Institute of Plant Nutrition
Justus Liebig University Giessen / Germany
Prof. Dr. Drs. h. c. Konrad Mengel

Total Soluble Iron in the Soil Solution of Physically, Chemically and Biologically Different Soils

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Submitted by
Tarek Ghassan Ammari
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Defense Committee:

Chairman: Prof. Dr. B. Honermeier.
1. Supervisor: Prof. Dr. K. Mengel.
2. Supervisor: Prof. Dr. W. Friedt.
1. Examiner: Prof. Dr. S. Schnell.
2. Examiner: Prof. Dr. H. Wegener.

Content

1. Introduction:

Iron (Fe) is very insoluble in aerobic environments at neutral and alkaline pH. The Fe(III) (hydr)oxides have solubility products ranging from 10^{-39} to 10^{-44}, limiting the Fe(III) aqueous equilibrium concentration to ca. 10^{-17} M, in the absence of complexing ligands (Hersman et al., 2001). Such conditions are particularly prevalent in semiarid, calcareous soils estimated to comprise over one-third of the world's land surface area (Crowley et al., 1987). Soluble Fe^{3+} decreases 1000-fold for every unit increase in pH, and is essentially unavailable above pH 4. Similarly, Fe^{2+} decreases in solubility 100-fold for every unit increase in pH. In contrast to Fe^{3+}, solubility of Fe^{2+} is also controlled by redox conditions, with the result that under reduced conditions, above pH 4, Fe^{2+} is potentially the most available form of soluble inorganic Fe (Crowley et al., 1987). Lindsay and Schwab (1982) have theoretically and experimentally determined that at neutral pH 7, pe+pH must be below 9 to support the soluble Fe^{2+} concentration critical for plant growth. In calcareous, aerated soils, these reduced conditions would occur only in oxygen-depleted microsites having high microbial activity, such as around organic matter particles or possibly in the plant root rhizosphere (Crowley et al., 1987).

The critical value required for plant growth is between 10^{-9} and 10^{-4} M Fe(III), a concentration that is two orders of magnitude higher than that expected in aerated soil solutions at equilibrium for the sum of all inorganic hydrolysis species of Fe^{3+} (Siebner-Freibach et al., 2003). In addition, most microorganisms require micromolar (10^{-6} M) concentrations of Fe to support growth. Thus, in aerobic environments, microorganisms are faced with a

discrepancy of ~10 orders of magnitude between available Fe (~10^{-17} M) and their metabolic requirement for Fe (Hersman et al., 2000).

The low solubility of inorganic Fe in neutral and alkaline soils has stimulated the search for the natural mechanisms by which Fe is made available to higher plants. Soil chemists have implicated natural organic chelates in the mobilization of Fe in soils (Powell and Szaniszlo, 1982). Iron concentration in soil solution is often higher than that expected from chemical equilibria equations of soil Fe minerals. This enhancement is partially ascribed to the presence of organic molecules exhibiting various extents of Fe-chelation abilities (Siebner-Freibach et al., 2004). The mobile forms of Fe, whose concentration in the soil solution may be between 1 and 10 μM, may be utilized provided the root can separate the Fe from the ligand at or very close to the site of uptake (Uren, 1984). Under conditions of Fe limitation, O'Connor et al. (1971) stated that at neutral to basic soil pH, inorganic Fe levels available for transport to the plant roots by both mass flow and diffusion are below plant requirements. It appears, therefore, that for plants growing in such soils, formation of soluble organic chelates is important in supplying Fe. These compounds include root exudates, natural chelators originated from the degradation of soil organic matter, metabolic products of microorganisms, or Fe chelate fertilizer added to the soil (Jurkevitch et al., 1988). Moreover, soil microbial activity may influence the growth of higher plants by various processes such as mineralization of organic N and S compounds, nitrification and sulfurication and also by the microbial production of chelates which solubilize Fe (Rroco et al., 2003). Among the most important of naturally-occurring, biosynthetic chelates are the great number and variety of siderophores produced by microbes and the

relatively few phytosiderophores produced by “Fe-efficient” grasses (Crowley et al., 1991).

Studies of Crowley et al. (1988, 1991) have shown that the production of chelating compounds by microorganisms increases Fe solubility in the rhizosphere and hence increase plant Fe acquisition. Bacterial and fungal siderophores and other chelating metabolites are assumed to serve as major sources of plant-available Fe in the rhizosphere (Masalha et al., 2000). Numerous prior studies have shown that a variety of microbial siderophores provide Fe to both graminaceous and dicotyledonous plants, including ferrichrome A for duckweed and tomato, ferrioxamine B (FOB) for cucumber (Powell and Szaniszlo, 1982), FOB or rhodotorulic acid (RA) for oat, tomato, sorghum, and sunflower, ferrichromes for oat, agrobactin for bean and pea, and pseudobactin for peanut, cotton and sorghum (Wang et al., 1993). Fe-rhizoferrin of *Rhizopus arrhizus* was found to be as effective as FeEDDHA for the remedy of chlorosis in tomato and provided Fe for barley and corn by ligand exchange with phytosiderophores. In addition to ligand exchange, uptake of Fe from Fe-chelate complexes can occur directly or after microbial degradation of the organic chelate by microorganisms in the rhizosphere which then releases the mineral Fe for subsequent uptake (Chen et al., 1998). It is now generally accepted that the transport of Fe across the plasmamembrane is closely linked to Fe^{III} reduction. Ferrous iron is then taken up and passes through a specific channel of the plasmamembrane (Mengel and Kirkby, 2001).

Soil Biota has the ability to alter the chemistry of soil environments through the synthesis of organic acids as well. Plants and associated microorganisms synthesize organic acids to detoxify the adjacent soil solutions or to enhance the fluxes of nutrients to the cell (Holmen and Casey, 1996). Naturally

occurring organic acids were observed to accelerate the dissolution of oxide and aluminosilicate minerals in both the laboratory and the field (Eick et al., 1999). Bacteria, lichens, and fungi in soils produce organic acids such as lactic, succinic, oxalic, citric, acetic and α-keto acids. These dissolved acids and other organic exudates can affect pH in weathering solutions and thereby promote or inhibit mineral dissolution. The dissolved organic molecules can also form surface complexes that affect weathered mineral surface characteristics by ligand-promoted dissolution or through inhibition of reactivity. Alternatively, organic ligands can complex cations in solution, inhibiting precipitation or lowering the saturation index in solution and enhancing dissolution indirectly (Kalinowski et al., 2000). In addition, the foremost attribute of soil humic substances and primarily to the fulvic fractions is that they can form complexes with metal cations such as Fe and mobilize them from solid particles in the soil to the root surface (Olmos et al., 1998), even under calcareous soil conditions.

The physiological requirements of Fe(III) by plants and the microorganisms, and the extreme insolubility of Fe-oxides at soil conditions ($4<pH<9$), makes siderophore secretion an important avenue for Fe acquisition by cells (Holmen and Casey, 1996). These siderophores, by definition, are more Fe(III)-specific and show higher association constants than low molecular weight organic acids such as oxalic acid (Kalinowski et al., 2000). Siderophores fall into several broad classes including the catecholates, hydroxamates, and amino carboxylate molecules. The hydroxamate siderophores are particularly interesting because they are highly specific for Fe(III); the complexation constants for ferric Fe are exceedingly high. Therefore, the hydroxamate siderophores will have a much larger effect on the cycling of Fe in soils than more conspicuous plant exudates, such as

oxalate, that are not as highly specific (Holmen and Casey, 1996). It has been shown that hydroxamate siderophores effectively chelate Fe over a wide range of pH and can provide Fe to plants at high pH (Reid et al., 1985). More than 200 siderophore compounds have been isolated (Hersman et al., 1995). Siderophores have been found to promote Fe solubilization from various soil minerals. The concentrations of siderophores in soil environments range quite broadly. Siderophore concentrations that were high enough to positively affect plant nutrition were found in soil extracts. In soils enriched with macronutrients as well as in the rhizosphere, which is enriched with plant exudates and organic matter, the concentrations of hydroxamate siderophores were found to be even higher (Siebner-Freibach et al., 2003, 2004) and in equilibrium with a much larger adsorbed pool which suggests resistance to both leaching and microbial decomposition (Cline et al., 1982). Hydroxamate siderophores concentrations are 10 to 50 times more abundant in the rhizosphere than in bulk soil (Cline et al., 1983). However, not all siderophores may be used by plants, and individual plant species and varieties have different abilities to utilize specific siderophore types (Crowley et al., 1988).

Lime-induced chlorosis is a common feature in fruit crops grown on calcareous soils. The extent of chlorosis and the resulting depression of yield are affected by many factors including the supply of water and nutrients, but the amount and properties of the soil carbonates with their associated control of pH and bicarbonate concentration has the most direct influence on the supply and utilization of Fe by crops (Mashhady and Rowell, 1978; Mengel et al., 1984). Citrus cultivation requires the use of rootstocks with high tolerances toward different plant pathogens and environmental stresses. One environmental stress that is common in many citrus growing regions is

alkaline, high carbonate soils with inadequate supplies of soluble inorganic Fe. It is generally believed that these soil conditions lead to Fe chlorosis in citrus, which left uncorrected, result in impaired plant growth and fruit production. Many of the commonly used citrus rootstocks are susceptible to Fe-deficiency. This is especially true of those rootstocks (mainly citranges) derived from the trifoliate orange (*Poncirus trifoliata*). There are, however, a small number of rootstocks that demonstrate significantly higher tolerance to low-Fe stress. These include mainly *Citrus macrophylla*, *Citrus jambhiri*, and several other rough lemon varieties. Yet these rootstocks are highly susceptible to other citrus diseases, and are used less frequently than the citranges and related rootstocks (Manthey et al., 1993).

The conventional approach to solving the problem by Fe supplementation is beset by high cost and inefficient application of Fe amendments (Hamze et al., 1986). Moreover, it was recently reported that synthetic chelates (i.e., EDDHA) can be leached out of the rootzone to deep soil layers contiguous to the water table, which might impose environmental and health hazards (Rombola et al., 2002). The introduction of certain plant species into the fields of fruit trees grown on calcareous soils might be an effective orchard floor management for improving the Fe nutritional status of these trees in comparison with those grown on bare soils.

Whatsoever the Fe solubility conditions in soils are, the most important factor for plant nutrition is the concentration of total soluble Fe, whether in its inorganic form or in its organically-bound form, in the soil solution because it controls the Fe transfer to plant roots by mass flow and diffusion. To our knowledge, until now no data are available about the total soluble Fe concentration in the soil solution and its relation to soil characteristics. This situation is due to the fact that concentrations of soluble Fe in the soil

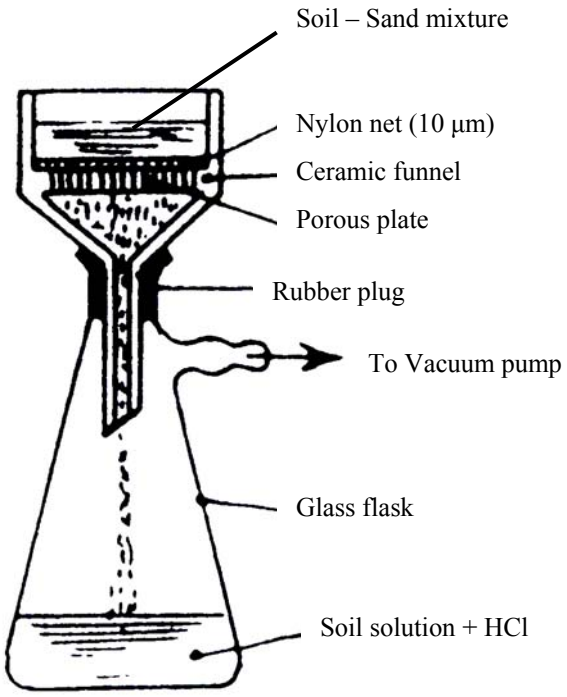
solution of some soils are low and the fact that obtaining soil solution meets with difficulties. Therefore, the objectives of this research were:

1. To develop an appropriate technique, the Buchner Funnel Technique (BFT), to nondestructively (temporal wise) and continuously extract the soil solutions of chemically and physically different soils to investigate the following:
    a. The concentration of the total soluble Fe.
    b. The relative Fe buffer power.
    c. The percentage of the organically-complexed Fe.
    d. The influence of soil microbial activity on the total soluble Fe concentration.
2. To adopt a reliable and reproducible analytical method for analyzing for the total soluble Fe concentration in the soil solutions.
3. To investigate the influence of the presence of grass and dicot plant species (*Festuca ovina*, *Festuca rossa* cv. *tricophylla*, *Poa nemoralis* and *Trifolium subterraneum*), grown on a calcareous soil, on the Fe nutritional status of Swingle Citrumelo (*Citrus paradisi* Macf. x *Poncirus trifoliata*), a susceptible citrus root stock to lime-induced Fe deficiency.

2. Materials and Methods:

2.1 General Description of the "Buchner Funnel Technique" (BFT):

The "Buchner Funnel Technique" (see the sketch below) was developed to nondestructively (temporal wise) and continuously extract the soil solutions under vacuum (-50kPa). In order to improve the extractability of particularly the heavy soils, soils were mixed with acid-washed sand at a ratio of 1:1 (100 g sand : 100 g air-dry soil). Nylon nets (10 μm pore diameter) were placed at the bottom of each Buchner funnel to prevent the suction of soil particles. Soils were incubated in an oven at 20ºC and daily watered up to 80% of its maximum water holding capacity and before and after each collection.



Because different soils varied in their textures, it was not possible to collect more than 1 ml soil solution particularly from clayey soils, therefore, 1 ml

soil solution (determined by weight) was collected in glass flasks which contained already 1 ml 0.5 M HCl. Thereafter solutions were immediately filtered through 0.2 μm disposable filter apparatus or centrifuged for 5 minutes at 13,000 rpm and analyzed for the total soluble Fe using the ferrozine-hydroxylamine hydrochloride method (see below).

All the tools and materials used in the conducted experiments were thoroughly soaked (washed) in 2 M HCl and rinsed with bi-distilled water before each use.

2.2 The Ferrozine-Hydroxylamine Hydrochloride Method:

This method was used to analyze for the total soluble Fe in the extracted soil solutions according to Viollier et al. (2000). All reagents were prepared in bi-distilled water.

2.2.1 Reagents:

1. Ferrozine (complexing agent) (monosodium salt hydrate of 3-(2-pyridyl)-5,6-diphenyl-1,2,4-triazine-*p,p'*-disulfonic acid) (FW: 492.47, 97%) 10^{-2} mol L^{-1} prepared in an ammonium acetate (CH_3COONH_4) solution of 10^{-1} mol L^{-1}.
2. Hydroxylamine hydrochloride (reducing agent) ($H_2NOH.HCl$, FW: 69.49, 99.9999%) 1.4 mol L^{-1} prepared in a solution of analytical grade hydrochloric acid of 2 mol L^{-1}.
3. Ammonium acetate (buffer) (FW: 77.08): A 10 mol L^{-1} solution adjusted to pH 9.5 with a solution of ammonium hydroxide (NH_4OH, 25%).
4. Standards were prepared in acidified bi-distilled water from 2 mM Fe(II) stock solution, which was prepared in 0.5 M HCl using $(NH_4)_2Fe(SO_4)_2.6H_2O$ (FW: 392.14) as Fe(II) source. The blank solution was acidified bi-distilled water.

The hydroxylamine hydrochloride was used as a reducing agent because it is a highly reactive Fe reductant that rapidly reduces any Fe(III) in solution (Holmen and Casey, 1996) according to the following reaction:

$$4Fe^{3+} + 2NH_2OH \rightarrow 4Fe^{2+} + N_2O + 4H^+ + H_2O$$

The 50 µL ammonium acetate buffer (pH 9.5) was enough to neutralize the amount of HCl added from the 0.5 M HCl used to acidify the soil solutions and from the hydroxylamine hydrochloride (the pH was checked with a pH meter provided with a pH micro-electrode).

2.2.2 Procedure:

The successive additions of the reagents were as follows:

1. 100 µL of ferrozine in 1-cm path length spectrophotometric cell.
2. 700 µL of filtered or centrifuged soil solution or standard solution.
3. 150 µL of hydroxylamine hydrochloride. All reagents were totally mixed.
4. Wait 10 minutes to complete the reaction (the reduction of iron to ferrous iron and the formation of the colored ferrozine-Fe(II) complex).
5. 50 µL of ammonium acetate buffer. All reagents were again totally mixed.
6. The absorbance at 562 nm was carried out using NOVASPEC II spectrophotometer. The absorbance values were corrected by multiplying them by 2 (because 1 ml soil solution was mixed with 1 ml 0.5 M HCl).

2.3 The Chemical and Physical Properties of the studied Soils:

Each air-dry soil was crushed and sieved through a 2-mm sieve.

Table 1. Chemical and physical properties of the studied 32 soils.

Soil Sampling Site (abbreviation)	Parameter							
	pH ($CaCl_2$)	$CaCO_3$ (g kg^{-1})	DTPA-Fe (mg kg^{-1})	O.C.* (g kg^{-1})	DOC* (g kg^{-1})	Sand (g kg^{-1})	Silt (g kg^{-1})	Clay (g kg^{-1})
1. Leinach (L)	7.65	523.8	6.2	26.6	0.229	5.5	404	250
2. Wuerzburg (W)	7.66	518.6	2.9	6.2	0.133	39.0	294	350
3. Wuerzburg/Stein (WS)	7.68	271.1	4.3	36.1	0.265	330.8	280	190
4. Uphusen (U)	7.00	63.2	137.8	39.9	0.362	56.0	816	50
5. Muschelkalk/Muehlhausen (M)	6.76	53.4	26.8	25.6	0.178	33.0	449	460
6. Keuper/Oberschwappach (O)	6.40	21.3	32.2	23.9	0.186	210.0	323	430
7. Langgoens Garten (L)	7.16	14.7	13.8	36.3	0.298	165.0	660	130
8. Soil** (H)	6.26	13.4	12.3	22.0	0.142	672.0	188	110
9. Ofenloch II (OII)	7.24	11.3	10.3	12.0	0.223	19.0	732	230
10. Trebur (T)	7.07	10.8	49.7	48.0	0.481	88.0	328	530
11. Eckekreuz (E)	7.27	6.9	16.0	10.6	0.182	42.0	713	230
12. Keuper/Koenigsberg (K)	6.73	6.9	49.6	18.9	0.117	145.0	422	410
13. Ofenloch III (OIII)	7.07	5.7	15.6	10.9	0.258	33.0	723	230
14. Gefaessversuchsst. Giessen (G)	6.53	5.4	33.6	34.8	0.362	433.0	399	130
15. Simeskopf (S)	6.75	4.6	48.3	17.4	0.265	251.0	399	330
16. Leopold (Le)	6.57	1.5	25.8	11.9	0.256	309.0	448	230
17. Muschelkalk/Am Stein (St)	6.29	1.5	37.0	16.9	0.156	272.0	450	260
18. Muensterstr. I (MI)	6.94	1.4	30.6	13.1	0.236	79.0	677	230
19. Feld E (F)	6.35	1.4	25.3	5.9	0.108	848.0	115	30
20. Emsteck (Em)	5.66	1.4	46.6	18.4	0.227	194.0	757	30
21. Schoene Aussicht (Sc)	6.69	1.3	46.8	12.3	0.212	36.0	721	230
22. Pfaffengraben II (PII)	7.09	1.2	60.3	11.4	0.235	35.0	723	230
23. Rand E (RE)	6.59	1.2	8.9	9.0	0.151	844.0	16	130
24. Acker an der Grenze Rodenbach (Ro)	6.32	1.0	52.8	19.5	0.205	592.0	258	130
25. Rastede (Ra)	5.77	1.0	210.9	22.5	0.264	772.0	175	30
26. Kirchhain (Ki)	4.54	0.9	135.4	28.5	0.455	38.0	403	530
27. Lang WieseRodenbach (RM)	6.53	0.8	99.5	17.1	0.244	646.0	206	130
28. Essen (Es)	4.95	0.8	55.4	20.8	0.139	808.0	141	30
29. Gladbacherhof (Gl)	7.25	0.6	35.6	9.3	0.454	36.0	724	230
30. Dorfprozeltener Predigtstuhl (P)	5.90	0.6	30.4	11.9	0.096	879.0	59	50
31. Klein-Linden (KL)	6.23	0.3	35.2	2.5	0.092	437.0	380	180
32. Grossgerau (Gr)	7.11	0.2	36.7	13.1	0.131	794.0	63	130

*O.C.: Organic carbon, DOC: Dissolved organic carbon

**The region was not identified.

The pH was determined with 0.01 M $CaCl_2$ solution according to Thomas (1996). The DTPA-extractable Fe was determined according to Lindsay and

Norvell (1978). The organic carbon % was calculated as the difference between the carbon in total carbon (Nelson and Sommers, 1996) and $CaCO_3$ (Loeppert and Suarez, 1996). The DOC was extracted by the electro-ultrafiltration (EUF-Nemeth, 1979) and measured by the Autoanalyzer II (Goulden and Brooksbank, 1974). Soil texture was determined according to (Gee and Bauder, 1986). The Silt fraction was calculated as the following: (100 – [Clay % + Sand % + Carbonate % + OC %]).

2.4 The Determination of Total Soluble Fe in the Soil Solution and the Fe Buffer Power of 32 Soils:

The Buchner Funnel Technique (BFT) and the ferrozine method were used in order to collect the soil solutions and to analyze for the total soluble Fe in the soil solutions of the above mentioned soils as well as to investigate the Fe buffer power of each soil. The Fe buffer power means the ability of each soil to maintain a constant Fe concentration in its solution after three consecutive extractions, which might give an indication about the type of Fe mineral with which the soluble Fe is in equilibrium, the ability of each soil to provide organic chelators for Fe and the amount of sorbed chelated Fe or the amount of the sorbed chelators, which might act as a reservoir for slow-released Fe. In this experiment 32 soils were used. Soils were incubated for 3 days (see the general description of the BFT) and the first collection of the soil solutions was carried out on the fourth day. A total of three consecutive collections were conducted. Each soil was replicated 5 times.

2.5. The Influence of Microbial Activity on the Concentration of the Total Soluble Fe in the Soil Solution:

The same methodology was employed in this experiment. To stimulate the soil microbial activity the following carbon sources were added to each soil:

a. Glucose (anhydrous) ($C_6H_{12}O_6$, FW: 180.2) was added at a rate of 15 g kg^{-1} soil.
b. Starch (soluble) $(C_6H_{10}O_5)_n$, FW: $(162.14)_n$ was added at a rate of 10 g kg^{-1} soil.
c. Cellulose (powder) was added at a rate of 5 g kg^{-1} soil.

These carbon sources were wet digested and analyzed for Fe to make sure that no Fe was added to the different soils. The control treatment was the same soils without carbon additions. Each treatment (with and without carbon additions) was replicated 3 times. For the collection of the soil solutions to analyze for total soluble Fe, the BFT was employed with and without carbon additions. Each treatment was replicated 4 times.

The microbial activity was measured using the gas chromatography (Perkin Elmer, Autosystem XL, ARNEL) and finally expressed as μg CO_2 per g soil per hour. A known weight from each replicate was incubated in a tightly closed glass bottle and the concentration of the evolved CO_2 was measured every two hours and a total of five measurements were made. Carbon dioxide evolution was used as an index for soil microbial activity because it is the best index for the whole metabolic activity of soil microbial populations.

2.6. The Determination of the Percentage of the Organically-Complexed Fe:

To measure the amount of the organically-complexed Fe present in each soil solution, the BFT combined with the ferrozine method were used. After the

collection of the soil solution, the concentration of the total soluble Fe was determined (using 700 µL aliquot) and the rest of the soil solution (1300 µL) was slowly passed through a strong cation-exchange column. Amberlite IR-120 (plus) (sodium form) was used as a strong ion-exchange resin. A total of 4 grams of Amberlite were used per column, which was sufficient enough to retain a high Fe(II) concentration. Each column consisted of a glass ball, glass wool (at the bottom of the column) and the cation-exchange resin. Each column was rinsed many times with bi-distilled water before being used. The soil solution after being passed through the cation-exchange column was analyzed for the total soluble Fe. The percentage of the organically-complexed Fe was calculated as follows:

% Organically-complexed Fe = ([Fe] after separation / [Fe] before separation) X 100

2.7. The Effect of Intercropping Swingle Citrumelo with Graminaceous and Dicotyledonous Plant Species on its Fe Nutritional Status:

One year-old Swingle citrumelo plants were transferred into plastic pots (2 plants per pot) end of February/2003. Before being transferred, shoots as well as roots were pruned to get a homogeneous vegetative growth. These plants were grown on a soil-sand mixture prepared by mixing the “Faenza” soil, the calcareous soil and sand at a ratio of 3:1:2, respectively. The “Faenza” and the calcareous soil have been previously sieved through a 10-mm sieve. The chemical and physical properties of the “Faenza” and the calcareous soils are shown in table 2.

Table 2. Chemical and physical properties of the "Faenza" and calcareous soils.

Parameter	"Faenza" Soil	Calcareous Soil
Sand (g kg^{-1})	30	500
Silt (g kg^{-1})	590	230
Clay (g kg^{-1})	380	270
Total Carbonate (g kg^{-1})	220	729
Active Carbonate (g kg^{-1})	110	120
pH	7.8	8.2
O.M. (g kg^{-1})	17	-
Exchangeable Na (meq 100 g^{-1})	0.2	-
Exchangeable K (meq 100 g^{-1})	0.5	-
Exchangeable Ca (meq 100 g^{-1})	23.5	-
Exchangeable Mg (meq 100 g^{-1})	4	-
Fe (mg kg^{-1})	20	7.8
Zn (mg kg^{-1})	1	5.9
Cu (mg kg^{-1})	-	59.1
Mn (mg kg^{-1})	5	-
P (mg kg^{-1})	4	-

Pots were filled with 25 kg of the soil-sand mixture. At the beginning of the experiment, plants were grown in a glasshouse (18-22ºC) in the experimental station of the University of Bologna (Italy). Under these conditions, the resumption of growth was fast and new vegetative growth was observed. Young leaves had an average of 45.6 SPAD unit (13/03/2003), however, later on youngest leaves showed Fe deficiency chlorosis. On 11th of March, plants were again pruned up to 8 young branches. Plants were, thereafter, transferred into a plastic greenhouse the 26th of April to avoid the tremendous increase in temperature inside the glasshouse. Treatments were as follows:

1. Control (Swingle citrumelo plants without grass or dicot plant species; grown on a bare soil).
2. Soil treated with Fe-chelate (FeEDDHA).

3. Plants intercropped separately with three perennial grass species (*Festuca ovina*, *Festuca rossa* cv. *tricophylla* and *Poa nemoralis*).
4. Plants intercropped with a dicot legume plant species (*Trifolium subterraneum*).
5. Plants treated with vivianite.

Each treatment was replicated 5 times. Plants were adequately irrigated and fertilized. Each intercropped Swingle citrumelo plant received during the period of the experiment an amount of 3.85 g N (in form of NPK 20-9-10 + Mg and S fertilizer; 11.5% NH_4-N and 8.5% NO_3-N) while each plant grown on a bare soil received 2.55 g N (Note: the annual dose is 10 g N $tree^{-1}$ $year^{-1}$). The grass species (*Festuca ovina*, *F. rossa* and *Poa nemoralis*) and the *T. subterraneum* were sown on the 13th and 14th of March. Three seeds per cm^2 were used (3825 seeds per pot). The *T. subterraneum* started to germinate and emerge between the 16th and 17th of March. Emergence of the three grass species was observed between the 20th and 22nd of March.

On the 31st of March, 100 ml per plant of Sequestrene NK 138 Fe solution were injected into the pots of the FeEDDHA treatment at a rate of 0.2 g L^{-1} (as a starting dose) and thereafter this was repeated in order to completely cure Fe deficiency chlorosis as follows:

Date	Quantity (ml) per plant	Concentration (g L^{-1})
11/04/2003	100	0.4
05/05/2003	100	1.0
13/06/2003	100	0.5
28/06/2003	100	1.0

On the 4th of April vivianite (90 g vivianite suspension L^{-1}) was injected into the pots of vivianite treatment in two points around each plant (10-15 cm deep) at a rate of 20 ml per plant. Vivianite ($Fe^{II}_3(PO_4)_2.8H_2O$) was prepared according to Rosado et al. (2002) as follows: 25 g of MAP ($NH_4H_2PO_4$) was

dissolved in 1 L then 75 g of $FeSO_4.7H_2O$ were gradually added while stirring. The second dose of vivianite (40 ml per plant) was added three weeks later. On the 8th of May, all grasses as well as the *T. subterraneum* were mowed. Thereafter, these grass and dicot plant species were allowed to grow. The re-growth of the *T. subterraneum* was weak.

The parameters that have been measured through out the experiment were:

- Chlorophyll concentration measured by chlorophyll meter (SPAD) (SPAD 502 Minolta Corp., Osaka, Japan) of Swingle citrumelo plants of the first 3 to 4 fully expanded young leaves.
- Leaf area (length and width) of Swingle citrumelo plants.

At harvesting (30/07/2003) the following parameters were measured:

- Leaf area, leaf fresh and dry weight of Swingle citrumelo plants.
- Chlorophyll concentration (Arnon, 1949).
- Leaf number and branch length.
- Young shoots' (those developed during the last 2 months of the experiment; June and July) fresh and dry weight as well as trunk and old shoots fresh weight.
- Leaf Fe concentration (Rosopulo et al., 1976) was measured by the AAS.
- Total root and fine roots ($\Phi \leq 2$-mm) fresh and dry weight.

2.8 Statistical Analysis:

Standard deviations were calculated using Excel and the mean separation was performed by the t-test ($\alpha = 0.05$ and 0.01) using Excel to compare between carbon-amended soils and unamended soils for both soil microbial activity and total soluble Fe concentration in soil solutions.

3. Results:

3.1 Spectrophotometric determination of total soluble Fe in the soil solution by the ferrozine-hydroxylamine hydrochloride method:

In order to test the reliability and reproducibility of this analytical method, the following tests have been conducted:

1. Test 1: Two sets of standard solutions (0-40 µM FeII) were prepared. The first set was prepared from the 2 mM Fe(II) stock solution and the second set was similarly prepared except that 0.5 ml soil solution was added to these standard solutions. To detect any possible interferences with the ferrozine method due to the presence of any possible substances or compounds in the soil solution, the slopes of the calibration curves of these two sets were compared as shown in Fig. 1.

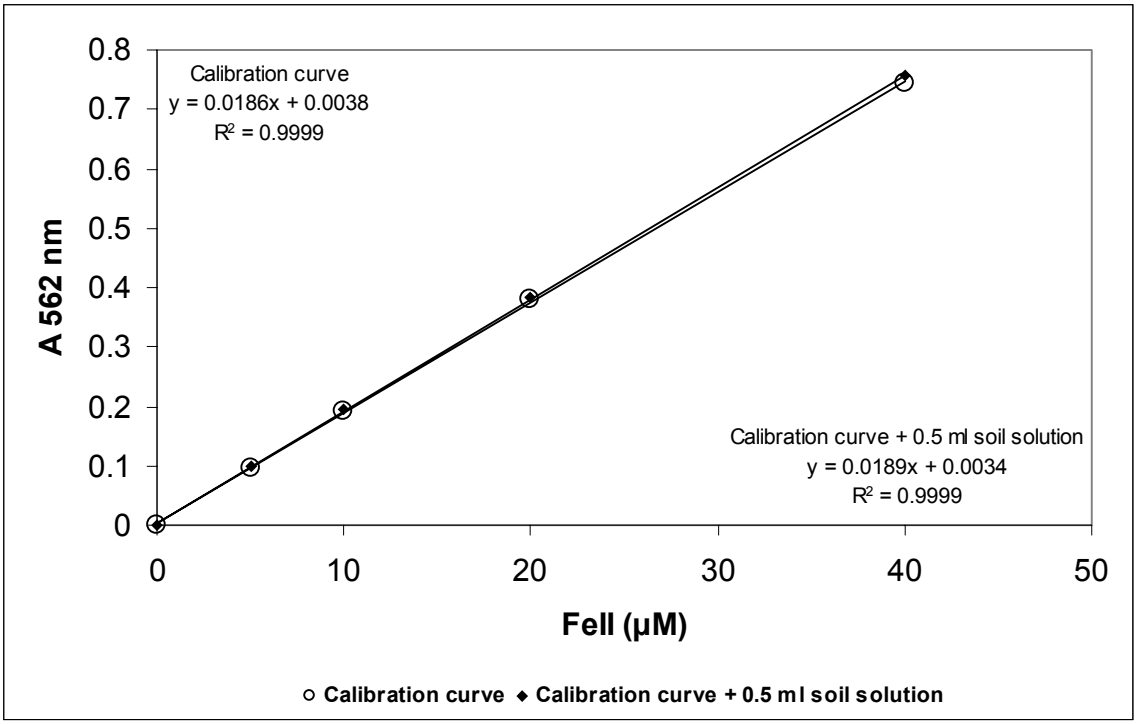


Figure 1. The calibration curve of the standard solutions prepared from 2 mM FeII stock solution or 2 mM FeII plus 0.5 ml soil solution.

The slopes of the two calibration curves are almost identical with constants of 0.0186 and 0.0189, respectively, which proved that the ferrozine method is a reliable analytical method. This method was not influenced by the possible presence of different substances or compounds in this soil solution.

2. Test 2: Two soil solution samples from different soils were mixed together at a ratio of 1 : 1 and re-analyzed for total soluble Fe. Before mixing the two soil solutions, the quantity used in the analysis was 700 μL from each soil solution. To conduct the test, 350 μL each (total of 700μL) were mixed together and re-analyzed for Fe. If the differences in the chemical composition of the different soil solutions interfered with the ferrozine method, we would expect discrepancy between the measured Fe concentration after mixing the two soil solutions and the theoretically calculated Fe concentration (([Fe] in soil solution no. 1 / 2)+([Fe] in soil solution no. 2 / 2)). However, the following results shown in table 3 proved again the reliability of the ferrozine method.

Table 3. A comparison between the measured and calculated Fe concentrations (test 2).

Test Number	Measured Fe concentration (μM)	Calculated Fe concentration (μM)	STD
1	30.2	29.8	0.3
2	20.1	20.3	0.1
3	22.0	22.2	0.1
4	20.0	20.0	0.0
5	26.9	26.6	0.2
6	6.0	6.2	0.2
7	4.5	4.4	0.1
8	5.0	5.1	0.1
9	6.3	6.3	0.0
10	6.0	5.9	0.1

3. Test 3: To further test the ferrozine method, 350 µL of 10 µM FeII was added to 350 µL of a soil solution and analyzed for Fe concentration. The theoretically calculated Fe concentration (([Fe] in soil solution/2) + 5 µM FeII) was compared with the measured Fe concentration. This test proved that the ferrozine method is definitely reliable according to the following results presented in table 4.

Table 4. A comparison between the measured and calculated Fe concentrations (test 3).

Test Number	Measured Fe concentration (µM)	Calculated Fe concentration (µM)	STD
1	6.5	6.4	0.1
2	8.0	8.0	0.0
3	8.9	8.9	0.0
4	6.2	6.2	0.0
5	5.9	5.8	0.1
6	6.7	6.8	0.1
7	6.6	6.6	0.0
8	7.4	7.4	0.0
9	6.3	6.3	0.0
10	11.4	11.3	0.1
11	11.5	11.5	0.0
12	7.4	7.3	0.1
13	13.8	13.9	0.1

3.2 The determination of total soluble Fe concentration in the soil solution and the Fe buffer power in 32 chemically and physically different soils:

3.2.1 The concentration of total soluble Fe in the soil solutions:

The concentration of the total soluble Fe in the soil solutions of 32 different soils (for chemical and physical properties, see materials and methods) was

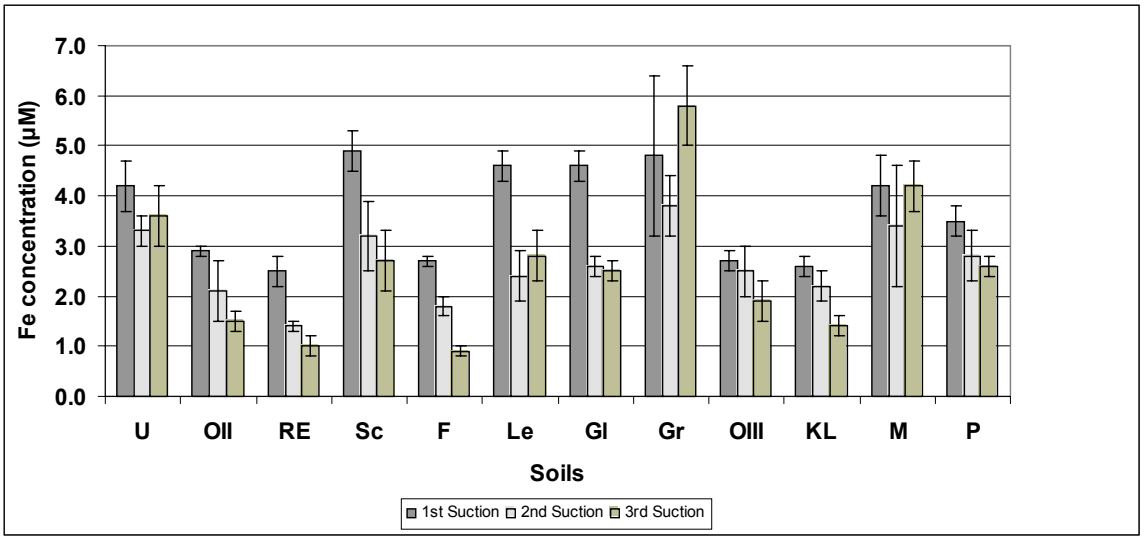
found to be in the micromolar range. The total soluble Fe concentration in the soil solutions of the first collection (Fig.2a and 2b) ranged from 2.5 to 188 µM for Rand E (RE) and Lang Wiese Rodenbach (RM), respectively. Surprisingly, the concentration of total soluble Fe in the soil solutions collected from three calcareous soils (pH 7.66 - 7.68) (Wuerzburg (W), Wuerzburg/Stein (WS) and Leinach (L)) ranged from 22 to 25.5 µM, which was similar or even much higher than that of other neutral and acidic soils (Fig. 2b). In the acidic pH range (pH < 7.0), the total soluble Fe concentration ranged from 2.5 to 188 µM for Rand E (RE) (pH 6.59) (Fig. 2a) and Lang Wiese Rodenbach (RM) (pH 6.53) (Fig. 2b), respectively. The Fe concentration in the most acidic soil (Kirchhain (Ki), pH 4.54) (Fig.2b) was 23.9. In the neutral and alkaline/calcareous pH range (pH ≥ 7.0), the total soluble Fe concentration ranged from 2.7 to 33 µM, for Ofenloch III (OIII) (pH 7.07) (Fig.2a) and Eckekreuz (E) (pH 7.27) (Fig. 2b), respectively.

3.2.2 The relationship between total soluble Fe concentration in the soil solution and soil chemical and physical properties:

No correlations were detected between the total soluble Fe concentrations in the soil solutions and the soil chemical and physical properties of the studied soils. Unexpectedly, the total soluble Fe in the soil solutions did not correlate with either the soil pH or the soil organic carbon, dissolved organic carbon, clay content and calcium carbonate content. The total soluble Fe concentration in the soil solution was weakly found to be correlated with the soil DTPA-extractable Fe ($r = 0.54$) after disregarding the Fe concentration in the soil solution collected from the Lang Wiese Rodenbach soil.

3.2.3 The Fe buffer power of the 32 different soils:

To investigate the ability of different soils to maintain a constant concentration of total soluble Fe in their solutions, a total of three consecutive collections have been conducted. Eleven soils (pH 4.54 – 7.68) (Fig. 2a and 2b) showed high Fe buffer power. Surprisingly, the three studied calcareous soils were among these eleven soils (Fig. 2b). The total soluble Fe concentrations in the soil solutions of the Wuerzburg soil (W) were 25.2, 39.8 and 27.2 µM, respectively, for the 1st, 2nd and 3rd collections. The Wuerzburg/Stein (WS) and Leinach (L) soils (calcareous soils) showed the same high Fe buffer power as the Wuerzburg soil. The Fe concentrations in the soil solutions of the Wuerzburg/Stein soil were 24.9, 20.1 and 24.0 µM and those of the Leinach soil were 22.0, 17.5 and 22.7 µM, respectively, for the 1st, 2nd and 3rd collections.



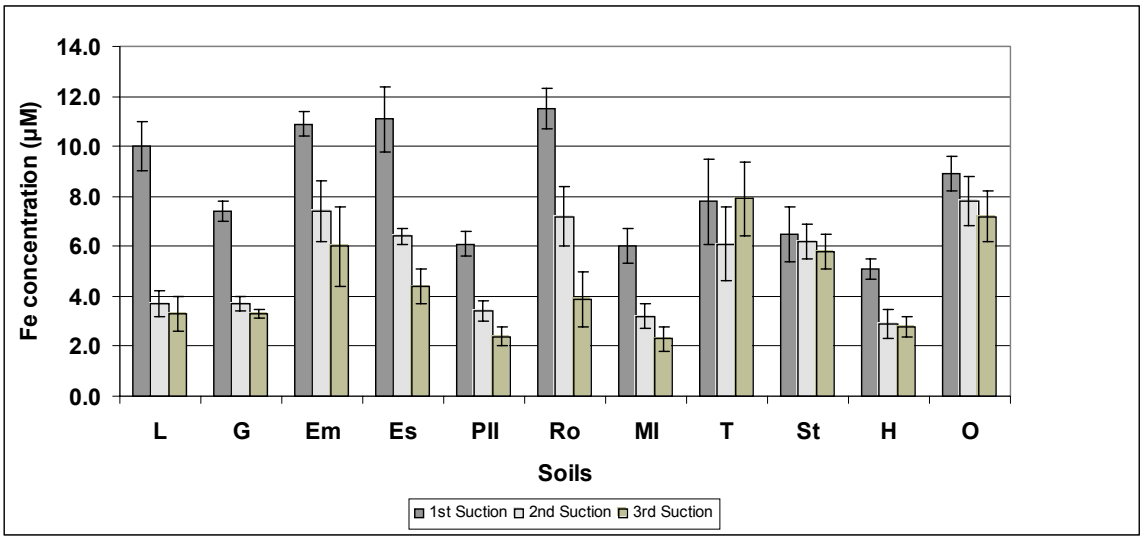
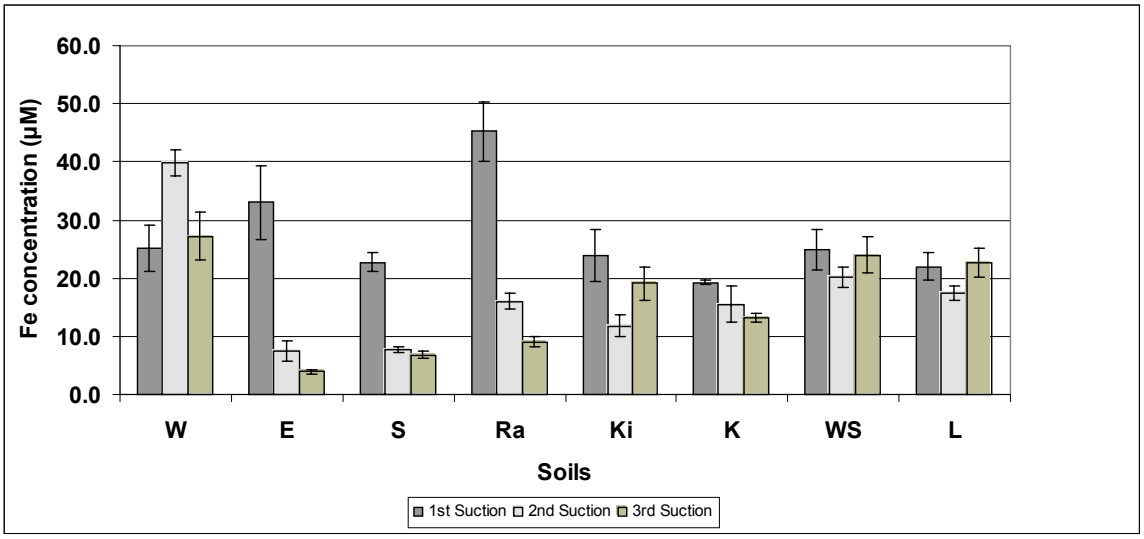


Figure 2a. Total soluble Fe concentrations (low concentrations) in soil solutions collected by three consecutive suctions.



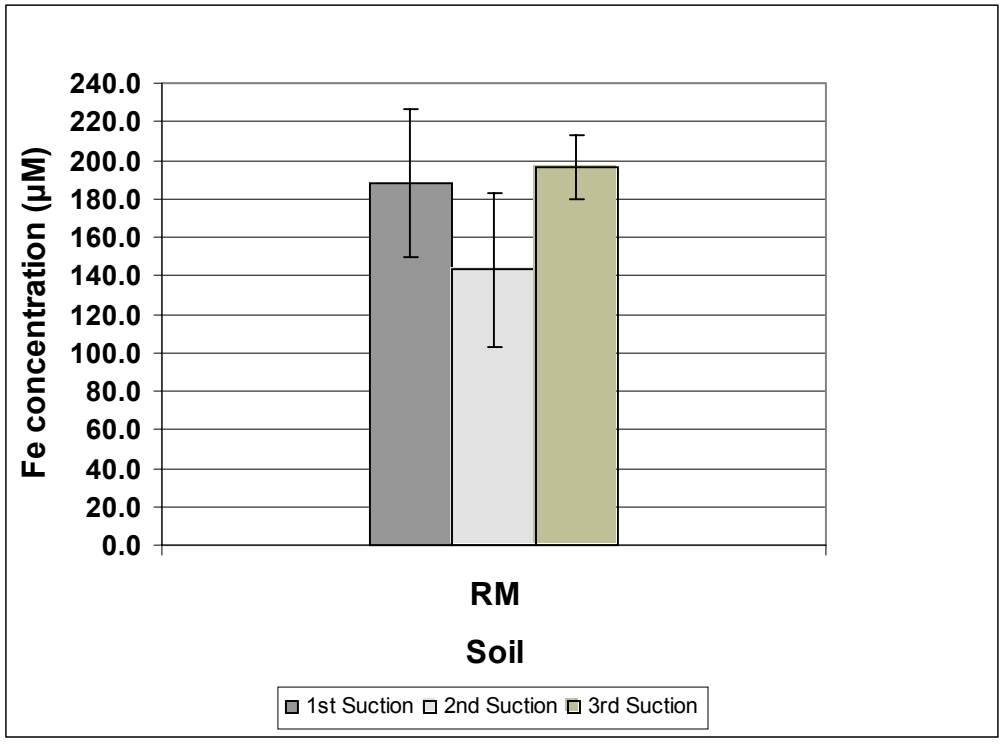


Figure 2b. Total soluble Fe concentrations (high concentrations) in soil solutions collected by three consecutive suctions. Lower part of the figure is meadow soil of Rodenbach.

3.3 The chemical form of the soluble Fe in the soil solution of 30 different soils:

The percentage of the organically-complexed Fe in the collected soil solutions ranged from 39.3 (Rand E soil) to 90.7% (Gladbacherhof) (Table 5).

Table 5. Organically-complexed Fe (%) in the soil solutions of 30 different soils.

Soil	Organically-complexed Fe (%)	STD
Wuerzburg	83.7	3.1
Eckekreuz	87.7	2.1
Langgoens Garten	85.3	2.5
Uphusen	84.7	1.5
Gefaessversuchsst. Giessen	71.7	1.3
Lang Wiese Rodenbach	66.4	4.5
Emsteck	60.8	4.8
Essen	55.5	5.5
Ofenloch II	80.0	2.7
Rand E	39.3	5.4
Pfaffengraben II	87.3	3.1
Simeskopf	75.0	3.6
Acker an der Grenze Rodenbach	71.7	3.1
Rastede	69.7	2.1
Schoene Aussicht	76.7	3.1
Muensterstr. I	81.7	2.5
Feld E	68.0	2.0
Leopold	77.7	2.5
Kirchhain	48.0	5.6
Gladbacherhof	90.7	2.5
Trebur	88.0	2.7
Grossgerau	84.3	2.5
Ofenloch III	88.7	2.1
Klein-Linden	66.7	1.5
Muschelkalk/Am Stein	76.0	2.0
Muschelkalk/Muehlhausen	80.3	2.5
Soil*	75.3	1.5
Dorfprozeltener Predigtstuhl	63.3	1.5

Keuper/Oberschwappach	73.3	1.5
Keuper/Koenigsberg	76.3	2.5

*The region was not identified.

Of particular interest is finding that the organically-complexed Fe (%) was found to be correlated with the soil pH (r = 0.77). If the value of the organically-complexed Fe found in the soil solution of Rand E soil (39.3%) is excluded, the correlation with the soil pH increased up to 0.93 (Fig. 3). The correlation between the organically-complexed Fe and the DOC was found to be very weak (r = 0.21). However, within the alkaline pH range, this correlation increased to 0.56.

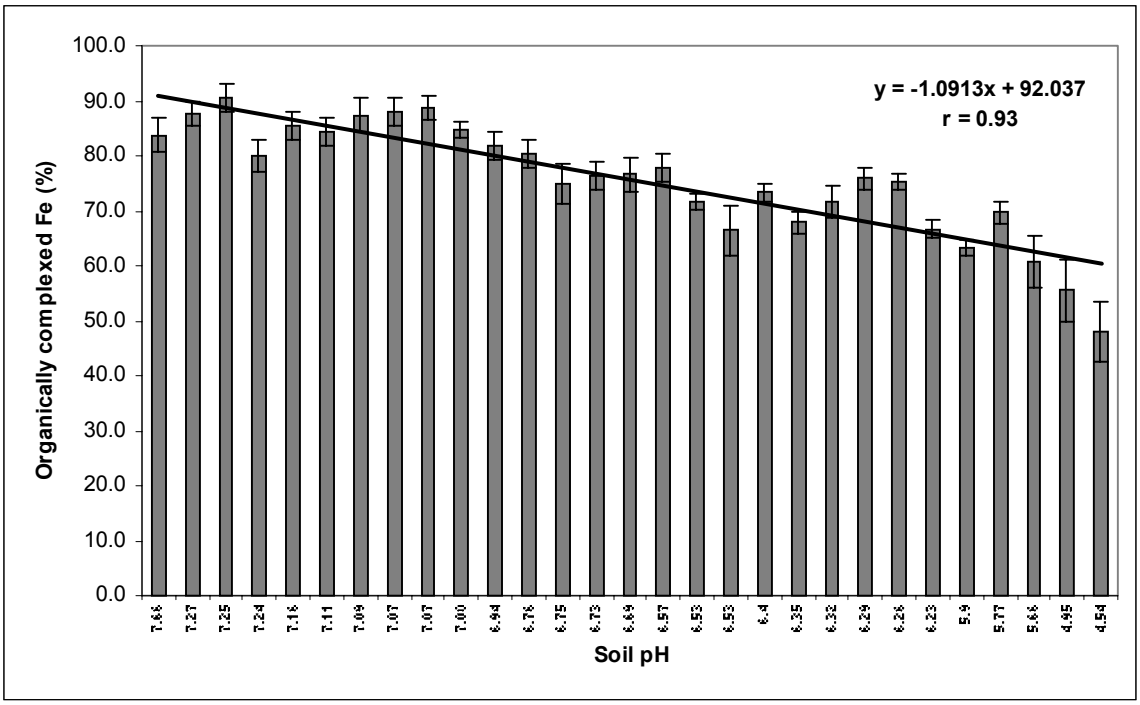


Figure 3. The correlation between the organically-complexed Fe and soil pH.

3.4 The availability of soil Fe as influenced by microbial activity:

The addition of glucose, starch and cellulose at a rate of 15, 10 and 5 g per kg soil, respectively, resulted in a higher soil microbial activity (μg CO_2 g^{-1} soil h^{-1}) in almost all the studied soils (Fig.4a, 4b and 4c). Interestingly, the higher microbial activity yielded higher total soluble Fe concentrations in the soil solutions of almost all the carbon-amended soils (Fig. 4a, 4b and 4c) even though they differ considerably in their chemical and physical properties. Although a higher microbial activity was detected in the carbon-amended Lang Wiese Rodenbach (RM), the total soluble Fe concentration in the soil solution of this soil decreased from 181.8 to 136.3 μM (Fig. 4c). Feld E (F) and Leopold (Le) soils did not respond to the additions of the above mentioned carbon sources. The microbial activity in these two soils did not increase as a result of carbon amendment. In parallel, the total soluble Fe concentrations in the soil solutions of these two soils did not increase as well (Fig. 4d). The higher microbial activity detected in the carbon-amended Kirchhain (Ki) soil, although not significant, resulted in a higher but not significant total soluble Fe concentration in comparison with the control (Kirchhain soil without carbon addition) (Fig. 4d). The highest microbial activity did not necessarily result in the highest total soluble Fe concentration in the soil solution. For an example, a 15 times higher microbial activity resulted in a 8.4 μM increase in the total soluble Fe concentration of Wuerzburg (W) soil, however, a 19.6 times higher microbial activity resulted in a 5.4 μM increase in the Fe concentration of the "unknown" (H) soil (Fig. 4a and 4b). The highest increase in the concentration of the total soluble Fe due to a higher microbial activity was recorded in the soil solutions of the calcareous soil, Wuerzburg (W) soil

(Fig. 4a), and Rastede (Ra) soil (pH 5.77) (Fig. 4a), although it is rich in DTPA-Fe (210.9 g kg^{-1}).

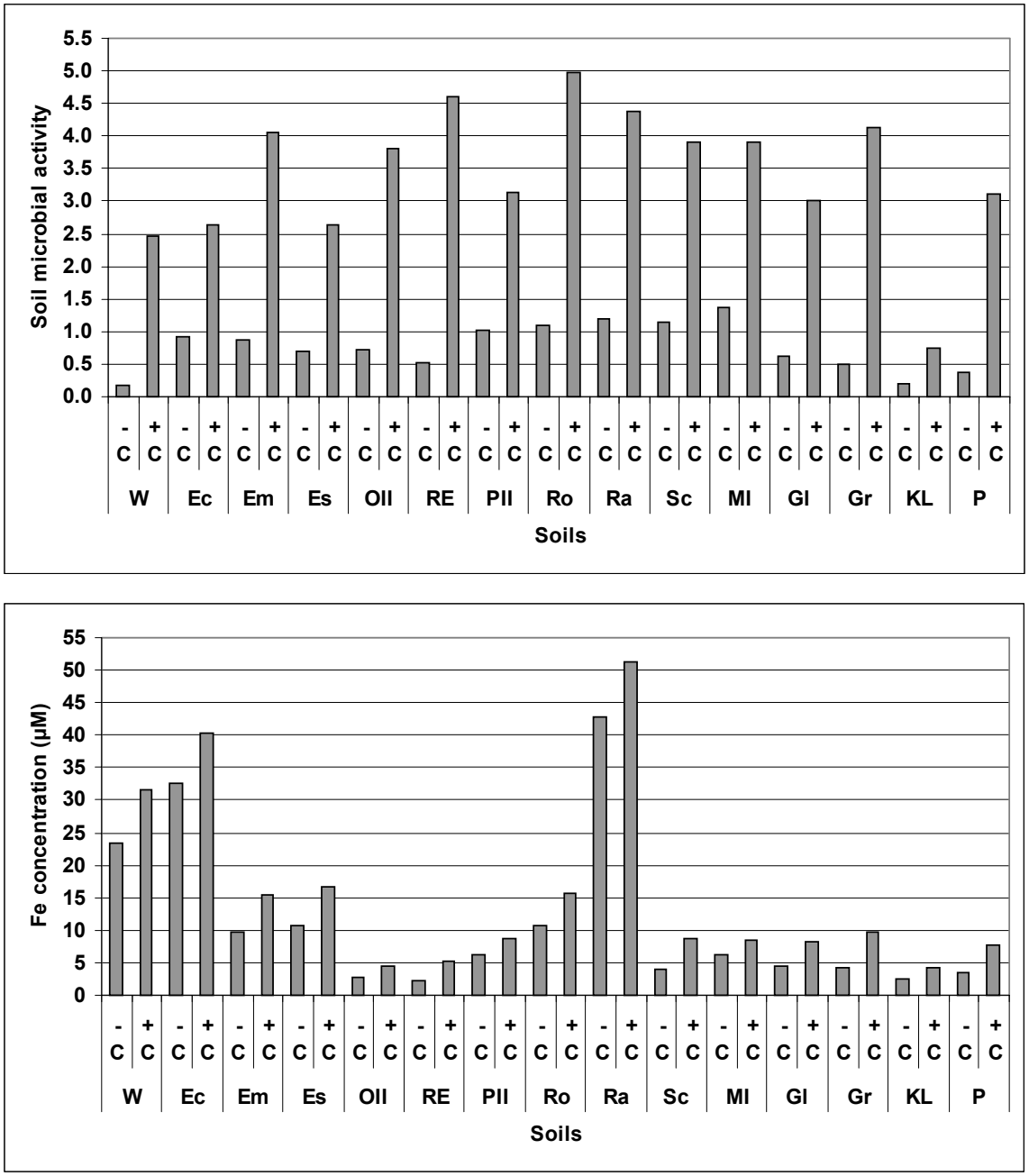


Figure 4a. Soil microbial activity (≤ 5 µg CO_2 g^{-1} soil h^{-1}) (upper part) and total soluble Fe concentration in the soil solution (lower part). All differences are significant at 0.01 except for Rastede (Ra) soil (only at 0.05) for the Fe concentration. +C and -C mean with and without carbon additions.

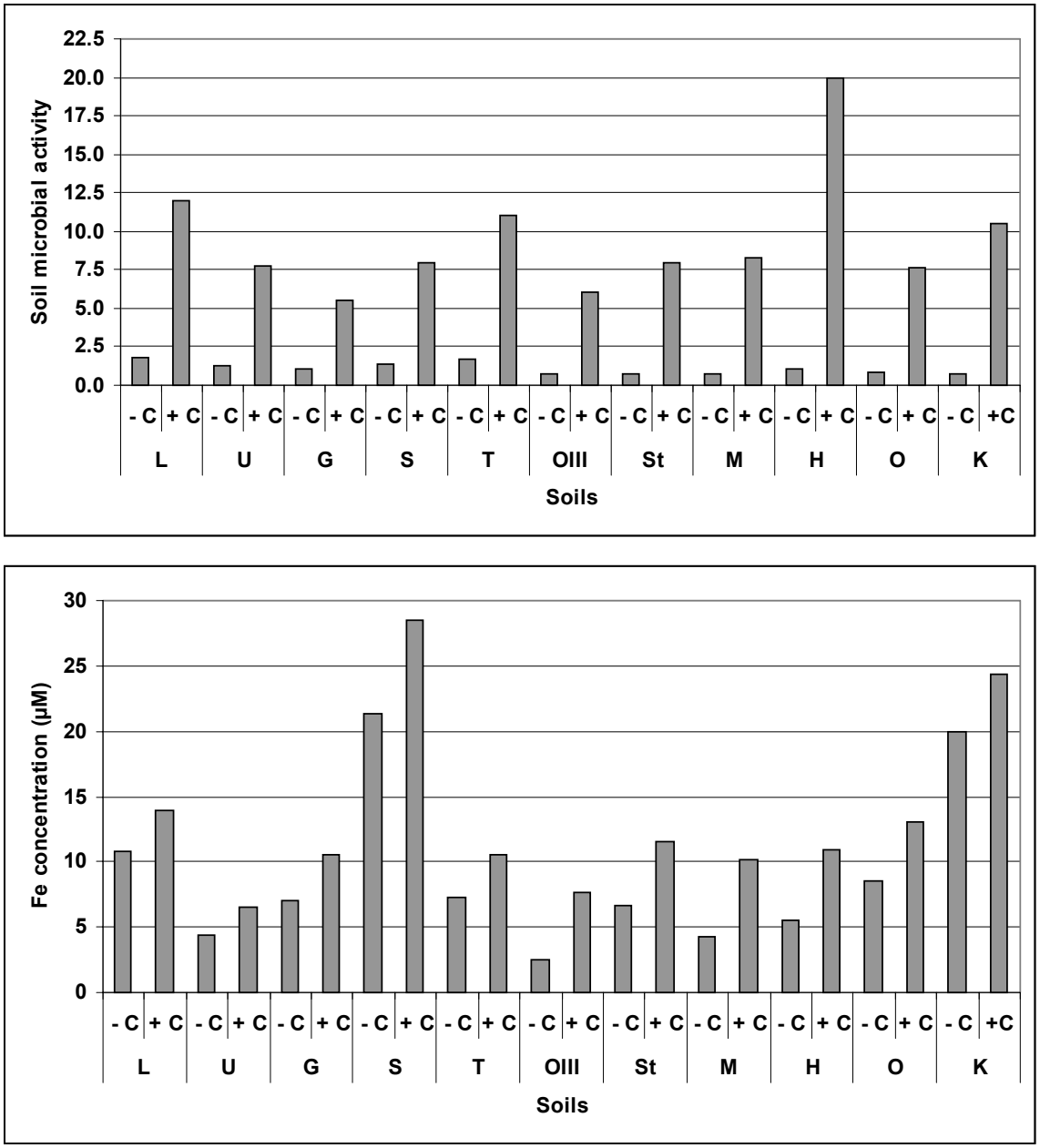


Figure 4b. Soil microbial activity (> 5 µg CO_2 g^{-1} soil h^{-1}) (upper part) and total soluble Fe concentration in the soil solution (lower part). All differences are significant at 0.01. +C and -C mean with and without carbon additions.

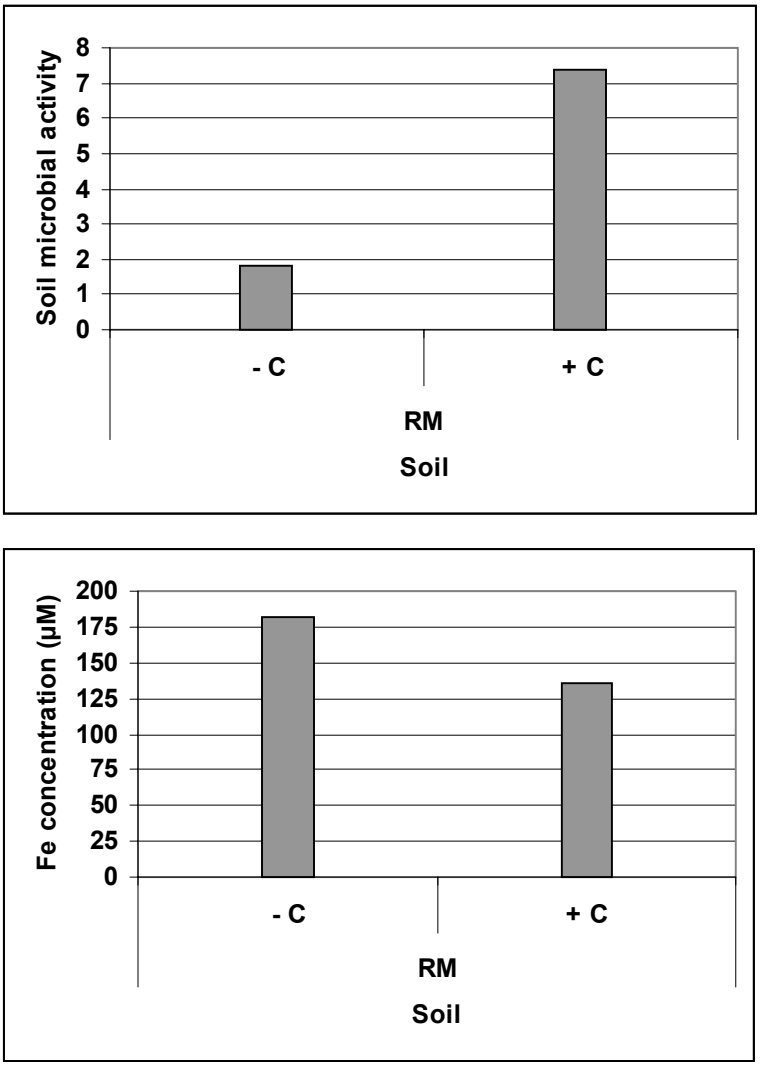


Figure 4c. Soil microbial activity (µg CO_2 g^{-1} soil h^{-1}) (upper part) and total soluble Fe concentration in the soil solution of Lang Wiese Rodenbach (RM) (lower part). Differences are significant at 0.05 for the Fe concentration and at 0.01 for the soil microbial activity. +C and -C mean with and without carbon additions.

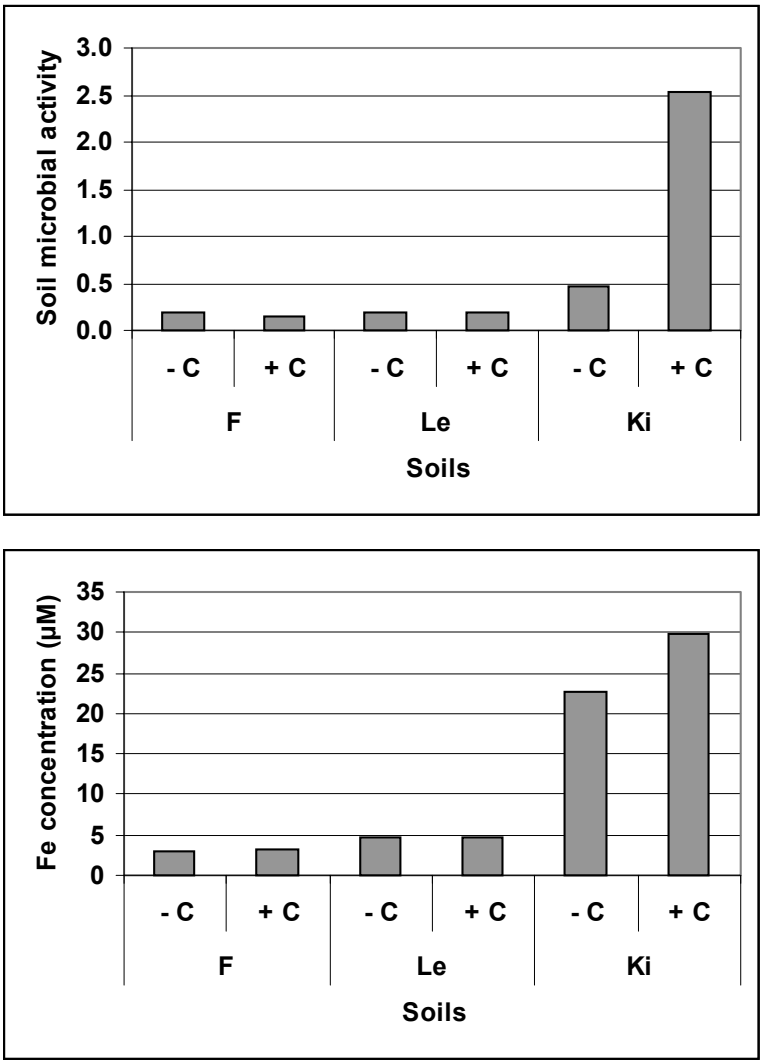


Figure 4d. Soil microbial activity (µg CO_2 g^{-1} soil h^{-1}) (upper part) and total soluble Fe concentration in the soil solution (lower part). Differences are not significant. +C and -C mean with and without carbon additions.

3.5The influence of intercropping swingle citrumelo with grass and dicot plant species on its Fe nutritional status:

The chlorophyll concentration of Swingle citrumelo plants treated with FeEDDHA differed statistically from the control (plants grown on a bare soil) after an amount of 160 mg FeEDDHA (9.6 mg Fe) per plant were added at three intervals (see materials and methods). However, those

intercropped with *Festuca rossa* showed statistically higher chlorophyll concentration starting from the 1st of April; at which the first SPAD readings were recorded (Table 6).

Table 6: Chlorophyll concentration (SPAD) of Swingle citrumelo plants (mean ± SD).

TRT	Date			
	01/04	11/04	26/04	08/05
Control	37.8±1.8	38.3±2.7	32.7±2.9	29.6±2.1
Fe-EDDHA	36.4±3.0	38.7±2.7	36.0±3.7	**36.4±1.7**
Vivianite	38.3±3.6	39.8±2.1	32.9±3.5	**35.0±2.2**
PN	39.6±2.3	41.4±2.1	35.1±2.2	**36.3±3.6**
FO	38.4±2.4	40.8±2.8	32.3±2.6	33.6±2.3
FR	**41.1±1.4**	**43.2±1.7**	38.7±3.8	**37.3±2.4**
TS	40.8±1.5	41.6±1.5	37.9±3.1	**35.9±3.0**

Numbers in bold are statistically different from the control.

This effect was interestingly persistent till the end of the experiment (Table 7). The another *Festuca* species, *F. ovina*, although known to be an "Fe-efficient" grass species, increased the chlorophyll concentration of the Swingle citrumelo plants in an intermittent rhythm and only after being mowed (Table 7). The influence of *Poa nemoralis* on the chlorophyll concentration of this citrus rootstock was positive before being mowed (Tables 6). However, after mowing this grass species, a decrease in the chlorophyll concentration of the Swingle citrumelo plants was recorded (Table 7).

Table 7: Chlorophyll concentration (SPAD) of Swingle citrumelo plants (after cutting*).

TRT	Date						
	11/06	18/06	25/06	09/07	16/07	23/07	29/07
Control	31.9±1.1	29.8±1.3	21.1±3.6	29.8±3.3	30.1±3.1	25.8±3.8	25.4±3.3
Fe-EDDHA	**37.0±1.0**	**37.2±1.8**	**32.8±1.6**	**37.6±1.9**	**37.4±1.5**	**38.7±1.2**	**40.9±2.3**
Vivianite	**35.4±1.8**	**34.5±2.2**	**30.9±3.1**	33.4±2.3	**35.5±2.2**	**35.5±2.4**	**36.4±2.7**
PN	**27.4±2.7**	28.8±2.3	**27.6±1.7**	27.1±2.9	**24.4±2.4**	25.4±3.6	29.6±3.2
FO	34.9±1.9	**33.2±1.3**	**29.2±1.9**	31.7±2.3	33.6±2.0	**34.0±2.6**	**33.6±2.4**
FR	**34.7±1.1**	**34.4±2.4**	**31.6±1.6**	**33.4±2.0**	**35.8±1.1**	**35.8±1.9**	**34.0±3.1**
TS	**36.9±0.3**	29.8±4.3	**28.6±3.0**	26.7±2.1	24.4±3.1	25.1±2.5	24.1±3.2

*values recorded after the cutting of the grass species and the *T. subterraneum*

At the end of the experiment, it was clear that Fe nutritional status of Swingle citrumelo plants intercropped with *P. nemoralis* was much suboptimal in comparison with the other treatments (Table 8). The *T. subterraneum* was as effective as the FeEDDHA and the other grass species, except the *P. nemoralis*, in improving the Fe nutritional status of this citrus rootstock (Table 6). After being mowed, its influence on the chlorophyll concentration of the Swingle citrumelo plants became inconsistent (Table 7). The low dose (20 ml per plant) of vivianite was not effective as Fe source for Swingle citrumelo plants. However, the application of another 40 ml per plant resulted in statistically higher chlorophyll concentration in comparison with control plants. Vivianite was interestingly as effective as the FeEDDHA (Tables 6 and 7).

Table 8: Swingle citrumelo leaf chlorophyll concentration (μg cm^{-2}) (30/07/03) (at the end of the experiment).

TRT	Chl Concentration
Control	10.0±1.8
Fe-EDDHA	**22.6±5.7**
Vivianite	**17.0±3.3**
PN	9.7±1.7
FO	**17.8±4.3**
FR	**17.8±2.9**
TS	9.5±2.9

Of particular interest is the finding that the positive effects on chlorophyll concentration and growth are not related to the Fe concentrations in leaves (Fig. 5). In contrast, the treatment (control) with a high Fe concentration was the poorest in chlorophyll concentration and growth, the treatment (*F. ovina*) with the highest growth and chlorophyll concentration had the lowest leaf Fe concentration. The Fe concentrations were not absolutely low and also that found in the *F. ovina* treatment was not very low. Obviously intercropping improved the Fe efficiency in Swingle citrumelo plants.

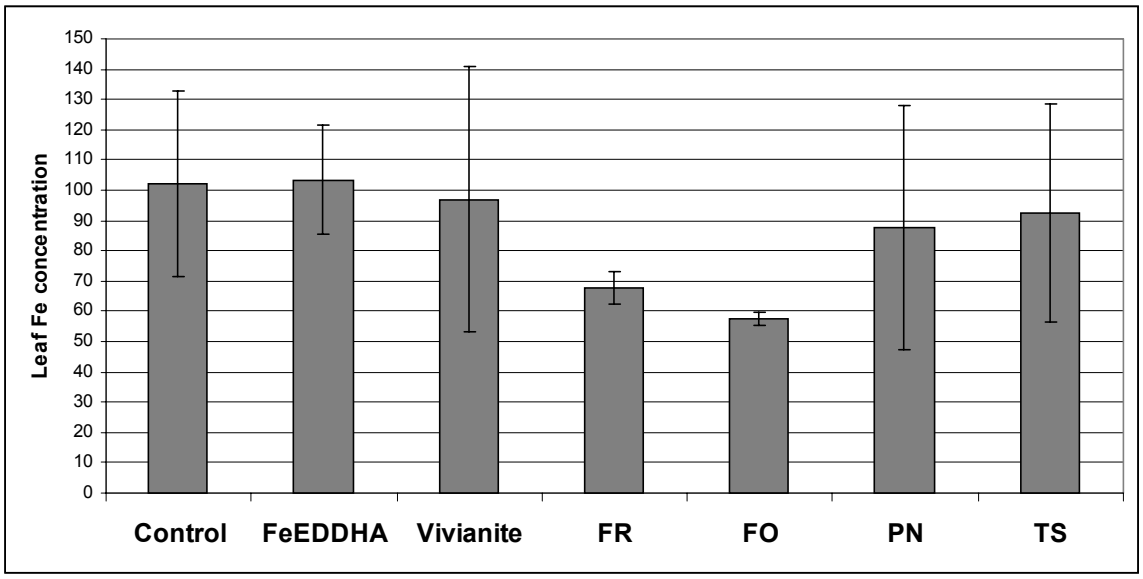


Figure 5: Leaf Fe concentration (µg g^{-1} DW) of Swingle citrumelo plants (30/07/03).

The leaf area (measured as the length and width of the leaf) of Swingle citrumelo plants was increased particularly by the FeEDDHA as well as the *Festuca* species compared with that of control plants (Tables 9 and 10). However, the presence of *T. subterraneum* resulted in leaf growth retardation (Table 9) only before being cut, which might explain the higher leaf chlorophyll content of the citrus rootstock (Table 6) in comparison with the control plants.

Table 9: Leaf Area (cm) of Swingle citrumelo (08/05/03).

Treatment	Length		Width	
	1st Leaf	2nd Leaf	1st Leaf	2nd Leaf
Control	6.6±0.4*	6.8±0.5	3.0±0.2	3.3±0.3
Fe-EDDHA	**7.7±0.5**	7.4±0.5	3.6±0.5	3.5±0.4
Vivianite	6.5±0.2	6.6±0.3	2.9±0.3	3.2±0.2
PN	6.7±0.3	6.8±0.4	3.1±0.3	3.3±0.2
FO	6.3±0.4	6.5±0.4	2.8±0.5	3.1±0.2
FR	6.8±0.5	6.8±0.5	3.2±0.4	3.3±0.3
TS	**5.7±0.3**	5.9±0.5	**2.5±0.2**	2.8±0.3

*Mean of two branches.

Table 10: Leaf Area (cm) of Swingle citrumelo (after mowing).

TRT	08/07		08/07		30/07	30/07
	L B1*	W B1	L B2	W B2	L	W
Control	6.4±0.3	3.1±0.3	6.5±0.4	3.1±0.4	5.8±0.9	2.6±0.5
Fe-EDDHA	**7.7±0.4**	3.8±0.4	**7.6±0.3**	**3.8±0.2**	**7.9±0.7**	**4.2±0.5**
Vivianite	7.2±0.7	3.5±0.5	7.0±0.6	3.2±0.3	6.8±1.1	3.3±0.7
PN	**7.4±0.4**	3.5±0.3	7.2±0.8	3.2±0.4	6.6±0.6	3.0±0.4
FO	**7.4±0.4**	3.4±0.4	6.7±0.5	3.0±0.2	**7.8±0.6**	**4.2±0.4**
FR	**7.4±0.4**	3.4±0.3	7.3±0.5	3.3±0.2	**7.9±0.2**	**4.0±0.3**
TS	6.6±0.3	3.0±0.2	6.4±0.4	2.8±0.2	6.2±0.5	2.7±0.4

*L: Length, W: Width, B1: Branch 1, B2: Branch 2

The application of FeEDDHA resulted in a significant increase in the vigor of Swingle citrumelo plants in terms of number of leaves, branch length, weights of trunk and old shoots, young shoots, and fully expanded young leaves (Tables 11, 12 and 13). Similarly, Swingle citrumelo plants intercropped with *F. rossa* showed an increase in the leaf number as well as branch length. The presence of both *Festuca* species increased weights of young shoots and fully expanded young leaves (Tables 12 and 13) in comparison with control plants and those treated or intercropped with vivianite or *P. nemoralis* and *T. subterraneum*, respectively. However,

vivianite treatment resulted in an increase in the dry weight of young shoots (Table 12) while the presence of *T. subterraneum* decreased the weight of trunk and old shoots (Table 12) in comparison with the control plants.

Table 11: Leaf Number and Branch Length (cm) of Swingle citrumelo plants (30/07/03).

TRT	Leaf Number	Branch (B) Length 1	B2	B3
Control	73±6.8	84±9.2	70±10.9	46±13.2
Fe-EDDHA	**100±5.6**	**107±9.6**	92±8.8	65±16.4
Vivianite	79±8.3	92±15.3	75±12.3	47±7.0
PN	80±6.6	92±9.8	71±10.8	49±13.9
FO	83±7.8	104±14.5	81±16.1	53±15.9
FR	**91±4.9**	**108±9.9**	83±16.3	54±8.8
TS	75±6.9	76±10.0	59±7.5	45±12.0

Table 12: Biomass (g) of Swingle citrumelo plants (30/07/03).

TRT	Young Shoots-Fresh Weight	Young Shoots-Dry Weight	Trunk + Old Shoots Weight
Control	52±6.9	18.2±3.2	202±13.1
Fe-EDDHA	**97±4.1**	**39.1±3.1**	**256±15.2**
Vivianite	65±9.3	**27.6±3.0**	209±9.6
PN	60±7.8	21.1±3.6	178±13.0
FO	**75±10.1**	**27.0±3.7**	195±16.4
FR	**96±4.4**	**32.6±1.5**	197±4.5
TS	54±9.1	18.2±3.9	**158±4.5**

Table 13: Leaf weight (g) of Swingle citrumelo (30/07/03).

TRT	Leaf Fresh Weight	Leaf Dry Weight
Control	0.4±0.2	0.2±0.01
Fe-EDDHA	**1.0±0.2**	**0.3±0.03**
Vivianite	0.6±0.2	0.2±0.04
PN	0.5±0.2	0.2±0.02
FO	**1.0±0.2**	**0.3±0.04**
FR	**1.0±0.2**	0.2±0.01
TS	0.5±0.1	0.2±0.02

Only the application of FeEDDHA and the intercropping of Swingle citrumelo plants with *F. ovina* increased the weight of both the total root system as well as the fine roots (Table 14) in comparison with the control plants and the other treatments.

Table 14: Total root and fine root fresh weights (g) of Swingle citrumelo (30/07/03).

TRT	Root Fresh Weight	Fine Root Fresh Weight
Control	112±12.9	71±12.3
Fe-EDDHA	**155±14.1**	**99±14.6**
Vivianite	124±13.9	89±20.3
PN	127±21.9	89±21.8
FO	**144±14.0**	**97±12.7**
FR	131±18.6	91±16.5
TS	105±10.8	73±7.0

4. Discussion

4.1 The Buchner Funnel Technique (BFT) and the ferrozine method

4.1.1. The Buchner funnel technique

The analysis of soil solution is an essential aspect of soil science. However, prior to analysis, soil solution must first be isolated from the soil. Soil solution can be collected by non-destructive and destructive methods. Non-destructive methods involve the installation of a soil solution collector (a lysimeter) that samples soil solution at the same point. Destructive methods involve soil sampling and subsequent extraction of soil solution in the laboratory. The sampling techniques used for monitoring soil solution are:

1. Tension lysimetry (soil water potential < 0)
2. Zero-tension lysimetry (soil water potential = 0)
3. Centrifugation of soil samples (centrifuge drainage)
4. Saturation extraction (Roades, 1982, Richards, 1941)
5. Suction cup solution samplers
6. Passive capillary samplers

These procedures differ considerably with respect to the soil solution fraction sampled, the effects of sampling on the site, as well as the extent to which they provide information about temporal and spatial variation in the properties of soil solution. The different soil solution fractions sampled by the first four techniques are shown in Fig. (6).

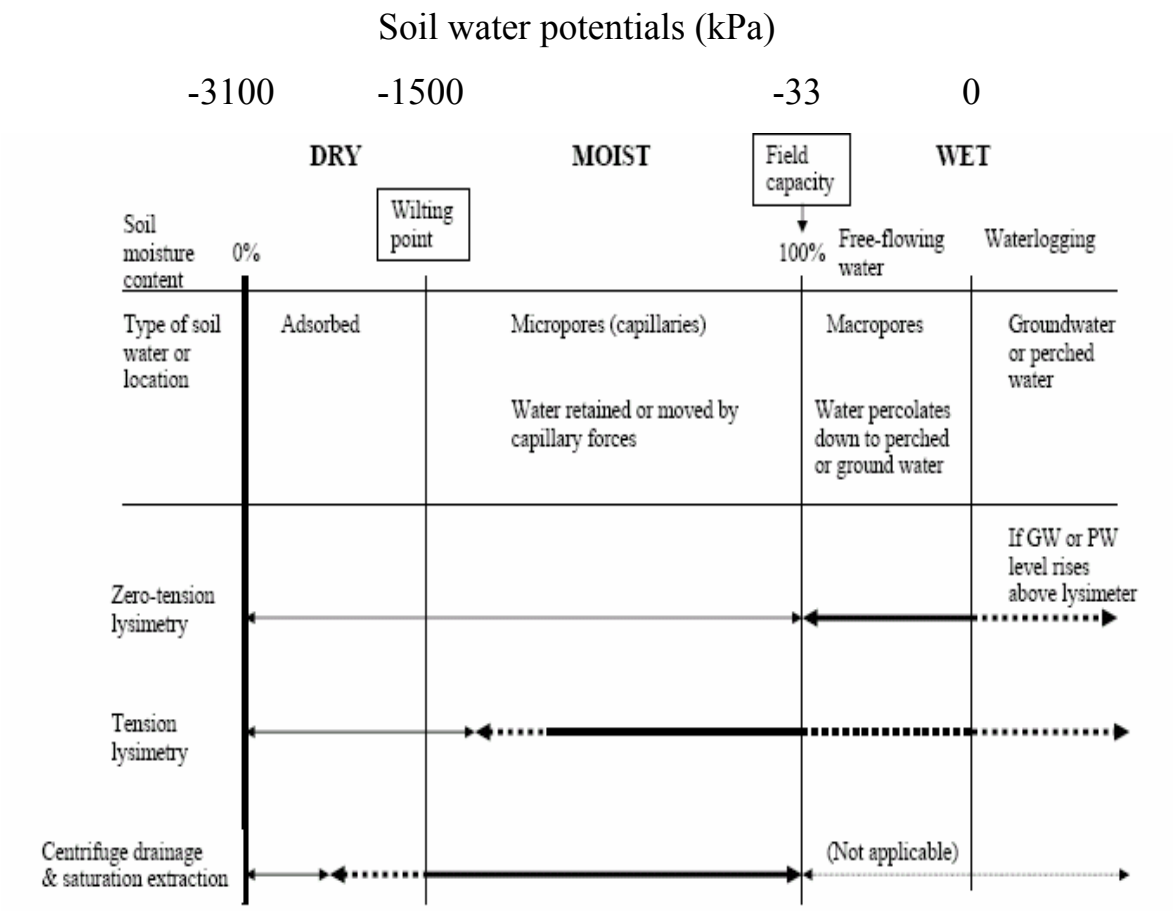


Figure 6. The soil water fractions sampled by zero-tension lysimetry, tension lysimetry and centrifuge drainage (thick lines). The thin lines indicate the fractions that cannot be sampled. The actual fractions sampled by tension lysimetry can vary depending on the size of the vacuum applied and the moisture content of the soil during sampling (dotted lines). Similarly the amount of adsorbed water sampled by centrifuge drainage depends on the centrifugation speed (GW: ground water, PW: perched water).

The main limitation of zero-tension lysimetry is that they only sample that fraction of soil water flux occurring under saturated soil conditions or during macropore flow. The tension lysimetry samples a relatively broad fraction of the soil solution. Soil solution samples are obtained by this technique only when the magnitude of the negative pressure (vacuum) applied exceeds that of the hydraulic forces holding the water in the soil. Tension lysimetry obviously also samples free-flowing water when it is present.

The use of the centrifuge drainage method is restricted by the same limitations associated with soil sampling, e.g. it is a destructive method, the information obtained is relevant only to the sampling time, and the determination of temporal variation requires successive samplings. The saturation extract method is a destructive technique and, as with centrifugation, a composite sample can be used. This method has the disadvantage of destroying the soil structure and of diluting the soil solution as a result of adding water to saturate the sample; anaerobic processes, e.g. denitrification and Fe reduction, may affect the solute concentration and composition.

Although suction cup samplers may be used to collect solution under unsaturated conditions, they do not provide a reliable estimate of soil water flux. In addition, suction must be applied manually to the sampler several hours or days prior to solution collection. This is problematic because the magnitude of tension exerted on soil water gradually decreases over time. Lastly, because of the small cross sectional area of the cup, multiple samplers are required to represent adequately soil variability. Passive capillary samplers provide an alternative means of sampling soil water in the field. As a result of their high conductivity and the tension exerted, they collect matrix and macropore flow under both saturated and unsaturated conditions. However, the passive capillary samplers could significantly alter measurement and speciation of soil solution chemistry (Goyne et al., 2000).

There are important differences between the chemical composition of soil solution obtained by the different techniques. The percolation water obtained using zero-tension lysimeters is the soil solution fraction that is primarily involved in soil formation processes. On the other hand, the chemical composition of soil solution obtained using tension lysimeters usually

represents the end result of e.g. buffering and neutralization processes in the different soil horizons.

The Buchner Funnel Technique (BFT) was used in our research to collect the soil solution at 80% of its maximum water holding capacity. This means that the soil is not anaerobic and solute concentrations and microbial activity are hardly affected by anaerobic processes. However, these conditions might have been developed in soil microsites. The tested soil samples were crushed, sieved through a 2-mm sieve and mixed with quartz sand; hence the soil structure is not identical with the *in situ* one. For this reason the water flow under natural conditions is not reflected in this technique. This, however, is not of primary interest since at a water potential around the field capacity (-33 KPa) solute transport depends much on following the water potential gradient in micro spaces and also the mass flow of water. Under plant growth conditions uptake of both water and solutes by roots provide the water potential gradient and a solute potential gradient. In our case also a gradient for Fe. Hence the solute transport towards the roots depends much on the solute concentration in the bulk soil solution. This is also true for Fe. The applied suction in our experiments (around -50 KPa) simulated the uptake of water and solutes by plant roots.

The relationship between the Fe bearing soil particles and the soil solution is not affected by the quartz sand particles with which the soil was mixed as these particles (acid-washed) contain no major amounts of soluble Fe and also their surfaces provide no major sites for Fe adsorption.

Under the conditions of this technique there was no considerable free-flowing water or macropore flow due to the fact that the water content of these soils were kept around the field capacity. Therefore, the soil solution from these soils was only collected when the magnitude of the negative

pressure (vacuum) applied exceeds that of the adsorption forces holding the water at soil surfaces. Although soils were mixed with sand to improve the water flow induced by suction, the solution collected by the BFT represents the aqueous liquid phase of the soil (mixed with sand) and its solutes, which means that this solution reflects the actual soil solution.

The total soluble Fe measured in these soil solutions represents the mobile (soluble) fraction of Fe *via* diffusion and mass flow, which is of utmost importance for the Fe nutrition of plants. Comparing these concentrations obtained by us with the very few data of Fe concentrations quoted in the literature, it becomes evident that the order of magnitude for Fe concentrations is the same for the various investigations. In this context it is of interest that Lindsay (1974) who discussed the Fe solubility in soils in a still valuable review paper came by theoretical consideration to the conclusion that the Fe concentration in the soil solution should be in the range of 1 µM in order to meet the plant demand. This is exactly the order of magnitude of Fe concentration we obtained with the BFT technique.

The Buchner Funnel Technique (BFT) is a reliable and reproducible tool to non-destructively (temporal wise) and continuously collect soil solutions. Our results showed that there was no considerable deviation of the values of the total soluble Fe concentration collected from 32 different soils from their means even when three replicates were only used. This definitely proves that the BFT is a precise tool to obtain the concentrations of Fe in the soil solution and most probably other elements as well. This method was further improved by adding 0.5 M HCl in the glass flasks where the soil solutions were collected in order to prevent the oxidation of Fe(II) if present, to maintain the oxidation-reduction state of Fe as it is in the soil solution before being collected and to facilitate the reduction of all chemical forms of Fe(III)

to Fe(II) by the hydroxylamine hydrochloride (Prof. Dr. S. Schnell, personal communications).

Moreover, the BFT was shown to be considerably reproducible. The same soils were tested at two different dates. The first date was when analyzing the 32 different soils for the Fe concentration in the soil solution and the second date when testing the influence of microbial activity on the concentration of total soluble Fe in the soil solution. Our results showed that there were no considerable differences in the concentrations of soluble Fe which provides strong evidence that this technique is highly reproducible.

As the type of soil solution sampling technique employed depends on the aim of soil solution monitoring, the following recommendations can be made for practical purposes:

1. Repeated soil sampling results in considerable disturbance to the site. Therefore, to produce a time series with short sampling intervals, a non-destructive method is the most appropriate.
2. For monitoring with large time intervals, repeated soil sampling is also appropriate, especially as this approach reduces spatial variation. If applied under field conditions, the BFT can be used for this purpose.

4.1.2 The Ferrozine method:

Our results clearly show that the ferrozine-hydroxylamine hydrochloride method is a reliable analytical method. The evidences can be presented as follows:

1. The slope of the calibration curve (FeII concentration versus absorbance) did not differ due to the addition of soil solution to the standard solutions.
2. Mixing different soil solutions with each other resulted in a reproducible measured concentration of Fe.
3. A known concentration of Fe(II) was detected after being added to different soil solutions.

These results proved that this analytical method is reliable and reproducible due to the absence of interferences forming any possible substances or compounds that might be differently present in the soil solutions. In addition, it can be concluded that the different chemical properties of the soil solutions did not interfere with the ferrozine method.

4.2 Total soluble Fe concentration in the soil solution and the Fe buffer power of different soils

The main source of iron (Fe) for plants in most soils is the Fe oxides. Under most soil conditions, i.e. at ambient temperature and pressure and a sufficiently high pO_2, Fe oxides are the most stable form of Fe.

In the common pH range of well aerated soils, the activity of Fe(III) ions is extremely low and would hardly be sufficient to meet the Fe requirement of plants. The low Fe(III) activity in soil solutions also excludes Fe(III) cationic species from ever comprising a substantial part of the exchangeable cations (Schwertmann, 1991).

The Fe oxides decrease in solubility by a factor of 3630 in going from amorphous $Fe(OH)_3$ to FeOOH (goethite) (Tables 15 and 16). Because of the extremely low solubility of the crystalline Fe oxides, equilibrium

relationships among them are not readily attained. This means that several crystalline Fe oxides may be present in soils simultaneously and persist for long periods of time without attaining equilibrium (Lindsay, 1995).

Table 15: Solubility product of soil Fe oxides

Oxide	[Fe] at pH 7.0 ($\mu M\ L^{-1}$)
Ferrihydrite (amorphous $Fe(OH)_3$)	$10^{-3.1}$
Soil-Fe ($Fe(OH)_3$)	$10^{-3.9}$
Maghemite (Fe_2O_3)	$10^{-5.0}$
Lepidocrocite (FeOOH)	$10^{-5.2}$
Hematite (Fe_2O_3)	$10^{-8.8}$-$10^{-5.8}$
Goethite (FeOOH)	$10^{-10.9}$-$10^{-5.9}$

Source: Schwertmann (1991)

Table 16: Dissolution rates in 0.5 M HCl at 25ºC for various Fe oxides

Oxide	g Fe h^{-1} m^{-2}
Lepidocrocite	6.4×10^{-4}
Magnetite	3.5×10^{-4}
Akaganeite	1.4×10^{-4}
Maghemite	0.99×10^{-4}
Hematite	0.13×10^{-4}
Goethite	0.05×10^{-4}

Source: Sidhu et al. (1981)

The initial precipitation of soluble ferric salts gives amorphous $Fe(OH)_3$. At pH 8.0 this solid supports only $10^{-21.46}$ M Fe^{3+} or 209 atoms of Fe^{3+} per L. Goethite supports only 0.56 atoms of Fe^{3+} per L at pH 8.0. This extremely low solubility helps to explain the difficulty of keeping Fe^{3+} soluble and mobile in soils of high pH. The minimum solubility of Fe occurs in the pH range of 7.5 to 8.5 where the major solution species is $Fe(OH)_3^{\circ}$, present at $10^{-10.4}$ M. This level of soluble Fe is approximately 250 fold less than the critical level of 10^{-8} M soluble Fe needed by rice and soybean. Iron deficiencies can be expected in well-aerated soils or nutrient solutions above

pH 5.0. The dominant Fe species in the pH range of 5.0 to 7.5 is $Fe(OH)_2^+$ which decreases 10-fold for each unit increase in pH while the activity of Fe^{3+} decreases 1000-fold. Calcareous soils are strongly buffered in the pH range near 8.0 where Fe reaches its minimum solubility; hence Fe chlorosis is appropriately referred to as lime-induced chlorosis (Lindsay, 1995).

Accordingly, it is obvious that the solubility of different Fe oxides *per se* and the chemical equilibria equations of soil Fe minerals (Fig. 7) cannot be used to explain our results found in this research. To be able to interpret our data, it is very important to understand that there are many factors affecting the solubility and the dissolution rate of the various Fe oxides. Schwertmann (1991) pointed out that because the solubility products of common soil

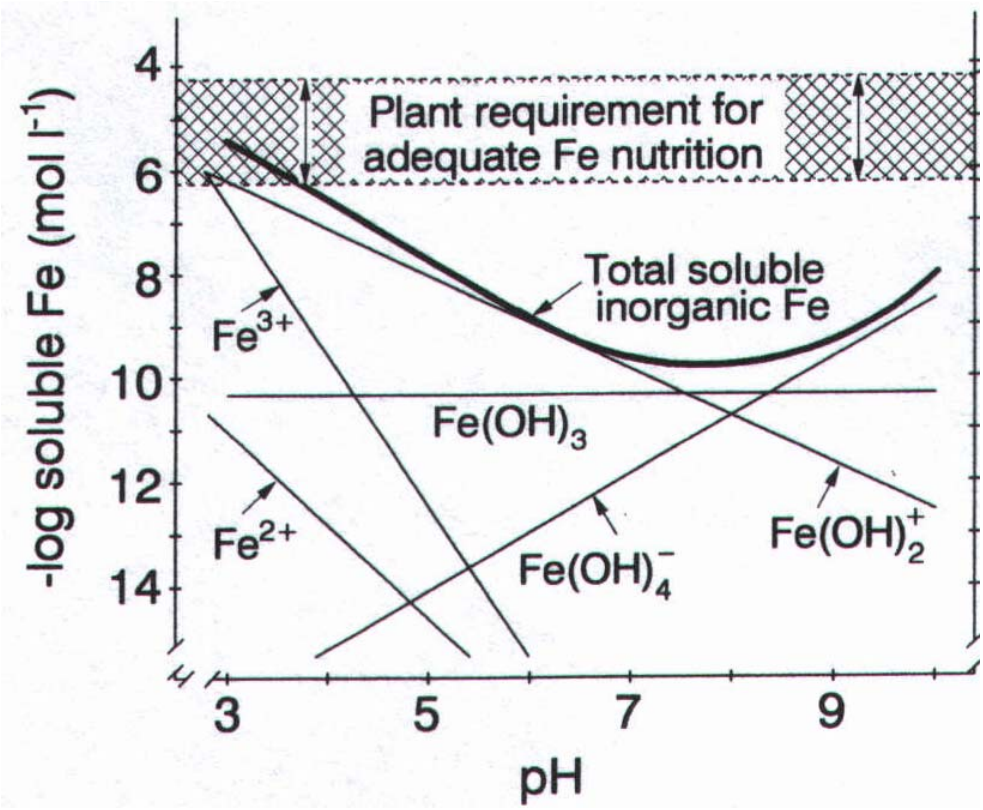


Figure 7. Solubility of inorganic Fe species in equilibrium with Fe oxides in well-aerated soils in comparison to the requirement of soluble Fe at the root surface (Source: Lindsay, 1974)

Fe(III)(hydr)oxides (Fe^{3+}).$(OH)_3$ are $\leq 10^{-37}$, the availability of Fe in aerobic soils must be governed by dissolution rate.

There are 3 principal reactions by which solid Fe oxides may release Fe into an aqueous solution: Protonation, reduction and complexation producing Fe(III) cations, Fe(II) cations and Fe(II) or Fe(III) complexes, respectively. In soil systems and particularly within the rhizosphere, complexation and reduction are much more important than protonation.

Factors that affect solubility and dissolution of Fe oxides are complicated and interrelated. Consequently, no one factor plays the major role in controlling the concentration of total soluble Fe in the soil solution of the tested 32 physically and chemically different soils.

Watteau and Berthelin (1994) studied the chemical dissolution of goethite in the presence of HCl and underlined that despite the low pH and the protons availability (at 1000 mM HCl), solubilization of goethite was not so much important. This result showed that the acidity alone and the exchange with protons alone were not sufficient to dissolve efficiently and fastly a well crystallized ferric oxyhydroxides. Holmen and Casey (1996) reported that proton-promoted dissolution becomes measurable as pH decreases below 2.5, but the Fe flux is small relative to that caused by reductive or ligand-promoted dissolution at higher pH conditions.

Besides pH, the electron activity (pe) is another and even more important parameter in determining the iron activity in soil solution. Whenever metabolic activity has consumed most of the available oxygen, electrons will be transferred to available electron acceptors and Fe(III) oxides will be readily reduced. At any given pH and pe the Fe^{2+} activity in equilibrium with a solid Fe(III) oxide depends on the thermodynamic stability of the latter. At pH 7 and pe = 3, as in weekly reducing soils, a_{Fe}^{2+} amounts to $10^{-11.0}$; $10^{-10.9}$

and $10^{-7.5}$ M L^{-1} for goethite, hematite and ferrihydrite, respectively. By comparison, Fe^{2+} concentration is 10^{-5} times less in well aerated soils (pe = 8). This underlines the importance of reductive mobilization for the Fe supply of the root (Schwertmann, 1991).

Weakly reducing soils appear to be in equilibrium with an Fe (III,II) mixed phase, called "hydromagnetite" $Fe_3(OH)_8$. The Fe^{2+} activity at pe = 3 and pH 7 would be $10^{-6.1}$ M L^{-1}; about 25 times higher than would be produced by equilibrium with ferrihydrite. Therefore, it is likely that similar mixed valency solids form in reduced microsites in soils where water content is high, O_2 is depleted, and redox is temporarily lowered. In such sites, Fe is solubilized, and upon subsequent oxidation, precipitates as amorphous $Fe_3(OH)_8$. Lindsay (1995) reported that so long as this solid persists, Fe solubility is maintained at an elevated level. Such amorphous solids may persist for several days or months and it could supply a critical level of 10^{-8} M Fe at pe+pH 12 whereas soil-Fe control of soluble Fe would require pe+pH to drop below 10. This can occur under natural conditions whenever O_2 is excluded and organic matter is available as an energy and electron source. Fluctuating moisture conditions help to regenerate these reduction microsites and prolong the persistence of this mixed valency solid. Schwertmann (1991) mentioned that natural goethites admixed with siderite ($FeCO_3$) dissolved to a larger extent in oxalate whereas those without siderite did not. Dissolution of siderite and the release of Fe(II) to solution induced simultaneous dissolution of normally insoluble goethite. Fe(II) exchanges electrons with surface Fe(III) which is then detached. Increasing the pH from 3.5 to 6.5 resulted in an increase of the adsorption of Fe(II) and thus an acceleration of dissolution. At pH higher than 6.5 Fe(II) became increasingly oxidized and it looses its catalytic effect. The field significance

of these results could be that once Fe(II) is produced in a weakly to strongly anaerobic environment, it may catalyze further dissolution and mobilization of Fe.

The solid phase properties of different Fe oxides are of central importance and they are extremely interrelated. It is not possible to separately discuss these properties. Simplistically, one might anticipate that the release of Fe from Fe oxides (ferrihydrite, hematite and goethite) would correlate to the relative solubility of these minerals; a fact that is absolutely not precise. For example, at circumneutral pH, ferrihydrite is the most soluble of the three, followed by hematite, which is slightly more soluble than goethite (Tables 15 and 16). However, the bacterium *Pseudomonas mendocina* obtained Fe from these minerals through ligand-promoted dissolution mechanism in contrast to their relative solubilities (Hersman et al., 2001). Thus, solubility cannot be used to explain these results.

It is commonly agreed that the solubility product of particles < 1 μm in diameter increases with decreasing particle size (Schwertmann, 1991). Consequently, it is believed that the rate of dissolution is usually a function of surface area. However, the A_s (m^2 g^{-1}) did not control Fe acquisition by *P. mendocina* from either goethite or hematite. It was suggested that the tendency of hematite and goethite to aggregate in neutral pH range makes standard measures of particulate surface area inappropriate (Hersman et al., 2001). In another study, Maurice et al. (2000) observed increased dissolution with decreasing particle length and decreasing aspect ratio (ratio of particle length to particle width). However, this particle length-dissolution trend cannot be explained by typical particle length-A_s relationships because A_s does not correlate inversely with particle length.

Structural order/disorder (degree of crystallinity) is also important for Fe oxides dissolution. However, microbial removal of Fe from ferrihydrite, hematite and goethite was not controlled by crystallinity (Hersman et al., 2001).

Nowack and Sigg (1996) showed the importance of the type of surface complex in the dissolution of goethite at different pHs. The dissolution of goethite by EDTA at various pH values was investigated. The dissolution rate increases with increasing pH yielding a maximum dissolution rate around pH 8.0. The maximum concentration of the mononuclear complex corresponds with the maximum dissolution rate. Others have postulated the hypothesis that a binuclear surface complex inhibits dissolution while a mononuclear complex enhances it. It was found that at low pH the binuclear surface complex dissolves with a slow rate. As the mononuclear complex becomes important at higher pH, the dissolution rate gets faster, decreasing again with decreasing concentration of surface complex. Schwertmann (1991) mentioned that in the acid range the Fe-EDTA surface complex is very stable and this hinders the detachment of the complex whereas in the alkaline range the complex is much weaker and may favor Fe detachment. Similarly, the dissolution of synthetic hematite with citrate and oxalate were compared (Schwertmann, 1991). Although citrate forms stronger complexes with Fe than oxalate much less Fe was dissolved by citrate, which was attributed to that more oxalate than citrate is adsorbed at the same pH or that fewer bonds have to be broken with oxalate than with citrate surface complex before Fe can be detached.

Many authors studied the effect of metals substituting for Fe in Fe oxides. A substantial decrease in dissolution rate between 0 and approximately 10 mol% Al substitution with goethites synthesized at 25ºC and at 70ºC was

observed. No such effect was, however, noticed in HCl at 60ºC. Other metals such as Cr have a strong effect on stabilizing goethite against dissolution in HCl, Ni has no effect and Co and Mn accelerated the dissolution (Schwertmann, 1991). In contrast, Hersman et al. (2001) found that microbial mediated dissolution of goethite was positively correlated with Al substitution. However, it should be taken into consideration that the level of Al substitution alters crystal size, texture, surface area, particle length and particle aspect ratio and other structural properties that influence the rates and mechanisms of goethite dissolution (Maurice et al., 2000, Cervini-Silva and Sposito, 2002). It was also reported that low Al substitution for Fe on goethite also leads to a decrease in proton- and ligand-promoted reductive dissolution rates due to fewer structural defects that promote abiotic dissolution (Cervini-Silva and Sposito, 2002). Quantification of defect densities is important because surface controlled, ligand-promoted dissolution of (hydr)oxide minerals is thought to occur preferentially at so-called reactive surface sites, many of which would be associated with structural defects (Maurice et al., 2000). Metal substitution for Fe in Fe oxides influences differently the various dissolution mechanisms. Maurice et al. (2000) reported that microbial dissolution rate increased with increasing Al content. Observations of increased anionic adsorption with increased Al substitution in goethite suggest that organic ligands produced by the bacteria may sorb more readily to the Al goethites and that negatively charged microorganisms also may attach more easily. Moreover, Maurice et al. (2000) speculated that perhaps the bacteria preferred shorter, lower aspect ratio crystals as attachment and dissolution sites, which increased with increasing Al substitution. However, a recent study of the microbial reductive dissolution of synthetic Al-goethite by

Clostridium butyricum, a Fe-reducing bacterium, showed a decrease in the rate of Fe release by reductive dissolution as Al substitution increased. The decrease in Fe release rate was attributed to the accumulation of Al at the goethite surface resulting in the blocking of reactive sites during enzymatic reduction. Another study of the microbial reductive dissolution of a natural Al-goethite sample by *Shewanella putrefaciens* found that Al sorption had no effect on dissolution. This latter result was explained by Al adsorption or precipitation at surface sites distant from those where reductive dissolution occurred (Cervini-Silva and Sposito, 2002).

Hersman et al. (2001) and Maurice et al. (2000) pointed out the importance of hydroxyl coordination or the type of surface hydroxyl groups. The coordinated hydroxyl groups, which are in effect the functional groups of Fe oxides, i.e., the chemically reactive entities at the mineral surface in an aqueous environment, consist of three main types: coordinated to one Fe atom (A), coordinated to two Fe atoms (B), and coordinated to three Fe atoms (C). Adsorption reactions are considered to involve only singly coordinated groups (type A). In general, singly coordinated hydroxyl groups are believed to be more common on the faces of goethite than on hematite. With increasing Al substitution, a greater proportion of the reactive type A sites are more available. This could help to explain at least in part the observed trend of increased microbial dissolution (ligand-promoted dissolution) of goethite more than hematite and with increased Al substitution, because dissolution is likely surface controlled.

The importance of the transients in Fe dissolution rates was pointed out by Hersman et al. (2001). Transients are nonstructural Fe sorbed to the mineral surface that are difficult to quantify and are ephemeral, in that once removed

by cleaning they may reform. Different Fe oxides may differ in the quantity of the sorbed nonstructural Fe.

Soil Biota has the ability to alter the chemistry of soil environments through the synthesis of organic acids. Naturally occurring organic acids have been observed to accelerate the dissolution of oxide and aluminosilicate minerals in both the laboratory and the field (Eick et al., 1999).

In natural systems, organic acids will compete with metal ions, oxyanions, and other organic ligands for reactive surface sites. It has been proposed that these competitive sorption reactions may inhibit or enhance dissolution depending on the type of the surface complex formed and the strength of the bond. For example, Bondietti et al. (1993) examined the influence of phosphate, arsenate, and selenite sorption on the EDTA-promoted dissolution of γ-FeOOH. The researchers found that the oxyanions inhibited dissolution at near neutral pH values, while they enhanced dissolution at pH values < 5. They hypothesized that mononuclear complexes (especially if they are bidentate) accelerate dissolution, while binuclear complexes inhibit dissolution. It was proposed that the inhibition of dissolution by binuclear complexes is due to the greater energy required to remove two metal atoms from the crystal lattice. Reductive dissolution of Fe oxides was also inhibited by the sorption of oxyanions.

Fendorf et al. (1997) observed that at low surface coverages a greater proportion of arsenate and chromate were observed to be associated with mononuclear complexes compared with a binuclear complex. At lower surface coverages a kinetic investigation (Grossl et al., 1997) indicated that mononuclear complexes were favored over binuclear complexes for chromate.

However, Eick et al. (1999) found that both chromate and arsenate were found to inhibit the oxalate-promoted dissolution of goethite at all pH values and surface coverages investigated except pH 6. Chemisorbed anions increase surface negative charge. This increase in surface negative charge is clearly demonstrated for the adsorption of chromate and arsenate on goethite. An increase in surface negative charge will result in a decrease in oxalate adsorption (due to electrostatic repulsion) and a decrease in dissolution rates. Oxyanions such as phosphate and arsenate pair strongly with Fe^{3+}, resulting in the precipitation of insoluble Fe(PO_4, AsO_4) phases. Consequently, adsorption of these oxyanions makes goethite resistant to organic ligand-promoted dissolution. Oxalate adsorption drops as the pH is raised from 3 to 7. The reduced adsorption coupled with the decrease in the activity of protons is reflected in the decrease in dissolution rates as the pH increases. But the surface complexation results indicated that the adsorption of both chromate and arsenate at pH 6 increases the net negative charge of the goethite surface (chemisorbed anions shift the PZC to lower pH values). This may be accompanied by an increase in the surface protonation. The dissolution of goethite requires protonation of an oxide or hydroxide ion adjacent to the removable Fe complex. At pH 6, the dissolution rate by oxalate alone is two orders of magnitude less than at pH 5. It is postulated that an increase in surface protonation accompanied by chromate and arsenate adsorption is responsible for the enhanced dissolution in the presence of the oxyanions. At pH 6, the reduced rate of oxalate promoted dissolution of goethite, in the absence of the oxyanions, is a function of the reduced activity of protons rather than a reduction in the surface concentration of oxalate.

One would expect ligands that reduce the rate of water exchange around a dissolved metal complex to also inhibit dissolution of the metal oxide. The rate of water exchange around many transition elements in outersphere $M(OH_2)_6^{+2}.(SO_4^{-2})(aq)$ complexes are slower than the rate of exchange around the $M(OH_2)^{2+}(aq)$. On the basis of this evidence, one would logically predict that the adsorption of sulfate would inhibit the dissolution of transition metal oxides.

A combined effect of reduction and complexation was demonstrated. Ascorbic acid was used as a reductant and oxalate as a complexant. The initial rate of dissolution of goethite increased markedly as the amount of adsorbed oxalate increased against a constant background level of ascorbic acid. The electron transfer to the surface-Fe and/or its detachment after reduction may be enhanced by adsorption of the oxalate ligand (Schwertmann, 1991).

Microbial reduction of Fe oxides cannot be totally ruled out. In experiments in which artificial nutrient solutions containing various Fe oxides were inoculated with *Clostridium butyricum* and *Bacillus polymyxa*, it was found that ferrihydrate was more readily reduced than goethite and hematite. In others, an aerobic Corynebacterium was used under controlled conditions and between 2% (for goethite and hematite) and 20% (for ferrihydrite) reduction in about 20-30 days were obtained (Schwertmann, 1991).

The ability of a ligand to chelate Fe cannot be determined merely by its stability constant for Fe^{3+}. Differences between this constant and stability constants for competing ions must be considered, along with differences in concentration between Fe^{3+} and competing ions (Cline et al., 1982). The concentrations of Ca and Mg occur at slightly higher levels in soils than in nutrient solutions. Thus chelating agents are likely to chelate less Fe in soils

than in nutrient solution of similar pH. This is also true because the total amounts of micronutrients (e.g., Zn, Cu) in soils generally exceed the amount of total ligand giving them the potential to completely displace Fe from the ligand. This differs definitely from one soil to another.

Moreover, at low pH in nutrient solutions in which total P exceeds total Fe, $Fe(OH)_3$ (amorphous) completely dissolves and inorganic Fe solubility is reduced since it is now controlled by $FePO_4$ (amorphous). Thus Fe has less ability to compete for chelating agents (Cline et al., 1982).

Organic matter improves Fe availability by combining with Fe, thereby reducing chemical fixation or precipitation of Fe as ferric hydroxide. This reduction in fixation and precipitation results in higher concentrations of Fe remaining in the soil solution, available for root absorption. Organic matter can also affect Fe availability by acting as an energy source for microorganisms that use up oxygen under waterlogged conditions. When microorganisms decompose organic matter, Fe previously tied up in organic compounds is released in forms available for plant uptake.

Iron chelates increase solubility and availability of Fe to plants in Fe-limited soils by increasing total Fe in solution and in hydroponic culture. In soils, chelates increase the pool of soluble Fe and diffusion of Fe from the bulk soil to depleted microsites in the rhizosphere. These compounds (chelates) may originate as root exudates, from the degradation of organic matter, as metabolic products of microorganisms, or as Fe chelate fertilizer added to the soil (Reid et al., 1984, Reid et al., 1986, Jurkevitch et al., 1986, Maurice et al., 2000).

Organic amendments are known to contain organic compounds capable of chelating Fe. These Fe chelators include humic acids, amino acids, phenolics, hydroxamates and catechol siderophores (Chen et al., 1998).

Soil humic substances have been widely regarded as playing a beneficial role in Fe acquisition by plants. This effect has been mainly attributed to the complexing properties of humic and fulvic acids increasing the availability of the micronutrient from sparingly soluble hydroxides. Difficulties in obtaining humic and fulvic acids free of Fe with the procedure usually employed for the fractionation of humic matter were taken as evidence for the formation of stable complexes (Pinton et al., 1998). Electrically charged sites on humic substances function to dissolve and bind trace minerals. Two negatively charged sites on the humic substance attract metal cations with two negative charges. As a result the cation binds itself to more than one charged anionic site. By forming organo metal claws these organic acids bring about the dissociation of primary and secondary minerals within the soil. These minerals then become available for uptake by plant roots. The greater the affinity of the metal cation for humic or fulvic acids, the easier is the dissolution of the cation from various mineral surfaces. Both the acidic effect and the chelation effects appear to be involved in dissolution of minerals and binding processes.

Indeed, the percentage of the organically-complexed Fe in the soil solutions was up to 90.7% (Table 5). Moreover, the percentage of the organically-complexed Fe was highly correlated only with soil pH ($r = 0.93$). This important finding implies that total soluble Fe in the soil solution becomes more organically complexed as the pH increases from 4.54 to 7.66. This fact definitely explains the absence of any correlations between the total soluble Fe concentrations in the soil solutions and soil chemical and physical properties of the studied soils. In addition, this finding totally confirms that the chemical equilibria equations of soil Fe minerals (Fig. 7) cannot be used to predict the real concentration of total soluble Fe in the soil solution

because these equations do not take into consideration that under certain conditions (in particular under alkaline and calcareous soil conditions; our findings) the most of the total soluble Fe present in the soil solution is complexed to soluble organic compounds. There is a great lack of information in the literature about the percentage of organically-complexed Fe in the soil solution. Uren (1984) reported that the percentage of organically-complexed Fe in soil solutions extracted at field capacity by centrifugation from a sandy loam soil limed to different pHs and equilibrated for 10 months ranged from 2% at pH 4.5 to 42% at pH 7.5. This was accompanied by an increase in the concentration of carbon in the extracted soil solutions (from 7.8 mM to 20.4 mM), which might explain the observed increase in the percentage of organically-complexed Fe. Our findings showed that the correlation between the organically-complexed Fe and the DOC was found to be very weak. However, within the alkaline pH range ($pH \geq 7.0$), this correlation increased to 0.56.

Kaiser and Zech (1999) found that sorption/desorption of natural organic matter (NOM) to soils and hydrous oxides is influenced by:

1. The solution-pH,
2. The solution composition (presence of inorganic oxyanions),
3. The degree to which the binding sites of the sorbents are occupied, especially by organic matter, and
4. The chemical composition and properties of the dissolved NOM itself. The influence of the latter results in a fractionation of NOM during the sorption process.

These authors reported that higher solution-pH released the greatest amount of NOM from the sorbents. The increase of the NOM released from goethite was approximately linear between pH 4.0 and 7.0 (Kaiser and Zech, 1999).

The strong desorption of NOM at high pH agrees well with the results on the desorption of humic acids from an iron oxide surface (Avena and Koopal, 1998). In addition, the desorption of NOM sorbed to mineral surfaces seems to be independent of the solution ionic strength. The same independence was found for the sorption of NOM on soils and hematite. In contrast, increasing concentrations of inorganic oxyanions that are known to compete with NOM for binding sites, such as SO_3^{2-} and $H_2PO_4^-$, resulted in increasing organic carbon release, but only $H_2PO_4^-$, an anion that forms strong bonds on Al and Fe oxide surfaces via surface complexation, released considerable amounts of sorbed NOM. Moreover, the hydrophobic NOM contains more acidic functional groups than the hydrophilic fraction, more ligands per molecule may form strong surface complexes (Kaiser and Zech, 1999).

It is of great importance that variations in critical soil chemistry parameters can induce shifts in the fractionation of micronutrients (Sims, 1986), which may result in a micronutrient deficiency or toxicity. Consequently, the effects of soil pH, Eh, exchange capacity, organic matter level, texture, oxide content, and clay mineralogy on the distribution of, for example, Mn, Cu, and Zn have received considerable research interest. Soil pH has been identified as a particularly critical parameter in the regulation of micronutrient availability. Decreases in exchangeable Zn as pH increased (Iyengar et al., 1981), were seen with associated shifts of Zn into organically complexed or oxide-bound pools. Increases were observed (McBride and Blasiak, 1979) in soil solution Zn at pH values > 7.5, due to the formation of soluble-Zn organic-matter complexes.

In the study of Sims (1986), four soils that varied widely in organic matter content (0.93 – 5.8% organic carbon), texture, and cation exchange capacity were studied. A fractionation scheme was utilized that partitioned Mn, Cu,

and Zn into the exchangeable (EX), organic (OM), Mn-oxide bound (MNOX), amorphous Fe-oxide bound (AFeOX), and crystalline Fe-oxide bound forms (CFeOX). Micronutrient distribution among these fractions was studied over a pH range of 4.0 to 7.7 in the soils. Soil pH markedly altered the distribution of Mn and Zn but had little effect on Cu. Although soil type did have some influence, exchangeable Mn and Zn were generally the dominant species of the elements below pH 5.2, while at higher pH values organically complexed and Fe-oxide bound forms were dominant (Table 17). These results, and probably our findings, can be attributed to the fact that solubilization of organic acids under alkaline conditions may result in increased complexation of Mn (and Zn) inhibiting their precipitation or occlusion by soil oxides. It was reported (McBride, 1982) that stronger bonding of Mn^{2+} by organic solids was induced by increases in soil pH.

Table 17: The influence of soil pH on Mn distributing in four soils:

Soil	O.C. ($g\ kg^{-1}$)	pH	EX	OM	MNOX	AFeOX	CFeOX
			% of total Mn				
Pocomoke	58.0	4.1	84	11	2	2	3
		5.0	48	39	5	4	3
		5.8	6	71	9	9	4
		6.9	8	60	12	17	4
		7.5	11	60	10	13	6
Rumford	9.3	4.8	50	17	5	14	13
		5.1	34	28	7	16	15
		5.8	22	34	13	16	15
		6.9	10	36	18	20	16
		7.7	5	34	11	29	21
Matapeake	13.0	4.7	12	47	15	22	5
		5.2	5	48	18	24	5
		5.6	3	56	11	22	6
		7.1	1	44	19	29	7
		7.5	1	74	9	13	4
Sassafras	13.0	4.8	19	43	8	23	7

		5.4	4	50	10	28	8
		5.9	3	49	13	26	9
		6.8	2	49	12	28	9
		7.5	2	45	10	29	13

Source: Sims (1986)

In our experiments concentration of the total soluble Fe in the soil solutions (of the first collection) ranged from 2.5 to 188 μM. These concentrations are higher than what it would be expected from the chemical equilibria equations of soil Fe minerals (Fig. 7) for the reasons previously mentioned. In the literature there are very scarce reports concerning the concentration of Fe in the soil solution. Mashhady and Rowell (1978) measured the Fe concentration in the soil solution of a sandy soil (85% sand, 1% organic carbon, pH 7.21-9.08). Yield of tomato plants decreased with increasing pH from 7.21 to 9.08. Plants did not develop chlorosis, and the foliar sprays had no significant effect on yield. Chlorosis due to deficiencies of Fe (and Mn) therefore did not occur in this soil. The authors suggested three possibilities exist for the supply of these nutrients:

1. some form of “contact exchange” or direct uptake from the solid particles in the rhizosphere;
2. poor soil physical conditions leading to waterlogging and reduction, and hence greater availability, of Fe (and Mn);
3. available chelated Fe (and Mn) in soil solution.

Excessive watering was avoided by bringing the soils only up to field capacity. This however does not preclude the possibility of anaerobic pockets (microsites) existing in the soil, and so supply of Fe (and Mn) in the reduced form may have occurred. However, D’Yakonova (1962) has shown that fulvic and humic acids from several soils were capable of dissolving considerable amounts of Fe which was then readily available, and Olomu et

al. (1973) found that almost all the Fe in the solutions from waterlogged soils was complexed with organic matter. At high pH this may be a particularly good source of Fe (and Mn), since it was noticed that drainage water from the pots was brown, presumably as a result of dispersed organic matter. Both Fe and Mn were present in the extracts of the soils to which Na_2CO_3 had been added, in amounts far greater than would be possible by solution of inorganic Fe and Mn compounds alone. The concentrations increased with increasing addition of Na_2CO_3; from 0.22 x 10^{-4} M Fe (22 μM Fe) at 0.0125 mol L^{-1} Na_2CO_3 to 7.34 x 10^{-4} (730 μM Fe) at 0.10 mol L^{-1}. If this Fe (and Mn) is available then the supply would be adequate. To test whether the Fe (and Mn) released after addition of Na_2CO_3 could supply the requirements of the tomatoes, addition of the soil extract to tomato plants grown in sand culture (100 ml of soil extract per pot given twice a week) removed all traces of chlorosis. It was concluded therefore that the soil extracts did supply the Fe (and Mn) requirements of the plants, but that some other factors reduced the yield. The most likely cause was a higher concentration of Na in the extracts (than in the corresponding nutrient solutions). In sodic soils the presence of Na_2CO_3 disperses organic matter, which chelates with Fe (and possibly Mn) and may supply the crop requirements for these nutrients. Where chlorosis does occur, then either the chelate supply may be low due to very low soluble organic matter levels, or there may be horizons rich in $CaCO_3$ which may result in lime induced chlorosis even though there are sodic characteristics in other parts of the profile.

In our experiments, unexpectedly, the total soluble Fe concentration in the soil solutions of the calcareous soils (this research) (pH 7.66-7.68) ranged from 22.0 to 25.5 μM (in the first collection), which was similar or even

higher than that of other neutral and acidic soils. Mengel et al. (1984) measured the Fe concentration in the soil solution of calcareous (pH 7.3, clay 43%, 8.3 mg DTPA-Fe kg^{-1}, 24.4% $CaCO_3$) and non-calcareous (pH 6.3, clay 14%, 8.1 mg DTPA-Fe kg^{-1}) soils as affected by different soil water contents (60% and 120%). It was found that the Fe concentration of the soil solution varied considerably throughout the experimental period. However, there were no major differences between treatments, except that the water saturated treatment of the non calcareous soil showed a lower Fe concentration in the soil solution for some weeks of the growth period (Table 18). The measured concentrations of total soluble Fe in the soil solution of the calcareous soils (this research) are very close to those measured in the study of Mengel et al. (1984).

Table 18: Fe concentration in the soil solution collected from a soil treated with different water contents

Fe concentration (µM)	Time
7-8	At the beginning
14-17 vs. 9*	1 week
13-15 vs. 11*	2 weeks
17-19 vs. 11*	3 weeks
≤ 14	4 & 5 weeks

*Calcareous soil (60 and 120%) and Non-calcareous soil (60%) vs. Non-calcareous soil (120%). Source: Modified from Mengel et al. (1984)

Chen et al. (1998) reported that chelators recovered from compost microorganisms (CCM) efficiently solubilize Fe from Fe_2O_3 particles at pH 7.5 in comparison with EDDHA. After four days, these chelators solubilized 25 µM Fe compared with 20 µM Fe solubilized by 52 µM EDDHA. The initial rate of Fe chelation and solubilization was greater for CCM than for

EDDHA. These Fe chelators include humic acids, amino acids, phenolics, hydroxamates and catechol siderophores.

Cesco et al. (2000) evaluated the capacity of water extractable humic substances (WEHS) to solubilize Fe in the soil using samples of three soils differing in their chemical characteristics. The presence of WEHS (1.7 mmol organic carbon per L) significantly increased the amount of Fe solubilized from all the three soils as compared to deionized water (Table 19).

Table 19: Solubilized Fe by WEHS and DTPA in different soils as percent of control (Fe solubilized by deionized water)

Soil	pH (H_2O)	Organic carbon (g kg^{-1})	$CaCO_3$ (g kg^{-1})	Deionized water	WEHS (1.7 mmol organic carbon L^{-1})	DTPA (100 µM)
Vertisol	8.34	23.3	110	100	154	122
Alfisol	6.86	12.4	<10	100	176	156
Leptosol	7.15	30.9	20	100	140	127

Source: Modified from Cesco et al. (2000)

Olmos et al. (1998) showed that addition of commercial humates increased the extractability of Fe from calcareous and non-calcareous soils (Table 20 and Fig. 8).

Table 20: Calcareous soil properties and the effect of two organic matter concentrations over Fe extraction (µg Fe g^{-1}) from this calcareous soil.

Texture	pH	$CaCO_3$ (g kg^{-1})	O.M. (g kg^{-1})	control	O.M. concentration (g kg^{-1})	
					0.2	0.8
Clay-loam	7.95	403	5.4	0.81 a	0.85 a	1.11 b

Source: Olmos et al. (1998)

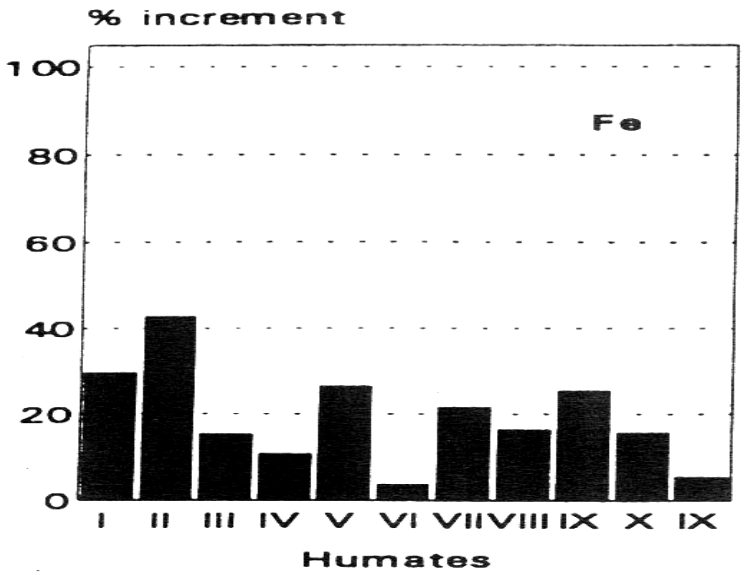


Figure 8. Increments of Fe availability expressed as percentage of element extracted after the interaction of calcareous soil with humates. Source: Olmos et al. (1998)

Pinton et al. (1999) found that Fe-WEHS could be a suitable Fe source for Fe-deprived cucumber plants even when the pH of the nutrient solution was buffered at 7.5. Essentially the same result was observed when the pH of the nutrient solution was buffered at a similar value by adding 1 g L^{-1} calcium carbonate (Mohamed et al., 1998).

Our data showed that eleven different soils were able to maintain an almost constant concentration of total soluble Fe in their solutions after being collected for three consecutive times. The studied calcareous soils showed a high Fe buffer power as well. These results are in agreement with the high Fe buffer power observed in the soils used in the work of Mengel et al. (1984) (Table 18). As far as Fe nutrition is concerned, humified organic matter may prevent precipitation of the micronutrient as ferrihydrite in the soil, providing a reservoir of Fe which can be utilized by plant root exudates

or microbial siderophores. In addition, the presence in soil solutions of low molecular weight fractions of the humic substances, which are capable of forming soluble complexes with Fe, due to their high content of oxygen-containing functional groups, suggests a more direct contribution of these molecules to Fe nutrition of plants (Pinton et al., 1999, Cesco et al., 2000). Cesco et al. (2000) found that the amount of ^{59}Fe-WEHS moving along the soil columns was smaller compared to Fe-EDDHA complexes. This effect was particularly evident when the calcareous Vertisol was considered. However, in this soil it was observed that addition of WEHS in the eluent mobilized ^{59}Fe from the soil column. These authors speculated that several factors might have contributed to the observed result, like polycondensation of water soluble humic molecules by formation of metal bridges. Another opportunity would be the adsorption of the Fe-WEHS to clay. Powell et al. (1980) suggested that binding to the clay and the organic matter particles could account for the recovery of only 3% of ferrioxamine B (microbial siderophore) added to soil. Such reservoir of Fe might be responsible for the high Fe buffer power observed in some soils tested in this research.

The concentration of total soluble Fe in the soil solution of the calcareous soils was found to be similar or even higher than that of the neutral and acidic soils. In addition, most of this Fe was found to be organically complexed. These findings support the hypothesis of Mengel (1995). According to this hypothesis, lime-induced chlorosis is not caused by the low solubility of Fe(III) oxides (or slow dissolution rates of these oxides), but is rather a problem of Fe utilization in the plant, in the root and leaf apoplast (physiological Fe deficiency), even when Fe in the soil is mobilized by natural chelators. It has been hypothesized by Mengel (1995) that Fe(III) chelates are transported to the root apoplast where chelates reduction may be

blocked due to the high apoplastic pH under alkaline conditions. Consequently, Fe is trapped in the apoplast, which leads to high Fe concentrations in the root apoplast.

Masalha et al. (2000) found that the Fe concentration of roots grown in the calcareous soil was about 5-10 times higher than those grown under acidic conditions in the organic soil. Similarly, Kosegarten and Koyro (2001) reported that, despite clearly lower amounts of DTPA-extractable Fe in the calcareous soil, the Fe concentration in the plant roots grown on this soil was 10 times higher than that of roots grown in the acidic soil. Bienfait et al. (1985) showed that, under calcareous soil conditions, the root apoplastic concentration of Fe was 2000 μg g^{-1} DW in comparison with only 200 μg Fe g^{-1} DW in acidic soil-grown maize roots. Under alkaline conditions, high pH levels prevail in the root apoplast, and as recently shown by fluorescence ratio imaging, fairly high apoplastic pH levels of around 7 occur in the hair zone of roots exposed to NO_3^-/HCO_3^- for a long period. In analogy to the findings of impaired Fe(III) reduction at apoplastic pH higher than 6 in intact leaves, Fe(III) reduction may also be substantially depressed in the epidermal root apoplast (Kosegarten and Koyro, 2001). Recently, Kosegarten et al. (2004) reported that in roots bathing in buffered outer solutions of different pH, a high pH sensitivity of apoplastic Fe(III) reduction was found, with the highest ferric Fe reduction rates at an apoplastic pH of 4.9; above an apoplastic pH of 5.3, no reduction was observed, the fact that confirms Mengel's hypothesis (Mengel, 1995) and supports our findings that under calcareous soil conditions, dissolution of Fe oxides is not the limiting factor and that the measured high concentrations of total soluble Fe in the soil solution of these soils are totally realistic.

4.3 The central role of microbial activity in increasing the concentration of total soluble Fe in the soil solution of different soils

An important feature of Fe both in soils and plants is the way it readily forms organic complexes with chelates which are called siderophores and are synthesized by bacteria, fungi and plants. They are of crucial importance for the Fe transport in soils and the Fe supply of plants (Mengel and Kirkby, 2001). Siderophores are highly Fe(III)-specific ligands excreted by aerobic and facultative anaerobic microorganisms to facilitate Fe uptake in aerobic environments. Siderophores form very stable soluble complexes with Fe(III). Fungal siderophores form complexes with Fe which have stability constants near that for the Fe(III)-EDTA complex (10^{30}), whereas bacterial siderophores can form complexes with stability constants near that for Fe(III)-EDDHA (10^{40}) (Hersman et al., 1995). More than 200 siderophore compounds have been isolated, the majority of which are either hydroxamates or phenolates-catecholates (Fig. 9) (Hersman et al., 1995).

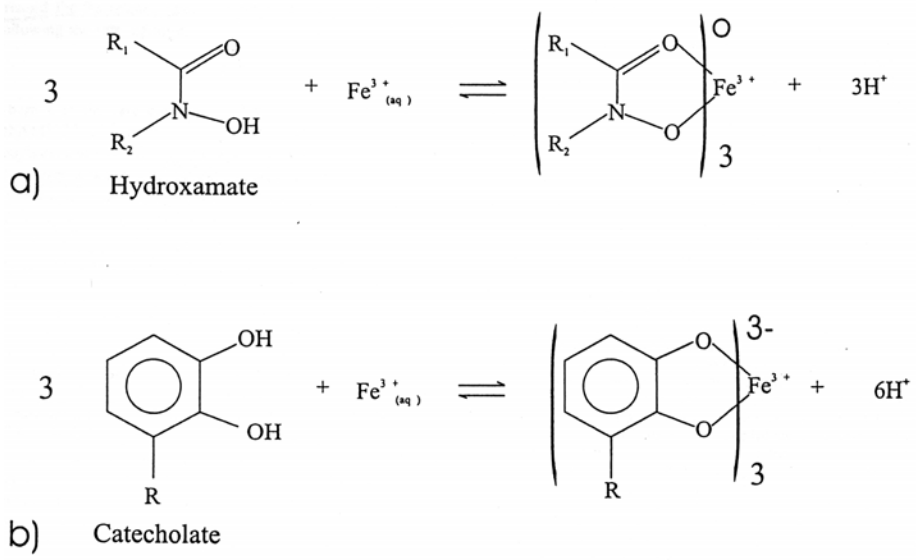


Figure 9. Fe(III) complexation reactions of a) hydroxamate and b) catecholate siderophores (Source: Kalinowski et al., 2000)

Siderophores could be adsorbed on the mineral and be responsible of an amorphisation of the surface (Watteau and Berthelin, 1994). According to Holmen and Casey (1996), the ligand-promoted dissolution mechanism by the acetohydroxamic acid (aHA) (smaller analogous ligand of DFOB) at pH > 4 involves 3 steps (Fig. 10):

1. Formation of the monohydroxamate surface complex,
2. Detachment of the Fe(III)-aHA complex with simultaneous movement and dissociation of water molecules to replace the eliminated oxygen sites on the mineral surface, and
3. Readsorption of aHA via a ligand exchange reaction.

The complex detachment and replacement of surface sites (step 2) is assumed to be the rate-controlling step.

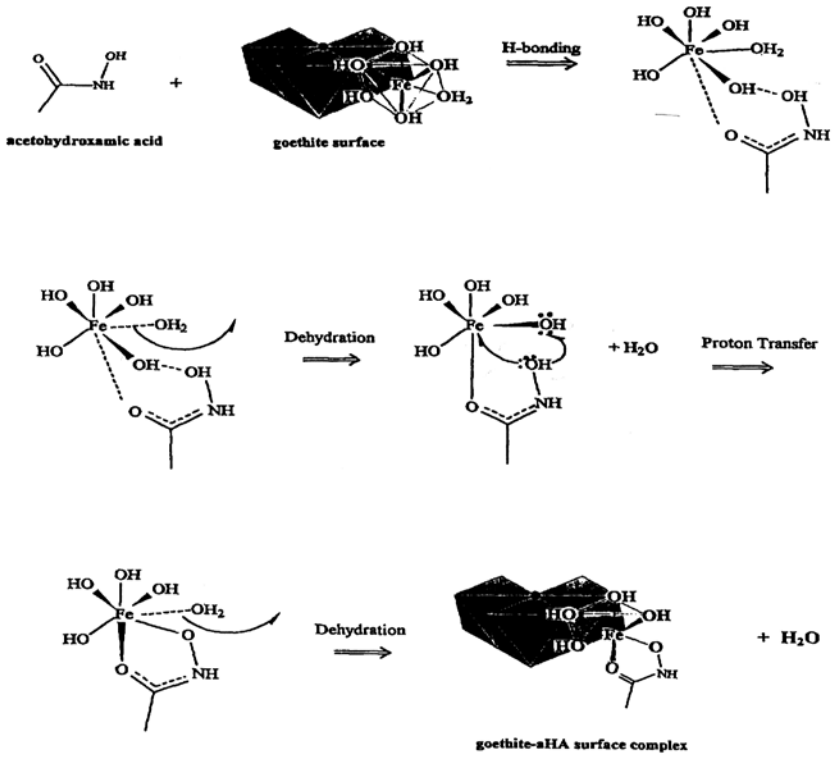


Figure 10. Formation of siderophore-Fe(III) oxide surface complex (Source: Holmen and Casey, 1996)

Our results clearly showed for the first time that the addition of different carbon sources to 30 soils resulted in higher microbial activity and consequently in an increase in the total soluble Fe concentration in the soil solutions collected from these soils using the Buchner funnel technique regardless of their markedly different chemical and physical properties These carbon sources (glucose, cellulose and starch) were added and mixed with these soils to simulate root exudates and the carbon enriched rhizosphere where microbial activity and its influence on plant Fe nutrition is supposed to be of pivotal importance. Total soluble Fe concentration in the soil solution of soils where no increase in the microbial activity after the addition of these carbon sources was detected, did not increase either. This provides evidence that the measured increase in Fe concentration was directly related to the increase in the microbial activity.

Most microorganisms require micromolar concentrations of Fe to support growth. With hydroponically-grown plant material, comparisons of physiological data for siderophore utilization by microbes and plants show that there is a striking parity between plant and microbial requirements for Fe; both require a concentration of 1 to 20 μM Fe for normal growth depending on the organism and source of chelated Fe. Studies with microorganisms show that as Fe falls below these levels, increasing concentrations of desferrisiderophore are rapidly accumulated in the culture media depending on the degree of Fe stress that is imposed (Crowley et al., 1991). Hersman et al. (2001) found that as the concentration of FeEDTA (as Fe source for microorganisms) decreased, so did microbial growth rate. The highest microbial growth was found to occur at 30 μM Fe and the lowest at 0.05 μM Fe and the control (no Fe added).

The concentrations of siderophores in soil environments range quite broadly. For bacteria, this corresponds to tens of micromoles to a few millimoles per liter (Hersman et al., 1995). Reid et al. (1984) found that the concentration of siderophores in the rhizosphere may exceed that in the bulk soil by as much as 50-fold. These investigations suggest that roots may encounter concentrations of siderophores in the micromolar range in soils.

Both soil and sand contained measurable quantities of siderophore groups. The concentrations of total hydroxamate siderophores (in 2 water : 1 soil (v/w) extracts) were at least 10 μM, a value that agreed well with ICP analysis for total soluble Fe. Because it was previously shown that much of the extractable hydroxamate siderophores in soil may be adsorbed to clay and organic matter, the concentration of hydroxamate siderophores that effectively mobilizes Fe is probably less than that extracted (Crowley et al., 1987). Kalinowski et al. (2000) mentioned that aqueous siderophores concentrations in nature may range from ~10 μM to 1-2 mM. Typical concentrations for other common chelators in soils, including oxalic and ascorbic acids, are 2-3 mM respectively. Association constants for Fe(III) for siderophores are many orders of magnitude greater than the association constants for these latter low molecular weight organic acids, however, so the effect of siderophores should be powerful.

Under Fe-stress conditions, very high concentrations of siderophores may be produced by common soil microbes when cultured in low Fe media. Concentrations of desferripyoverdin have been measured at 300 μM for *Pseudomonas putida* and 100 μM concentrations are not uncommon for other microbes. Although such concentrations are reported for pure cultures in nutrient media, it is likely that cell densities of 10^8 to 10^9 per mL are similar to microbial population densities in rhizosphere soil. For example,

total bacteria in the rhizosphere of oat have been estimated by direct microscopy to be 3 x 10^9 per gram. In addition to total microbial numbers, the metabolic activity of this population would also be important in determining the potential amount of siderophore that is produced. Since rhizosphere microbes depend on plant exudates for growth, microbial activity will almost certainly be related to plant growth and vigor. Application of mineral fertilizers or irrigation practices that result in a change in the composition or quantity of root exudate could significantly affect both the type and quantity of the various siderophores that are produced by different microbial populations (Crowley et al., 1991). Our results clearly showed that glucose, cellulose and starch (as carbon substrates) could increase the microbial activity in the tested soils and consequently, the siderophore production. Organic acids (from microbial or plant origin) are supposed to be good carbon substrates for siderophore production. Sharma and Johri (2003) reported that, in strain GRP3A, succinic acid was found to be a good substrate for siderophore production at pH 7.0. The siderophore production was recorded maximum in succinate medium (51.58 mg L^{-1}), which was 3-fold higher than malate medium (17.56) and 2.5-fold higher than citrate medium (20.33). Therefore, the kind of organic acids present in the soil, which might differ from one soil to another, would definitely influence the amount of produced siderophores and consequently the amount of solubilized Fe.

Bossier and Verstraete (1986) reported 70 μg kg^{-1} FOB equivalents in bulk grassland soils. Following soil amendments with sucrose and L-ornithine to simulate organic-substrate-enriched rhizosphere soil, siderophore levels increased to approximately 600 μg kg^{-1} soil, a value that converts to approximately 10 μM siderophore in solution. According to Crowley et al.

(1991), values obtained for siderophores in bulk soils range from 0 to 150 µg kg^{-1} soil. When converted to a concentration, this corresponds to 0 to 2.5 µM FOB equivalents. In soils amended with straw, siderophore concentrations of 10 to 80 µM FOB during organic matter decomposition were measured. When sucrose and ornithine are added to soil to simulate nutrient enrichment that occurs in the plant rhizosphere, ferric-hydroxamate siderophores have been measured at 616 µg kg^{-1} soil or approximately 9 µM FOB equivalents. This value is similar to the physiological concentrations that are produced and used by some microorganisms in pure culture and which may be effective for providing Fe to plants. These concentrations agreed very well with our ferrozine analysis for total soluble Fe in the 30 different soils used in our research. However, studies of disease suppressive soils indicate 50 µM desferri-pyoverdin soil applications can be used to obtain equivalent disease suppression provided by inoculation with a plant-beneficial *Pseudomonas* strain, suggesting that similar concentrations might be produced in rhizosphere soil.

Moreover, Crowley et al. (1987) measured the concentrations of microbial siderophores in calcareous sandy loam soil and in silica sand amended with 4% plant-litter organic matter (Table 21). At the 10 µM siderophore levels, siderophores would exceed soluble non-chelated Fe by 2.5 million to 1. Under aerated conditions at high pH, where diffusion of inorganic Fe is rate-limiting for plant uptake, it is probable that siderophores completely control the availability of Fe at the root surface.

Table 21: Concentrations of microbial siderophores detected in soil and sand amended with 4% plant-litter organic matter

Siderophore	μM Fe	
	4 weeks	8 weeks
Soil	13.56	11.36
Sand	33.40	14.00

Source: Crowley et al. (1987)

The magnitude of the increase in the concentration of total soluble Fe detected in the calcareous soil (used in our research) agrees well with the results of Crowley et al. (1987). Furthermore, Fe release from goethite, hematite and ferrihydrite by *Pseudomonas mendocina* appears to have occurred in excess. Microorganisms attached to the Fe oxides were removing enough Fe (in a micromolar concentration) to support not only their growth but also the growth of nonattached cells (Hersman et al., 2001). Similarly, Hersman et al. (2000) reported that not only is siderophore produced in the presence of hematite, but also it appears to have been produced in excess because more siderophore was produced than would be required to dissolve enough Fe for growth. These bacteria appear to have produced enough siderophore to dissolve ~4.9 times as much Fe as needed. It may be necessary for the cells to dissolve excess Fe to ensure growth. Perhaps this is related to the affinity of the Fe complex for the microbial surface sites (i.e., not all of the Fe in solution is accessible to the cells). Therefore, the overproduction of siderophore may be necessary to ensure that enough Fe becomes available for growth.

An example for the fungi which produce siderophores and live in intimate association with the roots of a wide variety of woody plants includes the Basidiomycetes *Amanita muscaria*, *Boletus edulis*, *Suillus* species, *Pisolithus tinctorius* and the imperfect species *Cenococcum geophilum*

(Powell and Szaniszlo, 1982). Among the large diversity of bacteria, pseudomonads are characterized, under iron limited conditions, by the production of siderophores, the pyoverdines or pseudobactins. In addition to these high affinity chelators, pseudomonads are also known to produce other lower affinity siderophores such as pyochelins. In *Pseudomonas putida*, heterologous siderophores can enhance the level of available iron (Sharma and Johri, 2003). The most common groups of siderophores are the hydroxamates and the catecholates, but novel groups of siderophores are still being discovered in nature (e.g. Amonabactin). Bacteria produce hydroxamate siderophores only in low-Fe surroundings and catecholates only in extremely low Fe surroundings. Hydroxamate siderophores form electrically neutral complexes when binding Fe(III), while catecholates form electrically (negatively) charged complexes (Fig. 9). The presence of two adjacent hydroxyl hydrogens (with high association constants) in catechol generally explains why catecholates are predicted to be more powerful ligands for Fe(III) than hydroxamates at non-acid pH values (association constants as high as 10^{52}). However, at acidic pH, hydroxamates are more powerful chelators than catecholates (Kalinowski et al., 2000). It is obvious that soil pH influences the type of siderophores produced and consequently the quantity of the solubilized Fe.

In the investigation of Crowley et al. (1987) (see above), 85% of the siderophores were in the ferrioxamine group, which includes ferrioxamine B and rhodotorulic acid as well as other hydroxamate siderophores.

However, bacteria, lichens, and fungi in soils also produce organic acids such as lactic, succinic, oxalic, citric, acetic and α-keto acids. These dissolved acids and other organic exudates can affect pH in weathering solutions and thereby promote or inhibit dissolution of minerals. The

dissolved organic molecules can also form surface complexes that affect weathered mineral surface characteristics by ligand-promoted dissolution or through inhibition of reactivity. Alternatively, organic ligands can complex cations in solution, inhibiting precipitation or lowering the saturation index in solution and enhancing dissolution indirectly. Insoluble extracellular polysaccharides can both increase and decrease dissolution of minerals under different conditions (Kalinowski et al., 2000). Similarly, in addition to the siderophores, Liermann et al. (2000) reported ion chromatographic evidence for production of formic, acetic, oxalic, and citric acids by the *Arthrobacter* sp. It is possible that the *Arthrobacter* sp. produces organic acids such as pyruvic, α-ketoglutaric, lactic, or succinic acids. Furthermore, gluconic, lactic and minor pyruvic and succinic acids from *Micrococcus halobius*, and bacteria commonly produce these acids were detected. The reported decreases in pH of solutions in growth experiments may therefore be presumed related to a mixture of these low molecular weight organic acids, as well as siderophore molecules. This decrease in pH might be of great importance for the root apoplastic pH and for the plasma-membrane ferric-chelate reductase and, consequently, for the reduction and uptake of Fe under calcareous soil conditions.

In addition, organic amendments are known to contain organic compounds capable of chelating Fe. These Fe chelators include humic acids, amino acids, phenolics, hydroxamates and catechol siderophores. Organic amendment addition also results in a rapid increase in microbial biomass size and activity and induces the development of a diverse bacterial community. The microbial composition of one organic amendment has been well characterized and includes many microorganisms that synthesize microbial siderophores (Chen et al., 1998). Chelators recovered from

compost microorganisms (CCM) efficiently solubilize Fe in comparison with synthetic chelates (25 μM solubilized Fe in case of CCM and 20 μM Fe in case of 52 μM EDDHA) from Fe_2O_3 particles at pH 7.5. The initial rate of Fe chelation and solubilization was greater for CCM than for EDDHA (Chen et al., 1998).

However, during a period of 21 days, the mobilization (solubilization and uptake) of Fe by the ectomycorrhizal fungus (*Suillus granulatus*) was very low, despite the production of aliphatic acids and the dramatic decrease in the pH (from 7 to 3). After 21 days, the Fe mobilization increased very significantly by solubilization in the nutrient medium and accumulation in the mycelium of large amounts of Fe provided by the ferric oxide. Such Fe uptake and solubilization occurred simultaneously to an important production of trihydroxamic siderophores, suggesting a relation between the production of these chelating compounds and the dissolution of ferric Fe from the well crystallized goethite. Such mechanisms of Fe dissolution and accumulation from different minerals occurred certainly in the rhizosphere of plants in order to increase Fe availability (Watteau and Berthelin, 1994). On a per cell basis, more siderophore was produced when Fe was supplied as hematite than in the Fe-free control. Thus, growth on hematite and the production of siderophore in the presence of hematite present compelling evidence that siderophore is produced as a mechanism to acquire Fe from hematite and that this is a successful mechanism (Hersman et al., 2000).

The inability of most of the naturally occurring organic acids to chelate Fe at high pH demonstrates the high degree of Fe specificity required to chelate Fe under these conditions. Only DFOB and a mixture of unknown hydroxamate siderophores exhibited sufficient Fe specificity to chelate Fe at all pH values tested (Cline et al., 1982). Three lines of evidence suggest that

chelation by siderophores is the dominant mechanism of Fe release. First, Zhang and Bloom (1999) have reported experiments investigating the dissolution of hornblende in the presence of low molecular weight organic acids. They have concluded that Al and Fe are preferentially released with respect to Si. In contrast, in experiments reported with bacteria, only Fe release was enhanced. Second, release of Fe is accelerated in the presence of the siderophore DFAM without bacteria. This provides evidence that siderophores can promote Fe release from hornblende. Third, release of Fe is accelerated when *Arthrobacter* sp. cultures are injected with DFAM. Therefore, although the effects of low molecular weight organic acids cannot be ruled out entirely, we conclude that chelation by siderophores is the dominant mechanism causing enhanced Fe release (Kalinowski et al., 2000). Crowley et al. (1987) pointed out that under conditions of low Fe solubility in soils, Fe chelates are extremely important for mobilizing Fe and increasing its availability to plants and microorganisms. However, with the exception of microbial siderophores, most of the many different chelating agents in soils are relatively nonspecific for Fe and readily chelate other more soluble metal ions. The naturally occurring organic acids, citrate and malate are unstable with Fe above pH 6, whereas, hydroxamate siderophores are stable with Fe over the entire pH range found in soils. But the *Pseudomonas* sp. siderophore is as powerful as typical carboxylic acids in promoting hematite dissolution at low pH (Hersman et al., 1995). Similarly, the dissolution rates measured in the presence of 10^{-3} M oxalate were higher by up to an order of magnitude (at pH 3.2) than the reported siderophore-promoted rates. However, in contrast to oxalate, the hydroxamate ligand is capable of maintaining elevated dissolved Fe(III) concentrations at neutral pH (Holmen and Casey, 1996).

Watteau and Berthelin (1994) found that after 2 days of incubation the amount of ferric Fe solubilized by the DFOB was much higher than the solubilization by the mixture of 3 organic acids (oxalic acid, citrate and malate) despite their higher concentration. This effect of the siderophore was always observed after 10 days of incubation.

To compare the efficiency of the aliphatic acids with the DFOB at the same molecular ratio, one millimole of DFOB has solubilized 500 μg of Fe as ferric Fe after 28 days, and that only 28 μg of ferric Fe has been dissolved by the effect of one millimole of aliphatic acids. This difference can be explained by the affinity of the DFOB for Fe^{3+} comparatively to those of other acids: affinity constants are $10^{30.5}$, $10^{11.9}$ and $10^{10.0}$ respectively for the DFOB, citric and oxalic acids.

It is also interesting to underline that despite the low pH and the protons availability (1000 mM HCl were added), solubilization of goethite in presence of HCl was not so much important. This result showed that the acidity alone and the exchange with protons alone were not sufficient to dissolve efficiently and fastly a well crystallized ferric oxyhydroxides.

Cline et al. (1983) reported that in an extract from a soil with pH 5.7 with citrate, supernatant Fe was measured at 2.73×10^{-4} M, indicating that 80% of the added citrate was chelated with Fe. No Fe chelation was detected for any other organic acids in this soil, and no detectable Fe chelation was measured for any of the organic acids in a soil with pH 7.5. DFOB, a siderophore produced by *Streptomyces pilosus* was shown to form a stable Fe-complex (FOB) in nutrient and soil solutions between pH 4 and 10 (Jurkevitch et al., 1988). Both DFOB and an unknown hydroxamate siderophores formed stable Fe chelates over the entire pH range (5-10). Measured chelated Fe remained unchanged when measured at increasing unit increments of pH

from pH 6.0-10.0 (Cline et al., 1982). When equilibrated in soil extracts of pH 5.7 and 7.5 for 24 h in the presence of excess Fe (10^{-3} M), hydroxamate siderophores (10^{-4} M) formed stable Fe chelates. At least 87% of DFOB, BEMX (*Botetus edulis* mix) and DFCA (desferrichrome A) were experimentally determined to be chelated with Fe in the soil extracts (Cline et al., 1983). It is concluded that hydroxamate siderophores should be effective Fe-chelating agents in acid and alkaline soils and that no other soil ions can compete favorably with Fe for the hydroxamate siderophore ligand.

Hydroxamate siderophores invariably present in a variety of soils at concentrations sufficiently high to affect plant Fe nutrition, and in equilibrium with a much larger adsorbed pool which suggests resistance to both leaching and microbial decomposition (Cline et al., 1982).

In addition, Jurkevitch et al. (1986) showed that, after a second growth cycle, a strong residual DTPA-Fe effect after the addition of the bacterial suspensions added along with their Fe-siderophore complexes and the Fe-siderophore added alone compared to the synthetic chelate FeEDDHA treatment was measured. Similarly, the Fe-siderophore amendments showed higher residual DTPA-Fe concentrations than the FeEDDHA treatment in which the available Fe left after the growth period is similar to that of the distilled water irrigated control (Jurkevitch et al., 1988).

Moreover, Fe was not found to be displaced by competing ions from the hydroxamate siderophores. When increasing amounts of soil were included in the equilibration media to provide labile solid phases for competing ions, decreasing amounts of Fe-hydroxamate siderophore were detected in the supernatant fluids. However, these decreasing values of Fe-hydroxamate siderophore appeared to be the result of adsorption of hydroxamate

siderophore to soil and not displacement of Fe from hydroxamate siderophore by competing ions (Cline et al., 1983).

According to our results, the highest increase in the concentration of total soluble Fe in the soil solution as affected by the addition of carbon sources and a higher microbial activity was observed in case of the calcareous soil of Wuerzburg (pH 7.66, OC 6.2 g kg^{-1} and DTPA-Fe 2.9 mg kg^{-1}) and a DTPA-Fe rich soil (Rastede soil, pH 5.77, OC 22.5 g kg^{-1} and DTPA-Fe 210.9 mg kg^{-1}). In the literature there are evidences which support our findings. Of potential ecological significance was the fact that the addition of $CaCO_3$ to the culture medium increased hydroxamate siderophore levels 5-fold whereas the same concentration of Ca (20 mM) added as $CaCl_2$ caused no significant increase (Powell and Szaniszlo, 1982).

In extracts of 19 soils, representing a wide range of pH, DTPA-Fe and organic matter content, hydroxamate siderophores concentrations ranged from 3.4 x 10^{-8} to 2.7 x 10^{-9} M DFOM equivalents. No simple relation between siderophores levels and either soil pH or DTPA-Fe was evident. Siderophores concentration was directly correlated with per cent organic matter (Powell et al., 1980). However, in another experiment, the FOB-values correlated positively with the organic carbon content of four soils and with the EDTA as well as DPTA extractable Fe but, in a second series of field samples, there was no correlation between siderophore concentration and organic carbon content of these soils. In addition, a correlation between the native siderophore concentrations and any other soil characteristic (clay and carbon content, EDTA-Fe, DPTA-Fe, moisture, microbial biomass) did not reveal a significant correlation. However, siderophore concentrations in those soils, after the addition of L-ornithine (carbon source), and the microbial biomass were inversely correlated (Bossier and Verstraete, 1986).

It is of pivotal importance that increasing the microbial activity resulted in higher total soluble Fe in the soil solution of almost all the 30 physically and chemically different soils. However, the highest microbial activity did not result in the highest total soluble Fe concentration in the soil solution. Moreover, only three soils did not respond to the carbon addition and in parallel the concentration of total soluble Fe did not increase.

There are many possible reasons that might explain the differences in the magnitude of the increased concentrations of the total soluble Fe observed in the tested soils as a consequence of higher microbial activity.

These reasons might be displayed as follows:

A. Kind of minerals and siderophore production:

Microorganisms such as fungi and bacteria should influence mineral dissolution reactions because they are ubiquitous (ever-present) inhabitants of mineral surfaces in natural weathering environments (Hersman et al., 1995). In a qualitative study of siderophore-promoted dissolution of minerals, it was concluded that the soil bacterium *Azotobacter vinelandii* produced increasingly powerful siderophores in the presence of the following minerals:

1. Pyrrhotite and marcasite;
2. Vivianite, olivine, and magnetite;
3. Hematite, siderite, pyrite, and goethite;
4. Ilmenite, micaceous hematite, and illite.

B. Degree of Fe starvation conditions and siderophore production:

Very little is known about siderophores produced by soil bacteria, and, under Fe starvation conditions, the bacteria may be stimulated to produce the most Fe-efficient chelator (Kalinowski et al., 2000). Other dissolution mechanisms might occur in the soil as well. Some Fe reduction was present when Fe was supplied as FeEDTA; this may be explained by the constitutive production of extracellular reductases by *Pseudomonas aeruginosa* and *Escherichia coli* (under "Fe-replete" conditions). These enzymes reduced and released Fe from a variety of ferric chelators (e.g., ferritin, transferrin, citrate, EDTA, and desferrioxamine) at rates several orders of magnitude faster than the release rates achieved by competitive chelation by siderophores. Under extreme Fe deprivation *P. mendocina* responds by both producing siderophore and exhibiting Fe reduction (Hersman et al., 2000).

C. Iron availability or extractability and siderophore production:

If siderophores are mainly used as Fe mobilizing agents, the increasing demand for Fe by the growing biomass could probably increase the need for siderophores in order to supply the newly formed microbial cells with this essential element. The relation between Fe availability or extractability in soil and siderophore production is not well established. To manipulate Fe availability and hence siderophore production Fe sources were added together with organic nutrients to the soil. In the $CaCO_3$-treated soil for instance some of the Fe added as citrate-Fe or EDTA-Fe will have been displaced from the chelator since the chelation of Fe by citrate and EDTA is unstable at pH 7.15. It has to be taken into consideration that this

displacement may require several weeks. However, siderophore production is not at all influenced by the addition of supplementary Fe sources. This suggests that in this soil the new biomass formed upon the addition of organic nutrients, had no difficulty in ensuring its Fe supply (Bossier and Verstraete, 1986). At first sight siderophore concentrations in soil are not inversely correlated with Fe extractability. Our results are in agreement with this concept. The observed increase in total soluble Fe concentration in the soil solution of even DTPA-Fe rich soils might be explained by the fact that siderophore concentrations in soil are not inversely correlated with Fe extractability. Possibly humus-bound Fe is the pool of Fe with which siderophores can exchange the metal. The chelating capacity of soil-humus with respect to Fe can be described by its stability constant (log K = 10) which is rather low compared to that of siderophores (log K = 30). Due to the week strength of humus-Fe bonds, siderophores may exchange Fe very rapidly. Indeed, the humus-bound Fe itself can most probably not be taken up by the microorganisms. Based on this hypothesis, siderophores are conceived as vehicles to transport Fe from the humus to growing microbial cells. This model is supported by the fact that low levels of Fe (5-10 mg kg^{-1} soil) in the form of Fe-citrate (log K = 10) and Fe-EDTA (log K = 25.1) are not able to increase Fe availability in soils and to repress siderophore production, because a larger pool of easily-exchangeable humus-bound Fe may be present. Fe availability might be limited due to the uptake of Fe by the growing plants and microorganisms depleting Fe in the vicinity of the rhizosphere. Under such conditions, microorganisms might use the root exudates to produce more siderophores stepping up the diffusion of Fe from the soil-humus complex to the point of Fe consumption. Possibly plants might in reaction to a reduced influx of Fe, change the composition of the

root exudates, facilitating the production of siderophores by the rhizosphere microorganisms (Bossier and Verstraete, 1986). Stevenson et al. (1994) pointed out that high molecular weight humic substances (e.g. humic acids) act as a reservoir of easily accessible Fe to organic ligands present in the soil, including plant and microbial siderophores.

D. Soil pH, organic matter and siderophore production:

Bossier and Verstraete (1986) found that both acidification and liming gave rise to an increase of the siderophore concentrations (88, 28 and 59 μg kg^{-1} soil for the acidified, untreated and limed soil, respectively). It is possible that the shift had made extra organic matter available for the microorganisms thus stimulating the synthesis of new biomass and concomitant formation of siderophores. This hypothesis was further examined by applying increasing amounts of sucrose (S) and L-ornithine (L) to the $CaCO_3$-treated soil. Siderophore production indeed turned out to be linked to the amount of organic substrate available (Table 22).

Table 22: Effect of carbon addition to $CaCO_3$-treated soil on siderophore concentration

Soil supplements (g kg^{-1} soil)	Siderophore concentration (μg kg^{-1} soil)
S: 0.90 L:0.37	196
S: 1.80 L: 0.75	382
S: 2.70 L: 1.13	616

Source: (Bossier and Verstraete, 1986)

E. Micronutrients and siderophore production:

In presence of Zn, Cu, and Mn metal ions, siderophore production was increased. Among the elements used, maximum siderophore levels were achieved with Zn supplementation (78.94 mg L^{-1}) followed by Cu and Mn (68.80 and 60.59, respectively) (Sharma and Johri, 2003).

F. Solid-phase properties and microbial dissolution of Fe oxides:

A strict aerobe, *Pseudomonas mendocina*, was grown with hematite, goethite, or ferrihydrite as a source for Fe. *P. mendocina* obtained Fe from these minerals in the following order: goethite > hematite > ferrihydrite at pH 7.2 (Hersman et al., 2001). There are many solid-phase properties that interfere with the microbial dissolution of Fe oxides. These interferences can be summarized as follows:

1. Amount of Fe oxides: increases in the amount of goethite, hematite, and ferrihydrite resulted in increased growth rates for this microorganism.
2. Surface area: while it appears that increased surface area resulted in increased growth, it does not appear that surface area (A_s: m^2 g^{-1}) alone controlled growth. The A_{eff} (m^2 L^{-1}) and not A_s controlled the microbial growth on Fe oxides. Furthermore, the growth of *P. mendocina* was affected differently by the different Fe oxides with the same A_{eff}.
3. Solubility and crystalline order: *P. mendocina* obtained Fe from these minerals in contrast to their relative solubilities. Microbial removal of

Fe from these minerals did not follow conventional wisdom because neither solubility nor crystalline order controlled acquisition.

4. Al substitution: dissolution was rather correlated positively with increased Al substitution. This result was counterintuitive because the stability of Fe oxides has been shown to increase with increasing Al substitution.
5. Hydroxyl coordination (the functional groups of Fe oxides): adsorption reactions are considered to involve only singly coordinated groups. In general, singly coordinated hydroxyl groups are believed to be more common on the faces of goethite than on hematite.
6. Transients: the effect of transients is responsible for initially rapid Fe dissolution rates. Transients are nonstructural Fe sorbed to the mineral surface that are difficult to quantify and are ephemeral, in that once removed by cleaning they may reform. These transients might be more common on the faces of goethite.

G. Al-substitution for Fe in Fe oxides and microbial dissolution of Fe oxides:

Maurice et al. (2000) reported that maximum microbial population increased with increasing Al substitution; hence, mineralogic variability of the type commonly observed in soil environments can be expected to alter rates of microbially mediated dissolution processes. For the Al goethites, however, a variety of characteristics change simultaneously. These characteristics might include:

1. The presence of a thin, highly reactive precipitate and/or adsorbed Fe on the surfaces of the Al-substituted goethites. The bacteria apparently were able to access this highly reactive Fe easily.
2. Quantification of defect densities is important because surface controlled, ligand-promoted dissolution of oxide minerals is thought to occur preferentially at so-called reactive surface sites, many of which would be associated with structural defects (due to Al-substitution).
3. It was observed that Al-substitution caused an increase in the microbial growth/microbial dissolution with decreasing particle length and decreasing aspect ratio; ratio of particle length to particle width. However, this particle length-dissolution trend cannot be explained by typical particle length-A_s relationships because A_s does not correlate inversely with particle length.
4. The surface hydroxyl groups on goethite which are coordinated to one Fe atom appear to be the most reactive. The particle aspect ratios decrease with increasing Al substitution, consequently a greater proportion of these reactive sites is available. This could help to explain at least in part the observed trend of increased microbial dissolution with increased Al substitution, because dissolution is likely surface controlled.
5. Bacteria preferred shorter, lower aspect-ratio crystals as attachment and dissolution sites.
6. Observations of increased anionic adsorption with increased Al substitution in goethite suggest that organic ligands produced by the bacteria may sorb more readily to the Al goethites and that negatively charged microorganisms also may attach more easily.

H. Surface concentration of siderophores and siderophore-promoted dissolution of Fe oxides:

Kraemer et al. (1999) found that after an initial fast reaction (at pH 6.5 and a total siderophore concentration of 240 μM), slow dissolution with a constant rate is observed. The dissolution rate in the presence of DFOD1 (0.17 μmol g^{-1} h^{-1}) was almost an order of magnitude higher than the dissolution rate in the presence of DFOB (0.02 μmol g^{-1} h^{-1}). (The amount of dissolved Fe was from 1-5 μM in case of DFOB and up to around 7.5 μM in case of DFOD1). The surface concentration of DFOD1 is about twice that of DFOB under these conditions, but the dissolution rate in the presence of DFOD1 is more than 8 times higher than in the presence of DFOB. Hence, the effect of these two ligands on the goethite dissolution rate is not linearly related to the adsorbed coordinating-ligand concentration.

I. pH and siderophore-promoted dissolution of Fe oxides:

Dissolution of goethite by catechol increased from 1.2 x 10^{-8} to 2.2 x 10^{-8} mol h^{-1} m^{-2} as pH increased from 5 to 9. Adsorption density of catechol on goethite increased from 3.1 x 10^{-7} to 7.0 x 10^{-7} mol m^{-2} as pH values increased from 5 to 8 (Yoshida and Nakashima, 2000).

J. Siderophores and diffusion of Fe in soil:

Diffusion caused by DFOB and EDDHA were similar at 10^{-4} M and at higher concentrations resulted in greater diffusion than that obtained with other solutions (citrate, EDTA and oxalate) at pH 7.5. At pH 5.2 ferrichrome

was even more efficient than DFOB. At pH 7.5, 10^{-3} M EDDHA resulted in a 4-fold increase in ^{55}Fe diffusion over that caused by the 10^{-3} M DFOB treatment, even though their stabilities with Fe^{3+} are similar. A probable explanation of this difference is the adsorption and immobilization of DFOB onto soil particles. The neutral charge on ferrated ferrichrome may help to explain its greater diffusion than the positively charged ferrated DFOB when equal molarities of the two compounds were used. The DFOB treatments, however, increased ^{55}Fe diffusion equally in the two soils. Increased H^+ concentration in a low-pH soil might outcompete with Fe^{3+} or FeEDDHA for adsorption sites, while the iron species might be more readily adsorbed in a high-pH soil. The soil particles might have the same affinity for the positively charged FeDFOB as they do for H^+, resulting in equal relative diffusion at all pHs (Reid et al., 1985).

K. Siderophore-promoted dissolution of Fe oxides in the presence or absence of the microorganism:

The solubilization of goethite in abiotic conditions, under the effect of aliphatic acids and DFOB, were not so efficient as those obtained in the incubation in presence of the fungus *Suillus granulatus*. This result can be explained by the ability of *Suillus granulatus* to accumulate Fe. Therefore it can be suggested that the equilibrium between the mineral and the solution was displaced and the dissolution reactions were increased. However, it was observed that increased concentrations of the hydroxamate siderophore, DFAM, increased the Fe release rate from hornblende non-linearly. Comparison of average initial Fe release rates over a week long period showed that the presence of *Streptomyces* sp. caused an approximately 5-

fold increase in the Fe release rate in buffered medium compared to cultures with hornblende only. However, when *Streptomyces* sp. was added along with DFAM, the presence of *Streptomyces* sp. resulted in a 2 to 3-fold rate increase over DFAM alone, regardless of DFAM concentration. It was suggested that the *Streptomyces* sp. may use the DFAM and recycle it (Kalinowski et al., 2000).

L. Synergism between siderophores and aliphatic acids and Al-substiution:

DFOB alone can modestly increase the rate of goethite dissolution in the absence of other ligands (ligand-promoted dissolution). However, in reality, for soils and other biologically active environments, a variety of organic ligands is always present, with oxalate being the most common. The siderophore-promoted Fe release rate increased both with level of Al substitution and with DFOB concentration up to about 100 μM, after which a plateau occurred, suggesting a saturation effect from DFOB adsorption as a precursor to dissolution. At concentrations above 200 μM, oxalate also enhanced the Fe release rate, which however was not influenced by Al substitution. For Al-goethites with mol % Al < 4, the Fe release rate in the presence of 40 μM DFOB together with varying concentrations of oxalate was typically greater than the corresponding sum of dissolution rates in the presence of the two ligands alone. This synergism may be the combined result of the ability of oxalate to adsorb strongly at the goethite surface, thus promoting Fe release, and of high selectivity of DFOB for Fe(III). Ferric oxalate complexes formed during dissolution will likely lose Fe^{3+} by ligand substitution with DFOB, leading to the production of $Fe(HDFO\text{-}B)^{+}$ and uncomplexed oxalate, the latter of which, in turn, could adsorb to the

goethite surface again. For Al-goethites with mol % Al > 4, synergism was not apparent (Cervini-Silva and Sposito, 2002).

Comparable rates of goethite dissolution are to be expected in the presence of either 500 μM oxalate or just 40 μM oxalate combined with only 10 μM DFOB, despite the fact that negligible dissolution occurs in the presence of 40 μM oxalate alone, and rather little in the presence of 10 μM DFOB alone. Oxalate adsorption onto goethite at 40 μM concentration is at about 70% of its maximal value, thereby providing a rich potential source of soluble Fe if the driving force for continual Fe-oxalate detachment from the goethite surface could somehow be increased. Adding a small concentration of predatory DFOB ligands, which have little propensity to be lost from solution by adsorption, can serve this purpose by depleting the aqueous solution phase of Fe-oxalate complexes, thus increasing the thermodynamic pressure for Fe-oxalate desorption. Very recently it has been shown that the dissolution rate of unsubstituted goethite at pH 5 in the presence of DFOB (≤ 80 μM) was doubled in the presence of oxalate (29 or 40 μM), whereas the dissolution rate in the presence of oxalate (0-200 μM) was increased by an order of magnitude when DFOB was present at 40 μM solution concentration. These results were thought to imply that if DFOB were present at large enough concentration to complex all dissolved Fe(III) released by oxalate-promoted goethite dissolution, it could displace oxalate from Fe-oxalate complexes, that may have formed and, as a result, enable the uncomplexed oxalate ligand to react again with goethite surface. The resulting synergy in the two-ligand system then suggests that the production of modest quantities of siderophores in the presence of low concentrations of oxalate would be an extremely effective mechanism for the microbial acquisition of Fe from goethite (Cheah et al., 2003).

M. Adsorption/desorption of siderophores on clay minerals; a reservoir of sorbed Fe complexes:

Clay minerals comprise a major part of the specific surface area in soils, thus their interaction with chelating agents and their Fe complexes is of great importance. Various studies have demonstrated a strong interaction of the cationic FOB with clayey soils (Siebner-Freibach et al., 2004). The adsorption of both DFOB and FOB to Ca- and Na-montmorillonite was rapid and high. FOB has the advantage for the sorption to Na-montmorillonite over that to Ca-montmorillonite. (Clays saturated with monovalent cations, such as Na^+, are well dispersed in solution and an extensive fraction of the adsorbing surfaces are exposed). Determination of DFOB and FOB adsorption to Ca-kaolinite revealed a very low affinity (Siebner-Freibach et al., 2004). Indeed the adsorption of both forms of the siderophore to Ca-montmorillonite was unchanged over a wide pH range (4.0-7.5) in accordance with their stable positive charge (Siebner-Freibach et al., 2004).

Desorption of siderophores can be brought about through cation exchange with, for example, Ca^{2+} or Na^+. At low solution concentration ($\leq$ 0.01 M), the efficacy of divalent cations (Ca^{2+}) in siderophore desorption was higher than that of the monovalent ones (Na^+). The differences in the desorbed amount cannot be explained by the differences in cation valency alone. However, at the highest concentration, desorption efficacy was higher for Na^+ than for Ca^{2+} in the initial washing cycles, probably due to the dispersion effects of Na^+ on montmorillonite platelets (Siebner-Freibach et al., 2004). At the highest concentrations of NaCl ($\geq$ 0.1 M), the desorbed fraction of DFOB was much higher than that of FOB.

By binding Fe from its environment, it can therefore form a reservoir of sorbed Fe complex, facilitating continuous Fe supply to plants according to the composition and concentration of the soil solution (Siebner-Freibach et al., 2004).

Low clay soils yielded almost twice as much hydroxamate siderophores as did high clay soils suggesting that adsorption might be an important determinant of hydroxamate siderophores concentration in bulk soil solution. Adsorption appeared to be correlated with clay and potassium. The difference in adsorptions between soils probably resulted from variations in number of exposed cation exchange sites on expansible lattice clays such as montmorillonite (Powell and Szaniszlo, 1982).

4.4 The improvement of Fe nutrition of swingle citrumelo by intercropping with perennial graminaceous and dicotyledonous plant species on a calcareous soil

Citrus rootstocks differ in their susceptibility to Fe deficiency, which causes economic losses through persistent leaf chlorosis and progressive necrosis of young shoots (Chapman, 1968). Many of the commonly used citrus rootstocks are susceptible to Fe-deficiency. This is especially true of those rootstocks (mainly citranges) derived from the trifoliate orange (*Poncirus trifoliata*). There are, however, a small number of rootstocks that demonstrate significantly higher tolerances to low-Fe stress. These include mainly *Citrus macrophylla*, *Citrus jambhiri*, and several other rough lemon varieties. Yet these rootstocks are highly susceptible to other citrus diseases, and are used less frequently than the citranges and related rootstocks (Manthey et al., 1993). Similarly, Hamze et al. (1986) grouped rootstocks according to their resistance to lime-induced chlorosis as follows:

1. *C. jambhiri* and *C. macrophylla* were highly resistant;
2. *C. aurantium*, *C. volkameriana*, *C. reticulata* and *C. limonia* were moderately resistant;
3. *C. sinensis*, *C. taiwanica*, troyer citrange (*C. sinensis* x *P. trifoliata*) and carrizo citrange were mildly resistant; and
4. *Poncirus trifoliata* and swingle citrumelo were non-resistant.

Lime-induced chlorosis is a common feature in fruit crops in calcareous soils. The extent of chlorosis and the resulting depression of yield are affected by many factors including the supply of water and nutrients, but the amount and properties of the soil carbonates with their associated control of pH and bicarbonate concentration has the most direct influence on the supply and utilization of Fe by crops (Mashhady and Rowell, 1978). According to Treeby and Uren (1993), the different citrus rootstocks appear to be using different mechanisms to maintain Fe supply to roots and prevent Fe chlorosis. These mechanisms might include:

1. Decreasing the pH of the nutrient solution – rough lemon, cleopatra mandarin and sour orange.
2. Releasing phenolic compounds – rough lemon and cleopatra mandarin.
3. Releasing reducing compounds – rough lemon, sour orange and trifoliate orange.
4. Increasing root-mediated reduction of chelated Fe(III) at pH 6.5 – rough lemon or at pH 8.0 – rough lemon and cleopatra mandarin.

Application of Fe in chelating forms represents a temporary remedy for Fe deficiency chlorosis, it is expensive, not feasible economically in the long term, and is only applicable to high value fruits. In addition, it was recently reported that synthetic chelates (i.e., EDDHA) can be leached out of the root

zone to deep soil layers adjacent to the water table, which might impose environmental and health hazards (Rombola et al., 2002). Therefore, the introduction of grasses as well as dicot plant species into fields of fruit trees grown on calcareous soils might be an effective ecological orchard floor management for improving the Fe nutritional status of these trees in comparison with those grown on bare soil, which is the most common practice particularly when irrigation water is a limiting factor.

Our results showed that there was a significant effect of the continuous Fe supply as FeEDDHA on various parameters such as growth vigor and chlorophyll concentration of the swingle citrumelo plants in comparison with the control (no FeEDDHA added) (Tables 6, 7, 8, 9, 10, 11, 12, 13, 14). The positive impact on growth vigor and chlorophyll concentration of swingle citrumelo plants caused by some of the other treatments compared to the control are, therefore, supposed to result from improving the Fe nutritional status of the plant.

It was necessary to apply the FeEDDHA many times to completely control the Fe deficiency chlorosis of swingle citrumelo plants grown on a calcareous soil. This can be attributed to the fact that this citrus rootstock is a non-resistant rootstock to lime-induced chlorosis (Hamze et al., 1986), which might imply that the concentration of the chelated Fe (FeEDDHA) should be high enough to compensate for the fact that the root-mediated reduction of chelated Fe(III) is not considerably increased in response to Fe deficiency. On the other hand, we cannot exclude the fact that FeEDDHA can also be degraded by soil microorganisms (Chen et al., 1998).

The effectiveness of the vivianite as a source of Fe in our research is shown for the first time in a citrus rootstock. Vivianite is a synthetic iron(II)-phosphate analogous to the mineral vivianite [($Fe_3(PO_4)_2.8H_2O$)]. This is in

agreement with Rombola et al. (2003b) who recently showed that vivianite prevented Fe deficiency chlorosis in kiwifruit grown on a calcareous soil. In addition, a long-lasting prevention of chlorosis has been achieved in field-grown pear and olive trees by soil application of vivianite (Iglesias et al., 2000, Rosado et al., 2002). Vivianite particles range between 2 to 10 μm in length and, unlike Fe-chelates, are hardly mobile through the soil profile and remain at the depth of application (25-30 cm under field conditions) where fruit trees' root density is the highest. According to Rosado et al. (2002), the long-term effectiveness of vivianite is caused by the poorly crystalline Fe oxides (ferrihydrite and lepidocrocite) resulting from the oxidation and incongruent dissolution of vivianite. The formation of these oxides mainly depends on the continuous removal of phosphate from vivianite. Precipitation and/or adsorption of phosphate on active soil surfaces and root uptake likely represent the main mechanisms of phosphate removal.

Our results showed that *Festuca rossa* was able to cure the lime-induced chlorosis of swingle citrumelo plants. Intercropping this citrus rootstock with *Festuca rossa* resulted in a clear and persistent significant influence on the chlorophyll concentration (Tables 6 and 7). In addition, this grass species had a positive impact on the leaf area and plant vigor (leaf number, leaf weight, branch length and weight of young shoots) (Tables 10, 11, 12, 13). The other *Festuca* spp. (*F. ovina*) showed a positive influence on the chlorophyll concentration only after being mowed (Table 7). Plant vigor (leaf area, leaf weight and weight of young shoots) was also significantly improved (Tables 10, 12, 13). However, at the end of the experiment, it was clear that both *Festuca* spp. were able to cure Fe deficiency symptoms of the citrus plants (Table 8). This would definitely present evidence that intercropping with grasses improved the Fe nutritional status of Fe

deficiency non-resistant citrus rootstock. The observed increase in the leaf area (Table 10) proved that Fe was physiologically more available for the citrus plants intercropped with these grasses compared with the control. In fact, an indispensable Fe containing enzyme for growth is ribonucleotide reductase which reduces the ribonucleotide diphosphate to desoxy-ribonucleotide. The latter is a building block for DNA and without these building blocks neither replication of DNA strands nor subsequent cell division can occur. This is in accord with the finding that insufficient Fe supply affects meristematic growth and the development of new leaves (Mengel and Kirkby, 2001). However, *Poa nemoralis* was not as effective as the other two grass species. The *Trifolium subterraneum* showed some positive effect immediately before and after being mowed. However, both *T. subterraneum* and *P. nemoralis* failed to either cure the Fe deficiency symptoms or show any positive impact on the vigor of the citrus plants at the end of the experiment, which might be due to an extreme competition for nutrients and/or an allelopathic effect that was already shown for some cereal crops (Perez and Ormeno-Nunez, 1991, Petho, 1993). The central question is “why did intercropping of swingle citrumelo with some grasses but also, to a lesser extent, with *Trifolium subterraneum* improve its Fe nutritional status?”

Our results are in agreement with the field observations which have indicated that Fe deficiency chlorosis symptoms in peanut are more severe and widespread in monoculture than intercropped with maize on calcareous soils. The chlorophyll and HCl-extractable Fe concentrations in young leaves of peanuts grown in mixture were much higher than those in monoculture, indicating that maize may have markedly improved the peanut Fe nutrition (Zuo et al., 2000). Most recently, Rombola et al. (2003a)

reported that intercropping kiwifruit plants with perennial grass species and a mixture of these grasses resulted in a prevention of Fe deficiency chlorosis (Table 23).

Table 23: Chlorophyll concentration and shoot biomass of kiwifruit plants intercropped with grasses.

Treatment	Chlorophyll concentration (SPAD unit)	Shoot biomass (g)
Control	15.1 ± 1.8	17.2 ± 1.5
FeEDDHA	29.2 ± 0.4	26.3 ± 2.3
Festuca	25.5 ± 1.3	21.9 ± 2.4
Lolium	28.3 ± 0.7	24.4 ± 1.8
Poa	25.8 ± 3.3	28.2 ± 2.7
Mixture	27.9 ± 0.6	21.7 ± 2.0

Source: Rombola et al. (2003a)

It is hypothesized that the introduction of some grasses and dicot plant species into fruit tree fields would enrich the rhizosphere with organic compounds (root exudates) and consequently, increase the rhizosphere soil microbial activity as well as the production of siderophores under calcareous soil conditions. Higher microbial activity was shown to result in a higher total soluble Fe concentration in the soil solution (Fig. 4a and 4b).

In the literature there is evidence that some plants, for example, Fe-stressed oat roots acquired Fe from five different siderophores. Among the five siderophores, oat demonstrated significant specificity for Fe acquisition from RA and FOB, which supplied 7- to 8-fold more Fe in comparison to FC, FCA, coprogen (Crowley et al., 1988b). This might imply that in an intercropping system the presence of various siderophores will be of great impact on the Fe nutrition of different plant species because not all plant species can equally utilize the same Fe-siderophore complex, which might

reduce competition among different plant species for a certain Fe-siderophore complex.

Grasses are known to produce phytosiderophores. The role of these compounds in providing the citrus rootstock with Fe cannot be ruled out. However, Masalha et al. (2000) pointed out that several lines of evidence have shed doubt on the proposed general function of phytosiderophores for Fe acquisition and thus the overall prevention of Fe chlorosis in grasses. Firstly, grasses show genotypical differences in the release of phytosiderophores; secondly, the release of phytosiderophores may prevent Fe chlorosis in grasses grown hydroponically, but not in the soil, because phytosiderophores are readily degraded by microbes. Accordingly, the ecological importance of phytosiderophores in natural habitats remains questionable. In addition, in the light of stability relationships for metal chelation by phytosiderophores, the possibility must be considered that production of phytosiderophores is a general physiological response to limited availability of trace metals at high pH. Such response might explain the finding that native grasses in soils actually accumulate greater concentrations of copper, zinc, and iron at pH 7.5 than at pH 6, despite the lower solubility of these metals at high pH (Crowley et al., 1987).

Of great interest is the finding (our research) that the positive effects on chlorophyll and growth are not related to the Fe concentrations in leaves (Table 8 and Fig. 4). In contrast, the control treatment with a high Fe concentration was the poorest in chlorophyll and growth. The treatment (*F. ovina*) with a higher growth and chlorophyll concentration than the control had the lowest Fe concentration. Obviously intercropping improved the Fe efficiency in swingle citrumelo plants. It is speculated that these grasses (considered to be Calcicoles) depleted the soil from nitrate and hence

swingle citrumelo was fed mainly on ammonium. In fact, according to Kirkby (1967), *Calcicoles* – plants with a preference for calcareous, high pH soils – utilize nitrate preferentially. In the literature there is evidence that in orchards under a grass cover, only low amounts of nitrate were found whereas in a similar fallow soil the nitrate concentration in the soil increased. In addition, shading and irrigation would aid in maintaining the desired moist conditions and consequently ammonia volatilization should be minimal. It is also of interest that intercropping swingle citrumelo plants with *F. ovina* resulted in a statistically higher weight of both the entire root system and the fine root (Table 14). This finding might have resulted in higher uptake of ammonium and probably siderophores, which, in turn, improved the Fe efficiency in the leaves by preventing any possible inactivation of Fe in the leaf apoplast. In fact, Hoffman et al. (1992) and Hoffman and Kosegarten (1995) measuring the pH of the leaf apoplast found that it increased with nitrate nutrition whereas it decreased with ammonium nutrition. From this finding it was hypothesized that at high apoplastic leaf pH Fe(III) reduction in the leaf apoplast is restricted and hence the uptake of Fe from the apoplast into the cytosol impaired (Mengel, 1994). Later Kosegarten et al. (1999) were able to confirm this hypothesis. These authors were able to show that at distinct microsites in the apoplast of sunflower leaves the pH was > 6 if the plants were fed exclusively with nitrate. Such microsites with elevated pH levels were not found if the plants were supplied with ammonium-N. In addition the authors showed that Fe(III) reduction in the leaf apoplast was greatly depressed at high pH. It is thus appears that under such conditions the Fe remains in the apoplast where it may even accumulate to high levels when at the same time the Fe

concentration in the cytosol is insufficient. Total leaf Fe concentration may be thus high but metabolically active Fe low.

5. Conclusions

The Buchner funnel technique (BFT) was developed to continuously and non-destructively collect the soil solution under vacuum (-50 KPa) to quantitatively and well reproducibly measure total soluble iron (Fe) concentrations in µM range in about 30 physically and chemically different soils collected from different regions in Germany. These soils were maintained at 80% of their maximum water holding capacity and incubated at 20ºC for three days before each collection. The collected soil solution represents the solution of soil and its solutes at about its field capacity.

Whatsoever the Fe solubility conditions in soils are, the most important factor for plant nutrition is the concentration of total soluble Fe, whether in its inorganic form or in its organically-bound form, in the soil solution because this controls the Fe supply to plant roots by mass flow and diffusion. From the presented results, the following conclusions can be drawn:

1. BFT is a reproducible method.
2. Fe concentrations in soil solutions in different soils vary within a wide µM range.
3. Soil O.M. and its turnover rather than inorganic Fe relationships in soils are decisive for the bioavailability of Fe in soils.
4. Based on our results, we assume that the widely spread assumption Fe chlorosis in plants results from the low Fe availability in calcareous soils is not correct. It is rather due to the effect of HCO_3^- on the root apoplastic pH. Under these conditions the reduction of the chelated Fe to Fe^{2+} and its uptake are restricted leading to the precipitation of Fe in the root apoplasm.

5. Soil microbial activity plays a much more important role than soil physical and chemical properties (i.e., soil pH) in controlling the concentration of soluble Fe in soil solutions.
6. Availability of Fe in soil solution is very important; however, it is not the only decisive factor from the plant nutrition point of view; the efficiency of Fe in plants grown on calcareous soils is of pivotal importance as well.

Zusammenfassung

Die wichtigste Komponente für die Bioverfügbarkeit von Eisen (Fe) in Böden ist die Fe Konzentration in der Bodenlösung. Sie ist in direktem Kontakt mit den Pflanzenwurzeln, und das in Lösung befindliche Fe ist maßgebend für die Anlieferung von Fe an die Wurzel, sei es über Massenfluss oder Diffusion. Die Aufnahme von Fe durch die Pflanzenwurzel bewirkt einen Fe-Konzentrationsgradienten, der zur Wurzel gerichtet ist. In der Literatur wurden seither noch keine Routineverfahren zur Bestimmung des Fe in der Bodenlösung beschrieben. Es war eine wesentliche Aufgabe der vorliegenden Thesis ein solches Routineverfahren zu erarbeiten und mit diesem Verfahren Faktoren und Prozesse zu ermitteln, welche für die Fe Konzentration in der Bodenlösung von Bedeutung sind.

Für die Extraktion der Bodenlösung wurde ein Verfahren entwickelt, das „Büchner Trichter-Technik (Büchner funnel technique)“ genannt wird. Hierbei werden 100 g trockener Boden mit 100 g Quarzsand gemischt und mit Wasser auf 80% der maximalen Wasserkapazität des Bodens eingestellt. Dieses Boden/Quarzsandgemisch wird auf einen Büchner-Trichter gebracht und durch Abdeckung vor Verdunstung geschützt. Nach einer Inkubationszeit von 3 Tagen wird die Lösung aus dem Boden/Quarzsandgemisch bei einem Vakuum von -50 kPa abgesaugt. Die Vermischung mit Quarzsand war notwendig, um einem Boden, dessen Wasserpotential niedriger als die Feldkapazität ist, noch Flüssigkeit zu entziehen. Die so vorliegenden Fe Konzentrationen reflektieren die Fe-Löslichkeit bei relativ trockenem Boden, was unter Freilandbedingungen die Regel ist. Der nicht an Wasser gesättigte Boden ist aerob, sodass kein lösliches Fe^{2+} gebildet wird. Die so gefundenen Fe-Konzentrationen entsprechen zwar nicht denen der Bodenlösung in situ, aber sie reflektieren die Löslichkeit von organischen und anorganischen Fe-Verbindungen im Bodenwasser.

Während der Extraktion wird das Gefüge des Boden/Quarzsandgemisches höchstens unwesentlich gestört; das Verfahren der Gewinnung von Bodenlösung ist also „non destruktiv“. Das im Extrakt vorliegende Fe wurde unter sauren Bedingungen mittels Hydroxylamin-Hydrochlorid zu Fe^{2+} reduziert, welches mit Ferrozine einen roten Farbkomplex bildet, dessen Intensität spektrophotometrisch bei einer Wellenlänge von 562 nm gemessen wird. Die Methode wurde eingehend überprüft. Es zeigte sich, dass die mit ihr erfassten Daten reproduzierbar sind, dass die Methode im µM Fe-Konzentrationsbereich anwendbar ist und damit die niedrigen, in der Bodenlösung vorkommenden Fe Konzentrationen quantitativ erfasst, was bei den seitherigen Methoden für die Bestimmung von Fe in der Bodenlösung nicht gegeben war.

Es wurden 30 verschiedene Böden mit o.a. Methode auf ihre Fe Konzentrationen in der Bodenlösung untersucht. Hierbei zeigten sich für die einzelnen Böden erhebliche Konzentrationsunterschiede , die in einem Bereich von 1 bis 188 µM Fe schwankten. Interessanterweise zeigten die sauren Böden die niedrigsten Fe Konzentrationen, während bei den Carbonatböden Konzentrationen im Bereich von 25 µM Fe gefunden wurden. Die seitherige Annahme, dass die Löslichkeit von Fe im Boden entsprechend der Löslichkeit amorpher und kristalliner anorganischer Fe Verbindungen im Boden mit dem Anstieg des Boden-pH abnimmt, wurde widerlegt. Diese Feststellung deckt sich mit dem Befund, dass der größte Anteil des in der Bodenlösung vorliegenden Fe als organischer Komplex vorliegt, dessen Anteil am Gesamt-Fe der Bodenlösung mit dem Anstieg des pH-Wertes zunimmt. Damit wird die seither weltweit verbreitete Annahme, die Fe-Chlorose bei Pflanzen auf Carbonatböden ginge auf eine unzureichende Fe-Löslichkeit im Boden zurück, widerlegt. Sie entspricht vielmehr den neuesten Befunden der Arbeitsgruppe von H. Kosegarten, dass HCO_3^-, ein in der Bodenlösung von Carbonatböden in relativ hoher

Konzentration vorliegendes Anion, die Reduktion von Fe(III)-siderophoren im Wurzelapoplasten hemmt und damit die Fe^{2+}-Aufnahme in das Cytosol blockiert (Kosegarten et al., 2004).

Die Volumin an Lösung, die bei den einzelnen Böden extrahiert wurden, waren unterschiedlich, bei den Sandböden höher als bei den tonreicheren Böden, bedingt dadurch, dass die Wasserbindung an den Boden mit den H_2O adsorbierenden Oberflächen zunimmt. Für die hier vorliegenden Untersuchungen aber war die Fe-Konzentration der wesentliche Parameter, da die Fe-Konzentrations-Unterschiede die Fe Diffusion zur Wurzel maßgeblich bestimmen. Der Diffusion kommt im Vergleich zum Massenfluss eine um so größere Bedeutung zu, je niedriger die Konzentrationen des betreffenden Elementes in der Bodenlösung sind. Bei Fe sind sie sehr niedrig. In einem weiteren Versuchsansatz mit 30 Böden konnte gezeigt werden, dass die Fe-Konzentration in der Bodenlösung von der mikrobiologischen Aktivität abhängt; eine Stimulierung der mikrobiologischen Aktivität durch die Zugabe von organischem C erhöhte die mikrobiologische Aktivität.

In einem Gefäßversuch wurde an einer Chlorose-empfindlichen Zitrusspecies Swingle citrumelo untersucht, ob die Anfälligkeit gegen Fe-Chlorose vom Bewuchs benachbarter Pflanzen beeinflusst wird. Insgesamt wurden 3 verschiedene Gräser und *Trifolium subterraneum* getestet. Den größten Einfluss hatte *Festuca*, hier waren Wachstum und Chlorophyll-Konzentration von Swingle citrumelo ebenso hoch wie in der Kontroll-Variante, die kontinuierlich mit einem Fe-Chelat versorgt wurde. Interessanterweise waren jedoch die Fe-Konzentrationen in den Blättern von Swingle citrumelo in der *Festuca*-Variante wesentlich niedriger als in der Kontroll-Variante. *Festuca* hatte also weniger die Fe-Aufnahme von Swingle citrumelo als die Fe-Effizienz in den Blättern erhöht. Diese Effizienz wird durch Nitrat vermindert.

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Curriculum Vitae

Personal Data:

Name : Tarek G. Ammari

Date of Birth : 16th of November 1973

Place of Birth : Amman-Jordan

Education:

Bachelor degree in Plant Production (Average: 86.5% - Excellent) – University of Jordan, Faculty of Agriculture.

M.Sc. in Agricultural Resources & Environment (Average: A (4.0) – Excellent) – University of Jordan, Faculty of Agriculture.

Publications:

Rombolà, A.D., Dallari, S., Quartieri, M., **Ammari, T.**, Scudellari, D. and Tagliavini, M. 2002. Effect of Foliar-Applied Fe Sources, Organic Acids and Sorbitol on the Re-greening of Kiwifruit Leaves Affected by Lime-Induced Iron Chlorosis. Acta Hort. 594: 349-355.

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