens showed statistically significant regional variation. The titanium content of angiosperms was studied in the first decade of the century. The findings suggesting a concentration variance from 20 $mg.kg^{-1}$ in red cabbage to 1900 $mg.kg^{-1}$ in the wood of *Quercus penduculata* (Geilmann 1920). In wild and cultivated plants, the data shows no difference in titanium concentration (Pellet & Fribourg 1905). It is interesting to note that much work has been done on titanium in plants, but there are few investigations which correlate the concentration of this element in the soil to that of the plant. One exception is the study conducted by Tonkonozhenko and Khlyupina (1974), who analysed the titanium concentration of roots, shoots and leaves of *Medicago sativa* together with the total content of the soil in various soil types in the Krasnodar territory. A correlation diagram that shows no link, neither between root and soil, nor shoot and soil, is represented in figure 2.1.

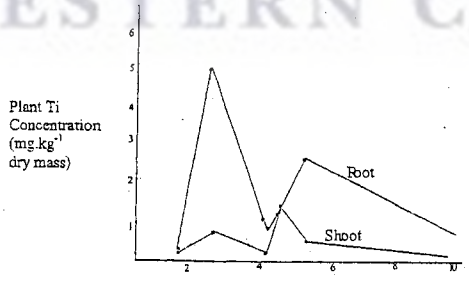


Figure 2.1 Total titanium concentration in the roots and shoots of *Medicago sativa* as related to that of the soil (after Tonkonozhenko and Khlyupina, 1974)

The lack of correlation is typical for almost all transition metals, because soil acidity and speciation of the transition metal, which determines metal uptake by plants, are weakly correlated with total soil concentration (Ernst 1985; Van der Werff 1981). It has been shown that the high solubility of titanium in a very acid sand soil (pH = 3) will increase the titanium concentration in plants growing in such soils. Dumon (1975) mentioned the findings of Ernst (1985), that *Corynephorus canescens* and *Rumex acetosella* leaves had titanium concentrations of 142 ± 22 mg kg^{-1} and 207 ± 18 mg kg^{-1} dry mass respectively, as compared to 2.4 and 4.8 mg kg^{-1} in the same plant species growing on a soil with a pH of 4.9. Contrary to these findings, Carrigan and Roger (1940) reported that even though titanium was detected in all the soils they studied, it was seldom found in the plant material. Studies carried out by Ruiz *et al.* (1946) found titanium in each of the 72 samples of food of vegetable origin. Labandeya (1942) also found titanium in all plants studied. The lowest value found was 0.5 mg kg^{-1} of fresh mass. Discrepancies found in the absence and presence of titanium in plants are probably related to the difference in sensitivity of the various methods used, and the ability of the plants to take up titanium as well as to variations in the titanium content of different soils. Since all soils contain some titanium, special precautions must be taken to eliminate surface contamination on plant parts such as leaves, roots, stem and trunks. Titanium like any other chemical element accumulates in a specific species and organ. For example in

alfalfa spp. the titanium content of the roots is higher than the shoots (Tonkonozenkho and Khlyupina, 1974). Gericke (1940) reported an enhancement of root growth by titanium, which has not been explained. In a North American study, titanium content in leaves is 50% higher than in twigs (Connor and Shacklette, 1975). This is clearly shown in Table 2.2 below.

Table 2.2: Concentration of Ti (mg kg^{-1} ash) in North American shrubs and trees (Connor and Shacklette, 1975)

Plant species	**Leaves**	**Twigs**	**# of analyses**	
			Leaves	**Twigs**
Acer rubrum	175	90	60	60
Diospyros virginiana	140	98	60	59
Liquidambar styraciflua	130	88	55	102
Nyssa sylvatica	220	145	60	60
Prunus serotina	155	120	60	60
Rhus coppalina	225	130	29	60

The findings of (Guha & Mitchell 1965 &1966) are in agreement with this pattern. In senescent leaves the titanium content was found to be 1.2 mg kg^{-1}. With respect to grain crops, mention should first be made of the data of Asmaeva (1969) who found 0.08 $mg.kg^{-1}$ titanium in the ashes of wheat grains. Looking at horticultural crops, an early

publication by Troitsky (1955) studied the composition of the soil, the vines grown on them, and the wines made from the grapes in various wine regions of the Soviet Union. His data suggest an average of 2000 $mg.kg^{-1}$ titanium in the soil, a maximum of 10$mg.kg^{-1}$ in the grapes and a few tenths of $mg.dm^{-3}$ in the wines. Dumon (1975) reported the accumulation of titanium in bark, as compared to the needles, of *Pinus maritima* to be 1.2 mg kg^{-1} dry matter. Konishi and Tsuge (1936) reported titanium to be more concentrated in the nodules of alfalfa.

Summary

In land plants, titanium accumulates in specific species or organs such as roots, shoots, stems, leaves or trunks, with increased concentrations in ageing leaves, the concentrations of titanium ranging up to 1900 $mg.kg^{-1}$.

2.2 Titanium compounds and plant growth

Titanium ascorbate and other titanium complexes such as titanium chloride, titanium sulphate, or the synthetic dicyclopentadienyl titanium chloride (Mrowca 1974) have been used extensively to regulate plant growth. With the exception of Blanck & Alten (1924) ; Hara *et al.* (1976), other investigators demonstrated the promotion of growth by these complexes, whether applied to the soil or as a foliar spray (Nemec & Kas 1923,

Terlikowski & Gornicki 1933, Young 1935, Baum 1939, Gericke 1940, Pais *et al.* 1969, Rutskaya 1971, Nautsch-Laufer 1974; Ram *et al.* 1983). Dobrolyubski (1961) was one of the first scientists to use titanium solutions as foliar sprays. He found that a titanium (III) solution increased the volume of yield and raised the sugar content of the grapes, but reduced the titrable acid content of the grape juice. He pointed out that by applying titanium solution to grapes, the yield increased and the quality of the product was better. Organoleptic tests revealed a favourable effect of titanium on the development of flavour and aroma in wines. Mrowca (1974) indicated the promotive effect of various compounds of titanium and zirconium, mostly cyclopentadienyl derivatives, on the yield and on the quality of wheat and other plants in his study with these compounds. An interesting paper by Gryzhankova *et al.* (1975) analysed some marine algae, *Lamina japonica* and *Ulva fenestrata* from the Japanese sea. They established, using chromatographic analyses that various metals, including titanium, have different dispersions in the lipophilic materials of plant cells and found a compound, which can be considered as a titanium-chelate of phosphopantotenic acid. This compound has a very low redox potential and may therefore be involved with reducing iron protein in redox-processes. Rutskaya (1971) and Rutskaya & Ruzhabskaya (1974) discovered that the addition of ammonium titanyl sulphate to soils influenced the development of sugar beet favourably. They found that the chlorophyll content of the leaves and the sugar content of the beet increased. These results were confirmed by

Wakamoto (1973) and Tsukamoto (1975), who stated that the iron and titanium compounds applied as fertilisers, promoted the nitrogen turnover of the soils, and exercised a favourable effect on the quantity and quality of soybean yield. Pais *et al.* (1969) investigated the role played by microelements, including titanium, on plant life. They grew the plants in containers, with synthetic materials (polyethylene or polystyrol), which are not much different from soil when viewed on ion-exchange basis. They used tomato as a test plant and observed favourable results regarding the chlorophyll content of leaves. Between 1972 and 1974, foliar nutrition experiments were carried out with tomatoes in the field (Pais & Hodossi 1975). An increase in yield, favourable changes in composition of the plant mass and an acceleration of fruit ripening was reported. The leaf spray for these experiments contained a number of macro- and micronutrients, and included titanium. The important role played by titanium in plant life is also seen by the results obtained by mass spectrography on the leaves of plants sprayed with titanium solution (Pais 1974). The data below (Table 2.3) indicates favourable effects of titanium manifested in the marked increase in the concentration of some essential microelements.

Table 2.3 . The effect of Ti in leaves on various metal concentrations ($mg.kg^{-1}$)

Sample		Ti	Zn	Mn	Cu	Ni	Co	Cr
Jonathan apple leaf	high Ti	210	350	8400	410	26	6.5	7.5
	Low Ti	43	83	1400	120	5	1.9	1.7
Ezerjo vine leaf	high Ti	72	2810	3440	4120	24	2.3	2.8
	Low Ti	19	1160	1080	1790	10	2.1	1.0

It was suggested that titanium enhanced photosynthetic processes in plants, and thus metal ion demand due to increased enzyme activities, which stimulated an increased ion uptake by the plant. During 1976 and 1977, large-scale experiments were conducted on the Kalocsa state farm with two American varieties of tomato plants: Yukon and Commander, with titanium solutions of varying concentrations (Pais 1983). The results showed no significant differences. In the same year (1977) a titanium solution was used at a concentration of 1 $mg.dm^{-3}$, producing positive results. Ram *et al.* (1983) compared the effectiveness of diclyclopentadienyl titanium chloride and titanium chloride at 0.25, 0.5 and 1 $mg.dm^{-3}$ levels of water-soluble titanium, applied as foliar sprays to beans. They found that titanium enhanced the chlorophyll content of leaves, suggesting its influence is to promote photosynthesis. They observed an increase in dry matter yield of beans by 5.6, 8.9 and 20 percent, and also found that both compounds had similar effects on beans. Maroti *et al.* (1984) conducted experiments on the role of titanium in plants, particularly its effect on the growth of tobacco callus. The titanium

was administered in the form of titavit (titanium ascorbate). The experimental plant material consisted of secondary tobacco tissue. The results showed a growth dependence on titanium concentration. This was displayed at a concentration of 0.02 $\mu mol.dm^{-3}$, stimulating the weight increase by 46% compared to the control. Simon *et al.* (1990) conducted similar experiments. The authors studied at the effect of titanium on growth and dry matter accumulation of *Bradyrhizobium japonicum* and *B. lupini*. They found that at 1 $\mu mol.dm^{-3}$ and 2 $\mu mol.dm^{-3}$titanium-ascorbate, the growth rate and dry matter production was enhanced. Whereas other organic titanium compounds such as L(+)-ascorbic acid had no effect on this plant. Martinez-Sanchez *et al.* (1993) found that titanium treatments *via* foliar spray on *Capsicum annum*, increased the ascorbic acid contents of leaves when compared to its corresponding untreated controls. Carvajal *et al.* (1994) conducted experiments on red pepper plants, using two titanium (IV) compounds, titanium ascorbate and titanium chloride. They investigated enzymatic activities such as catalase, peroxidase, lipoxygenase and nitrate reductase in seeds, embryos ,seedlings, and adult plants. The authors observed a stimulatory effect of titanium for every iron-depended enzyme studied at all developing stages, as well as for nitrate reductase. They concluded that titanium promotes iron effectiveness in cells, mainly in the cytoplasm and chloroplast, enhancing the Fe^{2+}/Fe^{3+} system (Dumon 1975).

During 1984 research was done to determine the effect of titanium on the germination

of wheat, maize, and sunflower seeds and on the growth of seedlings . In these experiments titanium was used at concentrations of 0.2 , 0.02 and 0.002 g Ti/kg seed. Seeds were also treated with corresponding concentrations of ascorbic acid solution, while the control was given ion-free water of the same volume. It was found that at the two lower concentrations of titanium, the rate of germination was accelerated by one or two days, and root growth and formation was more vigorous, with greater uniformity of the growth of the seedlings. The solution with the highest concentration of titanium caused no changes. The amount of titanium which plants require for growth stimulation cannot be defined as an absolute norm. For example 24 mg Ti as $TiCl_3.dm^{-3}$ nutrient solution promoted the growth of *Phaseolus vulgaris* cv saxa (Nautsch-Laufer 1974) whereas a similar concentration 30 mg kg^{-1} soil, decreased the yield of turnip plants (Kusaka *et al.* 1971) . Other plant species were even more sensitive. Only 4 mg Ti (given as $TiCl_3$) decreased the biomass production of the cabbage variety Shikidoru (Hara *et al.* 1976) and 1 mg Ti that of tomato (Pais *et al.* 1969). In *Phaseolus* (bean) and cabbage, the injury due to titanium was accompanied by high concentrations in the tested plants (Dumon 1975). Some of the growth promoting effects in soils may be associated with the stimulation of the nitrification process by titanium, with the promotion of nitrogen availability (Wakamoto 1973 ; Tsukamoto 1975).

Summary

Titanium compounds, when used as a fertilizer *via* roots or leaves in growth experiments, affects the growth of the plant, depending on species and concentration. It also has an effect on the nitrification process thereby promoting nitrogen availability.

2.3 The influence of titanium on physiological processes

The physiology of titanium can be better understood by looking at the plant make-up and cells. Titanium has a low mobility, which results in it being preferentially accumulated in roots, with very small amounts translocated to leaves. When the concentration of titanium in solution is increased, the concentration of titanium in roots increases much more than that of the shoots of *Phaseolus vulgaris* and *Zea mays* (Nautsch-Laufer 1974). To date, only one report has been published with regard to the distribution of titanium in plants and on the solubility of titanium in plants. The binding of titanium to the cell fraction in *Zea mays* increased with increasing titanium supply. At 144 mg $Ti.dm^{-3}$ nutrient solution, 65% of the cellular titanium of maize was found in the cell walls and 5.1% in the vacuoles of leaf cells. This stronger binding of titanium may be due to the formation of organic titanium compounds such as 4'- phosphopantetheine in algae or titanium flavi-complexes (Heimmerich & Lauterwein 1973).

2.3.1 **Enzyme activation and effect on chlorophyll content and photosynthesis**

Titanium enhances some enzymatic activities such as peroxidase, catalase, lipoxygenase and nitrate reductase. It also induces nutrient uptake. Furthermore, titanium produces a higher concentration of fruit pigments in which lipoxygenase is implicated, and in metabolites such as malic acid and ascorbic acid (Dumon & Ernst 1988). All the evidence leads to the conclusion that Ti acts indirectly through activation of Fe (Dumon and Ernst 1988). The evidence for catalase stimulation was noted in *Beta vulgaris* (Dumon 1975), for nitrate reductase in *Phaseolus vulgaris* and fructose 1,6-bi-phosphatase in *Anacystis nidulans* (Kiss *et al.* 1985). This stimulation of enzyme activities by titanium is increased with increasing titanium supply to the nutrient solution.

The effect of titanium on the chlorophyll content of plants has first been noted by Inman *et al.* (1935). They found that titanium could not be substituted for iron in chlorophyll formation. Its application increased the concentration of chlorophyll a, chlorophyll b, and total chlorophyll in beans, sugar beet and various other plants species (Pais *et al.* 1969, Pais *et al.* 1977 ; Ram *et al.* 1983). Kiss *et al.* (1985) reported that at moderately low concentration, titanium enhanced photosynthetic oxygen evolution. Gryzhankova and Boichenko (1975) stated that the titanium content of the leaves is much greater after illumination suggesting enhanced titanium transport in the light. They also

stated that the reducing activity of Ti^{4+} of titanium compounds is higher in the dark as compared with the process during photosynthesis. Contrary to this, Traetta-Mosca (1913) assumed a functional replacement of iron by titanium.

2.3.2 **Fixation of nitrogen by titanium dioxide**

Titanium dioxide has been reported to be involved in the nitrogen fixation in the nodules of legumes (Konishi & Tsuge 1936). They reported that potassium titanate, when added to solution cultures, improved the growth of alfalfa. The authors found a large amount of titanium in the leaves and stems. They also stated that titanium sulphate and potassium titanate increased the number of nodules, and caused earlier formation of nodules. Greater amounts of nitrogen were fixed where titanium was added to agar media and to soils. However, high concentrations of titanium sulfate were not beneficial. Anderson (1951), in a review article on nitrogen fixation, mentioned a possible relationship between titanium and nitrogen fixation by legumes. Two other reports, one by Dhar & Mukerji (1941) and other by Rao (1934), indicated that titanium oxide acts as a photocatalyst in the photochemical oxidation of nitrite to nitrate.

There appears to be only a single mechanism involved in biological nitrogen fixation. A molybdenum- and iron-containing enzyme reduces N_2 to $2NH_3$ in the only known simple

reaction of this type under ambient conditions. In 1934 this biological catalyst was named nitrogenase.

In nature three N_2-fixing agents have been intensively investigated at the molecular level, *viz Clostridium pasteurianium* (Hewitt 1983), *Azotobacter chroococcum* and *A. vinelandi* (Mckenna *et al.* 1970, Burns *et al.* 1971, Beneman *et al.* 1972; Nutman, 1976). Another element that is a co-factor for a few enzymes, is molybdenum (Mo), amongst which nitrogenase and nitrate reductase are most important. Nitrate reductase occurs in all plants. It converts nitrate (NO_3^-) into nitrite (NO_2^-), which then can be further reduced to ammonium-nitrogen for incorporation into amino acids. Nitrogenase is an enzyme required for nitrogen fixations (see Figure 2.2 below).

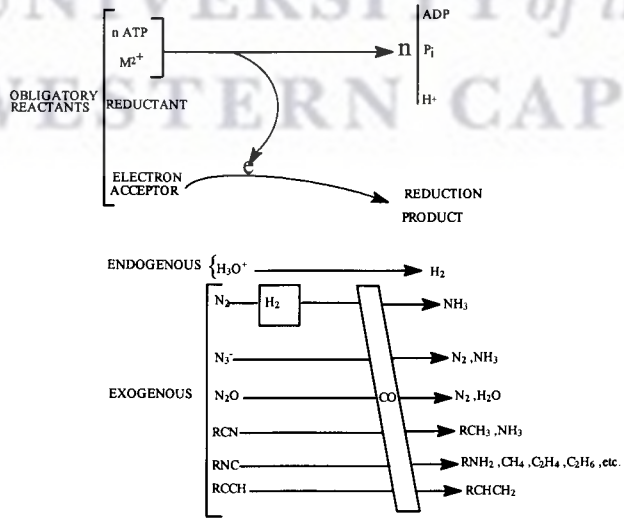


Figure 2.2: Reactions common to all nitrogenases

Titanium compounds have been used to enhance plant growth by the use of foliar sprays at 1 $mg.dm^{-3}$ levels. A series of experiments were carried out at the University of the Western Cape (UWC) to quantify this enhancement using tomato plants. The work involved use of the foliar sprays of titanium ascorbate, titanium oxalate, ascorbic acid, and oxalic acid in both nitrogen containing and nitrogen-deficient nutrient solutions. Root treatments with similar solutions were also employed.

Summary

Titanium as a trace element may increase the chlorophyll content of plants, enzyme activity and uptake of major and minor nutrients. It may have an effect on the fixing of nitrogen.

2.4 REFERENCES

Anderson, A.J. 1951. Proc. Spec. Conf. Plant and Anim. Nutri. 190-199.

Asmaeva, Z.I., Ilvitsky N.A. 1969. Isv. Vissh. Ucheb. Zaved. Pisch. Technol.

Arnon, D.I. & Stout, P.R. 1939. Plant Physiol. 14: 371-375.

Baum, T.H. 1939. Zur Einwirkung von Titan-Verbindungen auf Planzen. Diss. TH Muchen.

Beneman, J.R., McKenna, C.E., Lie R.F., Traylor T.G. & Kamen M.D. 1972. Biochim. Biophys. Acta. 264: 25-37.

Blanck, A. & Alten, F. 1924. J. Landwirtsch. 72: 103-110.

Bowen, H.J.M. 1966. Trace elements in biochemistry. Academic Press, London: 241.

Burns, R.C., Fuchsman, W.H. & Hardy, W.R.F. 1971. Biochem. Biophys. Res. Commun. 41: 353-358.

Carrigan, R.A. & Rogers, L.H. 1940. Soil Sci. Florida Proc. 2: 92-103.

Carvajal, M., Matinez-Sanchez, F. & Alcaraz , C.F. 1994. J. Plant Nutr. 17: 234.

Connor, J.H. & Shacklette, H.T. 1975. Geol. Surv. prof. Washington: 574F.

Dhar, N.R. & Mukerji, S.K. 1941. Ann. Agro. 11: 87-91.

Dobrolyubski, O.K.I. 1961. Mezkvuz. Sobeshch po mikroelement. II: 156-162.

Dumon, J.C. & Ernst, W.H.O. 1988. J. Plant Physiol. 133: 203-209.

Dumon, .J.C. 1975. Bull.Inst. Geol. Bassin d'Aquitaine. 18: 13-17.

Ernst, W.H.O. 1985. Schwermetalle, Al, Ti. Bielefelder Okol. Beitr. 1: 143-158.

Fiedler, H.J. & Rosler, H.J. 1987. Spurenelemente in der Umwelt. Gustav Fischer Jena.

Geilmann, J. 1920. J. Landwirtsch. 68: 107-124 .

Gericke, S. 1940. Umschan Wiss. Tech. 44: 597-599.

Goldberg, E.D. 1965. In : Riley, J.P and G. Skirrow (eds) Chemical Oceanography. Vo1. I. Acad. Press London.

Gryzhankova, L.N. Udel'Nova. & Boichenko, A. 1975. Izv. Akad. Nauk SSSR. Ser. Biol. 75: 76-82.

Guha, M..M. & Mitchell, R.L. 1965. Plant Soil. 23: 323-338.

Guha, M.M . & Mitchell R.L. 1966. Plant Soil. 24: 90-112.

Hardy, F. & Follet-Smith, R.P. 1931. J. Agric. Sci. 31: 739-761.

Hara,T., Sonoda, Y. & Iwai, I. 1976. Soil Sci. Plant Nutr. 22: 307-315.

Heimmerich, P. & Lauterwein, J. 1973. The structure and reactivity of flavin-metal complex. In : Eichitorn, G.I (ed). Inorganic Biochemistry. Elsevier Scientific Publ, Amsterdam: 1168-1190.

Hewitt, E.J. 1983. A perspective of mineral nutrition. Essential and functional metals in Plants. In : Robb, O.A., and Pierpoint W.S. (eds). Metals and micronutrients uptake and utilization by plants. Academic Press, Lodon: 277-323.

Inman, O.L, Barchlay, G. & Hubbard, M. 1935. Plant Physiol. 10: 821-822.

Jones, R.L. & Beavers, A.H. 1963. Soil Sci. 96 : 375-379.

Joffe, J.S. & Pugh, A.J. 1934. Soil Sci. 38: 254 –257.

Kaminskaya, S.E. 1941. Titanium in the principal zonal soils of the European part of the U.S.S.R. Compt. Rend. Acad.Sci, U.S.S.R. 33: 50-53.

Karim, A. 1953. J. Soil Sci. 4(1): 56 –58.

Kiss, F., Deak , G., Feher, M., Balough, A., Szabolsci , L. & Pais, I. 1985. J. Plant Nutr. 8 : 825.

Konishi, K. & Tsuge, T. 1936. On the mineral matters of certain leguminous crops I. II. mem. Coll. Agr. Kyoto. Imp. univ. 37: 1-36.

Kusaka, S., Maekawa, M., Sono, S. 1971. Toxicities of zinc, nickel, and titanium on turnip plants. Hyago Kenesitsu Nyogo Shhikenjo Kenkyn Hokoku, Japan. 18: 71-74.

Labandeya, H. 1942. Rev. Faculted Cienc. Quim (La Plata). 17: 257-264.

Lauchli, A. & Bieleski, R.L. 1983. (eds). Encyclopedia of Plant Physiology N.S. Vol. 15. Springer-verlag, Berlin.

Lee, C. 1943. Soil Sci. 55: 343-347.

Maroti, M., Pais, I. & Bognar, J. 1984. Acta Agron. Hung. 33: 315-322.

Martinez-Sanchez, F., Nunez , M., Moros, A.A., Gimenez, J.L. & Alcaraz, C.F. 1993. J.Plant . Nutr. 16: 975.

Mckenna, C., Beneman , J.R . & Traylor, T.G. 1970. Vanadium containing nitrogenase preparations: implications for the role of molybdenum in nitrogen fixation. Biochem. Biophys. Res. Comm. 41: 1501-1508.

McBride, M.B. 1981. Forms and distribution of copper in solid and solution phases of soil. In: Loneragn, J.F., Robson, A.D. & Graham R.D. (eds). copper in soils and plants, pp 25-45 . Academic Press, Sydney.

Mitchell, R.L. 1964. Trace elements in soils. In: Bear F.E. (editor). Chemistry of the soil. Reinhold, New York, N.Y., 2nd ed.

Mrowca, J.J . 1974. Cyclopentadienyl/ group IV-B metal/carbon sulfinates and sulfonates as agents for increasing crop yield. U.S. Patent. 3: 782,917.

Nautsch-Laufer, C. 1974. Die wirking von Titan auf der stoffwechsel von *Phaseoulus vulgaris* and *Zea mays*. Diss. Univ. Munster.

Nemec, A. & Kas, V. 1923. Biochem. Z. 140: 583-590.

Nutman, P.S. 1976. Symbiotic nitrogen fixation in plants. Cambridge University Press . Cambridge .

Pais, I., Somos, A., Duda , G., Tarjanyi, F. & Nagymihaly, F. 1969. Kuserletugyi Kozlem. 62: 25-401.

Pais, I., Feher, M. Farkas, E., Szabo, Z. & Cornides, I. Commun . 1977. Soil. Sci. Plant Anal. 8: 407-410.

Pais, I. & Hodossi, S. 1975. Kert.Egyet. Kozl. 39: 227-230.

Pais, I. 1974. Objective chemical experiments to clarify the role of some nutritive elements in horticultural crops. Ph.D. Thesis 185. Hungarian Academy of Sciences. Budapest.

Pais, I. 1983. J. Plant Nutr. 6 : 3.

Pratt, P.F. 1966. Titanium. In: Chapman, H.C. (ed). Diagnostic Criteria for plants and soils, pp.478- 479. Division of Agricultural Sciences, University of carlofornia, Riverside, California .

Pellet, H. & Fribourg, C. 1905. Ann. Sci. Agron (Paris) Ser 2: 19.

Ram, N., Veloo , M. & Cottenie, A. 1983. Plant Soil. 73: 285-290.

Rao, G.G. 1934. Soil Sci. 38: 143-159.

Riley, F.J. & Chester, R. 1971. Introduction to Marine Chemistry. Academic Press, London .

Ruiz, A., Guelbenzn , M. & Lopez De Azonia, J. 1946. Ann. Fis. Quim. Madrid. 42: 657-666.

Rutskaya, S.I. 1971. Znachenie Nov. Form. Udobr. Unelichenii Skh. Svekly : 254-256.

Rutskaya, S.I. & Ruzhabskaya, S.V. 1974. 6: 469-473.

Sachs, J.V. & Knop, W. 1860. Vegetationsversuche mit Ausschlub des Bodens uber die Nahrsotoff und sonstigen Eenahrungsbedingugen von Mais, Bohnen und anderen Pflanzen. Lanwirtsch. Versuchsstat. 2: 219-268.

Salomons, W. & Forstner, U. 1988. Management of mine tailings and dredged material. Springer Verlag, Berlin.

Satyanarayana, K.V.S. & Thomas, T.K. 1962. J. Ind. Soc Sci. 10: 211-222.

Sierp, F. 1967. Gewerbliche und industrielle Abwasser. Springer Verlag Berlin.

Silverman, M.P. & Munoz, E. P. 1971. Appl. Microbiol. 22: 923-924.

Simon, L., Balazsy, S., Balogh, A. & Pais, I. 1990. Acta Microbiol. Pol. 39: 51-57.

Swaine, D.J. 1955. Commonw. Bur. Soil Sci. Tech. Commun. No 48.

Swaine, D.J. & Mitchell, R.L. 1960. J. Soil Sci. 1: 347-368.

Stiles, W. 1958. Essential micro-(trace)elements. In : Ruhland W. (ed) Encyclopedia of Plant Physiology. Vol.IV. Mineral Nutrition of plants, pp 558-614. Springer Verlag, Berlin.

Takala, K. & Olkkonen, H. 1985. Ann. Bot. 22: 299-306.

Tamura, T..M., Jackson, L. & Sherman, G.D. 1955. Soil Sci .Soc. Amer. Proc.19: 435-439.

Terlikowski, F. & Gornicki. 1933. Z. Pflanzenernahr. Dung. Bodenkde. 31: 362.

Traetta-Mosca, F. 1913. Gazz. Chim. 43: 437-440.

Tonkonozhenko, E.V. & Khlyupina, M..I. 1974. Pochvovedenie. 3: 38-45.

Troitsky, E.P. 1955. Trudy Svsesojuz. Soveshchan: 379-388.

Tsukamoto, K. 1975. Japan. Kokai. 75 18: 242 (Cl .4A2, 4E0 , 4A0) .

Van der Werff, M. 1981. Ecotoxicity of heavy metals in aquatic and terrestrial higher plants. Ph.D Thesis. Free University Amsterdam.

Wakamoto, K. 1973. Japan. Kokai. 17 85: 354 (Cl.4 F0, 4AD).

Wallace, A., Alexander, G.V., Ghaudhry, F..M. 1977. Comm. Svil. Sci. Plant Anal. 8: 751-756.

Wells, N. 1960. J. Soil Sci. 11: 409-424.

Whitehead, J. 1984. Titan. In: Merian (ed). Metalle in der Umwelt. Verteilung.

Analytik and Biologische Relevanz , pp. 585-588. Verlag Chemie, Weinheim.

Woolhouse, H.W. 1982. Toxicity and tolerance in the response of plants to metals. Encyclopedia of Plant Physiology N.S. Vol. 12C, pp. 245-300. Springer Verlag , Berlin.

Young, R. S. 1935. Mem. Coenell Agricult. Exper. Stat: 174.

CHAPTER 3

THE EFFECT OF DIFFERENT TITANIUM SPRAYS ON THE GROWTH AND CHEMICAL COMPOSITION OF *Lycopersicum esculentum* cultivar Floridade .

3.1 INTRODUCTION

In the light of the interesting results found in experiments with titanium by various researchers such as Pais (1983), it was decided to repeat some of the work under controlled conditions. The effects of titanium on the growth of tomato plants using complete and nitrogen-deficient foliar sprays and soil treatments were studied. A water soluble complex of titanium (IV) with ascorbic acid as fully characterized (see p.17) during this study, was employed to prepare the nutrient solutions.

3.2 MATERIALS AND METHODS

3.2.1 Cultivation, treatment, and harvesting of plants

Lycopersicum esculentum (cultivar Floridade) plants were obtained from a reliable nursery. Seventy two healthy plants of similar size were selected and planted in 12,5 cm plastic pots filled with washed sand. The pots were placed in an experimental greenhouse in a randomized block design (eighteen different treatments replicated four times). In addition to the normal sunlight in the greenhouse, the plants were exposed to light of four 300 W Osram, ultra-vitalux sunlight lamps installed at a height of 1.6 meter above each group of plants, at a distance of 1,25 m from each other. The supplementary lights supplied light enriched in light of $\approx$ 610 nm, and were switched on at 6 am and off at

5 pm using a time switch. The greenhouse was maintained at 20 °C with no humidity control. The plants were sprayed once a week according to the treatments listed in Table 3.1 and watered on alternating days with distilled water and Hoagland's (Epstein 1972) Nitrogen Deficient (HND) solution for a period of 5 weeks.

Table 3.1: Foliar spray treatments:

3.1 Hoagland Complete Nutrient Solution (HCN)
3.2 Hoagland Half strength Nutrient Solution (HHN)
3.3 Hoagland Nitrogen Deficient Nutrient Solution (HND)
3.4 TiO_2 in water with conc = 10 $mg.dm^{-3}$ plus HND
3.5 TiO_2 in water with conc = 100 $mg.dm^{-3}$ plus HND
3.6 TiO_2 in water with conc = 1000 $mg.dm^{-3}$ plus HND
3.7 Ti-ascorbate in water with conc = 0.01 $mg.dm^{-3}$ plus HND
3.8 Ti-ascorbate in water with conc = 0.1 $mg.dm^{-3}$ plus HND
3.9 Ti-ascorbate in water with conc = 1 $mg.dm^{-3}$ plus HND
3.10Ti-oxalate in water with conc = 0.01 $mg.dm^{-3}$ plus HND
3.11Ti-oxalate in water with conc = 0.1 $mg.dm^{-3}$ plus HND
3.12 Ti-oxalate in water with conc = 1 $mg.dm^{-3}$ plus HND
3.13 Ascorbic acid in water with conc = 0.02 $mg.dm^{-3}$ plus HND
3.14 Ascorbic acid in water with conc = 0.2 $mg.dm^{-3}$ plus HND
3.15 Ascorbic acid in water with conc = 2 $mg.dm^{-3}$ plus HND
3.16 Oxalic acid in water with conc = 0.02 $mg.dm^{-3}$ plus HND
3.17 Oxalic acid in water with conc = 0.2 $mg.dm^{-3}$ plus HND
3.18 Oxalic acid in water with conc = 2 $mg.dm^{-3}$ plus HND

Table 3.2: The composition of the Nutrient Media (Hoagland solution)

Volume of each Stock solution cm^3 required per dm^3		
	+	N
M $Ca(NO_3)$	5	-
M KNO_3	5	-
M $MgSO_4$	2	2
M KH_2PO_4	1	1
1,3% FeEDTA	1	1
Micro Nutrient	1	1
M $CaCl_2$	-	5
M KCl	-	5

+ = complete nutrient solution , N= nitrogen deficient nutrient solution

The Hoagland solutions were prepared in 20 dm^3 plastic containers. Increases in plant height were measured, and the number of leaves counted, on a weekly basis for a period of 5 weeks. The plants were then harvested. The roots were excised, rinsed in distilled deionised water to remove sand particles and mineral nutrients on the surface, and oven dried to constant mass at 70°C. The shoots were measured, the number of leaves

counted, the fresh mass determined and the material was then dried to constant mass in an oven at 70°C.

3.2.2 **Preparation of PIXE samples**

Leaves and stems of plants, treated with titanium ascorbate solution with the highest concentration of titanium, were first cut into quarter pieces and then into cross sections with a glass blade and transferred to liquid isopentane, cooled by liquid nitrogen, and immediately freeze-dried. The samples were mounted on separate target frames and later carbon coated. Surface sections were made of the leaf tip and cross sections of the stem which was scoured as a whole, and finally at the stem surface. Elemental distribution maps were obtained using the elemental imaging system (Dynamic analysis) of the National Accelerator Center's nuclear microprobe. This instrument is suitable for measuring elemental distribution in plant tissues. Elemental scans were conducted using size ½Ca 4 x 4 μm^2 beam and a current between 200-600 pA. Two complementary techniques, proton- induced X-ray emission (PIXE) and back-scattering were used simultaneously . All reported elemental maps were obtained using PIXE , and maps for Ca , Mn and Ti were obtained.

3.2.3 **Plant analysis**

The dried plant samples were ground in a Wiley Intermediate Mill (60 mesh) and then digested in a sulfuric acid-peroxide mixture (Allen 1989). The digests were taken up in 20 cm^3 distilled water, and then made up to 100 cm^3 in volumetric flasks by addition of distilled water.

The following analyses were performed: Total nitrogen was determined by a micro-Kjeldahl method (Allen 1989), total titanium was determined using (Inductive couple plasma) and the distribution of titanium in plants was determined using PIXE (Proton induced X-ray emission).

3.2.3.1 **The micro-Kjeldahl method**

The ground plant material was digested in the digestive tubes using sulfuric acid-peroxide mixture. The tubes were then placed in a temperature controlled digestion block, which was made of aluminum with 5 x 4 holes per block.

3.2.3.2 **Inductively coupled plasma (ICP)**

Inductively Coupled Plasma became commercially available in 1974 with a promising future. The inductively coupled argon plasma derives its sustaining powers by induction from a high-frequency magnetic field. The ICP discharge is caused by the effect of a radio frequency field on a flowing gas. Argon gas flows upwards through a quartz-tube, wrapped around a copper coil or solenoid. Argon is not a conductor at room temperature but can be made electrically conducting by heating it. To initiate the ICP discharge, a discharge from the Tesla coil or a pilot spark is applied to the flowing argon (Christian 1980). The argon is quickly heated with a stable plasma being produced having a core temperature of about 9000-10000 °K. This type of excitation source has a number of advantages, i.e. the sample can be introduced in solution form through a spray chamber nebulizer, as in flame emission spectrometry. This makes sample handling much easier than in conventional emission spectroscopy. Sample introduction into the plasma sources can be categorised in three ways :

(i) There is a maximum 0.01μm drop , for introducing the sample into the atomiser .

(ii) The rate of solvent flow must fall within a specific range of values .

(iii) The analytical precision of an ICP system is strongly dependent on the control of these parameters .

This makes quantitative and sample handling much easier than in conventional emission spectroscopy. The introduction of liquid samples is equally important in ICP. In all cases the analyses can be only as good as the sample condition. The time required for the washout of the analyte from the ICP spray chambers is longer than that required for the atomic absorption spectrometer (AAS). This is due to the substantially lower gas and liquid flow rates (approximately 1 $dm^3 . min^{-1}$ and 1 $cm^3 . min^{-1}$ respectively) compared to (8 $dm^3 . min^{-1}$ and 6-8 $cm^3 . min^{-1}$) of AAS. The temperature of the plasma eliminates much chemical interference present in a flame, and most elements are readily excited. For many elements, ion line emission from ICP sources is considerably more intense than neutral atom line emission. The detection limits are very competitive with those using atomic absorption or flame emission spectrometry.

3.2.3.3 **Proton induced X- ray emission**

The nuclear microprobe is a multipurpose analytical instrument making use of different types of high-energy ions generated by accelerators, protons, deuterons, alpha particles or other ions. The following techniques are usually applied: proton induced X-ray emission (PIXE), particle back-scattering (BS), scanning transmission ion microscopy (STIM) and nuclear reaction analysis (NRA). Proton induced gamma-ray emission (PIGE) is a special kind of the last technique in which emitted gamma rays are detected. The size of the focused proton beam can be down to 0.5 $x10^{-6}$ m in diameter or even

less. The beam can be scanned over areas of up to 2.5 x 2.5 mm (Watt & Grime 1987, Tapper & Malmqvist 1991; Przybylowicz 1994).

(i) **Proton induced X-ray emission**

The analysed sample emits X-rays, which are excited by protons and measured with a Si (Li) or HPGe (High Purity Germanium) detector. This technique is similar to the EDX (Energy Dispersive X-ray Analysis), making use of a scanning microprobe, but has some differences which make proton excitation far superior to electron excitation. Both PIXE and EDX give information on the elemental composition in the form of two-dimensional elemental maps or point analyses. PIXE is capable of detecting elements at levels of 1ppm ($1mg.kg^{-1}$).

(ii) **Particle Back-scattering (BS)**

Information carried on the particles scattered from a sample at an angle of almost 180°C can be used to characterize sample composition and its real density. For example, this technique can be used to make maps of the light elements (C, O and N). This method allows the use of maps of light elements and the sample density. Back scattering can be used simultaneously with PIXE.

3.3 **Statistical Analysis**

A statistical analysis of variance was performed on the results obtained for shoots and roots using the SAS v6.12 computer package for statistical analysis software (SAS, 1990). The Shapiro-Wilk test was done to test for non-normality (Tables 3.3.1, 3.3.2 and Tables 3.4.1 and 3.4.2). The results in the tables above show that the lack of normality was due to kurtosis and not skewness and thus not important for our purpose (Shapiro & Wilk 1965). Least significant differences between treatments were calculated at 5% level to compare the treatment means.

Table 3.3.1 Analyses of varience (ANOVA) of growth parameters including all treatments

		Height cm		Leaf number		Shoot fresh mass (g)		Shoot dry mass (g)		Root fresh mass (g)		Root dry mass (g)	
Source	df	MS	P	MS	P	MS	P	MS	P	MS	P	MS	P
Block	1	30.407	0.05	1025.569	<0.01	72.178	0.153	3.151	0.051	51.262	0.214	0.15	0.096
Treat	17	591.723	<0.01	9948.68	<0.01	320.774	<0.01	15.404	<0.01	167.482	<0.01	0.409	<0.01
Error	51	10.927		183.049		39.501		1.143		33.203		0.067	
Corrected Total	71												
Non-Normality		p = 0.97		p = 0.79		p = 0.58		p = 0.70		p = 0.57		p = 0.72	

Table 3.3.2 Analyses of Varience (ANOVA) of element concentrations including all treatments

		Shoot nitrogen (mg/g)		Root nitrogen (mg/g)		Shoot Titanium (mg/g)		Root Titanium (mg/g)	
Source	df	MS	P	MS	P	MS	P	MS	P
Block	1	78.252	0.023	450.387	0.261	340568.7	0.03	76059.4	<0.01
Treatment	17	54.658	<0.01	400.414	0.282	641784.4	<0.01	18806.3	0.128
Error	51	22.731		327.683		104774.3		102.44	
Corrected Total	71								
Non-Normality		p = 0.92		p = 0.83		p = 0.95		p = 0.96	

Table 3.4.1 Analyses of varience (ANOVA) 0f growth parameters excluding controls

		Height cm		Leaf number		Shoot fresh mass (g)		Shoot dry mass (g)		Root fresh mass (g)		Root dry mass (g)	
Source	df	MS	P	MS	P	MS	P	MS	P	MS	P	MS	P
Block	1	24.048	0.0155	430.8	<.01	8.221	<0.01	0.27	<0.01	2.838	0.021	0.012	0.176
Treatment	14	5.508	0.5754	29.03	0.704	0.514	0.904	0.018	0.91	0.699	0.577	0.012	0.804
Error	42	6.194		38.24		0.975		0.0357		0.789		0.0069	
Corrected Total	59												
Non-Normality		p = 0.54		p = 0.909		p = 0.839		p = 0.4474		p = 0.6368			

Table 3.4.2 Analyses of Varience (ANOVA) of element concentrations excluding controls

		Shoot nitrogen (mg/g)		Root nitrogen (mg/g)		Shoot Titanium (mg/g)		Root Titanium (mg/g)	
Source	df	MS	P	MS	P	MS	P	MS	P
Block	1	82.796	0.035	696.072	<0.01	340568.7	<0.01	76059.4	<0.01
Treatment	14	46.701	0.077	188.878	0.285	641784.4	0.128	18806.31	0.128
Error	42	26.385		12.351		104774.3		10493.87	
Corrected Total	59								
Non-Normality		p = 0.92		p = 0.90		p = 0.95		p = 0.96	

3.4 RESULTS AND DISCUSSION

3.4.1 *Effects on growth*

Table 3.5.1 shows the effect of the different treatments on the growth of tomato plants. Hoagland Complete nutrient solution (HCN) (treatment 3.1, table 3.1) plants were highly significantly taller, had more leaves, greater shoot fresh and dry mass and larger root fresh and dry mass. Plants grown on Hoagland half strength nutrient solution (HHN) (treatment 3.2, table 3.1), were also significantly taller than plants grown on all other treatments, except for those grown in Hoagland Complete nutrient solution (HCN) (treatment 3.1, table 3.1). They were significantly shorter than those plants. A similar observation was made for the leaf number and shoot dry mass. The shoot fresh mass for Hoagland Complete nutrient solution (HCN) (treatment 3.1, table 3.1) plants was significant higher than plants grown in other treatments. Plants grown on Hoagland nitrogen deficient nutrient solution (HND) (treatment 3.3, table 3.1) did not differ significantly from plants grown on the lower titanium ascorbate (treatment 3.7, table 3.1) concentrations and the higher titanium oxalate concentrations (treatment 3.12, table 3.1). Again with the root dry mass, the only difference from the situation with the height, leaf number and shoot dry mass was that Hoagland half strength nutrient solution (HHN) (treatment 3.2, table 3.1) treated plants did not differ significantly from plants given titanium ascorbate at 0.1mg.dm^{-3} (treatment 3.7, table 3.1).

Thus in general the growth promotion with Hoagland Complete nutrient solution (HCN) treatment 3.1, (table 3.1) and Hoagland half strength nutrient solution (HHN) treatment 3.2, (table 3.1), plants supplied with nitrogen, was greater than for all other treatments. This was to be expected as nitrogen is an essential element and a macroelement and plays a very important role in plants

It is very interesting to see that the higher nitrogen supply for treatment 3.1, (table 3.1) and treatment 3.2, (table 3.1) nutrient solution did not result in the nitrogen concentrations of these plants being greater (Table 3.5.2) but rather translated into better growth. Moreover, most of the plants given titanium and oxalate or ascorbate treatments resulted in greater shoot nitrogen concentrations than were found in the nitrogen deficient treatment 3.3, (table 3.1). Only plants treated with high titanium ascorbate nutrient solution (treatment 3.9, table 3.1) had higher nitrogen levels in the roots than treatment 3.2, (table 3.1) (among plants not supplied with nitrogen). The plants with this treatment had higher shoot nitrogen concentrations (some 18 times higher).

The only difference in the shoot and root titanium concentrations was not surprisingly in those plants treated with titanium dioxide (treatment 3.6, table 3.1) where the titanium concentration was found to be significantly higher (Table 3.5.2).

Tables 3.6.1 and 3.6.2 show the results of the analysis excluding the extreme values of the controls. The shoot fresh and dry mass show no differences. There are differences in height, leaf number, root fresh mass and root dry mass but there appears to be no constant pattern (Table 3.6.1). Titanium ascorbate (treatment 3.9, table 3.1) resulted in a higher shoot and root nitrogen concentration than most other treatments, but not the titanium dioxide (treatment 3.6, table 3.1).

(Table 3.6.2). Titanium dioxide (treatment 3.5, table 3.1) in higher shoot and root titanium concentrations (Table 3.6.2).

In table 3.7 and 3.8 specific contrasts are tested. Once again the control plants clearly grew better than the other plants, and had significantly lower nitrogen concentrations (Table 3.7). The titanium ascorbate treated plants showed significantly higher shoot nitrogen than did the ascorbate treated plants (Table 3.7). Mozafar & Oetly (1993) found ascorbate did not increase plant growth, titanium may thus have resulted in a higher shoot nitrogen content. Carvajal *et al* (1994) also found that the effects on growth were due to titanium but not due to the ascorbate.

The controls were excluded from the comparison in Table 3.8. Once again plants supplied with titanium ascorbate gave a significantly higher shoot nitrogen concentration

than plants getting ascorbate did. The organic titanium complexes resulted in a higher root dry mass than the organic anions did, and specifically titanium ascorbate stimulated root dry mass more than ascorbate did. This results corresponds to the findings of Carvajal *et al* (1995) who observed that Ti-concentration was higher in the roots than in leaves when applied *via* roots. The titanium ascorbate treatment however resulted in a significantly lower root nitrogen concentration than in plants grown in titanium oxide treatments.

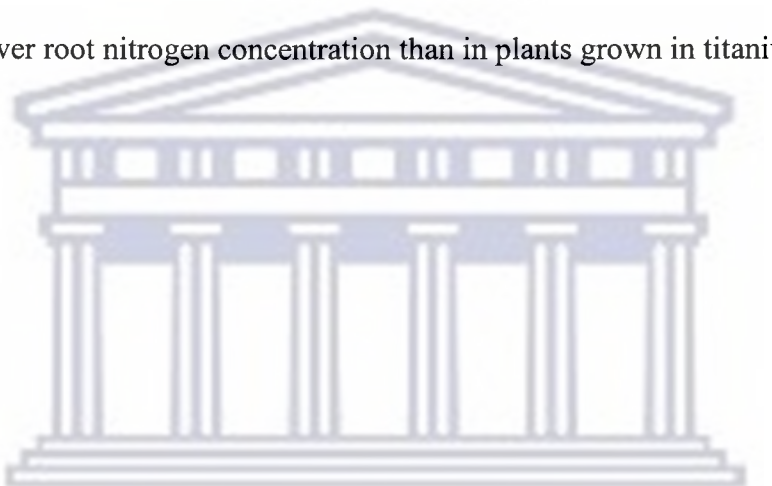


Table 3.5.2 **The effect of various titanium and nitrogen spray treatments on nitrogen and titanium content of tomato plants**

Treatments	Shoot Nitrogen		Root Nitrogen		Shoot Titanium		Root Titanium	
	mg/g	grouping	mg/g	grouping	mg/g	grouping	mg/g	grouping
3.1 Control 1 (HCN)	8.50	b	27.21	a b c	.	.	.	
3.2 Control 2 (HHN)	7.55	b c	42.86	a	.	.	.	
3.3 Control 3 (HND)	0.99	c	2.00	c	.	.	.	
3.4 TiO_2 (10mg.dm^{-3})	6.59	b c	8.68	b c	246.10	b	135.69	b
3.5 TiO_2 (100mg.dm^{-3})	8.44	b	15.79	b c	502.30	b	107.50	b
3.6 TiO_2 (1000mg.dm^{-3})	12.36	a b	18.43	a b c	1384.50*	a	292.41*	a
3.7 Tiasc(0.01mg.dm^{-3})	6.00	b c	6.36	b c	96.70	b	99.33	b
3.8 Tiasc (0.1mg.dm^{-3})	9.03	b	15.56	b c	157.50	b	99.87	b
3.9 Tiasc (1mg.dm^{-3})	18.67	a	28.86	a b	237.80	b	71.63	b
3.10 Tiox (0.01mg.dm^{-3})	10.21	b	6.15	b c	249.60	b	124.29	b
3.11 Tiox (0.1mg.dm^{-3})	12.15	a b	6.86	b c	106.20	b	76.12	b
3.12 Tiox (1mg.dm^{-3})	7.96	b	10.24	b c	384.90	b	71.31	b
3.13 Asc (0.02mg.dm^{-3})	6.27	b c	14.73	b c	.	.	.	
3.14 Asc (0.2mg.dm^{-3})	6.76	b c	23.66	a b c	.	.	.	
3.15 Asc (2mg.dm^{-3})	8.27	b	17.32	a b c	.	.	.	
3.16 Ox (0.02mg.dm^{-3})	12.20	a b	11.02	b c	.	.	.	
3.17 Ox (0.2mg.dm^{-3})	6.14	b c	23.70	a b c	.	.	.	
3.18 Ox (2mg.dm^{-3})	10.64	b	13.07	b c	.	.	.	
LSD (p=0.05)	6.77		25.70		472.39		149.50	

Asc = ascorbate
Ox = oxalate

Table 3.6.1 **The effect of variours titanium spray treatments on the growth tomato plants**

Treatments	Height		Leaf		Shoot fresh		Shoot dry		Root fresh		Root dry	
	cm	grouping	number	grouping	mass (g)	grouping	mass (g)	grouping	mass (g)	grouping	mass (g)	grouping
3.4 TiO_2 (10mgdm^{-3})	6.9	a b	31.50	a b	1.54	a	0.38	a	2.32	a b	0.14	b
3.5 TiO_2 (100mg.dm^{-3})	7.4	a b	28.75	a b	1.59	a	0.27	a	1.72	a b	0.12	b
3.6 TiO_2 (1000mg.dm^{-3})	7.8	a b	25.00	b	1.74	a	0.27	a	1.30	b	0.11	b
3.7 Tiasc(0.01mgdm^{-3})	5.9	a b	32.25	a b	2.46	a	0.38	a	2.49	a b	0.19	b
3.8 Tiasc (0.1mg.dm^{-3})	5.7	a b	34.25	a	2.55	a	0.47	a	2.66	a	0.33	a
3.9 Tiasc (1mg.dm^{-3})	8.6	a	30.00	a b	2.02	a	0.41	a	1.61	a b	0.22	a b
3.10 Tiox (0.01mg.dm^{-3})	4.4	b	29.25	a b	1.96	a	0.35	a	2.01	a b	0.16	b
3.11 Tiox (0.1mg.dm^{-3})	6.0	a b	32.50	a b	2.25	a	0.40	a	2.32	a b	0.17	b
3.12 Tiox (1mg.dm^{-3})	7.1	a b	34.00	a	2.66	a	0.42	a	2.84	a	0.22	a b
3.13 Asc (0.02mg.dm^{-3})	5.9	a b	30.50	a b	1.94	a	0.30	a	1.94	a b	0.12	b
3.14 Asc (0.2mg.dm^{-3})	5.7	a b	30.50	a b	1.61	a	0.24	a	1.81	a b	0.14	b
3.15 Asc (2mg.dm^{-3})	4.8	b	29.25	a b	1.86	a	0.30	a	1.89	a b	0.14	b
3.16 Ox (0.02mg.dm^{-3})	5.4	a b	30.25	a b	2.18	a	0.34	a	2.40	a b	0.16	b
3.17 Ox (0.2mg.dm^{-3})	6.3	a b	29.00	a b	2.25	a	0.38	a	1.86	a b	0.15	b
3.18 Ox (2mg.dm^{-3})	4.9	b	25.00	b	1.72	a	0.28	a	2.00	a b	0.14	b
LSD (p=0.05)	3.6		8.82		1.41		0.27		1.27		0.12	

Asc = ascorbate
ox = oxalate

Table 3.6.2 **The effect of variours titanium spray treatments on the nitrogen and titanium content of tomato plants**

Treatments	Shoot Nitrogen		Root Nitrogen		Shoot Titanium		Root Titanium	
	mg/g	grouping	mg/g	grouping	mg/g	grouping	mg/g	grouping
3.4 TiO_2 (10mgdm^{-3})	6.59	b	8.68	b	246.10	b	135.69	b
3.5 TiO_2 (100mg.dm^{-3})	8.44	b	15.79	a b	502.30	b	107.50	b
3.6 TiO_2 (1000mg.dm^{-3})	12.36	a b	18.43	a b	1384.50*	a	292.41*	a
3.7 Tiasc(0.01mgdm^{-3})	6.00	b	6.36	b	96.70	b	99.33	b
3.8 Tiasc (0.1mg.dm^{-3})	9.03	b	15.56	a b	157.50	b	99.87	b
3.9 Tiasc (1mg.dm^{-3})	18.67	a	28.86	a	237.80	b	71.63	b
3.10 Tiox (0.01mg.dm^{-3})	10.21	b	6.15	b	249.60	b	124.29	b
3.11 Tiox (0.1mg.dm^{-3})	12.15	a b	6.86	b	106.20	b	76.12	b
3.12 Tiox (1mg.dm^{-3})	7.96	b	10.24	b	384.90	b	71.31	b
3.13 Asc (0.02mg.dm^{-3})	6.27	b	14.73	a b	.	.	.	.
3.14 Asc (0.2mg.dm^{-3})	6.76	b	23.66	a b	.	.	.	.
3.15 Asc (2mg.dm^{-3})	8.27	b	17.32	a b	.	.	.	.
3.16 Ox (0.02mg.dm^{-3})	12.20	a b	11.02	b	.	.	.	.
3.17 Ox (0.2mg.dm^{-3})	6.14	b	23.70	a b	.	.	.	.
3.18 Ox (2mg.dm^{-3})	10.64	b	13.07	a b	.	.	.	.
LSD (p=0.05)	7.33	b	17.63	b	472.39		149.50	

Asc = ascorbate
ox = oxalate

Table 3.7 Contrast estimates between various treatments by means of meaningful comparison (including controls)

X vs Y	height	Leaf	Shoot fresh	Shoot dry	Root fresh	Root dry	Shoot	Root
	cm	number	mass (g)	mass (g)	mass (g)	mass (g)	nitrogen (mg/g)	nitrogen (mg/g)
Control vs Rest	370.3*	1379.3*	232.62*	49.70*	169.75*	8.59*	-56.48*	137.38
TiO vs Rest	17.8	-25.75	-5.97	-0.61	-4.45	-0.62	-4.7525.	-5.93
Tiasc ox vs asc ox	4.8	17.75	2.33	0.58	2.03	0.44	13.73	-29.47
Tiasc vs asc	3.8	6.25	1.62	0.42	1.12	0.34	12.39*	-4.92
Tiox vs ox	0.9	11.50	0.71	0.16	0.91	0.10	1.34	-24.55
Tiasc vs Tiox	1.1	5.50	1.45	0.32	1.54	0.16	9.01	-32.46
asc vs ox	-0.2	6.00	-0.74	-0.17	-0.63	0.06	-0.77	7.91
MSE	10.93	183.05	39.50	1.14	33.20	0.07	22.73	327.68
df_M	51	51	51	51	51	51	51	51

* significant at 5% level

Asc = ascorbate

Ox = oxalate

TiO = titanium dioxide

Table 3.8 Contrast estimates between various treatments by means of meaningful comparisons (excluding controls)

X vs Y	height	Leaf	Shoot fresh	Shoot dry	Root fresh	Root dry	Shoot Nitrogen	Root Nitrogen
	cm	number	mass (g)	mass (g)	mass (g)	mass (g)	mg/g	mg/g
TiO vs Rest	17.8	-25.75	-5.97	-0.61	-4.45	-0.62	-4.75	-5.93
Tiasc ox vs asc ox	4.8	17.75	2.33	0.58	2.03	0.44*	13.73	-29.47
Tiasc vs asc	3.8	6.25	1.62	0.42	1.12	0.34*	12.39*	-4.92
Tiox vs ox	0.9	11.50	0.71	0.16	0.91	0.10	1.34	-24.55
Tiasc vs Tiox	1.1	5.50	1.45	0.32	1.54	0.16	9.01	-32.46*
asc vs ox	-0.2	6.00	-0.74	-0.17	-0.63	-0.06	-7.67	7.91
MSE	6.19	38.24	0.98	0.04	0.79	0.01	26.39	152.56
df_M	42	42	42	42	42	42	42	42

* significant at 5% level

Asc = ascorbate

ox = oxalate

TiO = titanium dioxide

3.4.2 **Visual observations**

Three weeks after the start of the treatments, the plants grown in Hoagland nitrogen deficient nutrient solution (HND) (treatment 3.3, table3.1) were pale to brownish, which was a sign of nitrogen deficiency. Plants treated with titanium compounds, showed deficiency only at a later stage, unlike plants of treatment 3.3, (table3.1) which already showed signs of a lack of nitrogen after only 2 weeks, and as a result most of its leaflets started falling before those of plants grown in all other treatments. Plants grown in Hoagland Complete Nutrient solution (HCN) (treatment 3.1, table 3.1) and Hoagland Half Strength Nutrient solution (HHN) (treatment 3.2, table 3.1) were healthier and stronger than the rest of plants given other treatments.

3.4.3 **Titanium Content of plants using PIXE**

The total X-ray map in figure 3.1 below shows the outline image of the leaf surface including some leaf veins. Figure 3.2 is an example of the surface elemental distribution of the same area of *Lycopersicum esculentum* (cultivar Floridade) leaf as Figure 3.1 for Ca , Mn and Ti .

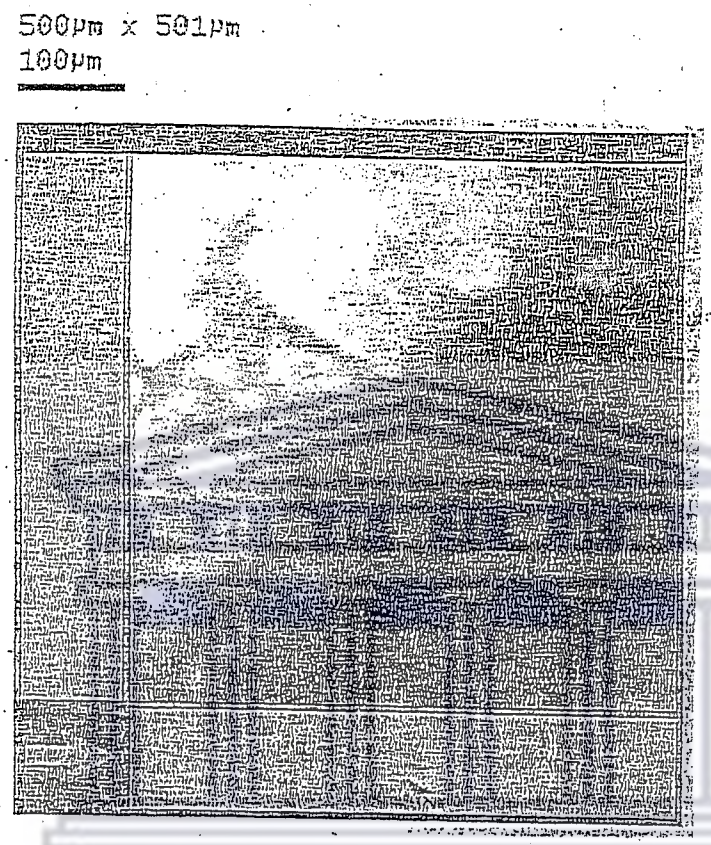


Figure 3.1 : The total X-ray image of a portion of the leaf surface of *Lycopersicum esculentum* (cultivar Floridade)

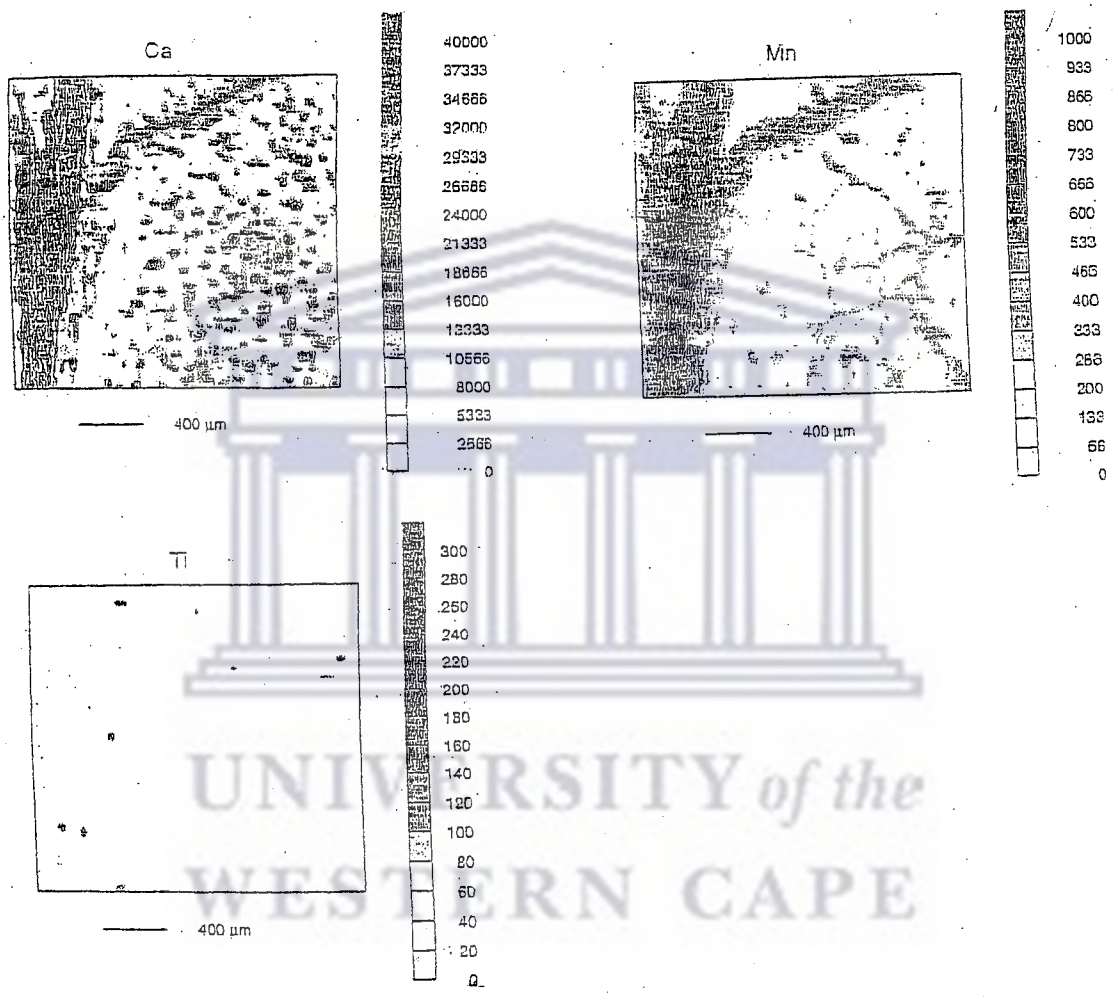


Figure 3.2: Elemental maps for Ca , Mn, and Ti on the leaf surface of *Lycopersicum esculentum* (cultivar Floridade). Scan size (800µm x 800µ m) analysed using 3MeV protons.

It is note noting that calcium occurs at a much higher concentration than the other metals. Calcium is more evenly distributed across the image than the other elements. Manganese is concentrated in the veins to a greater extent than Ca is. For Ti there are traces of titanium at the leaf vein and others on the leaf surfaces suggesting that titanium is somehow absorbed into the leaf.

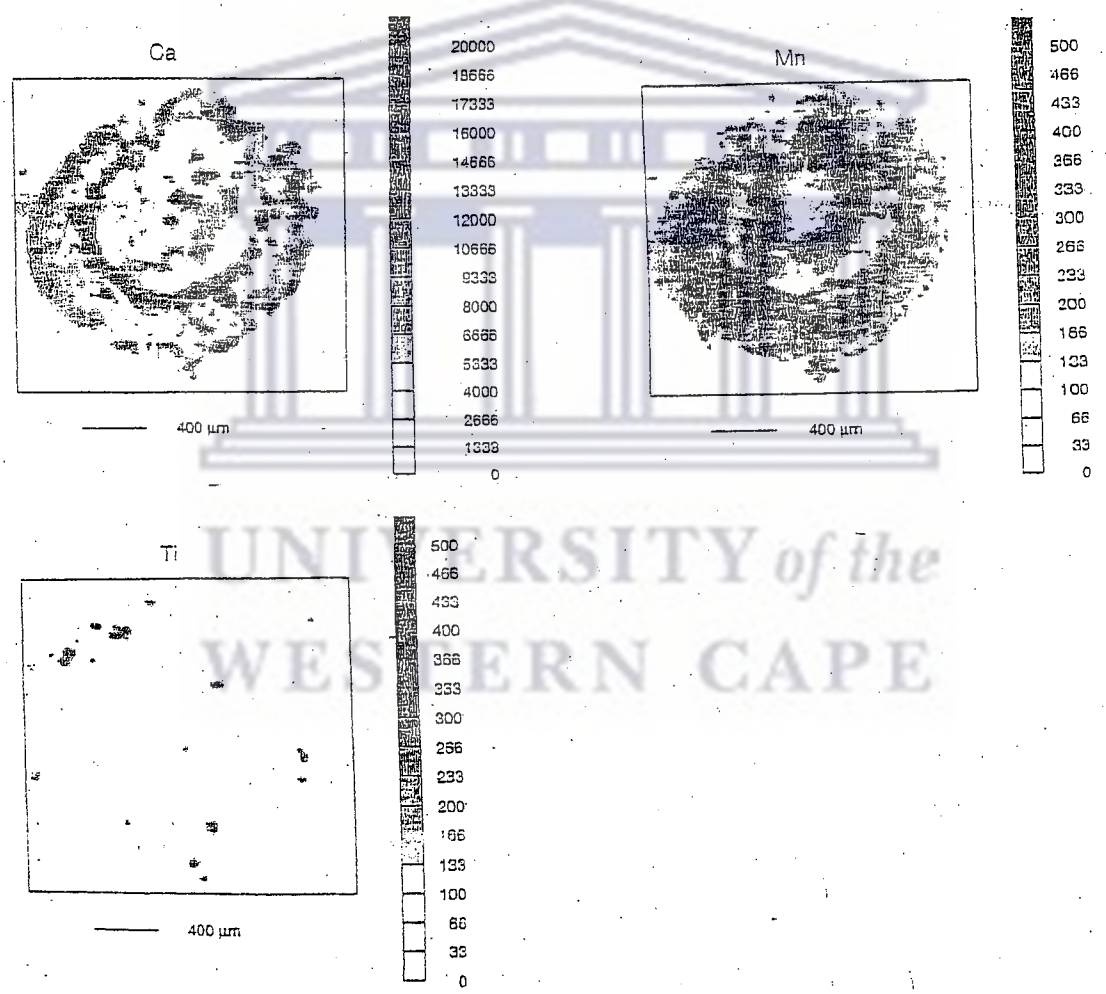


Figure 3.3 : Elemental maps showing the distribution of Ca , Mn, and Ti in the stem section of *Lycopersicum esculentum* cultivar Floridade. Scan size (400µm x 400µm) analysed using 3MeV protons .

The results in figure 3.3 show the difference in elemental distribution within the structure of the stem section, once again Ca is present at much higher concentrations than Mn and Ti. Calcium and Mn elemental maps show a clear differentiation between vascular tissues. The vascular tissue of the central cylinder exhibited a higher Mn concentrations as compared to Ca and Ti. Manganese was more concentrated in the outer regions of the central cylinder. Also, the distribution of Mn was more just below the inner epidermis than in the epidermis which was the case with Ca. Titanium was not evenly distributed in the cell. There were traces of titanium on the surface, which may be due to spraying on the outside. But there were traces of titanium in the central cylinder also, but no explanation for this could be found as titanium is regarded as an immobile element.

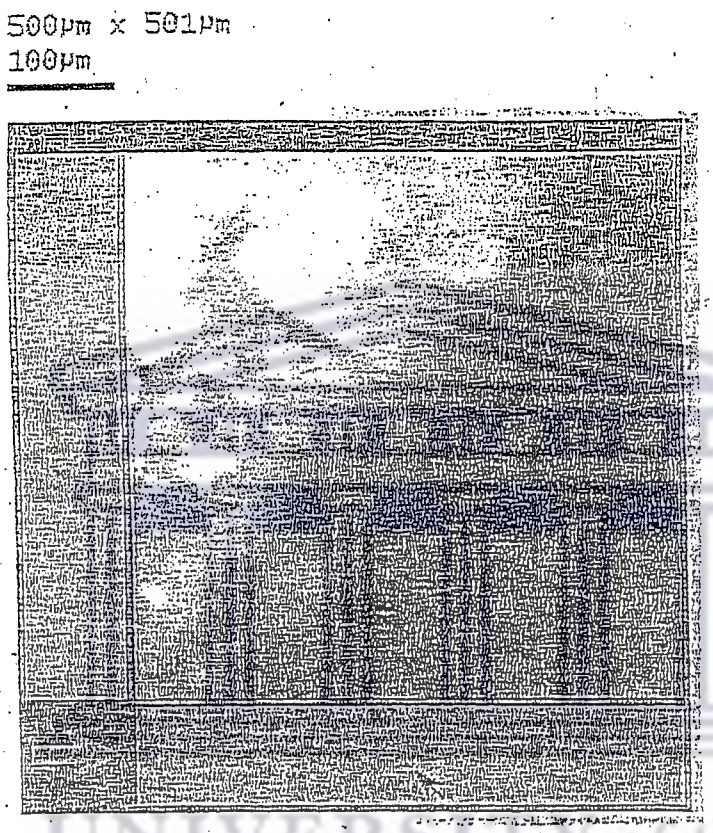


Figure 3.4 : The X-ray image of a surface portion of the stem cross section of *Lycopersicum esculentum* cultivar Floridade

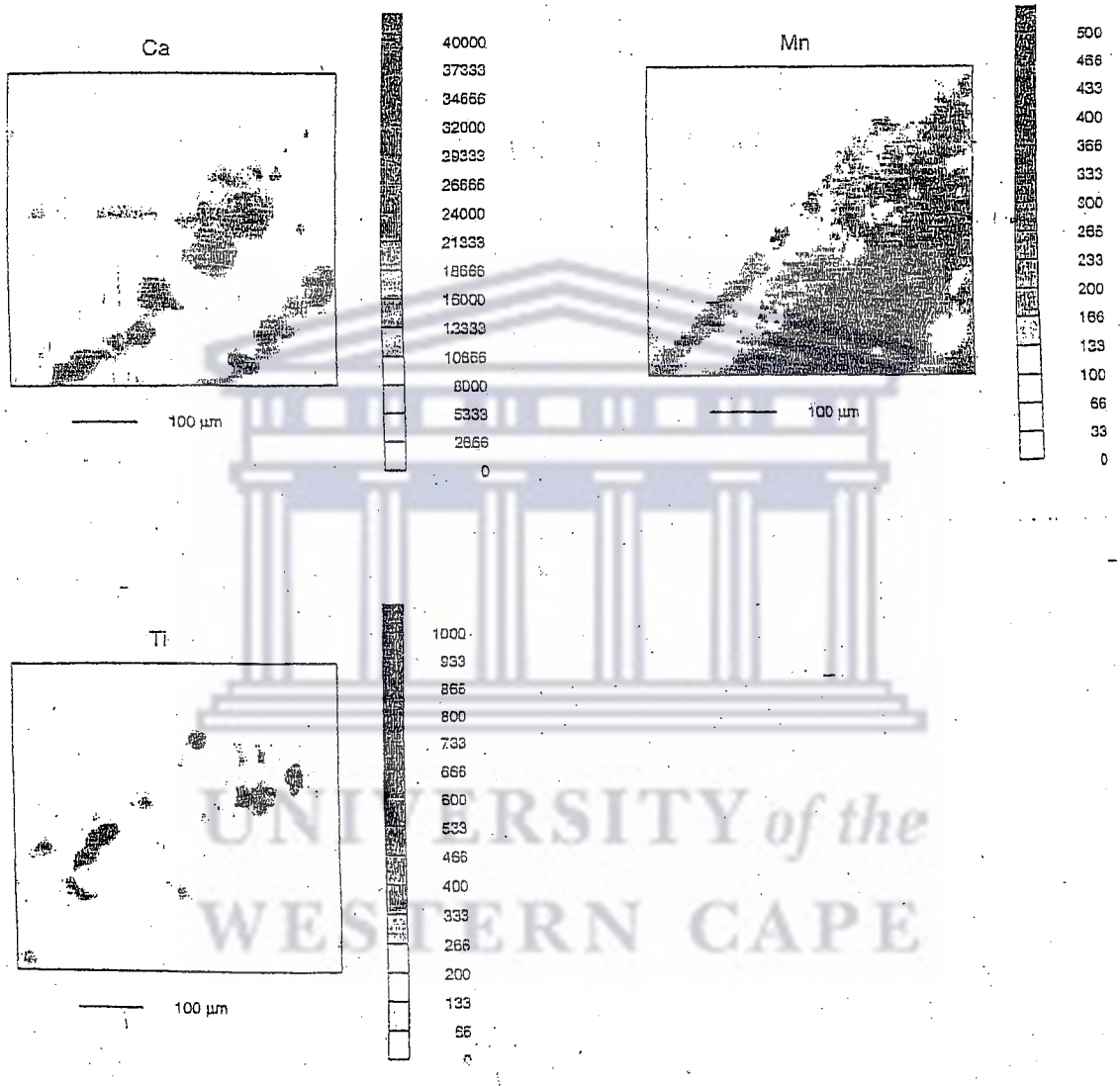


Figure 3.5: Elmental distribution of Ca, Mn, and Ti on the stem cross section of of *Lycopersicum esculentum* cultivar Floridade

Figure 3.4 shows the total X-ray image of the stem surface cross section including the vascular tissues. The results in figure 3.5 show the differences in the distribution of the elements within the stem cross section. The macro-element Ca has a higher concentration again as expected. Manganese is distributed more in the deeper tissues than on the outer epidermis, with Ca concentrated more in the epidermis and also on the outer part of the vascular region. There are traces of titanium in the outer part of the vascular region and also on the outer epidermis, the latter may be as a result of spraying the surface of the plant.

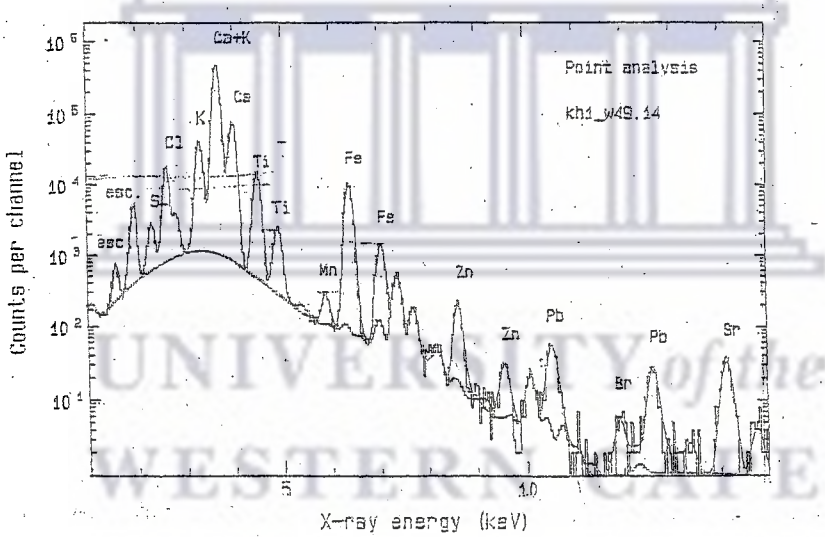


Figure 3.6 : The total X-ray energy spectrum of the elements for of *Lycopersicum esculentum* cultivar Floridade.

Figure 3.6 represents the total X-ray energy spectrum of the elements of *Lycopersicum esculentum* cultivar Floridade, which shows relatively high amounts of titanium metal as compared to lower amounts of Mn concentrations and other trace elements which are regarded as essential to plant growth.

3.5 **Conclusions**

In general, plants of treatments 3.1 and 3.2, (table 3.1) (Complete and Half strength Hoagland nutrient solution) did better than plants of all the other treatments, and plants of treatment 3.3, (table 3.1) Hoagland Nitrogen-deficient solution(HND) did worse than plants given all the other treatments. The PIXE maps for titanium show that there are some traces of titanium metal inside the cell which might be due to the absorption of titanium by plants: alternatively it may be an artifact due to sectioning.

3.5 **References**

Allen, S.E. 1989. Chemical Analysis of Ecological Materials. Blackwell Scientific Publication. London.

Carvajal, M., & Alcaraz, C.F. 1995. Phytochemistry. 39: 977-980.

Carvajal, M., Martinez-Sanchez, F. & Alcaraz, C.F . 1994. J. Plant Nutr. 17: 234.

Christian, I. 1980. Analytical Chemistry, 3rd Edition. John Wiley & Sons. New York :30.

Epstein, E. 1972. Mineral Nutrition of Plants: Principles and Perspective. John Wiley. New York.

Mozafar, A. & Oetly, J.J. 1993. J. Plant Physiol. 141: 316.

Pais, I. 1983. J. Plant Nutr. 6: 3.

Przybylowicz, W.J. 1994. The nuclear microprobe. A multi-purpose analytical instrument, unpublished. National Accelerator Centre Report

SAS.1990. SAS/STAT User's Guide, Version 6, Fourth Edition, Volume 2. SAS Institute Inc, SAS Campus Drive, Cary, NC 27513.

Shapiro, S.S & Wilk, M.B. 1965. An Analysis of Variance Test for Normality (complete samples). Biometrika. 52: 591-611.

Tapper, U.A.S. & Malmqvist, K.G. 1991. Analytical Chemistry. 63: 715A-725A.

Watt, F. & Grime, G.W. 1987. (eds). Principles and Applications of High Energy Ion Microbeams. Adam Hilger. Bristol.

CHAPTER 4

THE EFFECT OF DIFFFERENT TITANIUM SOIL TREATMENTS ON THE GROWTH AND CHEMICAL COMPOSITION OF *Lycopersicum esculentum* cultivar Floridade.

4.1 INTRODUCTION

The presence of titanium in the soil

Titanium is one of the most frequently encountered elements in the Earth's crust (Dumon & Ernst 1988), consequently, most soil samples that have been studied up to now contain 0.3-0.4 percent titanium. There have been no reports on titanium toxicity to plants. On the other hand the available titanium content in most soils is very low, less than 1ppm. In the soil, titanium is generally bound to silicates, so it is practically unavailable to plants. In this study the sand used for plant growth had no traces of titanium as this was tested in the previous year in similar experiments (Cerff 1995).

4.2 MATERIALS AND METHODS

4.2.1 Cultivation, treatment, and harvesting of plants

Lycopersicum esculentum (cultivar Floridade) tomato plants were obtained from a reliable nursery. Seventy two healthy plants of similar size and appearance were selected and planted in 12,5 cm plastic pots filled with acid washed sand. The pots were placed in an experimental greenhouse in a randomized block design (eighteen different treatments replicated four times). In addition to the normal sunlight available in the greenhouse, the plants were also exposed to four 300W Osram, ultra-vitalux sunlight lamps placed at a height of 1.6 meter, above each group of plants. The supplementary

lights supplied light enriched at 610 nm, and were switched on at 6 am and off at 5 pm using a time switch. The greenhouse was maintained at 20°C, with no control of the humidity. The plants were given treatments *via* the soil once a week according to the treatments listed in Table 4.1. and watered on alternating days with distilled water and Hoagland Nitrogen Deficient (HND) solution for a period of 5 weeks.

Table 4.1: Soil treatments

4.1 Hoagland Complete Nutrient Solution (HCN)
4.2 Hoagland Half Nutrient Solution (HHN)
4.3 Hagland Nitrogen Deficient Nutrient Solution (HND)
4.4 TiO_2 in water with conc = 10 $mg.dm^{-3}$ plus HND
4.5 TiO_2 in water with conc = 100 $mg.dm^{-3}$ plus HND
4.6 TiO_2 in water with conc = 1000 $mg.dm^{-3}$ plus HND
4.7 Ti-ascorbate in water with conc = 0.01 $mg.dm^{-3}$plus HND
4.8 Ti-ascorbate in water with conc = 0.1 $mg.dm^{-3}$ plus HND
4.9 Ti-ascorbate in water with conc = 1 $mg.dm^{-3}$ plus HND
4.10 Ti-oxalate in water with conc = 0.01 $mg.dm^{-3}$ plus HND
4.11 Ti-oxalate in water with conc = 0.1 $mg.dm^{-3}$ plus HND
4.12 Ti-oxalate in water with conc = 1 $mg.dm^{-3}$ plus HND
4.13 Ascorbic acid in water with conc = 0.02 $mg.dm^{-3}$ plus HND
4.14 Ascorbic acid in water with conc = 0.2 $mg.dm^{-3}$ plus HND
4..15Ascorbic acid in water with conc = 2 $mg.dm^{-3}$ plus HND
4.16 Oxalic acid in water with conc = 0.02 $mg.dm^{-3}$ plus HND
4.17 Oxalic acid in water with conc = 0.2 $mg.dm^{-3}$ plus HND
4.18 Oxalic acid in water with conc = 2 $mg.dm^{-3}$ plus HND

Any increases in plant height was measured, and the number of leaflets counted, on a weekly basis for a period of 5 weeks. After that the plants were harvested. The roots

were excised, rinsed in distilled deionised water to remove sand particles and surface mineral nutrients, and oven dried to constant mass at 70°C. The shoots were measured, the number of leaves counted, the fresh mass determined and the material was then dried to constant mass in an oven at 70°C .

4.2.2 **Plant analysis**

The dried plant samples were ground in a Wiley Intermediate Mill (60 mesh) and then digested in a sulphuric acid-peroxide mixture (Allen 1989). The digests were diluted with 20 cm^3 distilled water, and then made up to 100 cm^3 by further addition of distilled water.

The following analyses were performed on the digests: Total nitrogen was determined by a micro-Kjeldahl method (Allen 1989), and the total titanium was determined using the ICP (Inductive coupled plasma) instrument.

4.3 **Statistical Analysis**

A statistical analysis of variance was done on the shoots and roots using the SAS v 6.12 statistical software (SAS 1990; Table 4.2.1, 4.2.2 and 4.3.1, 4.3.2). The Shapiro-Wilk test was done to test for non-normality. The lack of normality was due to kurtosis and not skewness and thus not important for our purpose (Shapiro & Wilk 1965) see

(Tables 4.2.1 , 4.2.2 and Table 4.3.1, 4.3.2). Least significant differences between treatments were calculated at $p \leq 0.05$ levels to compare the treatment means.

Table 4.2.1 Analyses of Varience (ANOVA) of growth parameters including all treatments

		Height cm		Leaf numbers		Shoot fresh mass (g)		Shoot dry mass (g)		Root fresh mass (g)		Root dry mass (g)	
Source	df	MS	P	MS	P	MS	P	MS	P	MS	P	MS	P
Block	1	13.225	0.03	434.013	<0.01	13.1	0.375	0.7334	0.257	19.531	0.05	0.0858	0.015
Treatment	17	114.269	<0.01	1424.17	<0.01	965.29	<0.01	10.244	<0.01	104.515	<0.01	0.2443	<0.01
Error	51	4.125		65.719		12.379		0.5278		7.1432		0.0225	
Corrected Total	71												
Non-Normality		p = 0.98		p = 0.97		p = 0.97		p = 0.70		p = 0.85		p = 0.95	

Table 4.2.2 Analyses of Varience (ANOVA) of element concentrations including all treatments

		Shoot nitrogen (mg/g)		Root nitrogen (mg/g)		Shoot Titanium (mg/g)		Root Titanium (mg/g)	
Source	df	MS	P	MS	P	MS	P	MS	P
Block	1	116.856	0.523	288.6098	0.019	42.6781	0.819	10690.144	0.217
Treatment	17	227.928	0.141	325.5081	<0.01	120.6235	0.553	261924.31	<0.01
Error	51	154.327		80.9487		138.476		6723.921	
Corrected Total	71								
Non-Normality		p = 0.58		p = 0.94		p = 0.96		p = 0.87	

Table 4.3.1 Analyses of Varience (ANOVA) of growth parameters excluding controls

		Height cm		Leaf numbers		Shoot fresh mass (g)		Shoot dry mass (g)		Root fresh mass (g)		Root dry mass (g)	
Source	df	MS	P	MS	P	MS	P	MS	P	MS	P	MS	P
Block	1	7.196611	0.077	360.9944	<0.01	2.54668	0.279	0.09153	0.141	8.5541	0.047	0.03746	0.03
Treatment	14	4.27571	0.173	43.96428	0.619	2.27643	0.323	0.04751	0.473	2.42645	0.647	0.01077	0.523
Error	42	2.95102		52.06587		1.9244		0.04767		2.97174		0.01142	
Corrected Total	59												
Non-Normality		p = 0.98		p = 0.98		p = 0.97		p =0.98		p = 0.97		p = 0.97	

Table 4.3.2 Analyses of Varience (ANOVA) of element concentrations excluding controls

		Shoot nitrogen (mg/g)		Root nitrogen (mg/g)		Shoot Titanium (mg/g)		Root Titanium (mg/g)	
Source	df	MS	P	MS	P	MS	P	MS	P
Block	1	1.53157	0.929	228.8	0.074	42.6781	0.819	10690.14	0.217
Treatment	14	22.392	0.025	309.1312	<0.01	120.6235	0.553	261924.3	<0.01
Error	42	10.2365		92.4659		138.4768		6723.92	
Corrected Total	59								
Non-Normality		p = 0.98		p = 0.93		p = 0.96		p = 0.87	

4.4 RESULTS AND DISCUSSION

Table 4.4.1 gives the growth data for all treatments. It is clear that for all parameters given, plants grown in Hoagland Complete Nutrient solution (HCN) (treatment 4.1, table 4.1) and Hoaglands Half Strength nutrient solution (HHN) (treatment 4.2, table 4.1) had significantly results than plants grown in all other treatments, with (treatment 4.1, table 4.1) plants being significantly greater than (treatment 4.2, table 4.2) plants. The plants given the highest concentration of titanium oxide (treatment 4.6, table 4.1) were significantly taller than plants supplied with Hoaglands nitrogen deficient nutrient solution (HND) (treatment 4.3, table 4.1) as well as the other titanium oxide treatments, one of the titanium ascorbate treatments and two of the oxalate treatments. The plants getting the highest titanium ascorbate concentration (treatment 4.9, table 4.1) were taller than those getting one tenth of this concentration. Again the plants getting the highest TiO_2 (treatment 4.6, table 4.1) had more leaves than those with the intermediate titanium ascorbate treatments.

As far as nitrogen and titanium content are concerned, the results are shown in Table 4.4.2. The results indicate that plants grown in Hoagland complete nutrient solution (HCN) (treatment 4.1, table 4.1) and Hoagland Half strength nutrient solution (HHN) (treatment 4.2, table 4.1) had a higher shoot nitrogen concentration than those grown in Hoaglands nitrogen deficient nutrient solution (HND) (treatment 4.3, table 4.1), and

plants grown Hoaglands half strength nutrient solution (HHN) (treatment 4.2, table 4.1) had a higher shoot nitrogen concentration than plants grown in all other treatments but for in Hoaglands complete nutrient solution (HCN) (treatment 4.1, table 4.1). The picture for root nitrogen was much more complex, with the highest concentrations of each group of treatments tending to higher root nitrogen concentrations.

The plants with the highest TiO_2 (treatment 4.6, table 4.1) had greater shoot titanium concentrations than those with the intermediate titanium oxalate treatment. When it comes to root titanium concentrations, the plants with the highest TiO_2 (treatment 4.6, table 4.1) resulted in a higher concentration than the second highest, which was in turn higher than plants grown in all other treatments.

In Table 4.5 we have a re-analysis of the data in Table 4.4 excluding the control treatments. Table 4.5.1 sheds little light on the effects on plant height and leaf number, but does show effects on mass not evident in Table 4.4.1. Plants grown in titanium oxide (treatment 4.6, table 4.1)) resulted in a greater shoot fresh and dry mass than plant grown in (treatments 4.8, 4.11, 4.16 and 4.18, table 4.1). Similarly it resulted in a greater root fresh and dry mass than plants grown in (treatments 4.8 and 4.18, table 4.1) did.

Once again, from Table 4.5.2 it can be seen that each type of treatment at high concentration tended to result in increased shoot and root nitrogen concentrations. (It may be that those plants treated with titanium have overall a higher nitrogen total content than those without titanium). Those plants receiving TiO_2 (treatment 4.6, table 4.1) have a higher titanium shoot concentration than treatment 4.11, (table4.1); similarly treatment 4.6, (table 4.1) resulted in a higher root titanium concentration than plants receiving treatment 4.5, (table 4.1), which in turn was higher than those of all other plants receiving titanium treatments.

The direct comparison given in Table 4.6, which includes the control treatments, shows significant differences between the controls and all other treatments. The measured parameters were greater in controls in all cases but for the root nitrogen concentrations where the controls were lower. The only other significant difference shows that the plants grown in TiO_2 treatments had higher root nitrogen concentrations than plants grown in all other treatments.

In Table 4.7 the control plants are excluded from the comparison and the results indicate that plants grown in titanium ascorbate nutrient solution resulted in lower shoot nitrogen concentrations than all the other plants grown in ascorbate and oxalate treatments. The

plants receiving titanium ascorbate treatments also resulted in lower shoot nitrogen concentrations than the plants given titanium oxalate treatments.

Table 4.4.1 **The effect of various titanium and nitrogen soil treatments on the growth, nitrogen and titanium content of tomato plants**

Treatments	Height		Leaf		Shoot fresh		Shoot dry		Root fresh		Root dry	
	cm	grouping	number	grouping	mass (g)	grouping	mass (g)	grouping	mass (g)	grouping	mass (g)	grouping
4.1 Control 1 (HCN)	26.3*	a	106*	a	68.20*	a	7.10*	a	25.11*	a	1.21*	a
4.2 Control 3 (HHN)	12.3*	b	60.5*	b	15.48*	b	1.96	b	11.84*	b	0.59	b
4.3 Control 2 (HND)	4.1	d e	27.50	c d	2.25	c	0.21	c	3.85	c	0.15	c
4.4 TiO2 (10mgdm^{-3})	4.1	d e	28.25	c d	2.15	c	0.32	c	3.89	c	0.17	c
4.5 TiO2 (100mg.dm^{-3})	4.0	d e	28.25	c d	2.52	c	0.34	c	3.92	c	0.19	c
4.6 TiO2 (1000mg.dm^{-3})	7.3	c	37.50	c	4.68	c	0.68	c	6.29	c	0.34	c
4.7 Tiasc(0.01mgdm^{-3})	5.7	c d e	31.25	c d	3.04	c	0.44	c	4.76	c	0.25	c
4.8 Tiasc (0.1mg.dm^{-3})	2.9	e	24.25	d	1.67	c	0.28	c	3.68	c	0.18	c
4.9 Tiasc (1mg.dm^{-3})	6.0	c d	35.00	c d	3.47	c	0.50	c	4.67	c	0.29	c
4.10 Tiox (0.01mg.dm^{-3})	4.5	c d e	32.00	c d	2.73	c	0.46	c	4.99	c	0.25	c
4.11 Tiox (0.1mg.dm^{-3})	4.8	c d e	32.75	c d	2.57	c	0.36	c	4.56	c	0.23	c
4.12 Tiox (1mg.dm^{-3})	5.2	c d e	30.75	c d	2.51	c	0.39	c	4.23	c	0.21	c
4.13 Asc (0.02mg.dm^{-3})	5.6	c d e	32.50	c d	3.05	c	0.46	c	4.29	c	0.23	c
4.14 Asc (0.2mg.dm^{-3})	4.9	c d e	32.25	c d	2.76	c	0.41	c	4.44	c	0.22	c
4.15 Asc (2mg.dm^{-3})	5.1	c d e	34.00	c d	3.40	c	0.50	c	5.05	c	0.25	c
4.16 Ox (0.02mg.dm^{-3})	4.3	d e	28.50	c d	2.29	c	0.36	c	4.08	c	0.21	c
4.17 Ox (0.2mg.dm^{-3})	5.0	c d e	33.25	c d	3.60	c	0.58	c	5.88	c	0.32	c
4.18 Ox (2mg.dm^{-3})	4.0	d e	28.25	c d	1.98	c	0.31	c	3.46	c	0.16	c
LSD (p=0.05)	2.9		11.51		4.99		1.03		3.79		0.21	

Asc = ascorbate
Ox = oxalate
TiO = titanium dioxide

Table 4.4.2 **The effect of various titanium and nitrogen soil treatments on the nitrogen and titanium content of tomato plants**

Treatments	Shoot Nitrogen		Root Nitrogen		Shoot Titanium		Root Titanium	
	mg/g	grouping	mg/g	grouping	mg/g	grouping	mg/g	grouping
4.1 Control 1 (HCN)	21.54*	a b	23.53	a b c d	.	.	.	.
4.2 Control 2 (HHN)	36.46*	a	19.86	b c d e f g	.	.	.	.
4.3 Control 3 (HND)	1.68	c	4.53	i	.	.	.	.
4.4 TiO2 (10mgdm^{-3})	7.26	b c	15.13	e f g h i	23.21	a b	65.74	c
4.5 TiO2 (100mg.dm^{-3})	10.96	b c	32.00	a b	27.79	a b	262.31*	b
4.6 TiO2 (1000mg.dm^{-3})	9.52	b c	31.04	a b	35.02*	a	822.01*	a
4.7 Tiasc(0.01mgdm^{-3})	8.44	b c	17.80	c d e f g h	27.02	a b	54.43	c
4.8 Tiasc (0.1mg.dm^{-3})	11.30	b c	28.36	a b c d	28.26	a b	56.17	c
4.9 Tiasc (1mg.dm^{-3})	8.99	b c	30.87	a b	18.21	a b	63.99	c
4.10 Tiox (0.01mg.dm^{-3})	9.29	b c	6.92	h i	26.10	a b	60.23	c
4.11 Tiox (0.1mg.dm^{-3})	8.17	b c	16.93	d e f g h i	17.82	b	54.67	c
4.12 Tiox (1mg.dm^{-3})	4.82	b c	27.07	a b c d e	20.96	a b	40.74	c
4.13 Asc (0.02mg.dm^{-3})	7.95	b c	10.50	g h i	.	.	.	.
4.14 Asc (0.2mg.dm^{-3})	10.48	b c	24.29	a b c d e f	.	.	.	.
4.15 Asc (2mg.dm^{-3})	13.21	b c	19.79	a b c	.	.	.	.
4.16 Ox (0.02mg.dm^{-3})	7.90	b c	13.02	f g h i	.	.	.	.
4.17 Ox (0.2mg.dm^{-3})	7.13	b c	16.51	d e f g h i	.	.	.	.
4.18 Ox (2mg.dm^{-3})	13.78	b c	33.36*	a	.	.	.	.
LSD (p=0.05)	17.64		12.70		17.17		119.67	

Asc = ascorbate
Ox = oxalate
TiO = titanium dioxide

Table 4.5.1 **The effect of various titanium and nitrogen soil treatments on the growth of tomato plants**

Treatments	Height		Leaf		Shoot fresh		Shoot dry		Root fresh		Root dry	
	cm	grouping	number	grouping	mass (g)	grouping	mass (g)	grouping	mass (g)	grouping	mass (g)	grouping
4.4 TiO2 (10mgdm^{-3})	4.1	b c	28.25	a b	2.15	b	0.32	b	3.89	a b	0.17	b c
4.5 TiO2 (100mg.dm^{-3})	4.0	b c	28.25	a b	2.52	b	0.34	b	3.92	a b	0.19	a b c
4.6 TiO2 (1000mg.dm^{-3})	7.3*	a	37.50*	a	4.68*	a	0.68*	a	6.29*	a	0.34*	a
4.7 Tiasc(0.01mgdm^{-3})	5.7	a b	31.25	a b	3.04	a b	0.44	a b	4.76	a b	0.25	a b c
4.8 Tiasc (0.1mg.dm^{-3})	2.9	c	24.25	b	1.67	b	0.28	b	3.68	b	0.18	b c
4.9 Tiasc (1mg.dm^{-3})	6.0	a b	35.00	a	3.47	a b	0.50	a b	4.67	a b	0.29	a b c
4.10 Tiox (0.01mg.dm^{-3})	4.5	b c	32.00	a b	2.73	a b	0.46	a b	4.99	a b	0.25	a b c
4.11 Tiox (0.1mg.dm^{-3})	4.8	a b c	32.75	a b	2.57	b	0.36	b	4.56	a b	0.23	a b c
4.12 Tiox (1mg.dm^{-3})	5.2	a b c	30.75	a b	2.51	b	0.39	a b	4.23	a b	0.21	a b c
4.13 Asc (0.02mg.dm^{-3})	5.6	a b	32.50	a b	3.05	a b	0.46	a b	4.29	a b	0.23	a b c
4.14 Asc (0.2mg.dm^{-3})	4.9	a b c	32.25	a b	2.76	a b	0.41	a b	4.44	a b	0.22	a b c
4.15 Asc (2mg.dm^{-3})	5.1	a b c	34.00	a b	3.40	a b	0.50	a b	5.05	a b	0.25	a b c
4.16 Ox (0.02mg.dm^{-3})	4.3	b c	28.50	a b	2.29	b	0.36	b	4.08	a b	0.21	a b c
4.17 Ox (0.2mg.dm^{-3})	5.0	a b c	33.25	a b	3.60	a b	0.58	a b	5.88	a b	0.32	a b
4.18 Ox (2mg.dm^{-3})	4.0	b c	28.25	a b	1.98	b	0.31	b	3.46	b	0.16	c
LSD (p=0.05)	2.5		10.30		1.98		0.31		2.46		0.15	

Asc = ascorbate
Ox = oxalate
TiO = titanium dioxide

Table 4.5.2 **The effect of various titanium and nitrogen soil treatments on the nitrogen and titanium content of tomato plants**

Treatments	Shoot Nitrogen		Root Nitrogen		Shoot Titanium		Root Titanium	
	mg/g	grouping	mg/g	grouping	mg/g	grouping	mg/g	grouping
4.4 TiO2 (10mgdm^{-3})	7.26	c d	15.13	d e f	23.21	a b	65.74	c
4.5 TiO2 (100mg.dm^{-3})	10.96	a b c	32.00*	a	27.79	a b	262.31	b
4.6 TiO2 (1000mg.dm^{-3})	9.52	a b c	31.04	a b	35.02*	a	822.01	a
4.7 Tiasc(0.01mgdm^{-3})	8.44	c d	17.80	b c d e f	27.02	a b	54.43	c
4.8 Tiasc (0.1mg.dm^{-3})	11.30	a b c	28.36	a b c d	28.26	a b	56.17	c
4.9 Tiasc (1mg.dm^{-3})	8.99	b c d	30.87	a b	18.21	a b	63.99	c
4.10 Tiox (0.01mg.dm^{-3})	9.29	a b c d	6.92	f	26.10	a b	60.23	c
4.11 Tiox (0.1mg.dm^{-3})	8.17	c d	16.93	c d e f	17.82*	b	54.67	c
4.12 Tiox (1mg.dm^{-3})	4.82	d	27.07	a b c d	20.96	a b	40.74	c
4.13 Asc (0.02mg.dm^{-3})	7.95	c d	10.50	f	.		.	.
4.14 Asc (0.2mg.dm^{-3})	10.48	a b c	24.29	a b c d	.		.	.
4.15 Asc (2mg.dm^{-3})	13.21	a b	29.79	a b c	.		.	.
4.16 Ox (0.02mg.dm^{-3})	7.90	c d	13.02	e f	.		.	.
4.17 Ox (0.2mg.dm^{-3})	7.13	c d	16.51	c d e f	.		.	.
4.18 Ox (2mg.dm^{-3})	13.78*	a	33.37*	a	.		.	.
LSD (p=0.05)	4.57		13.72		17.17		119.67	

Asc = ascorbate
Ox = oxalate
TiO = titanium dioxide

Table 4.6 Contrast estimates between various soil treatments by means of meaningful comparison (including controls)

X vs Y	height	Leaf	Shoot fresh	Shoot dry	Root fresh	Root dry	Shoot	Root
	cm	number	mass (g)	mass (g)	mass (g)	mass (g)	nitrogen (mg/g)	nitrogen (mg/g)
Control vs Rest	140.25*	501.25*	385.552*	39.956*	135.825*	6.259*	159.205*	-94.018*
TiO vs Rest	3.375	1.250	4.365	0.282	2.325	-0.006	-0.495	57.232*
Tiasc ox vs asc ox	0.175	-2.750	-1.080	-0.1952	-0.305	-0.005	-0.445	0.472
Tiasc vs asc	-1.125	-8.250	-1.020	-0.1512	-0.670	0.011	-2.912	12.452
Tiox vs ox	1.300	5.500	-0.060	-0.0440	0.365	-0.0163	-6.533	-11.980
Tiasc vs Tiox	-1.075	-3.250	-1.397	-0.1550	0.005	-0.0185	-9.367	-13.657
asc vs ox	2.375	8.750	1.338	0.111	0.360	0.002	2.835	1.678
MSE	4.126	65.719	12.379	0.528	7.143	0.023	154.327	80.080
df	51	51	51	51	51	51	51	51

* significant at 5% level
Asc = ascorbate
Ox = oxalate
TiO = titanium dioxide

Table 4.7 Contrast estimates between various soil treatments by means of meaningful comparisons (excluding controls)

X vs Y	height	Leaf	Shoot fresh	Shoot dry	Root fresh	Root dry	Shoot Nitrogen	Root Nitrogen
	cm	number	mass (g)	mass (g)	mass (g)	mass (g)	mg/g	mg/g
TiO vs Rest	3.375	1.250	4.365	0.282	2.325	-.005	-0.500	57.23
Tiasc ox vs asc ox	0.175	-2.750	-1.080	-0.195	-0.310	-.005	-9.450*	0.47
Tiasc vs asc	-1.125	-8.250	-1.020	-0.151	-0.670	0.011	-2.910	12.45
Tiox vs ox	1.300	5.500	-0.060	-0.044	0.365	-0.015	-6.530	-11.98
Tiasc vs Tiox	-1.075	-3.250	-1.397	-0.155	0.005	-0.021	-9.367*	-13.66
asc vs ox	2.375	8.750	1.338	0.111	0.36	0.002	2.825	1.68
MSE	2.951	52.065	1.924	0.047	2.972	0.01	10.236	92.47
df	42	42	42	42	42	42	42	42

* significant at 5% level
Asc = ascorbate
ox = oxalate
TiO = titanium dioxide

4.4.1 **Visual observations**

Plants grown in Hoagland Complete nutrient solution (HCN) (treatment 4.1, table 4.1) and Hoagland Half Strength nutrient solution (HHN) (treatment 4.2, table 4.1) were much healthier and stronger than plants grown in all the other treatments. Titanium ascorbate plants, as compared with those given titanium dioxide and titanium oxalate, had leaves which were not as yellowish as leaves obtained with the latter treatments. Plants started loosing most of their leaflets only after the third week of treatment.

4.5 **Conclusion**

Plants supplied with nitrogen clearly grew better than those that were not. The nitrogen concentration in the plant was not directly related to plant size. Plants supplied with the two higher concentrations of titanium oxide had significantly high root titanium concentrations, but it did not result in high shoot concentrations suggesting titanium is not readily translocated. Overall, all other treatments resulted in a higher plant nitrogen content than the Hoagland nitrogen deficient solution (HND) (treatment 4.3, table 4.1) irrespective of the presence or absence of titanium.

4.6 **References**

Allen, S.E. 1989. Chemical Analysis of Ecological Materials. Blackwell Scientific Publication. London.

Cerff, B.R . 1995. The Use of Titanium Compounds in the Photocatalytic Fixation of Dinitrogen and Plant Growth Enhancement. Mini-Thesis in Chemistry Department, University of Western Cape.

Dumon, J.C & Ernst, W.H.O. 1988. J. Plant Physiol. 133: 203-209.

SAS. 1990. SAS/STAT User's Guide, Version 6, Fourth Edition, Volume 2. SAS Institute Inc, SAS Campus Drive, Cary, NC 27513.

Shapiro, S.S & Wilk, M.B. 1965. An Analysis of Variance Test for Normality (complete samples)., Biometrika. 52: 591-611.

CHAPTER 5

THE EFFECT OF DIFFERENT TITANIUM FOLIAR AND SOIL TREATMENTS ON THE GROWTH AND CHEMICAL COMPOSITION OF *Lycopersicum esculentum* Cultivar Floridade.

5.1 INTRODUCTION

In order to verify the work of Pais *et al.* (1983), experiments involving complete nutrient solutions both as foliar spray and soil treatment, were carried out simultaneously in the greenhouse. The experiments were conducted under much more carefully controlled conditions than those used by Pais *et al.* who established that under normal circumstances titanium ascorbate enhanced the plant growth and also increased the production of tomato plants.

5.2 MATERIALS AND METHODS

5.2.1 Cultivation, treatment, and harvesting of plants

Lycopersicum esculentum (cultivar Floridade) plants were obtained from a reliable nursery. Seventy-two healthy plants of similar size were selected and planted in 12,5 cm plastic pots filled with washed sand. The pots were placed in an experimental greenhouse in a randomized block design (eighteen different treatments and two modes of application replicated once). The normal sunlight in the greenhouse was enriched by the light of four 300W Osram, ultra-vitalux sunlight lamps that were placed at a height of 1.6 meter above each group of plants. This supplementary light of 610 nm was switched on at 6 am and off at 5 pm using a time switch. The greenhouse was maintained at 20°C, with no humidity control.

The plants were subjected once a week to the treatments listed in Table 5.1 as either foliar spray or soil treatment, and watered on alternating days with distilled water plus Hoagland Complete Nutrient solution (HCN) solution for a period of 5 weeks.

Table 5.1 : **Full nutrient Foliar spray treatment :For Foliar Spray and Soil Treatments**

5.1 Hoagland Complete Nutrient Solution (HCN)
5.2 Hoagland Half Strength Nutrient Solution (HHN)
5.3 Hoagland Nitrogen Deficient nutrient solution (HND)
5.4 TiO_2 in water with conc = 10 $mg.dm^{-3}$ plus HCN
5.5 TiO_2 in water with conc = 100 $mg.dm^{-3}$ plus HCN
5.6 TiO_2 in water with conc = 1000 $mg.dm^{-3}$ plus HCN
5.7 Ti-ascorbate in water with conc = 0.01 $mg.dm^{-3}$ plus HCN
5.8 Ti-ascorbate in water with conc = 0.1 $mg.dm^{-3}$ plus HCN
5.9 Ti-ascorbate in water with conc = 1 $mg.dm^{-3}$ plus HCN
5.10Ti-oxalate in water with conc = 0.01 $mg.dm^{-3}$ plus HCN
5.11 Ti-oxalate in water with conc = 0.1 $mg.dm^{-3}$ plus HCN
5.12 Ti-oxalate in water with conc = 1 $mg.dm^{-3}$ plus HCN
5.13 Ascorbic acid in water with conc = 0.02 $mg.dm^{-3}$ plus HCN
5.14 Ascorbic acid in water with conc = 0.2 $mg.dm^{-3}$ plus HCN
5.15 Ascorbic acid in water with conc = 2 $mg.dm^{-3}$ plus HCN
5.16 Oxalic acid in water with conc = 0.02 $mg.dm^{-3}$ plus HCN
5.17 Oxalic acid in water with conc = 0.2 $mg.dm^{-3}$ plus HCN
5.18 Oxalic acid in water with conc = 2 $mg.dm^{-3}$ plus HCN

For convenience, the pots were labeled in terms of the concentrations of the solution. The Hoagland solutions were prepared in 20 dm^3 plastic containers. Plant height was measured, and the number of leaflets counted on a weekly basis for a period of 5 weeks. After that the plants were harvested. The roots were excised, rinsed in distilled deionised

water to remove sand particles and mineral nutrients, and oven dried to constant mass at 70°C. The shoots were measured, the number of leaves counted and the fresh mass determined. They were then dried at 70°C to constant mass. The dry mass of both roots and shoots was determined.

5.3 **Plant analysis**

The dried plant samples were ground in a Wiley Intermediate Mill (60 mesh) and then digested in a sulfuric acid-peroxide mixture (Allen 1989). The digests were diluted with 20 cm^3 distilled water, and then made up to 100 cm^3 by further addition of distilled water. The following analysis were performed: Total nitrogen was determined by a micro-Kjeldahl method (Allen 1989), the total titanium was determined using the ICP (Inductive couple plasma).

5.4 **Statistical Analysis**

A statistical analysis of variance was done on the shoots and roots using the SAS V6.12 statistical software (SAS, 1990; Tables 5.2.1and 5.2.2). Shapiro-Wilk test was done to test for non-normality. The lack of normality was due to kurtosis not skewness and thus not important for our purpose (Shapiro & Wilk 1965) as shown in Tables 5.2.1 and 5.2.2.

Table 5.2.1 Analyses of Varience (ANOVA) of growth parameters including all treatments

		Height cm		Leaf numbers		Shoot fresh mass (g)		Shoot dry mass (g)		Root fresh mass (g)		Root dry mass (g)	
Source	df	MS	P	MS	P	MS	P	MS	P	MS	P	MS	P
Block	1	1.869	0.69	618.35	0.023	285.98	<0.01	11.908	<0.01	450.6	<0.01	1.062	<0.01
application	1	5.78	0.49	110.01	0.32	26.48	0.22	0.0205	0.44	4.44	0.56	0.049	0.42
Treatment	17	41.36	<0.01	379.2	<0.01	65.52	<0.01	0.566	0.94	16.651	0.27	0.124	0.101
Treatment x Application	17	10.895	0.56	210.87	0.049	15.87	0.56	0.362	0.411	10.957	0.65	0.192	<0.01
Error	35	11.901		109.2		17.29		0.336		13.235		0.075	
Corrected Total	71												
Non-Normality		p = .991		p = .996		p = 0.983		p = 0.996		p = 0.992		p = 0.959	

Table 5.2.2 Analyses of Varience (ANOVA) of element concentrations

		Shoot nitrogen (mg/g)		Root nitrogen (mg/g)		Shoot Titanium (mg/g)		Root Titanium (mg/g)	
Source	df	MS	P	MS	P	MS	P	MS	P
Block	1	442.18	0.02	260.83	0.01	102672.2	0.52	39974.67	0.25
application	1	77.23	0.324	36.75	0.32	392562.8	0.21	28939.68	0.33
Treatment	17	769.56	<0.01	689.63	<0.01	215407.1	0.52	679303.8	0.03
Treatment x Application	17	213.92	<0.01	255.46	<0.01	196552.7	0.58	456775	0.11
Error	35	77.076		36.801		235174.5		29.365	
Corrected Total	71								
Non-Normality		0.994		0.966		0.571		0.677	

5.5 RESULTS AND DISCUSSION

5.5.1 *Effects on growth*

Table 5.3.1 gives the growth data for all treatments. The plants given Hoagland Complete Nutrient nitrogen (HCN) (treatment 5.1, table 5.1) were taller and had a greater shoot fresh mass than the plant receiving most other treatments. The plants getting the highest titanium ascorbate concentration were taller than the plants grown on other treatments. The results also show increases in leaf numbers as compared to the plants of other treatments. The plants given oxalate (treatment 5.17, table 5.1) had a larger root dry mass than all the other plants. The plants receiving Hoagland nitrogen deficient nutrient solution (HND) (treatment 5.3, table 5.1) were shorter than all the other plants.

As far as nitrogen and titanium contents are concerned, the results are shown in Table 5.3.2. The plants grown in Hoagland Complete Nutrient solution (HCN) (treatment 5.1, table 5.1) had a higher shoot and root nitrogen concentration than the plants receiving Hoagland Half Strength Nutrient solution (HHN) (treatment 5.2, table 5.1) and Hoagland Nitrogen Deficient Nutrient solution (HDN) (treatment 5.3, table 5.1) and the plants getting most other treatments. The plants supplied with ascorbic acid at the middle concentration (5.14, table5.1), had a higher nitrogen concentration in the roots than plants

with other treatments. The plant given the highest Ti O_2 (treatment 5.6, table 5.1) had a greater shoot titanium concentration than plants grown in other treatments.

The direct comparisons given in Table 5.4 which includes the control treatments shows differences between controls and other treatments. The plants grown in the control solutions had lower leaf numbers than the rest of the plants on other treatments. The nitrogen content of the shoots and roots were also lower than in plants on treatments. The measured parameters were greater in plants with titanium ascorbate and oxalate than in plants with ascorbate and oxalate. Titanium ascorbate treated plants are shown to result in increased shoot fresh mass when compared to the ascorbate and oxalate treated plants. Plants receiving TiO_2 treatments resulted in a lower shoot dry mass and root fresh and dry mass than the rest of the plants getting other treatments. The nitrogen content of TiO_2 treated plants was increased in the roots above that of the plants getting other treatments. Titanium ascorbate treated plants also showed a lower root nitrogen concentration than titanium oxalate treated plants. The plants supplied with ascorbate showed larger increases in nitrogen in roots than plants treated with oxalate. The results also showed a higher nitrogen content for the titanium ascorbate treated plants than for plants receiving ascorbate, oxalate and titanium oxalate treatments.

In Table 5.5, It is clear that there was no significant difference between the applications of foliar spray and soil treatments.

Table 5.3.1 **The effect of soil and shoot applications of various titanium and nitrogen treatments on growth, nitrogen and titanium content of tomato plants**

Treatments	Height		Leaf		Shoot fm		Shoot dm		Root fm		Root dm	
	cm	grouping	number	grouping	g	grouping	g	grouping	g	grouping	g	grouping
5.1 Control 1 (HCN)	18.3	a b	77.50	b	26.72	a	2.51	a	14.79	a	0.60	b
5.2 Control 2 (HHN)	10.4	e f	55.50	c d	9.24	d e	1.12	d	9.52	b c	0.42	b
5.3 Control 3 (HND)	7.8	i	49.50	d	8.64	e	1.03	c d	7.37	a b c	0.41	b
5.4 TiO2 (10mgdm^{-3})	10.0	e f	68.25	b c	13.54	c d e	1.49	b c d	10.38	a b c	0.46	b
5.5 TiO2 (100mg.dm^{-3})	11.6	d e f	77.75	b	16.19	b c	1.79	a b c d	11.78	a b c	0.51	b
5.6 TiO2 (1000mg.dm^{-3})	14.8	b c d e	71.50	b	15.06	b c d	1.40	b c d	9.61	a b	0.46	b
5.7 Tiasc(0.01mgdm^{-3})	13.5	b c d e	70.75	b	19.89	b	2.04	a b	13.08	a b	0.60	b
5.8 Tiasc (0.1mg.dm^{-3})	17.7	a b c	81.25	a b	20.57	b	2.04	a b	14.17	a b	0.62	b
5.9 Tiasc (1mg.dm^{-3})	20.7	a	94.25	a	16.97	b c	2.09	a b	14.08	a b	0.62	b
5.10 Tiox (0.01mg.dm^{-3})	15.7	a b c d	77.25	b	16.99	b c	1.91	a b c	13.61	a b	0.64	b
5.11 Tiox (0.1mg.dm^{-3})	14.9	b c d e	77.00	b	19.08	b c	2.07	a b	14.65	a b	0.69	b
5.12 Tiox (1mg.dm^{-3})	13.0	c d e	68.75	b c	16.44	b c	1.80	a b c d	11.23	a b c	0.55	b
5.13 Asc (0.02mg.dm^{-3})	13.6	b c d e	79.25	a b	17.61	b c	1.98	a b	13.05	a b	0.65	b
5.14 Asc (0.2mg.dm^{-3})	13.4	b c d e	67.00	b c	13.72	c d e	1.48	b c d	11.14	a b c	0.49	b
5.15 Asc (2mg.dm^{-3})	10.6	e f	77.50	b	15.13	b c d	1.62	b c d	12.63	a b	0.55	b
5.16 Ox (0.02mg.dm^{-3})	11.6	d e f	72.25	b	14.78	b c d	1.71	a b c d	13.44	a b	0.67	b
5.17 Ox (0.2mg.dm^{-3})	11.3	d e f	70.25	b c	17.01	b c	2.13	a b	13.70	a b	1.21	a
5.18 Ox (2mg.dm^{-3})	13.5	b c d e	70.50	b c	16.03	b c	1.96	a b	12.87	a b	0.64	b
LSD (p=0.05)	4.95		15.00		3.93		0.83		5.22		0.39	

Asc = ascorbate
Ox = oxalate
TiO = titanium dioxide

Table 5.3.2 The effect of soil and shoot applications of various titanium and nitrogen treatments on the nitrogen and titanium content of tomato plants

Treatments	Shoot N mg/g	Shoot N grouping	Root N mg/g	Root N grouping	Shoot Ti mg/g	Shoot Ti grouping	Root Ti mg/g	Root Ti grouping
5.1 Control 1 (HCN)	66.44	a	56.58	a	.	.	.	.
5.2 Control 2 (HHN)	19.18	g h i	21.01		.	.	.	.
5.3 Control 3 (HND)	14.38	i	18.72		.	.	.	.
5.4 TiO2 (10mgdm^{-3})	29.30	f g h	39.86	d e	69.00	b	142.10	b
5.5 TiO2 (100mg.dm^{-3})	43.00	c d e	47.75	b c d	117.30	b	95.80	b
5.6 TiO2 (1000mg.dm^{-3})	53.92	a b c	55.99	a b	754.70	a	496.10	a
5.7 Tiasc(0.01mgdm^{-3})	34.96	e f g	24.34		43.40	b	55.00	b
5.8 Tiasc (0.1mg.dm^{-3})	46.86	b c d e	34.51		44.20	b	54.90	b
5.9 Tiasc (1mg.dm^{-3})	52.25	b c d	44.27	c d e	43.00	b	61.30	b
5.10 Tiox (0.01mg.dm^{-3})	36.47	e f g	19.01		35.00	b	57.10	b
5.11 Tiox (0.1mg.dm^{-3})	46.16	b c d e	29.23		157.10	b	47.60	b
5.12 Tiox (1mg.dm^{-3})	58.36	a b	50.11	a b c	47.40	b	40.70	b
5.13 Asc (0.02mg.dm^{-3})	23.97	g h i	56.18	a b	.	.	.	.
5.14 Asc (0.2mg.dm^{-3})	38.17	e f	57.29	a	.	.	.	.
5.15 Asc (2mg.dm^{-3})	39.66	d e f	38.46	e	.	.	.	.
5.16 Ox (0.02mg.dm^{-3})	29.10	f g h	43.22	c d e	.	.	.	.
5.17 Ox (0.2mg.dm^{-3})	36.09	e f g	41.08	d e	.	.	.	.
5.18 Ox (2mg.dm^{-3})	52.66	b c	33.45		.	.	.	.
LSD (p=0.05)	12.60		8.71		723.48			

Asc = ascorbate
Ox = oxalate
TiO = titanium dioxide

Table 5.4 **Contrast estimates between various treatments by means of meaningful comparison (including controls)** with various apllications

X vs Y	height	Leaf	Shoot fm	Shoot dm	Root fm	Root dm	Shoot N	Root N
	cm	number	g	g	g	g	mg/g	mg/g
Control vs Rest	-23.80	-211.25*	-26.73	-4.20	-31.06	-2.23	-120.94*	-133.15*
TiO vs Rest	-24.18	-36.25	-25.76	-4.09*	-30.60*	-2.20*	10.18	103.26*
Tiasc ox vs asc ox	21.60*	32.25	14.97*	1.07	3.96	-0.50	55.41*	-68.21*
Tiasc vs asc	1.30*	22.25	10.98*	1.10	4.51	0.14	32.27*	-48.82*
Tiox vs ox	7.28	10.00	3.99	-0.03	-0.55	-0.64*	23.14*	-19.40*
Tiasc vs Tiox	5.98	-1.00	6.06	0.70	2.66	0.18	39.17*	-53.58*
asc vs ox	1.30	11.00	-2.07	-0.73	-3.22	-0.82	-16.03	34.18*

* significant at 5% level
Asc = ascorbate
Ox = oxalate
TiO = titanium dioxide

Table 5.5 : The effect of application on growth and nitrogen content of tomato plants

	Height	Leaf	Shoot fm	Shoot dm	Root fm	Root dm	Shoot N	Root N
	cm	number	g	g	g	g	mg/g	mg/g
Foliar	13.20	73.80	16.91	1.84	12.04	0.57	41.09	38.79
Soil	13.70	71.30	15.75	1.73	12.53	0.63	39.02	40.22
Application	0.49	0.32	0.22	0.44	0.57	0.42	0.32	0.32

***Significant at 5%Level**

5.5.2 **Visual observations**

Plants on all treatments had strong and thick stalks with green leaves and they all looked healthy. The plants treated with titanium compounds had much greener leaves than the plants given Hoagland complete nutrient solution (HCN) (treatment 5.1, table 5.1). Plants receiving treatment 5.8 were more prolific than plants receiving treatments 5.6 and 5.10, (table 5.1). These treatments were more prolific than the plant receiving treatment 5.1, (table 5.1). The root growth was also enhanced for titanium treated plants. The roots of the plant receiving treatment 5. 8, (table 5.1) and treatment 5.11, (table 5.1) were more prolific than those of the plant receiving Hoagland complete nutrient solution (HCN) (treatment 5.1, table 5.1). see Figure 5.2 below.

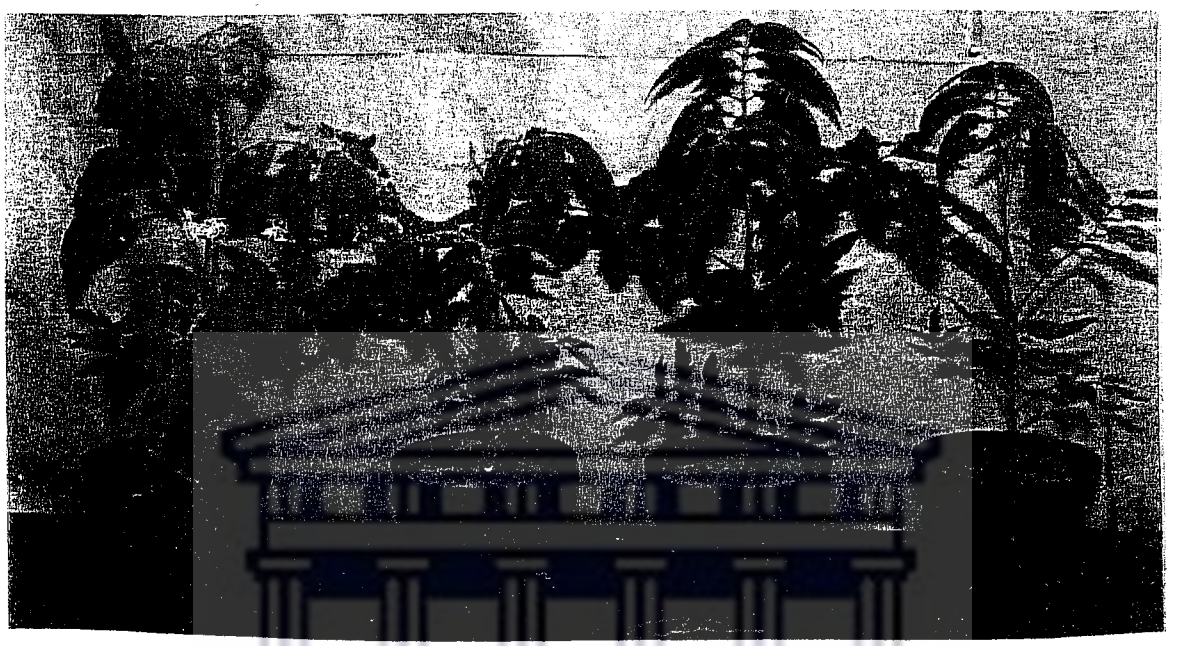


Figure 5.1: The effect of titanium on the chlorophyll content of tomato plants : The plant on the right was treated with titanium dioxide (treatment 5.6, table 5.1) titanium ascorbate (treatment 5. 8, table 5.1) and titanium oxalate (treatment 5.10, table 5.1) while the plant on the left was given Hoaglands complete nutrient solution (HCN) (treatment 5.1, table 5.1)

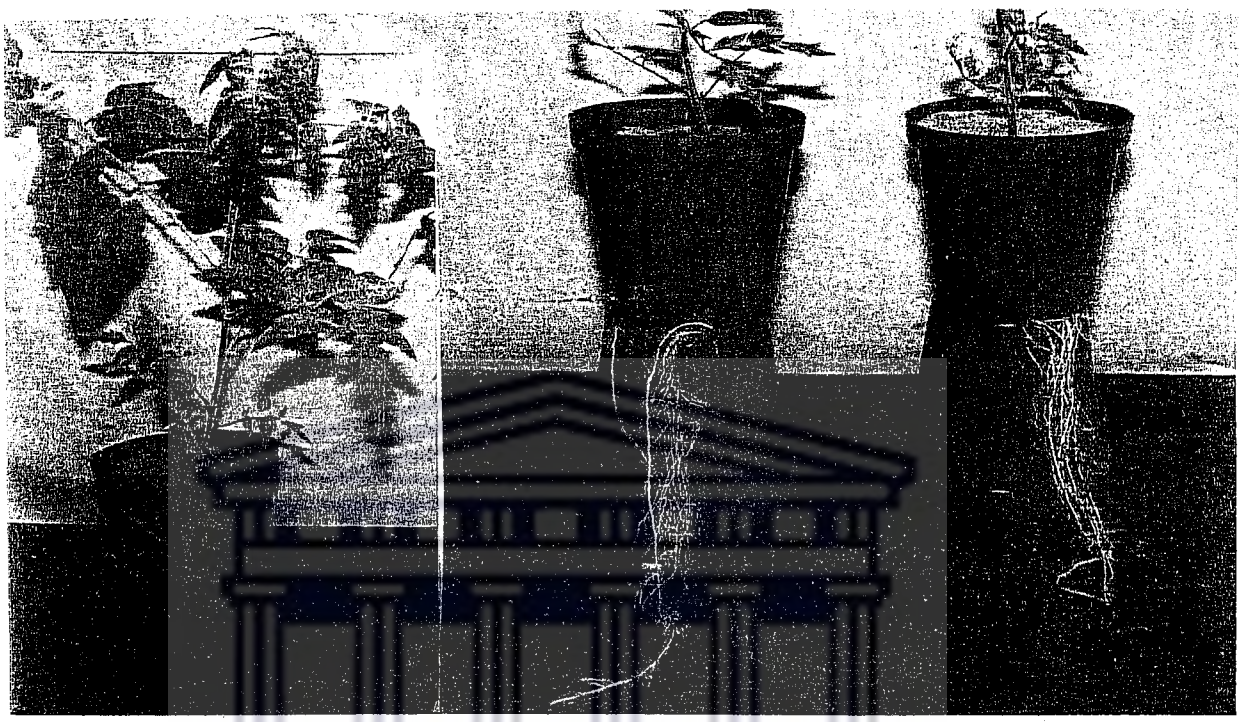


Figure 5.2: The enhancement of root growth by titanium : The plants on the right was treated with titanium oxalate (treatment 5.11, table 5.1) and titanium ascorbate (treatment 5.8, table 5.1) while the plant on the left was given Hoaglands complete nutrient solution (HCN) (treatment 5.1, table 5.1).

5.6 **Conclusion**

The work of Pais *et al* was repeated, but his results were not confirmed. The following observations were made. There was no difference in plant growth and nitrogen content due to the form of application (spray or irrigation) of the different treatments. Caution must be exercised in the interpretation of the results in Table 5.4. Although treatments titanium compounds resulted in higher shoot and root nitrogen contents and more leaves

than the control plants, it must be remembered that they received complete nutrient solution whereas two-thirds of the control plants did not. The titanium oxide treatments resulted in enhanced root growth.

5.7 REFERENCES

Allen, S.E. 1989. Chemical Analysis of Ecological Materials. Blackwell Scientific Publication. London.

Pais, I. 1983. J. Plant Nutr. 6: 3.

SAS. 1990. SAS/STAT User's Guide, Version 6, Fourth Edition, Volume 2. SAS Institute Inc, SAS Campus Drive, Cary, NC 27513.

Shapiro, S.S. & Wilk, M.B. 1965. An Analysis of Variance Test for Normality (complete samples). Biometrika. 52: 591-611.

SUMMARY

The nature of the coordination of titanium to L(+)-ascorbic acid poses a challenge for future studies. In this work a viable method of forming solid titanium (IV) complexes of ascorbic acid from a non-aqueous solution was devised which avoided the use of heat and repeated extraction. This method allows a complete ligand exchange when using $TiCl_4$ as a starting material. The presence of co-ordinated ascorbic acid species was confirmed using infrared spectrophotometry. The use of this method for further studies is recommended as it does avoid nitrogen free conditions and it works out cheaper and hopefully it will be possible to obtained crystals of titanium (IV) ascorbate species upon which x-ray crystal analysis can be carried out. The nuclear microprobe proved to be a very useful tool in these studies. More work in this area still needs to be done. While some titanium compounds appeared to enhance some growth parameters in some instances, titanium did not consistently result in improved growth or increased nitrogen concentration within tomato plants.

APPENDIX . 1

2 . Preparation of titanium oxalate

A similar procedure as in p16 (chapter 1) was followed for the preparation of titanium oxalate complexes. In place of L(+)-ascorbic acid, oxalic acid was used (13.79 g , 0.109 mol). The white amorphous solution was precipitated with ethanol and analysed.

3. PREPARATION OF SAMPLES FOR INSTUMENTAL USE

3. 1 Preparation of ICP samples

0.01-0.1g of foliage and roots were weighed out into 250 ml conical flasks and digested with 4 ml HNO_3 followed by 4 ml H_2SO_4. The mixture was heated on the hot plate with a magnetic stirrer. The resulting solution was diluted to 100 ml with deionised water and the titanium content was determined using inductively coupled plasma, model ICAP-9000 spectrometer. The wavelength was maintained at 336.121 nm, detection limit 1 ppm, the plasma flow rate was at (1Lmin) and the nebulizer flow rate at (1mL/min). The current was set at 15 Am.

3.2 Preparation of samples for Kejdahl method

Samples of foliage and roots were weighed out separately into digestion tubes, which were randomly placed on the digestion block. 4ml H_2SO_4 solution containing salicylic acid per liter of distilled water was added followed by 0.5 g sodium thiosulphate and 1 kjeldahl tablet. The samples were digested at temperatures ranging from the intervals 220°C, 250° C , 280° C and 300° C and at 40 min intervals and thereafter followed by 350° C for 1.5 hrs until there was a colourless solution . The solution was cooled for 10 min with concomitant addition of water to stop solidification of the acid. Standard solutions ranging from 0.2-1 mg Nm^{-1} to 2-10 mg Nm^{-1} were prepared from the stock solution stock solution.

The entire analyte was transferred to a Buchi boiling tube for distillation. A phenolphthalein indicator was added together with excess NaOH and 5 ml of 2% boric acid solution and titrated with 0.01 M HCl solution under controlled conditions. The results were recorded.