ORGANIC ACIDS AND TRACE ELEMENT EXTRACTABILITY IN SEWAGE SLUDGE-TREATED SOILS

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Tese apresentada à Universidade Federal de Lavras, como parte das exigências do Programa de Pós-Graduação em Agronomia, área de concentração Solos e Nutrição de Plantas, para a obtenção do título de "Doutor".

Orientador
Eng. Agr. PhD. Luiz Roberto Guimarães Guilherme

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SUMMARY

ABSTRACT

MARCHI, Giuliano. **Organic acids and trace element extractability in sewage sludge-treated soils**. 2005, 104p. Thesis (Doctorate in Soil Science and Plant Nutrition)[*]

The prediction of trace elements plant availability in sewage sludge-treated soils by extractants in a single extraction is seldom achieved and depends on the soil and sewage sludge properties, as well as the plant and environmental factors. The method of trace elements extraction proposed in this work tries to mimic the rhizosphere environment by using both mono and divalent ions, different organic acid compositions and several successive extraction kinetics to assess the total trace element "availability" that should be achieved after several plant growths in the same spot until that "available" fraction is depleted. The experiments were conducted at the Environmental Sciences laboratories at the University of California, Riverside, between 2003 and 2004. The trace elements Cd, Zn, Cr, Ni, and Cu extraction by organic acids solution from a Red Distroferric Latosol treated with sewage sludge from Franca treatment station was studied. The plots were fitted with the first order kinetics model and the kinetics parameters for Zn, Cd, Ni, Cr, and Cu were compared. The Zn ultimate metal release (C_o) values due to the first order dissolution reaction from the 1, 10, and 100 mmol L^{-1} complete organic acid solution (COAS) presented the same magnitude. The more appropriated extraction method was found to be the use of one and a half-grams of soil in 30 mL of 0.1 mol L^{-1} of a modified organic acid solution (MOAS), pH 4.8±0.1, performing 15 successive extractions, each one every 24 hours. Following the proposed methodology, four organic acid mixtures (0.1 mol L^{-1}) and three conventional extractants (DTPA, Mehlich I, and NH_4OAc) were added in 50 mL centrifuge tubes along with 1.5 g sewage sludge-treated soil (4 mmol L^{-1} $CaSO_4$ and 0.05 mmol L^{-1} NaCl) in three replicates. A sequential extraction was also performed as a post kinetics study onto the remaining soil. For Cd and Zn, the extractants DTPA and Mehlich I extracted almost the entire extractable fraction in the first extraction. The NH_4OAc extracted much less than all the other extractants for all trace elements. The plant uptake kinetics results for Zn were close to the kinetics results of all

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[*] Guidance committee: Luiz Roberto Guimarães Guilherme – UFLA (Adviser), Andrew C. Chang – UCR (Adviser).

organic acids used in this experiment. The Mehlich I kinetics had also a close result to the plant uptake.

RESUMO

MARCHI, Giuliano. **Ácidos orgânicos e extratabilidade de elementos-traço em solos tratados com lodo de esgoto**. 2005, 104p. Tese (Doutorado em Solos e Nutrição de Plantas)[*].

A predição da disponibilidade de elementos-traço em solos tratados com lodo de esgoto por extratores em uma única extração é dificilmente conseguida e depende das propriedades do solo e do lodo, bem como da planta e de fatores ambientais. O método de extração de elementos-traço proposto neste trabalho tenta reproduzir a rizosfera pelo uso de íons mono e divalentes, diferentes composições de ácidos orgânicos e cinética de extrações sucessivas para acessar a total "disponibilidade" de elementos-traço que é verificada após vários cultivos sucessivos no mesmo local até que a fração "disponível" seja esgotada. Em experimentos realizados nos laboratórios de ciências ambientais da Universidade da Califórnia em Riverside, entre os anos de 2003 e 2004. A extração dos elementos-traço Cd, Zn, Cr, Ni e Cu por soluções de ácidos orgânicos foi estudada em um Latossolo Vermelho distroférrico tratado com lodo de esgoto proveniente da estação de tratamento de esgoto de Franca. Os dados foram descritos pelo modelo de cinética de primeira ordem e os parâmetros para Zn, Cd, Ni, Cr e Cu foram comparados. Os valores para a liberação de Zn final (C_o) calculados pela equação de primeira ordem para a solução de ácidos orgânicos (1, 10 e 100 mmol L^{-1}) apresentaram a mesma magnitude. O método de extração mais apropriado foi aquele com o uso de 1,5 gramas de solo em 30 mL de uma solução "modificada" de ácidos orgânicos 0,1 mol L^{-1}, pH 4,8±0.1, perfazendo 15 extrações sucessivas, uma a cada 24 horas. Seguindo a metodologia proposta, quatro misturas de ácidos orgânicos (0,1 mol L^{-1}) e três extratores convencionais (DTPA, Mehlich I e NH_4OAc) foram adicionados em tubos de centrífuga de 50 mL com 1,5 g de solo tratado com lodo de esgoto (4 mmol L^{-1} $CaSO_4$ e 0,05 mmol L^{-1} NaCl) em três repetições. Extrações seqüenciais também foram realizadas em um estudo pós-cinética no solo remanescente. Para Cd e Zn, os extratores DTPA e Mehlich I extraíram quase a totalidade da fração "disponível

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[*] Comitê orientador: Luiz Roberto Guimarães Guilherme – UFLA (Orientador), Andrew C. Chang – UCR (Orientador estrangeiro).

total” na primeira extração. O NH_4OAc extraiu as menores quantidades de elementos-traço que os outros extratores.

CHAPTER 1

1 GENERAL INTRODUCTION

In environmental research there is a need for data concerning trace elements in soils and a demand for suitable extractants able to give information on the amounts of these elements in soils and their availability to plants (Anderson, 1975). Then, for much time the environmental scientists come up with researching methods that could provide a clear picture of the trace elements phytoavailable portion in soils, sewage sludges, and, for the most part, sewage sludge-treated soils. The searching for the most suitable method lead these researchers to embrace methods of extraction such as DTPA, EDTA, NH_4OAc, Mehlich I and Mehlich III, and several other chemicals in a single extraction procedure with the hope they could screen those materials for potential food-chain contamination.

Even though these procedures can give some information about trace elements under definite situations, the chemical root/soil interface situation is diverse from the line of attack of those trace element conventional methods of extraction. In the rhizosphere, unlike in the soil, there is a particular pH, a release of chemicals such as organic acids that are the components of utmost reactivity in the soil, and, on top of it, the microorganisms interact with all chemicals, soil components, roots, and trace elements modifying the entire environment.

A good trace element extractant from soil should be able to extract those elements from the same pools the plant does, and at a similar rate. A synthetic mixture of organic acids, similar to those released by the plant roots, seems to be able to fulfill this task. However, plants do not extract trace elements from the soil at once, they extract along the time, slowly dissolving, solubilizing, chelating, and uptaking them from the soil solution. Additionally, these processes are under the microorganisms shade and depend on the soil properties as well as the ionic strength, and ion interaction that can affect the rhizosphere's reaction thermodynamics.

The trace element extraction kinetics using a rhizosphere like solution could be able to predict the total phytoavailability of trace elements along the time until the available portion become depleted. A complete set of thermodynamic calculations would then be quite useful to understand these kinetics reactions and a sequential extraction from the soil or sewage sludge could shed some light on the available pool issue.

The aim of this work was to evaluate the more appropriate procedure to extract trace elements using organic acids to determine the availability of trace elements in a sewage sludge-treated soil. Additionally, the trace elements extractability kinetics from this soil by organic acid mixtures and by chemical methods commonly used, such as DTPA, Mehlich I, and NH_4OAc was compared, and the sequential extraction, the modifications on the trace elements pools for sewage sludge-treated soils after kinetics extractions was evaluated.

2 LITERATURE REVIEW

2.1 Soil

Soils are, by nature, very complex multicomponent systems, in which many different ionic species in solution and solid phases with different surface properties are present (Kretzschmar and Voegelin, 2001). It is not only a geochemical sink for contaminants, but also acts as a natural buffer controlling the transport of chemical elements and substances to the atmosphere, hydrosphere, and biota. However, the most important role of soils is its productivity, which is basic for the survival of humans. Thus, maintenance of the ecological and agricultural functions of soils is the responsibility of mankind (Kabata-Pendias and Pendias, 2001).

Soil-surface active sites include both constant negative charge sites associated with phyllosilicate clay minerals as well as variable charge sites associated with oxides, (oxy)hydroxides and humic material. Both inner- and outer-sphere complexes can be formed between the metallic ions and these reactive sites on soil particles (Evans et al., 1995).

2.2 Contaminants

The persistence of contaminants in soils is much longer than in any other compartments of the biosphere, and contamination of soil, especially by trace elements, appears to be virtually permanent. Metals accumulated in soils are depleted slowly by leaching, plant uptake, erosion, or deflation. Several estimates have clearly indicated that the complete removal of metallic contaminants from soils is nearly impossible (Kabata-Pendias and Pendias, 2001).

Plants affect contaminant chemistry in soil at the soil-root interface through a number of processes (McLaughlin et al., 1998):

i) Plant uptake may reduce ion activity and desorb contaminants from surfaces, or convective flow of solution to the root may move additional contaminant to the rhizosphere, leading to sorption,

ii) Plant-induced changes in solution chemistry can affect sorption, e.g., pH, ionic strength, macronutrient cation concentrations (e.g., Ca),

iii) Plants excrete organic ligands that may increase or decrease the total concentration of contaminant ions in solution depending on whether free activity is well buffered or poorly buffered, respectively,

iv) Living or dead plant material in the rhizosphere can act as new sorbing surfaces for contaminants, and

v) Microbial activity, stimulated by plants, can also affect contaminant behavior by the above processes.

Regardless of their origins and the reasons for the increase of their concentration in soils, trace elements are liable to contaminate food chains by migrating toward groundwater or by accumulating in plants (Bermond and Ghestem, 2001). Trace elements in soils can be involved in a series of complex chemical and biological interactions including oxidation-reduction, precipitation and dissolution, volatilization, and surface and solution phase complexation. Knowledge of the time-dependence nature of their behavior is important (Selim and Amacher, 2001).

In many cases the harmful effect of metal on the ecosystem is related to metals dissolved in soil solution, especially uncomplexed free ions. However critical limits and soil quality standards are expressed mostly in total (or "reactive") metal content in soil. Therefore one has to use an empirical relationship (transfer functions) linking trace elements concentration (or activity) in soil solution with metal content in soil and soil characteristics to calculate a correspondent dissolved metal concentration (and activity) (Pampura et al., 2003).

Trace elements contamination is so important that some researchers are looking for alternatives for sewage sludge disposal. Some believe incineration is the only way to dispose a trace elements loaded sewage sludge, but this alternative has high costs and also is an air polluting technique. The removal of trace elements from the sewage sludge by extraction with organic acids to be able to use this residue in agriculture with lower risk of soil contamination was proposed by Veeken and Hamelers (1999). Despite of that, any alternative seems better than dispose crude sewage sludge on rivers, where the pollution could be disastrous. Unfortunately it is a common practice in developing countries. Brazil has just started to preserve rivers and obligate cities to build treatment stations. The next step will certainly be what to do with the sewage sludge generated by these stations.

In contrast to the problem of trace elements excesses resulting from pollution, agricultural land in many parts of the world, including Brazil, has been found to be deficient in one or more micronutrients. This includes the trace elements/metallois: Cu, Mn and Zn, which are essential for plants and animals, and Co, Cr, and Se, which are only necessary to animals (Alloway, 1995).

2.3 Sewage sludges

The final destination of the large quantity of sewage sludges produced from urban-industrial sites become a problem and the amount of sewage sludge produced by the human activity is growing more and more. This sewage sludge processed by the treatment stations aiming its safe use on agricultural lands is also called biosolid (Juliatti et al., 2002). This denomination is used to decrease public rejection when the material is used to provide organic matter and plant nutrients to home gardens and other public related activities.

Sewage sludge is the residue produced from the treatment of domestic and industrial waste waters and large amounts are produced worldwide. Sewage sludges might be a significant source of plant nutrients and organic matter. However, the beneficial properties of sewage sludges are limited by their contents of potentially harmful substances such as trace elements (Alloway, 1995). The incorporation of these trace elements in the soil can lead to their accumulation which may result in a potential threat to human health (Silva et al., 2002). Elevated concentrations of trace elements in human diet constitute a potential health hazard in the long term. Nonetheless, agricultural products represent an important pathway for movement of potentially toxic trace elements from soil to human beings (Srivastava et al., 1999). After ingested, the toxic trace elements can form strong complexes with biomolecules, and their presence, even in small amounts, can be hazardous to the health of animals (Evans et al., 1995).

Sewage sludges from municipal and industrial sources are frequently applied to soils to provide plant nutrients for crop production. However this practice has the potential of creating environmental pollution problems because many sludges present very high content of toxic trace elements (Evans et al., 1995). Thus, the effects of sewage sludge applications on soil composition are of great environmental concern and have been the subject of many studies and much legislation. The presence of trace elements in the sludge creates a potential for the formation of organometallic compounds by biosynthetic processes. Thus, an understanding of all factors influencing the phase distribution of metals in soils is a prerequisite for estimating the critical loads of trace elements and their effect on soil organisms (Kabata-Pendias and Pendias, 2001).

The addition of sewage sludges increases the organic matter content in the soil. The organic matter amendments to metal-contaminated soils can have an ameliorative effect due to the increase in the surface area and in the number of adsorption sites (Shuman et al., 2002), allowing some trace elements with high affinity for organic materials to be complexed strongly in high energy sites. The organic matter provided by sewage sludge applications is also rich in nutrients and improve the soil.

As a matter of fact, sewage sludge application to coarse-textured soils may result in trace elements accumulation in the soil and increased trace elements uptake by crops. Thus, if the sewage sludge is to be utilized as fertilizer for crop production, sludge rates and frequency of application should be based on the trace element content of the sewage sludge, the type of soil receiving the sewage sludge and the kind of crop grown (Gaynor and Halstead, 1976). However, the total trace element concentration present in the sewage sludge does not reflect the phytoavailability originated from its application to soils. The speciation of trace metals in wastes appears to be of greater environmental concern than their total concentration (Planquart et al., 1999).

2.4 Trace elements

Trace element species entering soils with sewage sludges differ based upon the source and the kind of the sludge treatment. The forms associated with sesquioxides and with compounds bound to organic residual fractions usually predominate in sludges of municipal origin. When waters are mixed with some industrial effluents, however, the speciation of metals greatly differs based upon the discharged forms from factories (Kabata-Pendias and Pendias, 2001).

The mobile fraction of trace elements behaves like divalent cations in soil phases and is controlled by dynamic equilibria between solid and liquid phases. However, the complexity of all possible reactions in natural heterogeneous soil systems needs more data for an appropriate prediction of ecological consequences of the soil pollution with trace elements. The fate of these elements in soils depends upon many soil processes, which can be generalized as follows (Kabata-Pendias and Pendias, 2001):

i) Dissolution
ii) Sorption
iii) Complexation
iv) Migration
v) Precipitation
vi) Occlusion
vii) Diffusion (into minerals)
viii) Binding by organic substances
ix) Absorption and sorption by microbiota
x) Volatilization

All these processes are governed by several soil properties, of which soil pH and redox potential are known to be the most important parameters. Thus, the solubility of trace elements is often shown as a function of pH affected by the amount and kind of organic matter. Also soil CEC, and components as carbonates, Fe and Mn hydrous oxides, clay minerals, and fine granulometric fractions are known to play significant roles in the behavior of trace elements (Kabata-Pendias and Pendias, 2001).

Sorption of trace elements in most soils is highly pH dependent, due to the effects of pH on the variable charge sorption sites and to the tendency for metallic contaminants to hydrolyze in solution. With the exception of metals that form oxyanions (e.g., Cr, Se, and As), the metal retention in soil particles is low at low soil solution pH and as this pH increases, metals sorption increases dramatically the so-called “pH sorption edge”. Given the dramatic changes in solution pH that can occur in the rhizosphere, plant root activity is likely to have a marked effect on metal availability through changes in soil solution pH (Mclaughlin et al., 1998).

Cadmium

There is a crescent environmental concern about Cd as one of the most ecotoxic metals that exhibit highly adverse effects on the soil biological activity, the plant metabolism, and the health of humans and the animals (Kabata-Pendias and Pendias, 2001).

As for most other trace elements, toxicity of cadmium is generally associated with the inhibition of enzyme systems. Cadmium strongly interferes with metalloproteins, metalloenzymes, metallothioneins and phospholipids. For humans the two main target organs of acute and short-term cadmium exposure are the gastrointestinal tract after ingestion and the lungs after inhalation; the critical organ for long-term exposure is the kidney, where the average biological half-life of cadmium has been estimated to be 17.6 years (Förstner, 1986).

Plants take up cadmium relatively easily, and in soils there are several reactions and situations, relatively common in agricultural systems, which may increase food chain transfer e.g. acidity, salinity, or zinc-deficiency. (McLaughlin, 2003). Since the chelation of trace elements in soil disturbs the equilibrium between the labile metal on the solid phase and soil solution and thus enhances the release of the former to the soil solution, the metal chelates in the soil-root interface zone replenish the metal ion taken up by the plant. Removal of a complexed metal by plant uptake establishes a diffusion gradient to transport more chelated metal toward the root surface (Krishnamurti et al., 1997).

Cadmium "phytoavailability" diagnostic tests usually involve the determination of Cd in soil test extracts obtained by equilibrating the soil with (i) dilute extractants such as water and neutral electrolyte solution (e.g. 0.01 mol L^{-1} $CaCl_2$), (ii) strong extractants such as mineral acids (e.g. 0.1 mol L^{-1} HCl), or (iii) metal chelating extractants such as 0.005 mol L^{-1} DTPA. However, the Cd found in dilute extractants represents only the concentration of Cd in equilibrium with Cd in solid phase and it does not account for the ability of a soil to buffer or replenish Cd in solution phase. Conversely, strong acid extractants may change the regular soil conditions to others very different from those where plants grow and cause soil minerals to dissolve. Amounts of Cd extracted under such circumstances may have little relationship with the forms of soil Cd available to plants (Risser and Baker, 1990).

Cadmium is more soluble than Zn^{2+} in acidic oxidizing solutions, and has medium to high mobility in well-drained acid soils. The high mobility is attributed to the fact that Cd^{2+} adsorbs rather weakly on organic matter, silicate clays, and oxides unless the pH is higher than 6 (Pierangeli et al., 2005). Above pH 7, Cd^{2+} can co-precipitate with $CaCO_3$ or precipitate as $CdCO_3$, and Cd phosphates which limit its solubility as well. Therefore, the mobility and bioavailability of Cd in neutral to alkaline soils is low. Liming acid soils is an effective mean of limiting the uptake of Cd by plants (McBride, 1994).

Zinc

In general, the effect of the different types of soils and other factors on Zn availability may be summarized as follows (Alloway, 1995):

i) In some highly leached acid soils, total Zn levels may be very low, resulting in low available contents.

ii) Availability of Zn decreases at increasing pH values of the soil, due to the lower solubility of Zn minerals and increasing adsorption of Zn by negatively charged colloidal soil particles.

iii) In low organic matter content in soils, Zn availability is directly affected by the content of organic complexing or chelating ligands, originated from decaying organic matter or root exudates.

iv) Low temperatures and light intensities, generally, decrease Zn availability, mainly because of restricted root development.

v) High P levels in the soil may decrease Zn availability and uptake by plants. The Zn-P antagonism is one of the best-known nutrient interactions in soil chemistry and plant nutrition.

vi) Interactions with other nutrients may decrease Zn availability, mainly Zn-Fe antagonism. Additionally, Zn-Cu, Zn-N and Zn-Ca interactions are widely known.

In solution, divalent zinc ions, similar to other metals, tend to be surrounded by six water molecules arranged in an octahedron which may be written as $Zn(H_2O)_6^{2+}$. Such ions are multiprotic acids because the water molecules may lose protons. The first step gives $ZnOH(H_2O)_5^+$ the monovalent cation – more simply written $ZnOH^+$. Further steps give uncharged species, and later monovalent anion (Barrow, 1993).

Soil organic matter binds Zn forming stable compounds; therefore, Zn accumulation in organic soil horizons and in some peats is observed. However, although the stability constants of Zn-organic matter compounds in soils are relatively low, a high proportion of Zn is bound to organic matter in mineral soils. The addition of sewage sludges to these soils modifies the distribution pattern of Zn, increasing significantly two Zn species-easily, the soluble and the exchangeable (Kabata-Pendias and Pendias, 2001).

In acid, aerobic soils, Zn has medium mobility, being held in exchangeable forms on clays and organic matter. The rather high solubility potential of Zn^{2+} in acid soils, and its high-concentration in industrial wastes and sewage sludges, make it a potential agent for phytotoxicity from the land application of wastes (McBride, 1994).

The immobilization of Zn in soils rich in Ca and P, in well-aerated soils with S compounds, and in soils containing enhanced amounts of certain Casaturated minerals such as allophone, imogolite, and montmorillonite, as well as hydrous oxides, has an important practical impact on the Zn deficiency of plants. Zinc deficiencies, most frequently, result from management practices in crop production (e.g., overliming, P fertilization, organic matter amendment) (Kabata-Pendias and Pendias, 2001).

The sorbed forms of Zn in soils (Zn-soil) are, normally, more stable than most Zn minerals except franklinite ($ZnFe_2O_4$), which could be an important factor controlling the solubility of Zn depending on the Fe^{II} concentrations (Alloway, 1995).

Nickel

Nickel can exist in the oxidation states ranging from -1 to +4 (Dojlido and Beat, 1993). The +2 oxidation (nickelous) state is the only stable form of nickel in soil environments. The Ni^{2+} cation is almost as electronegative as Cu^{2+}; this fact and its electronic structure favor the formation of complexes with organic matter that are comparable in stability to those of Cu^{2+}. Bioaccumulation of Ni fits easily into octahedral sites, co-precipitating readily into Mn and Fe oxides in soils. Chemisorption on oxides, noncrystaline aluminosilicates, and layer silicate clays is enhanced above pH 6,whereas lower pH favors exchangeable and soluble Ni^{2+}. Because solubility decreases markedly at higher pH, mobility of Ni is medium in acid soils, becoming very low in neutral to alkaline soils. Under reducing conditions, Ni^{2+} is incorporated into sulfides that restrict mobility to very low levels (McBride, 1994).

The natural chemistry of cadmium, zinc and, nickel is restricted to their forms of divalent cations since the reduction of them to the metallic state is unlikely to occur in soils. But the behavior of chromium and copper is more complex (Davies, 1980).

Chromium

In the pH range of agricultural soils, chromium is unique among the trace elements because of its existence in two environmentally important oxidation states: trivalent (Cr^{III}) and hexavalent (Cr^{VI}) (Srivastava et al., 1999). These forms have sharply contrasting chemical properties: Cr^{IV} is fully hydrolyzable and exists in water as an oxyanion. The content of the weak acid and its conjugated base is dependent on the dissociation constant, K_a of the acid and its oxyanion (Evans et al., 1995).

$$HCrO_4^- \rightarrow CrO_4^{2-} + H^+, \quad pK_a = 6.51 \qquad [1.1]$$

Chromium VI is more readily extracted from soil and sediment particles and is the more toxic form of Cr for humans, being highly toxic even in small concentrations. On the other hand, Cr^{III} can be considered an essential element for the human being, when present in a proper concentration range, being toxic only in very high concentrations (Chen et al., 2001).

The chromate ion is in pH-dependent equilibrium with other forms of Cr^{VI} ions such as $HCrO_4^-$ and dichromate ($Cr_2O_7^{2-}$). Chromium III, on the other hand, is much less mobile and adsorbs more strongly to particulates. The solubility of Cr^{III} decreases above pH 4 and above pH 5.5 complete precipitation occurs (Alloway, 1995). The Cr^{III} oxides are chemically inert, and their dissolution proceeds via slow surface reactions with dissolved chemical agents (H^+, OH^-, ligands) (Grygar, 1998).

Therefore, the Cr^{III} d^3-configuration exhibits a tendency to form inert complexes. The reaction kinetics of such complexes is very slow, and can take from a few minutes up to several days (Dyg et al., 1990). However, chromium pollution problems will arise if Cr^{III} becomes mobilized by any means. Chromium-organic acid interactions in the soil-plant system are important for solubilization/binding of metals from the highly insoluble soil mineral phase (Srivastava et al., 1999).

Chromium is an immobile element, difficult to extract from soils even by aggressive chemical agents. Toxicity of Cr to plants is occasionally seen in unusually Cr-rich soils formed from the parent rock, serpentinite, or under high pH conditions favorable to Cr^{III} oxidation (McBride, 1994). The Cr^{III} form is a very immobile cation that complexes strongly with organic matter and chemisorbs on oxides and silicate clays, even at quite low pH (McBride, 1994).

Copper

Copper can exist in four valence states: the native element Cu^0 and the ions $^{+1}$, $^{+2}$ and $^{+3}$ (Dojlido and Best, 1993). It occurs in soil, solids and solutions almost exclusively as the divalent cation Cu^{2+}. Organically complexed Cu^{2+} is bound more tightly than any other divalent transition metal; this fact is most evident at low metal loadings in humus when very selective complexing groups (amines or polyphenols) are involved. Lability of these complexes is rather low, limiting bioavailability (McBride, 1994).

Copper in Earth’s crust is most abundant in mafic and intermediate rocks and has a tendency to be excluded from carbonate rocks. Copper is present in several minerals where the common primary minerals are simple and complex sulfides. These minerals are quite easily soluble in weathering processes and release Cu ions, especially in acid environments. Therefore, Cu is considered one of the more mobile heavy metals in hypergenic processes. However, it is a very versatile trace cation and, in soils or depositional material, exhibits a great ability to chemically interact with mineral and organic components of the soil. The Cu ions also readily precipitate with various anions such as sulfide, carbonate, and hydroxide. Thus, Cu is a rather immobile element in soils and shows relatively little variation in the total content in soil profiles (Kabata-Pendias and Pendias, 2001).

Under most physic-chemical conditions encountered in soils, the adsorbed forms of Cu (soil-Cu) are more stable than any Cu minerals except in strongly reducing conditions when cuprous ferrite ($Cu_2Fe_2O_4$) is more stable than soil-Cu (Alloway, 1995).

Total Cu in soils includes six ‘pools’ classified according to their physic-chemical behavior. The pools are: soluble ions and inorganic and organic complexes in soil solution; exchangeable Cu; stable organic complexes in humus; Cu adsorbed by hydrous oxides of Mn, Fe and Al; Cu absorbed on the clay-humus colloidal complex; and the crystal lattice-bond Cu in soil minerals (Alloway, 1995).

Extractable Cu, sometimes called ‘available’, refers to the amount of this element in soil that correlates statistically with the concentrations absorbed and assimilated by plants. The ‘availability’ of Cu to plants refers to the readiness with which the available ion $[Cu(H_2O)_6]^{2+}$ is absorbed by plants in acid soils and $Cu(OH)_2^0$ in neutral and alkaline soils. It is related to the chemical potentials (analogous to pH) of the respective species in the soil solution. The level and distribution of total and extractable Cu in the soil profile varies with soil type and parent material. Copper is specifically adsorbed or ‘fixed’ in soils, making it one of the trace metals that move the least (Alloway, 1995). However, the key soil factors affecting Cu movement are the organic chelation and complexing. These reactions govern Cu behavior in most soils. The ability of the organic soil constituents to bind Cu is well recognized and is of particular relevance to the transfer of these elements into biological systems.

Availability

The term trace element availability has been used to describe the extracted amounts of trace element from the soil or other materials by chemical extractants and other mechanisms; the results of those extractions are also called potentially available to plants. The "bioavailability" has been used to compare trace elements extracted from those materials by some live organism, as plants and biota. "Phytoavailability" is specifically related to the extraction of those elements by plants.

The term "metal bioavailability" is the fraction of the total metal burden present in a given matrix, which can be utilized by the biota. In most cases, this is equated with solubility, although there are exceptions; some microorganisms can extract metals directly from a solid matrix using extra-cellular secretions; root exudates can directly and indirectly solubilise metals; and re-entrained particulates can be inhaled or ingested then solubilized within a target organism. Bioavailability is a direct function of particle dissolution in the digestive system or lungs without the intervention of secondary factors. Direct measurement of bioavailability is possible in these circumstances. Problems arise when measuring bioavailability in complex ecosystems where metal solubility is a function of interacting biological, chemical and physical factors. Transfers of bioavailable metals depend on the assemblage and complexity of the biota. Measurements are modified by restricted transfer at each level, making identification of a target organism a fundamental process in estimating bioavailability (Lepp, 2003).

The phytoavailability of a metal in the soil customarily is defined by the amount of metal absorbed by growing plants or by the concentration in the harvested plant tissue. As plants grown on sewage sludge-treated soils typically absorb << 1% of the soil metals, it would take a long time to exhaust sewage sludges-borne metals that are available for plant absorption in those soils.

Therefore, phytoavailability of sewage sludges-borne metals must account not only for the plant uptake in one growing season but also for the total amounts available over time. Neither the plant uptake nor the concentration of metals clearly reflects the labile pool of the soil in just one extraction. It should be extracted all the phytoavailable pool, and this can only be achieved with several plant growing cycles on the same spot, and not only one.

Trace elements in the soil system may be part of the liquid and solid phases. In the latter, they are distributed among the various soil components and associate with them to generate chemical forms that determine their mobility. These elements may be found in soils in the liquid phase as free cations or complexed with inorganic and organic ligands, and in the solid phase retained at exchange sites or at specific sites of oxides, organic matter and in structures of primary or secondary minerals (Krishnamurti et al., 1997; Ahumada, 2001). A free ion in the soil solution will precipitate when the solubility product of the compound produced by it with any reaction partner is attained. Precipitates may occur with hydroxides, carbonates, phosphates, sulphides, molybdates, and with several other anions, including humates, fulvates and other organic ligands (Alloway, 1995).

As an example, in arid zone soils, the presence of carbonate minerals effectively immobilizes trace elements by providing an adsorbing or nucleating surface and by buffering pH at high values where precipitation takes place (Moral et al., 2002). In tropical, humid zone soils as Oxisols, normally the topsoil presents low pH and the carbonate minerals were dissolved long ago.

Among the factors affecting trace elements availability in soils, the soil parameters, such as total concentration, pH, organic matter, adsorption site, microbial activity, and moisture regime play an important role. Other factors, such as climatic conditions and interactions between them and other macro-and micronutrients at the soil level and on the plant level also largely affect their availability (Alloway, 1995).

The establishment of soil screening levels for risk assessment for both bioavailability and the protection of groundwater rely on an understanding of the lability of chemicals in soils. Thus, identification of the major soil parameters affecting metal lability in soils is a requisite to prediction of metal behavior and for the establishment of appropriate soil screening levels (Impellitteri et al., 2001).

2.5 Mobility

The mobility of trace elements is related to their capacity to pass from one soil compartment to another where the element is bound more or less energetically. The ultimate mobile compartment is the soil solution, which determines the bioavailability of trace elements (Moral et al., 2002).

The mobility of metals, typically for systems involving solution/solid interactions, reflects the flux of metal species in a certain medium, which contains both accelerating and inhibiting factors and processes. The accelerating factors comprise effects as pH lowering, redox changes, inorganic and organic complexation, and microbially mediated species transformations such as biomethylation. Among the spectrum of “barriers”, physical processes include adsorption, sedimentation, and filtration; chemical barriers comprise mechanisms such as complexation and precipitation; biological barriers are often associated with membrane processes, which can limit translocation of metals, for example from plant roots to the shoots and fruits. “Complexation” in its various forms can both inhibit and accelerate metal fluxes, particularly in biological systems consisting of different types of membranes (Förstner et al., 1990).

It can be expected that even at relatively small percentages of organic substrates these materials are primarily involved in metabolic processes and, thus, may constitute the major carriers for the transference of trace elements within the food chain (Calmano et al., 1990).

McBride (1994) proposed the following order for the chelation of metal by soil organic matter:

$$Cu^{2+} > Ni^{2+} > Pb^{2+} > Co^{2+} > Ca^{2+} > Zn^{2+} > Mn^{2+} > Mg^{2+}$$

Many soils are affected by the utilization of organic wastes such as sewage sludges that could be inputs of pollutants and especially trace elements to the soil. These elements may be retained by soil components in the surface soil layer by reacting with organic compounds, may exchange, or precipitate or co-precipitate as sulfides, carbonates and/or Fe or Mn oxides or hydroxides (Moral et al., 2002). In arid zone soils, the presence of carbonate minerals effectively immobilizes trace elements by providing an adsorbing or nucleating surface and by buffering pH at high values where precipitation takes place (Moral et al., 2002). Chelation regulates to a large degree the solubility and availability of trace elements to plants (Laurie and Manthey, 1994).

A number of chemical reactions in solution can enhance the solubility of trace elements (inorganic complexation, including hydrolysis, and organic complexation, both with natural and synthetic molecules). Chloride is likely, under natural and polluted aquatic conditions, the most efficient inorganic complex former for solubilizing trace elements. High concentrations are nevertheless required (typically greater than 0.01 mol L^{-1} Cl^{-}, but this depends on the metal). Natural organics and synthetic multidentate chelators are also very powerful complex formers (Bourg, 1995).

Soluble, exchangeable and chelated species of trace elements are the most mobile in soils, and govern their migration and phytoavailability. The behavior of trace elements in soils reflects in their speciation and depends greatly upon the added forms or compounds (Kabata-Pendias, 1995). A highly significant correlation between metal contents of plants and concentration of their mobile species in soils is generally observed. However, the uptake by roots is a complex metabolic and/or nonmetabolic process and is controlled by several plant and soil factors. There is a great diversity in plant ability either to accumulate or to exclude several trace elements from root media. The stress of both deficiency and excess of these elements alters plant reactions, and is known to produce even mutagenic changes in plants. Among several soil factors the origin of trace elements has also significant impact on their availability (Kabata-Pendias, 1995).

2.6 Dissolution and desorption of trace elements

The dissolution of a trace element can be defined as the detachment of the trace element ions from the surface of a mineral and their subsequent transport to the bulk solution (Robarge, 1999). The dissolution-precipitation equilibria control the trace elements solubility while the presence of ligands in natural waters and soil solutions can accelerate these processes. However, nonequilibrium is likely the rule rather than the exception. Nevertheless, the chemical and biological liability of specific trace elements and ligands are often controlled by precipitation-dissolution reactions (Hayes and Traina, 1998).

Therefore, the fate and impact of dissolved trace elements are strongly influenced by the formation of complex ions with organic and inorganic ligands. Many metals and ligands exist in soil solution as complex ions, and not dissociated ionic species (Hayes and Traina, 1998).

The most important variables affecting metal partitioning in soils are also the factors that affect desorption/dissolution of trace elements in soils. Metals in soil solids may enter the soil solution by desorption and dissolution (Sparks, 1995). Metal precipitates, which may represent higher concentrations of metal in soil, will dissolve to maintain equilibrium concentrations in the solution phase. Desorption processes primarily depend on the characteristics of the solid, on the complexation of the desorbing metal, on the system pH, on the ionic strength of solution, on the matrix type, species of possible exchanging ions in solution, and kinetics effects (Impellitteri et al., 2001).

The most important factor governing the desorption of metals from clay minerals in tropical soils is the system pH for minerals with predominantly pH-dependent charges, such as kaolinite (Impellitteri et al., 2001). Rainfall, evaporation, and plant transpiration can change trace element concentration in soil solutions more than tenfold, whereas the observed variations for major ions (Ca^{2+}, Mg^{2+}, K^+, Na^+, NO_3^-, and PO_4^{3-}) are much less. The acidification increases the intensity of trace metals mobilization in soils (Kabata-Pendias and Pendias, 2001).

Soluble major ions greatly influence the quantities of soluble trace elements. Solutions of most soils contain an excess of Ca, which in many soils constitutes more than 90% of the total cation concentration. Calcium is, therefore, the most important cation in governing the soluble stage of trace elements in soils. There are examples, however, of soils in which complexing trace cations prevent higher than normal concentrations of dissolved metal ions in solutions and the ready uptake of trace elements by plants usually is related to the formation of complexes (Kabata-Pendias and Pendias, 2001). Oxalate has the tendency to precipitate in the presence of Ca^{2+}. While this will reduce its potential complexing ability with some nutrients it may be important for the release of P from Ca^{2+} containing minerals such as apatite (Jones, 1998). Calcium oxalate may be an important form of Ca in the solid phase that could control Ca^{2+} activity in certain environments (Inskeep and Comfort, 1986).

Differing tendencies of cations complexation to interact with ligands can be explained by the rules of coordination chemistry. It is possible, therefore, to predict that certain cations complex, more readily, a particular ligand. In the soil aqueous phase, organic compounds and water are the most abundant ligands; therefore, hydrolysis and organic complexation are the most common reactions in soil solutions. These reactions are pH sensitive and can be correlated with the size and charge of the cations. Higher ionic potentials usually indicate a higher degree of hydration in the solution, thus an easier precipitation. The solubility of trace elements in soils depends on complex formation. However, most of the species of trace elements, especially cations, are slightly soluble, and only small proportions occur in the aqueous phase (Stumm and Morgan, 1996).

2.7 Rhizosphere

The ability of the roots to uptake trace elements is based on the processes at the soil-solution and the solution-root interfaces. The access of an element to plants is determined primarily by its origin, which influences its chemical forms, and by its association with soil components (Kabata-Pendias, 1995).

The supply of trace elements in the soil solution to roots is determined to a large extent by the soil characteristics, and takes place through one or more of three processes (Jarvis, 1981):

i) mass flow with the soil solution;
ii) diffusion through the soil solution and;
iii) root interception.

Plant themselves may be responsible for changes in the form of trace elements in the soil solution. For example, as soil pH changes, not only the quantity and the nature of the ligands present in solution change, but also the degree of complexing may be changed (Jarvis, 1981). Rhizosphere pH may differ from that of the bulk soil by up to two units, depending on plant and soil factors. The most important factors for root-induced changes in rhizosphere pH are imbalance in the cation/anion uptake ratio and the corresponding differences in net excretion of H^+ and HCO_3^- (or OH^-), the excretion of organic acids, and indirectly, the microbial acid production from root release of organic carbon, and enhanced CO_2 production (Marschner, 1993).

Root exudates released into the soil surrounding the root have been implicated in many mechanisms for altering the level of soluble ions and molecules within the rhizosphere (Jones et al., 1996).

The biological activities of microorganisms in the rhizosphere to a large degree mediate the solubility of metal ions at root surfaces (Manthey et al., 1994). However, it depends on the microorganism activity. As an example, low concentrations of some trace elements (e.g. copper, chromium, molybdenum, nickel, selenium, zinc, etc.) are essential to the healthy functioning and reproduction of microorganisms, plants and animals (including man). On the other hand, at high concentrations, these same essential elements may cause direct toxicity or reproductive deffects. Some trace elements are not essential (e.g. arsenic, lead, mercury, etc.) for plants and animals and even low concentrations of these elements in the environment can cause toxicity to microorganisms and both plants and animals (McLaughlin, 2003).

In the terrestrial environment, there are several barriers to the bioaccumulation and biomagnification of trace elements. In uncontaminated environments, the bioavailability of many trace elements is often low, due to the presence of highly insoluble or occluded forms in soil. Then, transfer through the food chain is limited by low concentrations in soil solution. If trace elements are added to soils in a soluble, highly bioavailable form, reactions with soil surfaces and solutes may also render insoluble precipitates or strongly adsorbed species, also keeping risks from foods chain transfer low. Lead (Pb) is a good example of such an element, since even if the total or added concentrations are hazardous, food-chain risks may be low when fate and transport issues are considered. A further issue to be considered is the propensity of some organisms to actively exclude some elements from their uptake, or mitigate transferring them to organs consumed by higher trophic levels (McLaughlin, 2003).

Plant roots either absorb the chelated metals or extract the metals from the chelate. It is common for metals such as Cu, Pb, and Cd, after absorption by plants from the soil, to accumulate in (or on) roots. The organic ligands in particular increase the "carrying capacity" of soil solutions for strongly complexing metals such as Cu^{2+}, increasing total metal solubility (McBride, 1994).

The significant role of plants cycling the trace elements and transferring them to the food chain has been well illustrated for various ecosystems and was published in numerous papers. Plants can accumulate trace elements in or on their tissues due to their great ability to adapt to variable chemical properties of the environment: thus, plants are intermediate reservoirs through which trace elements from soils, and partly from waters and air, move to man and animals (Kabata-Pendias and Pendias, 2001).

The exudation of soluble organic compounds by plants is perhaps one of the most important processes likely to affect retention of contaminants by soil in the rhizosphere. Soluble organics can affect retention of metal ions on mineral surfaces through (Mclaughlin et al., 1998):

i) Competition for sorption sites or blocking of sorption sites in the mineral colloid,

ii) Increased retention due to formation of a strongly sorbed metal-ligand complex,

iii) Decreased retention due to complexation of metal in solution in a non-sorbing form or lowered free metal activity leading to desorption of surface-bound metal,

iv) Increased retention through the increase in the surface negative charge, which is dependent on the solution pH and the isoelectric point of the surface,

v) Increased retention through the increase in the surface area and charge due to the structural distortion of minerals during formation,

vi) Dissolution of clay minerals leading to the decrease in the retention of contaminants.

The conclusion from a large number of studies on copper absorption by plant roots is that chelating substance enhances Cu and Zn solubility but inhibits Cu absorption by plants (Laurie and Manthey, 1994).

Organic acids released by the roots have the capacity to complex metals in solution. The degree of complexation, however, depends on the particular organic acid involved (number and proximity of carboxyl groups), the concentration and type of metal and the pH of the soil solution. Organic acids with only one carboxyl group (lactate, formate and acetate) have very little metal-complexing ability (Jones, 1998). Any resulting complex of a metal and a ligand (organic acid) might have a net charge that can be negative, positive or neutral (Nigam et al., 2001).

The stability constants of chelates with metals tend to be in the following decreasing order: Cu > Fe = Al > Mn = Co > Zn. Low-molecular-weight organic ligands, not necessarily humic in origin, can form soluble complexes with metals and prevent them from being adsorbed or precipitated (Alloway, 1995).

2.8 Extractant

Natural organic ligands capable of binding metals and adsorbing onto surfaces are abundant in the environment (Vasudevan and Stone, 1998). The dominant organic ligands in soil solutions are best described by their functional groups. These are dominated by carboxyl, carbonyl, amino, imidazole, phenolic OH, alcoholic OH, and sulfhydryl groups (Hayes and Traina, 1998).

In plant nutrition, as well as in pedology or environmental research, it is of great interest to know the soil's content of different elements. However, for many elements the total content is of little biological or ecological interest since a great deal is firmly bond in crystal structures, and released only to the extent that mineral particles are altered by chemical weathering, which is a slow process. Some elements may also be firmly bound in the humus and become released only when the humus it is mineralized (Anderson, 1975).

The classical and essentially empirical approach is to correlate extracted amounts from soils with the content present in plants grown on the same soil (Merckx et al., 1986). The techniques most widely used for soils employ a single extractant and if the extracted amount of one element correlates with the amount absorbed by the plant it can be used to predict plant uptake or the likelihood of deficiency or toxicity symptoms occurring in plants. Such approach is well established in soil science for predicting the uptake, at natural concentrations, of essential trace elements such as Cu, Co, Mn, and Zn and of potentially toxic elements such as Mo and Ni. For the trace elements associated with pollution from sewage sludges or industrial wastes and effluents or industrial wastes elevated concentrations, extractant methodology is not well established and few predictions of uptake from soil extraction analysis can reliably be made (Ure, 1995).

The Zn quantity extracted from soils may vary depending on the used extractant, revealing different degrees of success on the evaluation of toxicity or deficiency to plants (Nascimento et al., 2002). Furthermore conversion equations between results from an extractant do not present satisfactory adjustment (Pereira et al., 2001). Thus results from one extractant cannot be converted into another. Ribeiro-Filho et al. (2001) describes that DTPA and

Mehlich I extractions, concerning phytoavailability, depend on the soil characteristics or previous treatments. Abreu et al. (1995) argument that Mehlich I, Mehlich III, and the DTPA methods presented low efficiency to evaluate the Pb, Cd, Cr and Ni phytoavailability in not contaminated soil samples.

A method that accurately predicts trace elements bioavailability is essential in evaluating risks from using sewage sludges in agricultural soils. Several have been developed; most are based on chemical extractions. Some suggest the use of neutral solutions as reagents for extraction, for example water or unbuffered salt solutions with an ionic strength that mimic that of the soil, i.e. $CaCl_2$, $MgCl_2$, $NaNO_3$, and NH_4NO_3. The use of metal chelating extractants containing DTPA (+TEA) or EDTA is another approach. Strong acid solutions are also used, such as HCl, Mehlich III, HNO_3, and aqua regia. Chelating agents solutions are believed to extract potentially mobile portions of metals. Neutral salt solutions have been introduced as simulating the natural soil solution and therefore are useful to evaluate the ecological relevance of metals. Acid extractants, depending on the strength and soil mineralogical composition, can extract nearly total amounts of trace metals. However, environmental attributes that influence plant uptake are dynamics and the methods employed might be effective for particular conditions but not universal for all situations (Kabata-Pendias and Pendias, 2001; Pires et al., 2003).

In the soil, metals may react with soil components through processes that include complexation, adsorption, and/or precipitation (Alloway, 1991). At the end, metals in solid and solution phases should approach equilibrium. Roots behave as a sink that absorbs metals from soil solution and equilibria would be reestablished through desorption and dissolution of metals from solid phases. The reaction kinetics thus may affect the availability of metals. Based on this reasoning, an infinite metal sink in sewage sludges-treated soils may mimic the kinetics of these reactions and reflect metals availability (Lee and Zheng, 1993).

More meaningful extraction schemes are based on the idea that the uptake of ions from the soil solution might be controlled or at least mediated by the plant root itself by releasing specific components that bind these ions through chelation or complexation reactions (Lindsay, 1974, cited by Merckx et al., 1986).

The extractant solution composed by organic acids found on rhizosphere developed by Pires et al. (2004) was considered capable to evaluate trace elements phytoavailability.

Chelating agents form soluble complexes with metal ions in solution, favoring the uptake of macronutrients by roots as well as their replacement in solution from the surrounding soil (Knezek and Ellis, 1980). Generally, the extractability of trace elements from calcareous soils is best determined with DTPA. This may reflect the chelating capacity of the Lindsay-Norvell solution while maintaining soil pH at alkaline values to optimize cation extraction. For the non-calcareous soils smaller differences between extractants were observed although ammonium chloride was the most effective extractant of trace elements under acid conditions. The influence of soil pH and electrical conductivity on soluble-exchangeable trace elements was noticeable for Cd, Ni, and Co for all the extractants and especially for chloride salts (Moral et al., 2002).

2.9 Kinetics

Observations from kinetics and transport experiments often indicate a slow and continued trace metals release at low concentrations for extended periods of time. Such slow release or excessive tailing or breakthrough curves is often difficult to describe and single reaction type models fail to quantify the results (Selim and Amacher, 2001).

The uptake by plants, which determines the plant tissue concentration and phytotoxicity of a metal, is determined by the kinetics of metal mobilized by rhizosphere soil solution (i.e. root exudates + background matrix of soil solution). In this manner, the phytoavailability of sewage sludges-borne metals may be defined in terms of a capacity factor (metals solubilized by root exudates), which describes "how much" of the metals are available. Therefore "how long" metals in sewage sludges-treated soil will last will depend on the rate at which metals are solubilized. Based on this approach, the availability of sewage sludges-borne metals in the soil may be expressed in the following manner (Koo, 2001):

$$M_t = C \times e^{-kt} \qquad [1.2]$$

where M_t is the cumulative metal removal by crops in t years, C is the capacity factor representing the labile metal pool of the sewage sludges-treated soils, and k is the intensity factor representing the rate that metals dissolve in the rhizosphere and, therefore, become available for plant absorption (Koo, 2001).

Field data may be used to establish C and k for sewage sludges-treated soils. However, long-term field based metal absorption data is hard to acquire. The phytoavailability of metals needs to be estimated. The dissolution behavior of sewage sludges-borne metals in the root exudates may define the total root exudate-extractable metals and the metal dissolution rate (Koo, 2001).

The rates of chemical reactions are found to be a function of the extent to which the reaction has proceeded. That is, the rate is a function of the reactant concentrations; it is found in all cases to be directly proportional to the amounts or concentrations of one or more of the reactants raised to some power. The value of that power, or, in more complex reactions, the sum of the values of the powers, is called the order of the reaction. The proportionality constant is called the rate constant of the reaction, and is normally indicated by the symbol k (Plambeck, 1996).

For a zero-order reaction, the rate of reaction is a constant. When the limiting reactant is completely consumed, the reaction abruptly stops. For a first-order reaction, the rate of reaction is directly proportional to the concentration of one of the reactants (Blaush, 2003). The equations are commonly used to describe reactant consuming, however can also be used to describe cumulative increases in products, as trace elements solubilized from soil materials.

Then, kinetics data from trace element extraction reagents could contribute towards the creation of a tool for predicting risks to the environment due to the presence of trace elements in soil (Bermond et al., 1998).

3 GENERAL MATERIAL AND METHODS

3.1 Soil material

For one set of experiments, samples from the 0-20 cm depth of a Dark Red Distroferric Latosol from Jaguariúna field plot experiments established on 1999 (Rangel, 2005) (located in São Paulo State, Brazil, 22º41' S, 47º W, and 570 m altitude) were collected. These samples were analyzed to assess their physical and chemical characteristics (pH_{H_2O} = 5.5; Ca^{2+} = 27.5 $mmol_c$ dm^{-3}; Mg^{2+} = 8.5 $mmol_c$ dm^{-3}; P (Mehlich I) = 3.5 mg dm^{-3}; K^+ = 1.51 $mmol_c$ dm^{-3}, Al^{3+} = 1 $mmol_c$ dm^{-3}; $H+Al^{3+}$ = 35 $mmol_c$ dm^{-3}, organic matter = 25.5 g kg^{-1}, and clay = 450 g kg^{-1}; Silva, 1999).

One sewage sludge from the Franca Sewage Sludge Treatment Station (Franca sewage sludge) (Table 1.1) was added and mixed to this soil. The treatment station is from São Paulo State, Brazil.

The soil treatments scheme was based on Franca (F) sewage sludges application on the field plots as follows (Table 1.2): 1) control, sewage sludge-free (F_0); 2) sewage sludges application based on the N required for the maize crop, 90 kg N ha^{-1} (Raij et al., 1996) (F_{1N}); 3) two times the required N (F_{2N}); 4), 4 times (F_{4N}), and 5) 8 times (F_{8N}). Maize was cultivated during five growing seasons. Each growing season was preceded by one sewage sludge application. These samples were, then, air-dried and ground to pass through a 2-mm sieve, homogenized, and stored for subsequent analysis.

TABLE 1.1 Franca sewage sludge chemical characteristics applied on the five maize crops.

Attribute	Unity[‡]	First growth	Second growth	Third growth	Fourth growth	Fifth growth
P	g kg^{-1}	16.0	21.3	12.9	13.8	27.3
K	g kg^{-1}	1.0	0.99	1.0	1.5	1.0
Na	g kg^{-1}	0.5	0.6	0.9	0.5	0.4
As	mg kg^{-1}	<1	<1	<1	<0.01	<0.1
Cd	mg kg^{-1}	3.32	2.0	2.05	1.14	0.6
Pb	mg kg^{-1}	199.6	118	1405	78.6	43.0
Cu	mg kg^{-1}	239.8	359	240.9	187.1	196.0
Cr	mg kg^{-1}	633.8	1325	1230.3	202.0	182.4
Hg	mg kg^{-1}	<0.01	<1	<0.01	<0.01	<0.1
Mo	mg kg^{-1}	<0.01	<1	<0.01	<0.01	<0.1
Ni	mg kg^{-1}	54.7	74	72.4	63.9	49.5
Se	mg kg^{-1}	<0.01	<1	<1	<0.01	<0.1
Zn	mg kg^{-1}	1230	1590	1198	773.0	890.6
B	mg kg^{-1}	40.7	7.1	19.7	10.4	13.6
OC	g kg^{-1}	305.1	374	382.4	370.9	475.4
pH		6.3	6.4	5.4	8.9	8.3
Humidity	%	66.4	80.2	71.2	79.5	76.69
VS	%	60.5		72.5	67.0	58.65
N-Kjeldahl	g kg^{-1}	47.0	50.8	55.2	47.4	43.1
N- NH_4	mg kg^{-1} [§]	4803.2	119	2094.1	2330,6	2008.8
N-NO_3^- - NO_2^-	mg kg^{-1} [§]	22.0	54.8	43.9	56.4	38.3
S	g kg^{-1}	16.3	13.3	15.7	9.3	10.1
Mn	mg kg^{-1}	349.3	267	232.5	439.8	712.9
Fe	mg kg^{-1}	33,793	31,700	24,176	39,895	64,900
Mg	g kg^{-1}	2.2	2.5	2.2	2.7	5.0
Al	mg kg^{-1}	32,564	33,500	23,317	18,189	21,672.2
Ca	g kg^{-1}	29.2	16.8	24.8	13.3	11.5

[‡] Dry mass basis; OC = organic carbon; VS = volatile solids.
[§] Data for N-NH_4 and N-NO_3-NO_2 were determined on natural samples.
Data source: Dias (2005) and Bettiol (personal communication).

TABLE 1.2 Amount of sewage sludge and mineral fertilizers (MF, N, P_2O_5, K_2O) applied on the five maize growths.

	Sewage sludge (kg ha^{-1}, dry mass basis)					N sown+ N cover (kg ha^{-1})					P_2O_5 (kg ha^{-1})					K_2O (kg ha^{-1})				
Growth	1º	2º	3º	4º	5º	1º	2º	3º	4º	5º	1º	2º	3º	4º	5º	1º	2º	3º	4º	5º
Control																				
MF						16+34	18+72	18+82	20+70	20+80	80	90	90	70	70	64	72	72	56	70
F_{1N}	3014	3504	3766	4432	4300											28	33	58	96	63
F_{2N}	6028	7008	7533	8863	8700											25	29	45	90	54
F_{4N}	12057	14017	15065	17726	17400											17	23	18	75	36
F_{8N}	24113	26033	30131	35452	34800												11		42	3

MF = mineral fertilizer; F_{1N}, F_{2N}, F_{4N} e F_{8N} = Franca sewage sludge doses applied to supply one, two, four and eight times the N requirement for the maize.
Data source: Dias (2005).

3.2 Sewage sludges properties

TABLE 1.4 Total trace element concentrations* (mg kg^{-1}) of sewage sludges and sewage sludge-treated soils.

Material	Cd	Zn	Ni	Cr	Cu
			Sewage sludge		
Franca	1.42	1131.75	53.96	243.93	236.31
			Sewage sludge-treated soils		
F_{8N}	0.21	71.74	15.90	58.12	37.44

* USEPA 3052.

3.3 Soil saturation extracts

Soil saturation extracts were prepared mixing 200 g of soil (field moist weight) with deionized water (DI) and, then, the watery soil (sealed with a plastic cover) was equilibrated for 24 h (Rhoades, 1996). Three replications were performed. The watery soil solutions were extracted by vacuum and the soil saturation extracts were split in 3 portions for the following analysis. Then the extracts were acidified with 1% of concentrated HNO_3 and the total soluble cations were analyzed. All the samples were stored in a cold room at 4°C prior to analysis and then analyzed within one week. The soil saturation extracts were analyzed, for cations, by ICP-emission spectroscopy, and for anions by ion chromatography (NO_3^-, Cl^-, SO_4^{2-}, PO_4^{3-}) (Table 1.5). The soil solution pH was determined and the ionic strength (I) was calculated from the electrolytic conductivity (EC, Table 1.6).

TABLE 1.5 Soil saturation extract compounds found for the control and sewage sludge-treated soils§.

Soil	pH	I#	Na^+	Ni^{2+}	Pb^{2+}	Sr^{2+}	Zn^{2+}	Al^{3+}	Ba^{2+}	Ca^{2+}	Cu^{2+}	Fe^{2+}	K^+	Mg^{2+}	Mn^{2+}	Cl^-	SO_4^{2-}	NO_3^-
		mmol L^{-1}	--------------------								-log [ ]‡ --------------------							
C	4.65	1.34	3.755	Nd	Nd	6.411	6.175	4.021	6.680	3.624	Nd	4.699	4.127	3.629	5.853	4.873	4.873	5.991
F_{8N}	6.09	10.63	3.523	Nd	5.831	5.789	5.448	3.639	6.332	2.697	Nd	4.387	3.840	2.723	5.099	3.335	3.052	4.190

§ Data represents mean value of 3 samples.
‡ [ ] denotes concentration of a given species in mol L^{-1} on the extracts, respectively.
# Calculated I from the data on table.
Nd means not detected values, below the detection limits.
C = control soil; F = sewage sludge-treated soil

TABLE 1.6 Soil saturation extracts properties for the control and sewage sludge-treated soils§

Soil	Sum cations#	EC	I (EC) x 1000‡	[Ca + Mg]*	[Na + K]*	$CaSO_4$Π	NaClΠ	Iφ
	$mmol_c$ L^{-1}	dS m^{-1}	--------------------		mmol L^{-1}		--------------------	
C	1.195	0.141	1.795	0.47	0.25	0.5	0.25	2.25
F_{8N}	8.248	0.703	8.928	3.90	0.44	4.0	0.50	16.5

§ Data represents mean value of 3 samples.
# Sum of the major mono and divalent cations (Ca + Mg + Na + K) concentrations from the extracts analysis. This value must match the electrolytic conductivity (EC) value (dS m^{-1}) (Rhoades, 1996).
‡ Estimated ionic strength calculated from the EC (dS m^{-1}), by multiplying this value by the constant 0.0127 (Griffin and Jurinak, 1973).
Π The $CaSO_4$ and NaCl concentrations selected to compose the background solution for the subsequent experiments.
φ Ionic strength calculated from the $CaSO_4$ and NaCl concentration.
* Sum of the major mono and divalent cation concentrations.
C = control soil; F = sewage sludge-treated soil

All the following experiments were equilibrated with background solutions containing 4 mmol L^{-1} $CaSO_4$ + 0.5 mmol L^{-1} NaCl to match the F_{8N} (sewage sludge-treated soil) main soil matrix. To model this background solution, the major cations were summed (mol L^{-1}) as follows: divalent ($[Ca^{2+} + Mg^{2+}]$), and monovalent ($[Na^+ + K^+]$). Then the major anions (mono and divalent) were chosen to compose the background solution (SO_4^{2-} and Cl^-). These sums were further used to calculate the amount of salts applied to the solutions as $CaSO_4$ and NaCl as background components. Then, the development of the trace element extractants was based on the maize root exudates found in Koo (2001).

3.4 Organic acids extractant composition

In order to determine all the extractant conditions that could fit in this work, we started looking for studies in the literature about root exudates composition and metal dissolution in soils. Most likely, the work from Koo (2001) was the most complete found for *Zea mays* L. and was selected to be the raw basis for the extractant development.

The average composition of organic acids recovered from the rhizosphere grown on sewage sludges-treated medium (Table 1.7) was taken and a synthetic composition was prepared.

TABLE 1.7 Properties of organic acids collected in root exudates of *Zea mays* L.[1]

Organic acid	Molecular weight	Formula	COOH mole fraction ratio	COOH groups
Acetic	60.05	$C_2H_4O_2$	0.287	1
Butyric	88.11	$C_4H_8O_2$	0.209	1
Glutaric	132.12	$C_5H_8O_4$	0.004	2
Lactic	90.08	$C_3H_6O_3$	0.366	1
Maleic	116.07	$C_4H_4O_4$	0.042	2
Oxalic	90.04	$C_2H_2O_4$	0.043	2
Propionic	74.08	$C_3H_6O_2$	0.010	1
Pyruvic	88.06	$C_3H_4O_3$	0.0004	1
Succinic	118.09	$C_4H_6O_4$	0.006	2
Tartaric	150.09	$C_4H_6O_6$	0.032	2
Valeric	102.13	$C_5H_{10}O_2$	0.001	1

[1] Data from Koo (2001).

Oxalic acid was suppressed from the synthetic solution because calcium oxalate ($CaC_2O_4.H_2O$) pK_{sp} is about 8.4 (Dean, 1992), presenting very low solubility in water.

The pH 4.8 used on the extractant study was the same used by Koo (2001). The pH selection was based on the literature review of rhizosphere pH from several studies and on the pH effects on the metal dissolution in sewage sludges-treated soils. Accordingly (Uren and Reisenauer, 1988; Evans et al., 1995; Bermond et al., 1998 and Koo, 2001), the trace elements dissolution rate is significantly similar when the pH is between 4.0-5.0.

3.5 Organic acids and trace elements iteration

The iterations among the trace elements and the extractant depends on each organic acids concentration and their log K. Then the log K is a temperature dependent constant, for this reason, the speciation calculations were made at a fixed temperature of 25°C (room temperature).

TABLE 1.8 Organic ligands and their stability constants (Log K) with Cd[1].

Ligand‡	Cd					
	ML/M.L#	ML$_2$/ML.L	ML$_3$/ML$_2$.L	MHL/MH.L	MH$_2$L$_2$/MH$_2$L.L	MHL$_2$/MHL.L
Ace	1.92	2.72	2.99			
Glu	2.87					
Lac	1.26*	1.98*	2.5*			
Mal	2.36*	3.6**	3.8**	1.34*		
Oxa	3.89	4.94				
Pro	1.6	2.57				
Pyr	0.78	1.18*				
Suc	2.62	3.5	3.38	7.04		
Tar	2.73	4.08		5.78	10.87	7.37

‡ Species: Ace = acetate^{-1}, But = butyrate^{-1}, Glu = glutarate^{-1}, Lac = lactate^{-1}, Mal = maleate^{-2}, Oxa = oxalate^{-2}, Pro = propionate^{-1}, Pyr = pyruvate^{-1}, Suc = succinate^{-2}, and Tar = tartrate^{-2}.

M = Cd; L = ligand.

* NIST database 46.7 (2003); ** Smith and Martell (1989).

[1] Data from Visual Minteq (Gustafsson, 2004).

TABLE 1.9 Organic ligands and their stability constants (Log K) with Zn[1].

Ligand‡	Zn			
	ML/M.L#	ML$_2$/ML.L	ML$_3$/ML$_2$.L	MHL/MH.L
Ace	1.57	1.91		
But	1.43			
Glu	2.45	2.56		6.75
Lac	1.86*	3.75*	3.4*	
Mal	2.93*	5.32*		
Oxa	4.87	7.69		
Pro	1.44	1.93		
Pyr	1.26*	1.98*		
Suc	2.52	2.82		7.15
Tar	3.43	5.48		5.91

‡ Species: Ace = acetate^{-1}, But = butyrate^{-1}, Glu = glutarate^{-1}, Lac = lactate^{-1}, Mal = maleate^{-2}, Oxa = oxalate^{-2}, Pro = propionate^{-1}, Pyr = pyruvate^{-1}, Suc = succinate^{-2}, and Tar = tartrate^{-2}.

M = Zn; L = ligand.

* NIST database 46.7 (2003).

[1] Data from Visual Minteq (Gustafsson, 2004).

TABLE 1.10 Organic ligands and their stability constants (Log K) with Ni[1].

Ligand[‡]	Ni			
	ML/M.L[#]	ML_2/ML.L	ML_3/ML_2.L	MHL/MH.L
Ace	1.44	2.4		
But	6.91			
Glu	2.47			
Lac	2.2*	2.76*	3.1**	
Mal	3.9*			1.83*
Oxa	5.16	8.44		
Pro	1.19			
Pyr	1.12**	0.95*		
Suc	2.5			6.94
Tar	3.46			5.89

[‡] Species: Ace = acetate^{-1}, But = butyrate^{-1}, Glu = glutarate^{-1}, Lac = lactate^{-1}, Mal = maleate^{-2}, Oxa = oxalate^{-2}, Pro = propionate^{-1}, Pyr = pyruvate^{-1}, Suc = succinate^{-2}, and Tar = tartrate^{-2}.

[#] M = Ni; L = ligand.

*NIST database 46.7 (2003); ** Smith and Martell (1989).

[1] Data from Visual Minteq (Gustafsson, 2004).

TABLE 1.11 Organic ligands and their stability constants (Log K) with Cr[1].

Ligand[‡]	Cr			
	ML/M.L[#]	ML_2/ML.L	ML_3/ML_2.L	MHL/MH.L
Ace	1.8	2.92		
Lac	3.301***	2.398***	2.699***	
Mal	5.4[§]	3[§]	1.9[§]	
Oxa	4.71			
Pro	4.7	7.04	9.7*	
Tar	2.96			6.37

[‡] Species: Ace = acetate^{-1}, Lac = lactate^{-1}, Mal = maleate^{-2}, Oxa = oxalate^{-2}, Pro = propionate^{-1}, and Tar = tartrate^{-2}.

[#] M = Cr; L = ligand.

* NIST database 46.7 (2003); ***Christensen and Izatt (1983); [§] Pierrin (1979).

[1] Data from Visual Minteq (Gustafsson, 2004).

TABLE 1.12 Organic ligands and their stability constants (Log K) with Cu[1].

Ligand[‡]	Cu					
	ML/M.L[#]	ML_2/ML.L	ML_3/ML_2.L	MHL/MH.L	M2HL2/M2HL.L	M2L2/M2L.L
Ace	2.21	3.4	3.94			
But	2.14					
Glu	3.24					
Lac	2.52	4.08*	4.7*			
Mal	3.4[§]	4.9[§]	6.2[§]	2.44*	4.16*	5.15*
Oxa	5.72	10.23				
Pro	2.22	3.5				
Pyr	2.2*	4.9*				
Suc	3.4			7.87		
Tar	3.97	5.2		6.29		
Val	2.12					

[‡] Species: Ace = acetate^{-1}, But = butyrate^{-1}, Glu = glutarate^{-1}, Lac = lactate^{-1}, Mal = maleate^{-2}, Oxa = oxalate^{-2}, Pro = propionate^{-1}, Pyr = pyruvate^{-1}, Suc = succinate^{-2}, Tar = tartrate^{-2}, and Val = valerate^{-1}.

[#] M = Cu; L = ligand.

* NIST database 46.7 (2003); ** Smith and Martell (1989), [§] Pierrin (1979)

[1] Data from Visual Minteq (Gustafsson, 2004).

3.6 Trace element analysis

After the equilibration, all the slurries were centrifuged for 20 minutes at 8,000 rpm to separate the solution and solid phases. The solution phase was passed through a 0.45-µm filter with a PVFD membrane (Millex, Millipore) into 50 mL flasks. The filtrates were acidified with 1% (v/v) concentrated HNO_3.

The metal contents of the supernatants were determined using, for Cd, Zn, Cr, Cu, and Ni, the Perkin-Elmer AAnalyst 800 Atomic Absorption Spectrometers (Perkin-Elmer, Bodenseewerk, Germany) with an AS-800 Autosampler. The solutions used in this experiment were all checked for purity and the quality control was assured by the Standard Reference Material (SRM) 1640 trace elements in natural water from NIST (National Institute of Standards and Technology) or the reference material Lab Performance Check Standard 1 (LPC-1-100/500) from SPEX (based on SRM from NIST). Most of the chemicals used in the experiment were trace metal-grade (TMG) and used without further purification. In this study lead was not studied as it was extracted in low concentrations, very near the detection limits for the atomic absorption.

3.7 Kinetics studies

Several authors (Pohlman and McColl, 1986) have studied which model the trace elements extraction kinetics follows. These approaches give a good indication of the model in which the trace element extraction kinetics by organic acids should fit.

Under conditions where neither the ligand nor the solid is significantly depleted during the reaction, steady state dissolution kinetics (i.e., a constant rate of dissolution over time) should be observed where the dissolution rate is proportional to the concentration of the surface complex (Hering, 1995). However, when the trace elements labile pools are greatly depleted, and other (harder to extract) pools start to be assessed, the kinetics is not a steady state dissolution. Normally it is a pseudo first order kinetics, which includes several dissolution constant rates. A multiple first-order model (equation 1) could be applied in the way to simplify the mathematic calculation that describes the dissolution.

Because the reagents used in this study were in excess, the kinetics reactions can fit, therefore, on a multiple first-order model where the rate of desorption of an ion from the soil particles is given by Equation 1.3 (Bermond and Ghestem, 2001):

$$C_t = C_o \,(1 - e^{-kt}) \qquad [1.3]$$

Where:

C_o denotes the ultimate metal release due to the first order dissolution reaction (mg kg^{-1}). C_o represents, therefore, a prediction, i.e., a future estimative calculated by plotting the equation from the data;

k denotes the kinetics constant rate related to the ion extraction (ln (mg kg^{-1}) t^{-1});

C_t is the concentration of the extracted ion (measured in the solution) at time t (mg kg^{-1}).

The model can be rearranged as (Koo, 2001):

$$M_Q = a\,(1 - e^{-bQ}) \qquad [1.4]$$

Where:

M_Q represented the cumulative metals removed (mg kg^{-1}) at Q;

Q is the amount of cumulative organic acid mixture (mol of COO^-) for extraction;

a is the ultimate metal removal (mg kg^{-1}) and

b is the removal constant (ln (mg kg^{-1}) mol of $COO^{- -1}$).

The kinetics curves from the trace elements extraction studies were plotted by the Sigma Plot software according to model 1.4, using C_o and C_t as substitutes for M_Q and a. All calculations and statistics were made by this program fitting the scatter plot to the exponential rise to the maximum model (equation 1) and the variables were found on the program reports.

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CHAPTER II

EXTRACTION OF TRACE ELEMENTS FROM A SEWAGE SLUDGE-TREATED SOIL

CHAPTER 2

ABSTRACT

MARCHI, Giuliano. **Extraction of trace elements from a sewage sludge-treated soil**. 2005, 104p. Thesis (Doctorate in Soil Science and Plant Nutrition).[#]

The prediction of trace elements plant availability in sewage sludge-treated soils by extractants in a single extraction is seldom achieved and depends on the soil and sewage sludge properties, as well as the plant and environmental factors, such as temperature, humidity, and light. The method of trace elements extraction proposed in this work tries to mimic the rhizosphere environment by using both mono and divalent ions, different organic acid compositions and several successive extraction kinetics to assess the total trace element "availability" that should be achieved after several plant growths in the same spot until that "available" fraction is depleted. The plots were fitted with the first order kinetics model and the kinetics parameters for Zn, Cd, Ni, Cr, and Cu were compared. The Zn ultimate metal release (C_o) values due to the first order dissolution reaction from the 1, 10, and 100 mmol L^{-1} complete organic acid solution (COAS) presented the same magnitude. The more appropriated extraction method was found to be the use of one and a half-grams of soil in 30 mL of 0.1 mol L^{-1} of a modified organic acid solution (MOAS), pH 4.8±0.1, performing 15 successive extractions, each one every 24 hours.

[#] Guidance Committee: Luiz Roberto Guimarães Guilherme – UFLA (Adviser), Andrew C. Chang – UCR (Adviser).

RESUMO

MARCHI, Giuliano. **Extração de elementos-traço de um solo tratado com lodo de esgoto**. 2005, 104p. Tese (Doutorado em Ciência do Solo e Nutrição de Plantas).[#]

A predição da disponibilidade de elementos-traço em solos tratados com lodo de esgoto por extratores em uma única extração é dificilmente conseguida e depende das propriedades do solo e do lodo, bem como da planta e de fatores ambientais, tais como temperatura, umidade e luz. O método de extração de elementos-traço proposto neste trabalho tenta reproduzir a rizosfera pelo uso de íons mono e divalentes, diferentes composições de ácidos orgânicos e cinética de extrações sucessivas para acessar a total “disponibilidade” de elementos-traço que é verificada após vários cultivos sucessivos no mesmo local até que a fração “disponível” seja esgotada. Os dados foram descritos pelo modelo de cinética de primeira ordem e os parâmetros para Zn, Cd, Ni, Cr e Cu foram comparados. Os valores para a liberação de Zn final (C_o) calculados pela equação de primeira ordem para a solução de ácidos orgânicos (1, 10 e 100 mmol L^{-1}) apresentaram a mesma magnitude. O método de extração mais apropriado foi aquele com o uso de um e meio gramas de solo em 30 mL de uma solução “modificada” de ácidos orgânicos 0.1 mol L^{-1}, pH 4.8±0.1, perfazendo 15 extrações sucessivas, uma a cada 24 horas.

[#] Comitê Orientador: Luiz Roberto Guimarães Guilherme – UFLA (Orientador), Andrew C. Chang – UCR (Orientador estrangeiro).

1 INTRODUCTION

The trace elements extraction from sewage sludge-treated soils have been tested in several works by a number of chemical extractants such as DTPA (Lopez-Valdivia et al., 2002), EDTA (Wu et al., 2003), Mehlich I (Gartley et al., 2002), Mehlich III (Cancela et al., 2002), Ammonium acetate (Ure et al., 1993) and others as single extraction methods of analysis. These methods extract trace elements from several pools from the soil but do not reproduce the rhizosphere reactions. Other methods such as sequential extractions were not able to predict the phytoavailable pool as the plant extracts trace elements from several different pools in the soil.

The chemical forms or oxidation states of an element are dependent on the physic-chemical properties of both the trace element and the surrounding environment. Not least as important in this respect is the composition and concentration of competing ligands and the affinity of the metal ion for these ligands (Smith et al., 1992), which will increase their solubility and mobility in the soil solution.

The trace elements solubilization in the soil by root exudates is one of the main mechanisms that increase the trace elements uptake by plants. Once the chemical composition of this exudates is identified, these substances can be synthesized in laboratory and, then, utilized as an extractant able to evaluate the available trace element quantities to the plants on the same way the chemical extractants do (Abreu et al., 2002). Several reports have shown the chemical composition of the exudates however, Koo (2001) brings a complete essay for the maize crop. Despite of that, the trace element dissolution by substances found in the root environment takes a long time and its uptake by plants is slow.

For this reason, the assessment of total organic acid extractable trace elements by kinetics studies would be a good indication of the total amount of metals potentially available to plants (Koo, 2001). Then, the trace elements extraction kinetics from a contaminated soil by a synthetic organic acid solution, similar to that found in the rhizosphere, can reproduce the rhizosphere environment and foresee the total phytoavailable trace elements after some extractions.

The aim of this work was to develop a procedure using organic acids for metals extraction in a sewage sludge-treated soil.

2 MATERIAL AND METHODS

2.1 Kinetics of trace elements dissolution

Basically the following assay was used as a preliminary experiment to determine the appropriate solution concentration, soil:solution ratio, and number of extractions to be used in the succeeding experiments.

2.2 First assay

Two grams of the sewage sludge-treated soil (air-dried-wt. basis) were mixed with 20 mL of the complete organic acids solution (COAS) (Table 1.7) in 50 mL Teflon test tubes. The contents were shaken and allowed to equilibrate at 298K using a rotary mixer, SA-12 Motor Speed Control (B & B Motor and Control Corp. Long Island City, NY), which rotated the capped test tubes head to tail at approximately 2 rpm for 24 hours each extraction. A total of 10 extractions were performed for the metal dissolution kinetics studies. The metal concentrations were determined at the 0, 24, 48, 72, 96, 120, 144, 168, 192, 216, and 240 hours for the accomplishment of the kinetics studies. The rotation speed (2 rpm) was maintained constant in all treatments. The pH of the system was 4.8±0.1. Three COAS concentrations 0, 0.001, 0.01, and 0.1 mol L^{-1} in 4 mmol

L^{-1} $CaSO_4$ and 0.05 mmol L^{-1} NaCl along were tested. Each treatment combination was replicated four times. One percent v/v of chloroform was added to each bulk solution to control microbial activity and prevent organic acid decomposition during the course of the equilibration.

2.3 Kinetics of trace elements dissolution by a modified organic acid mixture

On the first assay, the organic mixtures showed some precipitation, caused mainly by Ca-oxalate crystallization, as revealed by some tests, and by Visual Minteq calculations (Gustafsson, 2004). Any precipitation is undesirable because it make difficult to be sure about how many carboxylic groups will be interacting with the trace elements over varying analytical conditions. To fix this problem some changes in the original solution were performed (Table 2.1) to find out the best approach and continue the experiments.

Table 2.1. Modified organic acid solution (MOAS) composition.[1]

Organic acid	Molecular weight	Formula	COOH mole fraction ratio	COOH groups
Acetic	60.05	$C_2H_4O_2$	0.2998	1
Butyric	88.11	$C_4H_8O_2$	0.2183	1
Glutaric	132.12	$C_5H_8O_4$	0.0042	2
Lactic	90.08	$C_3H_6O_3$	0.3823	1
Maleic	116.07	$C_4H_4O_4$	0.0439	2
Propionic	74.08	$C_3H_6O_2$	0.0104	1
Pyruvic	88.06	$C_3H_4O_3$	0.0004	1
Succinic	118.09	$C_4H_6O_4$	0.0063	2
Tartaric	150.09	$C_4H_6O_6$	0.0334	2
Valeric	102.13	$C_5H_{10}O_2$	0.0010	1

[1] Adapted from Koo (2001).

On this experiment, the same set up used in the first assay was changed to fit the experimental needs. The soil:solution ratio was changed from 1:10 to 1:20 (1.5 g of soil in 30 mL solution) to allow to store more solution to posterior analysis and the 48 hours extraction time was added in order to evaluate the rate of extraction. Also, the number of extractions was increased to 15 to picture the kinetics until the equilibrium was reached. This experiment was conducted just with two concentrations (0.05 and 0.1 mol L^{-1}) of the MOAS (Table 2.1) and the extraction kinetics of Cd, Zn, Cr, Cu, and Ni was performed.

3 RESULTS AND DISCUSSION

For this set of experiments, the rhizosphere conditions (pH, ionic strength, table 1.6, and the most reactive chemicals in the rhizosphere, the low molecular weight organic acids) were reproduced synthetically. However, the concentration of these organic acids in the rhizosphere can change naturally depending on, mainly, the soil humidity. Then the first order model can be used to picture the available trace element concentration in the soil after a kinetics of dissolution under different concentrations of these organic acids normally found on the rhizosphere (between 0.001 and 0.1 mol L^{-1}). This wide concentration range used on the experiment is regarding to wet and dry cycles in the soil as well as to a possible gap about underestimation in the real organic acids concentration in the soil due to the interaction of organic acids with the soil’s solid phase and the utilization of organic acids by the soil microbial community as pointed by Jones et al. (2003).

3.1 First assay

On this first assay the dissolution kinetics of the trace elements reveal some important information about the trace element behavior under the organic acid extractant reaction. The cadmium total available pool (C_o) pattern (Figure 2.1), differ from Zn C_o (Figure 2.2) when comparing the three chosen concentration (0.001, 0.01, and 0.1 mol L^{-1}) results. For Cd, these C_o values were quite different from each other (from 49.75 to 75.54 μg Cd kg^{-1} soil) while for Zn, there is a certain similarity, chiefly on the lower and the higher concentrations (C_o around 36 mg Zn kg^{-1} soil). This behavior can be explained based on the trace element affinity by the extractant solution (Table 2.2).

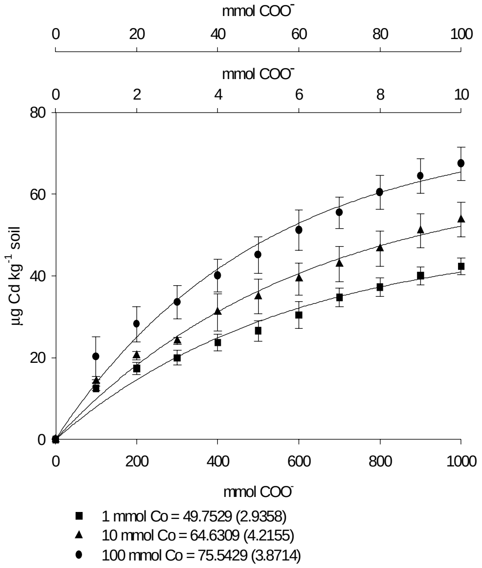

FIGURE 2.1 Cadmium extraction from the F_{8N} soil by the complete organic acid mixture kinetics in three concentrations (1, 10, and 100 mmol L^{-1}) (C_o denotes the Cd concentration at the COO^- concentration, Ct). Values between parentheses are the standard error (n = 4).

The use of kinetics data for the extraction of trace elements from soils by organic acid mixtures cannot give the speciation (localization) of these elements in terms of soil compartments. One should argue this first-order kinetics could be able to classify trace elements extracted into labile metals (quickly extracted) and non-labile metals (less quickly extracted), however, as found by Bermond et al. (1998) the benefits of doing so in terms of predicting the bioavailability or mobility of trace elements in the environment still have to be demonstrated. Furthermore, the kinetics in the present experiment represent the total trace elements pool the plant would uptake from the sewage sludge treated soil along the extraction time and, thus, it is better achieved by the element release factor without separating it into fractions as it could compromise the trace element availability predicted by this method.

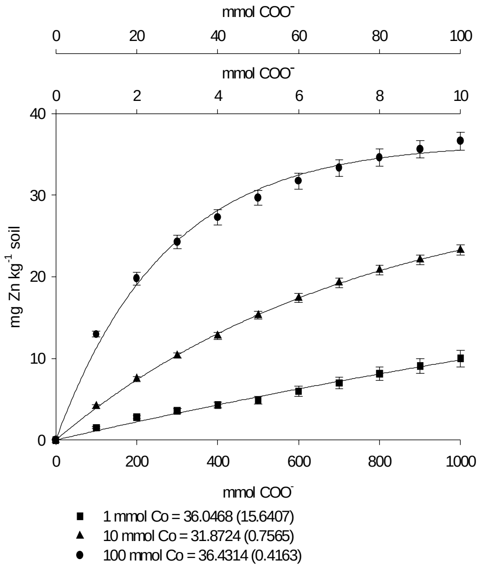

FIGURE 2.2 Zinc extraction from the F_{8N} soil by the complete organic acid mixture kinetics in three concentrations (1, 10, and 100 mmol L^{-1}) (C_o denotes the Cd concentration at the COO^- concentration, Ct). Values between parentheses are the standard error (n = 4).

The organic acid mixture is not expected to change free cation concentrations in solution (Mench and Martin, 1991). The increases in trace element concentrations observed in the kinetics with the rise in the extractant concentration are most likely due to the formation of complexed cations, which disturb equilibria. As the total phytoavailable trace elements, in the soil sample, are present in “trace” amounts, a speciation (Table 2.2) can be calculated using the ultimate trace element release value (C_o) and the initial extractant concentration (0.1 mol L^{-1}). When using the first extraction results, approximately, the same speciation results would be achieved.

TABLE 2.2 Speciation of the complete organic acids mixture 0.1 mol L^{-1} (Table 1.7), background solution (Table 1.4), and the trace elements concentration (C_o values) extracted by kinetics studies (Figures 2.1 and 2.2) from the F_{8N} soil[§].

Species[‡]	Ace	But	Glu	Lac	Val	Pro	Pyr	Suc	Tar	Mal	Oxa	Cl^-	SO_4^{2-}	Na^+	Ca^{2+}	Cd^{2+}	Zn^{2+}
									%								
Ace	99.5													0.8	6.2	27.8	0.3
But		99.7													2.5		0.2
Glu			99.1												0.1	0.3	
Lac				98.0											12.0	13.2	96.4
Val					99.9												
Pro						99.8									0.1	0.4	
Pyr							99.5										
Suc								99.2							0.1	0.3	
Tar									89.2					0.4	8.8	5.8	1,0
Mal										94.8				0.2	5.7	1.9	0.3
Oxa											72.6				0.1	0.6	0.2
Cl^-												99.8				0.9	
SO_4^{2-}													95.2	0.9	10.0	7.7	0.2
Na^+											0.1		0.1	97.5			
Ca^{2+}	0.4	0.2	0.9	0.6	0.5	0.2	0.5	0.7	10.2	5.0	20.7	0.1	4.7		54.2		
Cd^{2+}																41.0	
Zn^{2+}				1.3					0.3		6.5						1.3
Total	99.9	99.9	99.9	99.9	99.9	99.9	99.9	99.9	99.8	99.9	99.9	99.9	99.9	99.9	99.9	99.9	99.9

[§] Visual Minteq (Gustafsson, 2004).

[‡] Species: Ace = acetate^{-1}, But = butyric^{-1}, Glu = glutaric^{-1}, Lac = lactic^{-1}, Val = valeric^{-1}, Pro = propionate^{-1}, Pyr = pyruvate^{-1}, Suc = succinate^{-2}, Tar = tartrate^{-2}, Mal = maleate^{-2}, and Oxa = oxalate^{-2}. The amount of finite solids is 2.14 mmol L^{-1} as Ca-oxalate.H_2O.

Cadmium and oxalate can form very strong complexes into solution (Table 1.8). For this reason the amount of Cd-oxalate complexes should account for 27%, and 87% of all Cd should be hold into solution (speciation not shown). In spite of that, if the Ca-oxalate is allowed (in the Visual Minteq program, Gustafsson, 2004) to precipitate, the Cd-oxalate interaction will be overturned. Thus only 0.5% of the Cd will be complexed with oxalate and the COAS will be able to complex only 58.9% of the total Cd (Table 2.2). This effect is due to the oxalate concentration drop into solution when Ca-oxalate precipitates.

Cadmium and Zn, however, play opposite roles into the COAS. The first is weakly complexed (27.8% by acetate as a main complexant; table 1.8) while the second is strongly complexed (96.3% by lactate as a main complexant; table 1.9; 98.7% by the COAS; table 2.2). Those trace elements remaining in solution as free cations (Cd^{2+}, Zn^{2+}, Ni^{2+}, Cr^{2+}, and Cu^{2+}, table 2.2) were not retained by those organic acids present into the solution, playing another iterations (as sorption and desorption) with soil particles or just as free cations.

When using a 10 mmol L^{-1} COAS (speciation not shown), instead of 100 mmol L^{-1} (Table 2.2), the amount of Cd complexed decrease from 58.9 to 30.8% while for Zn it just reduces from 98.6 to 90.4%. Consequently, by changing the extractant concentration from 100 to 10 mmol L^{-1}, the cadmium C_o values won’t be near each other, but for Zn, the C_o values will. Likewise, the amount of Cd complexed using a 1 mmol L^{-1} COAS decreased to 24.5% and, for Zn, it was also a large drop (48.2%), but not enough to change significantly the C_o values.

This Visual Minteq calculation (Table 2.2) reveal what is happening with our solution, then several calculations and conclusions can be made from the results. The calculations above were based on total amounts extracted by the COAS. Figure 2.1, reveal that only 23.7, 30.8, and 35.9% were recovered from the total Cd content of the F_{8N} soil for the 1, 10 and 100 mmol L^{-1} treatments, respectively, while, for Zn, only 50.2, 44.4, and 50.8% were recovered by COAS in the same concentrations used above. These values represent the total amount (10^2 C_t/total) of trace element extractable by our COAS.

The C_o value being on the same magnitude (Figure 2.2) for 1, 10 and 100 mmol L^{-1} means that, for Zn, any COAS concentration could reach a result of the same magnitude (extracts the "available" fraction), depending on the time the soil is exposed. When using a COAS 100 mmol L^{-1} the bioavailable fraction is depleted fastly, while with a COAS 1 mmol L^{-1}, it takes a long time (we cannot see the stability "plateau" point on the curve 2.1). Then, the plant should be able to uptake the total available Zn fraction from the treated-soil, estimated by C_o value, despite the organic acids concentration into the rhizosphere, depending just on the time, and thus, the plants, after several years growing in the same place, should be able to uptake the same amount as the C_o found by our proceeding.

3.2 Kinetics of trace elements dissolution by the modified organic acid mixture

For extractant development purposes, the COAS concentrations 1 and 10 mmol L^{-1} were discarded from the next experiment because they are too far from equilibrium ("plateau") points (Figures 2.1 and 2.2). Also, to demonstrate that all curves converge to a C_o of the same magnitude, five additional extraction points were included along with the 50 mmol L^{-1} concentration. This is because C_o values extrapolates the number of plotted points in the curves, thus the statistics show no reliable confidence limits for these values. For this reason it is needed to add additional points to the plots and use a high extractant concentration to reach the curve equilibrium portion easier. This will allow discussing the kinetics last point (C_t) within 5% confidence limits.

Greater amounts of Zn, Ni, Cr and Cu were extracted by 100 than with 50 mmol L^{-1} by Pires (2003) using a mixed organic acid solution composed by acetic, citric, lactic and oxalic acids by a extraction procedure with seven steps. The Zn and Cd C_o values for the 50 and 100 mmol L^{-1} concentrations were close each other (Figures 2.3 and 2.4) while the Ni, Cr and Cu C_o values showed a discrete relationship (Figures 2.5 to 2.7). From these results we can foresee that, among these trace element extractions, perhaps the COAS can predict best C_o values for Zn (under assorted environmental conditions). The COAS can also predict C_o values for Cd, but, based on the figure 2.1, this will happen only under very definite conditions (soil type, humidity, high organic acids concentration in the rhizosphere). Also, small modifications on the COAS changing the organic acids concentrations or excluding one or more organic acid from the mixture could lead to a different Cd C_o result.

In spite of that, the Cd extractable by the modified organic acid solution (MOAS) C_o values (Figure 2.3) represent from 20.61 to 23.75% of the total Cd into the sewage sludge-treated soil for the 50 mmol L^{-1} extraction in the 24 and 48 hours, respectively. For the 100 mmol L^{-1}, the C_o values represent from 23.65 to 26.89% of the total sewage sludge-treated soil Cd in the 24 and 48 hours, respectively. The difference between these values (around 3%) for 24 and 48 hours extraction time is small and this show that, for Cd, a 24-h time is enough for each extraction kinetics. The difference between these values for Zn, Ni, Cr, and Cu (5.50, 1.37, 1.28, and 0.97%, respectively; figures 2.4 to 2.7) for 24- and 48-h extraction time are also small and as for Cd, for the other trace elements, 24 hours is also considered enough for each extraction kinetics.

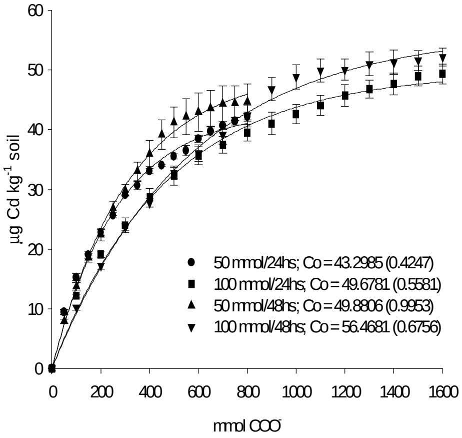

FIGURE 2.3 Kinetics of cadmium extraction from the F_{8N} soil in response to the modified organic acid mixture kinetics in two concentrations (50 and 100 mmol L^{-1}) and two different equilibrium times (24 and 48 hours). C_o denotes the Cd concentration at the COO^- concentration, Ct. Values between parentheses are the standard error (n = 4).

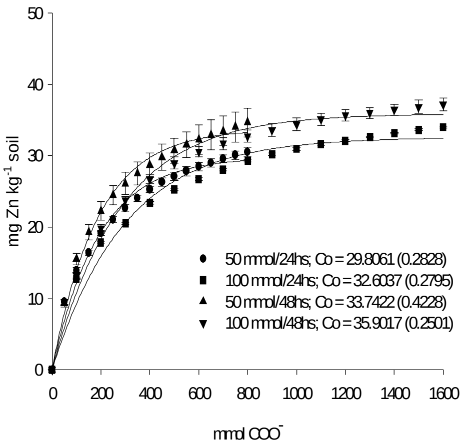

FIGURE 2.4 Kinetics of zinc extraction from the F_{8N} soil in response to the modified organic acid mixture in two concentrations (50 and 100 mmol L^{-1}) and two different equilibrium times (24 and 48 hours). C_o denotes the Cd concentration at the COO^- concentration, Ct. Values between parentheses are the standard error (n = 4).

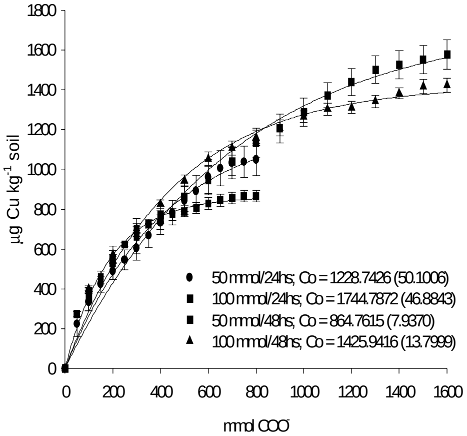

FIGURE 2.5 Kinetics of nickel extraction from the F_{8N} soil in response to the modified organic acid mixture kinetics in two concentrations (50 and 100 mmol L^{-1}) and two different equilibrium times (24 and 48 hours). C_o denotes the Cd concentration at the COO$^-$ concentration, Ct. Values between parentheses are the standard error (n = 4).

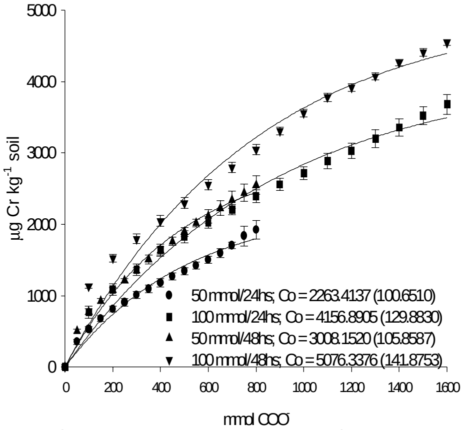

FIGURE 2.6 Kinetics of chromium extraction from the F_{8N} soil in response to the modified organic acid mixture in two concentrations (50 and 100 mmol L^{-1}) and two different equilibrium times (24 and 48 hours). C_o denotes the Cd concentration at the COO$^-$ concentration, Ct. Values between parentheses are the standard error (n = 4).

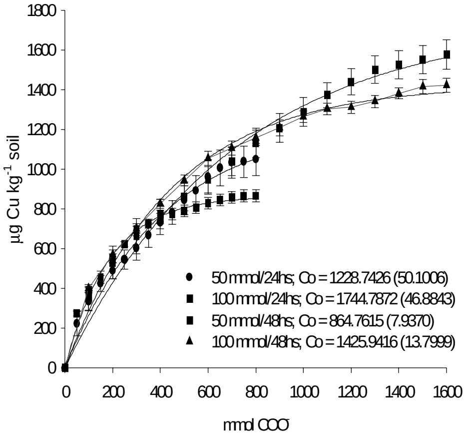

FIGURE 2.7 Kinetics of copper extraction from the F_{8N} soil in response to the modified organic acid mixture in two concentrations (50 and 100 mmol L^{-1}) and two different equilibrium times (24 and 48 hours). C_o denotes the Cd concentration at the COO^- concentration, Ct. Values between parentheses are the standard error (n = 4).

Between the concentrations 50 and 100 mmol L^{-1}, there is no apparent reason to choose one over the other. In this case, we chose the 100 mmol L^{-1} by convenience. This concentration is high enough to maintain the extractant stable, and reach, by kinetic, a “plateau” curve very suitable to “extractable” trace element amounts prediction.

The percentage of trace elements retained on solution (speciation not shown) changes in a very small proportion from 100 to 50 mmol L^{-1} for all trace elements studied. For this reason, when the C_o ratio was calculated from the 50 and 100 mmol L^{-1} (50:100 C_o ratio) and compared this ratio found on 24 with that, on 48 hours (time for each extraction on the kinetics), the results were quite analogous for Cd, Zn, Ni, and Cr. However, the analysis of the C_o values found at 48 hours showed a significant increase when compared to 24 hours for all elements except for Ni and Cu in both 50 and 100 mmol L^{-1}. For Cu and Ni this fact may occur because they have a great affinity for organic matter and, after a dissolution, a slow second reaction could be occurring, probably due to a precipitation with organic matter in the soil, mainly after the seventh extraction (Figures 2.5 and 2.7), or perhaps, when soluble/exchangeable Cu or Ni is a little bit depleted on the soil binding sites.

As suggested by Lehmann and Harter (1984) from Cu adsorption studies, after the organic acids compete for the exchange sites with Cu, displacing Cu from low energy sites, this element is then complexed into solution. Subsequently during a long extraction time (24 or, mainly, 48 h) a re-sorption of Cu onto the high-energy sites could be happening. This approach was also discussed by Amacher et al. (1988) for Cr^{VI} and Cd, however, the differential binding energy (low and high) was related to the trace element concentration into solution. Thus for lower concentrations of Cr^{VI} and Cd as in this experiment, they are normally retained by sites where they are more strongly held.

4 CONCLUSIONS

1 – Based upon the factors necessary to reach a plateau, which could indicate a “total” trace elements availability, the extraction procedure suggested for this soil is the use of one and a half-grams of soil in 30 mL of the 100 mmol L^{-1} modified organic acid solution (Table 2.1), pH set as 4.8±0.1, performing 15 successive extractions, each one every 24 hours;

2 – The Zn kinetics extraction C_o values from 1, 10 and 100 mmol L^{-1} by the COAS presented the same magnitude.

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CHAPTER III

COMPARISON OF TRACE ELEMENTS EXTRACTANTS IN A SEWAGE SLUDGE-TREATED SOIL

CHAPTER 3

ABSTRACT

MARCHI, Giuliano. **Comparison of trace elements extractants in a sewage sludge-treated soil**. 2005, 104p. Thesis (Doctorate in Soil Science and Plant Nutrition).#

The kinetics of organic acid extractants solutions in the extraction of trace elements from soils that can mimic the rhizosphere was studied in order to find the best extractant method. The method was used to extract Cd, Zn, Ni, Cr, and Cu. For the kinetics study, four organic acid mixtures (0.1 mol L^{-1}) were added in 50 mL centrifuge tubes along with 1.5 g sewage sludge-treated soil (4 mmol L^{-1} $CaSO_4$ and 0.05 mmol L^{-1} NaCl) in three replicates. Three conventional extractants (DTPA, Mehlich I, and NH_4OAc) were also used to compare the results. A sequential extraction was also performed as a post kinetics study onto the remaining soil. For Cd and Zn, the extractants DTPA and Mehlich I extracted almost the total extractable fraction in the first extraction. The NH_4OAc extracted much less than all the other extractants for all trace elements. The plant uptake kinetics results for Zn were close to the kinetics results of all organic acids used in this experiment. The Mehlich I kinetics had also a close result to the plant uptake.

Guidance Committee: Luiz Roberto Guimarães Guilherme – UFLA (Adviser), Andrew C. Chang – UCR (Adviser).

RESUMO

MARCHI, Giuliano. **Comparação de extratores de elementos-traço em solo tratado com lodo de esgoto**. 2005, 104p. Tese (Doutorado em Ciência do Solo e Nutrição de Plantas).[#]

A cinética de extração por soluções de ácidos orgânicos com intuito de reproduzir a rizosfera foi estudada de forma a encontrar o melhor método de extração para elementos-traço tais como Cd, Zn, Ni, Cr e Cu. Para o estudo de cinética, quatro misturas de ácidos orgânicos (0,1 mol L^{-1}) foram adicionados em tubos de centrífuga de 50 mL com 1,5 g de solo tratado com lodo de esgoto (4 mmol L^{-1} $CaSO_4$ e 0,05 mmol L^{-1} NaCl) em três repetições. Também foram utilizados três extratores convencionais (DTPA, Mehlich I e NH_4OAc) para a comparação de resultados. Extrações seqüenciais também foram realizadas em um estudo pós-cinética no solo remanescente. Para Cd e Zn, os extratores DTPA e Mehlich I extraíram quase a totalidade da fração "disponível total" na primeira extração. O NH_4OAc extraiu quantidades menores de elementos-traço que os outros extratores.

[#] Comitê Orientador: Luiz Roberto Guimarães Guilherme – UFLA (Orientador), Andrew C. Chang – UCR (Orientador estrangeiro).

1 INTRODUCTION

The total amount of trace elements in soils is distributed among the free ions and organic complexes in the soil solution, adsorbed and exchangeable in the colloidal fraction, composed of clay particles, humic compounds and iron, manganese and aluminum hydroxides, and secondary mineral and insoluble complexes in the solid phase of the soil (Smith et al., 1992). In order to comprehend the environmental chemistry of these elements it would be necessary to characterize in full the proportions and chemistries of all its various forms under the diverse range of conditions possible in natural systems (Ure and Davidson, 1995). Thermodynamic calculations are particularly useful when there is a lack of reliable data on metal speciation in solution, or when analytical techniques do not permit accurate assessment of the free ionic concentration and metal complexes formed with the various ligands in the system (Behel et al., 1983).

The identification of the formed species can have great implications for understanding the behavior of trace elements in natural and polluted systems, providing the level of information necessary for their safe management (Bourg, 1995). The possible mobility and bioavailability are the result of the reactivity of trace elements in soils, in other words, their localization in different soil components, which is now usually called speciation (Bermond et al., 1998).

In the sewage sludge additions to the soils the majority of trace elements added will be found in insoluble forms which are extractable by chelates and acids rather than in water soluble or exchangeable forms (Silviera and Sommers, 1977).

The acid and chelant solutions, such as the Mehlich I and DTPA extractants were always the preferred to the trace elements availability evaluation in soils because of their great extraction capacity, making it easy to determine the elements by instrumental techniques used some time ago. However, nowadays, the detection limits have been reduced due to the development of new equipments such as atomic absorption with graphite furnace (electrothermal), ICP, atomic fluorescence and others making it possible to analyze small trace element amounts in soil extracts, such as the amounts the plants absorb in a short time. Nonetheless, the major critic about chemical extractants is that these compounds cannot simulate reactions occurring near the roots, mainly those taking place at the rhizosphere level (Abreu et al., 2002).

This work used extraction kinetics to compare trace elements extractability by organic acid mixtures with that obtained by using DTPA, Mehlich I, and NH_4OAc extractants. Additionally, a sequential extraction procedure was used to assess changes in the trace element pools in a sewage sludge-treated soil after those kinetics extractions.

2 MATERIAL AND METHODS

2.1 Speciation studies

A computer simulation using the program Visual Minteq (Gustafsson, 2004) was used to predict the iterations on the following experiment and, thus, be able to calculate a simplified organic acid mixture (Table 3.1) that could extract the same amount that the modified organic acid mixture (Table 2.1). The Visual Minteq program was updated by the NIST – Standard Reference Database, 46, version 7.0 (2003), Smith and Martell (1989), Christensen and Izatt (1983), and Pierrin (1979) for those iterations not contemplated on the program such as the stability constants (Tables 1.7 to 1.11) for the maleate^{-2} (cis-butenedioic acid), lactate^{-1} (2-hydroxypropanoic acid), pyruvate^{-1} (2-

oxopropanoic acid), and others, versus H^+, Na^+, Ca^{2+}, Cd^{2+}, Zn^{2+}, Ni^{2+}, Cr^{2+}, and Cu^{2+}.

TABLE 3.1 Simplified organic acid mixture extractant composition.

Organic acid	Molecular weight	Formula	COOH mole fraction ratio	COOH groups
Acetic	60.05	$C_2H_4O_2$	0.4190	1
Lactic	90.08	$C_3H_6O_3$	0.5343	1
Tartaric	150.09	$C_4H_6O_6$	0.0467	2

2.2 Kinetics study

On this experiment, 7 extractants were used on the trace elements dissolution kinetics study. Four of these extractants were composed by low molecular weight organic acids as follows:

i) modified organic acids mixture (MOAS, table 2.1),
ii) simplified organic acid mixture (SOAS, table 3.1),
iii) lactic acid, and
iv) acetic acid.

One and half-grams of the sewage sludge-treated soil (air-dried-wt. basis) was mixed in 30 mL of those mixtures described above (1:20 soil:solution ratio; concentration, 0.1 mol L^{-1}; background solution, 4 mmol L^{-1} $CaSO_4$ and 0.05 mmol L^{-1} NaCl) into 50 mL Teflon test tubes. The contents were shaken and allowed to equilibrate at 298K using a rotary mixer, SA-12 Motor Speed Control (B & B Motor and Control Corp. Long Island City, NY), which rotated the capped test tubes head to tail at approximately 2 rpm for 24 hours for each extraction. The pH of the system was 4.8±0.1. One percent v/v of chloroform was added to each bulk solution to control microbial activity and prevent organic acid decomposition during the course of the equilibration.

The other 3 extractants used on the kinetics were adapted from methods normally found for trace elements extraction:

v) Mehlich I (HCl 0.05 mol L^{-1} + H_2SO_4 0,0125 mol L^{-1}; 7.8 g of the sewage sludge-treated soil (air-dried-wt. basis) mixed with 30 mL, mixed for 5 minutes for each extraction; Nelson et al., 1953),

vi) ammonium acetate (pH 7; 1.875 g of the sewage sludge-treated soil (air-dried-wt. basis) mixed with 30 mL, mixed for 14 h for each extraction; Ure et al., 1993), and

vii) DTPA (dietilenetriaminepentacetic acid 0.005 mol L^{-1}, TEA (trietanolamine) 0.1 mol L^{-1} and $CaCl_2$ 0.1 mol L^{-1}, pH 7.3 (Lindsay and Norvell, 1978); 10 grams of the sewage sludge-treated soil (air-dried-wt. basis) mixed with 20 mL; mixed by 2 h for each extraction).

The contents (treated soil and extractant solution) were shaken into 50 mL Teflon test tubes and allowed to equilibrate at 298K. A total of 15 extractions were performed for the metal dissolution kinetics studies. The metal concentrations were determined at 0, 24, 48, 72, 96, 120, 144, 168, 192, 216, 240, 264, 288, 312, and 336 hours for the kinetics studies.

2.3 Sequential extraction procedure

The sequential extraction was performed as a post kinetics study on the remaining sewage sludge-treated soil. This procedure, adapted from Ahnstrom and Parker (1999) and Bertoni (2003), was taken place after the MOAS, lactic, acetic, and NH_4OAc treatments.

Duplicate 2-g portions (oven-dried-wt. basis) were weighed into 50 mL Teflon centrifuge tubes and sequentially treated to obtain the following four operationally defined fractions:

Fraction 1: “soluble/exchangeable.” Each sample was reacted with 10 mL of 0.1 mol L^{-1} $BaCl_2$ in a reciprocating shaker (2400 oscillations per h) for 2 h at room temperature. Extraction efficiency for Fraction 1 was increased by conducting two successive treatments for each soil material.

Fraction 2: “oxidizable.” The residue was mixed with 5 mL of 5% NaOCl (adjusted to pH 8.5 with trace metal grade (TMG) HCl) and reacted in a water bath (90 – $95^{o}C$) for 30 min. After 15 min the cap was opened and tightened again and the slurry briefly vortex-mixed.

Fraction 3: “reducible.” The residue was mixed with 20 mL of 0.2 mol L^{-1} oxalic acid 0.2 mol L^{-1} NH_4oxalate 0.1 M ascorbic acid (adjusted to pH 3.0 with NH_4OH TMG). The slurry was placed in a water bath (90 – $95^{o}C$) for 30 min with periodic mixing as for fraction 2. This procedure was conducted two times successively for each sample.

Fraction 4: “residual.” The residue was lyophilized (freeze dry), and mixed. Then the residue was analyzed following the EPA 3052 method.

Between each step the residue was suspended in 5 mL of 0.1 mol L^{-1} NaCl to displace entrained solution, minimize sample dispersion, and limit resorption. These rinse solutions were pooled with the preceding extracts rather than discarded.

3 RESULTS AND DISCUSSION

Basically this set up was used to determine the simpler organic extractant that match best with the complete organic acid mixture and compare it with other methods found in the literature currently used in most of the environmental laboratories.

As the MOAS or the COAS are too complex to prepare, it was needed to develop a simplified organic acid solution (SOAS) easy to prepare at any laboratory. Three main acids were chosen, primarily by the amount of trace elements it could complex (Table 3.2) and by its representativeness on the COAS. For this reason butyric acid, even present in a large concentration in the COAS, was not added to the SOAS because it complexes trace elements weakly. In its place it was chosen tartaric acid, whose complexes formed with those elements were stronger than those formed by the butyric acid (Table 3.2). The other two organic acids: acetic and lactic (Table 3.3), were used to prepare two even simpler extractants than the SOAS. The first composed by acetic acid (Table 3.4), was also based on its representativeness on the COAS solution and the second, composed by lactic acid (Table 3.5), was based on its complexation strength (log Ks, tables 1.8 to 1.12, and 3.2) with trace elements.

TABLE 3.2 Speciation of modified organic acids mixture 0.1 mol L^{-1} (Table 2.1), background solution (Table 1.4), and the trace elements concentration (C_o values) extracted by kinetics studies (Figures 3.5 to 3.9) on the F_{8N} soil[§].

Species[‡]	Ace	But	Glu	Lac	Val	Pro	Pyr	Suc %	Tar	Mal	Cd^{2+}	Zn^{2+}	Ni^{2+}	Cr^{3+}	Cu^{2+}
Ace	97.3										27.7	0.4	3.8	0.7	9.4
But		99.5										0.2	0.4		4.8
Glu			98.3								0.3		0.1		0.1
Lac				97.2							13.1	96.6	44.9	42.5	63.7
Val					99.9										
Pro						99.6					0.4		0.1		0.3
Pyr							99.1								
Suc								94.9			0.3		0.1		0.3
Tar									71.3		3.9	0.7	8.3	0.2	10.6
Mal										85.2	1.5	0.2	21.3	54.6	2.7
Cl^-											0.8				
SO_4^{2-}											5.3	0.2	1.8		0.8
Na^+	1.9							3.7	13.9	5.1					
Ca^{2+}	0.8	0.5	1.6	1.3	0.1	0.4	0.9	1.4	14.3	8.3					
Cd^{2+}											46.7				
Zn^{2+}				1.4					0.3	0.1		1.6			
Ni^{2+}													19.1		
Cr^{3+}				0.1						1.3				1.5	
Cu^{2+}				0.1					0.2						7.4
Total	99.9	99.9	99.9	99.9	99.9	99.9	99.9	99.9	99.9	100.0	99.9	99.9	99.9	99.6[#]	99.9

[§] Visual Minteq (Gustafsson, 2004).

[‡] Species: Ace = acetate^{-1}, But = butyric^{-1}, Glu = glutaric^{-1}, Lac = lactic^{-1}, Pro = propionate^{-1}, Pyr = pyruvate^{-1}, Suc = succinate^{-2}, Tar = tartrate^{-2}, and Mal = maleate^{-2}. The sum of species activity was calculated as 0.8418 mol L^{-1}.

[#] $CrOH^{2+}$ should be included as 0.4% of the total Cr species in this simulation.

TABLE 3.3 Speciation of simplified organic acids mixture 0.1 mol L^{-1} (Table 3.1), background solution (Table 1.4), and the trace elements concentration (C_o values) extracted by kinetics studies (Figures 3.5 to 3.9) on the F_{8N} soil[§].

Species[‡]	Ace	Lac	Tar	Ca^{2+}	Na^{+}	SO_4^{2-}	Cl^{-}	Cd^{2+}	Zn^{2+}	Ni^{2+}	Cr^{3+}	Cu^{2+}
							%					
Ace	97.3			8.4	1.1			33.7	0.4	5.4	1.2	9.5
Lac		98.3		16.7				16.5	97.6	63.2	94.7	74.8
Tar			72.1	8.1	0.4			4.7	0.7	11.2	0.5	10.1
Ca^{2+}	0.8	1.3	13.9	60.2		6.5	0.2					
Na^{+}	1.9		13.9		97.8	12.2	2.1					
SO_4^{2-}				6.6	0.7	81.2		4.4	0.1	1.8		0.55
Cl^{-}							97.7	0.7				
Cd^{2+}								39.9				
Zn^{2+}			0.1						1.1			
Ni^{2+}										18.5		
Cr^{3+}											2.5	
Cu^{2+}												5.0
Total	99.9	99.6	99.9	100.0	100.0	99.9	100.0	99.9	99.9	99.9	99.4[#]	99.9

[§] Visual Minteq (Gustafsson, 2004).

[‡] Species: Ace = $acetate^{-1}$, Lac = $lactic^{-1}$, and Tar = $tartrate^{-2}$.

[#] $CrOH^{2+}$ should be included as 0.6% of the total Cr species in this simulation.

TABLE 3.4 Speciation of acetic acid 0.1 mol L^{-1} (Table 3.1), background solution (Table 1.4), and the trace elements concentration (C_o values) extracted by kinetics studies (Figures 3.5 to 3.9) on the F_{8N} soil[§].

Species[‡]	Ace	Ca^{2+}	Na^{+}	SO_4^{2-}	Cl^{-}	Cd^{2+}	Zn^{2+}	Ni^{2+}	Cr^{3+}	Cu^{2+}
					%					
Ace	97.1	23.6	2.6			67.6	45.1	43.1	62.7	84.6
Ca^{2+}	0.9	68.4		7.9	0.3					
Na^{+}	1.9		96.7	12.5	2.1					
SO_4^{2-}		7.9	0.7	79.5		3.4	5.6	5.2		1.6
Cl^{-}					97.6	0.5				
Cd^{2+}						28.5				
Zn^{2+}	0.1			0.3			49.3			
Ni^{2+}								51.7		
Cr^{3+}									29.6	
Cu^{2+}										13.8
Total	99.9	100.0	100.0	99.9	100.0	99.9	99.9	99.9	92.3[#]	99.9

[§] Visual Minteq (Gustafsson, 2004).

[‡] Species: Ace = $acetate^{-1}$.

[#] $CrOH^{2+}$ should be included as 7.7% of the total Cr species in this simulation.

TABLE 3.5 Speciation of lactic acid 0.1 mol L^{-1} (Table 3.1), background solution (Table 1.4), and the trace elements concentration (C_o values) extracted by kinetics studies (Figures 3.5 to 3.9) on the F_{8N} soil[§].

Species[‡]	Lac	Ca^{2+}	Na^{+}	SO_4^{2-}	Cl^{-}	Cd^{2+}	Zn^{2+}	Ni^{2+}	Cr^{3+}	Cu^{2+}
					%					
Lac	97.7	15.7				20.9	98.3	69.7	95.9	89.4
Ca^{2+}	1.7	75.1		9.2	0.3					
Na^{+}			99.3	12.8	2.2					
SO_4^{2-}		9.2	0.7	77.9		8.7	0.2	2.9		1.2
Cl^{-}					97.5	1.3				
Cd^{2+}						68.9				
Zn^{2+}	0.5						1.5			
Ni^{2+}								27.4		
Cr^{3+}									3.2	
Cu^{2+}										9.4
Total	99.9	100.0	100.0	99.9	100.0	99.9	99.9	99.9	99.1[#]	99.9

[§] Visual Minteq (Gustafsson, 2004).

[‡] Species: Lac = $lactate^{-1}$.

[#] $CrOH^{2+}$ should be included as 0.9% of the total Cr species in this simulation.

For Cd and Zn, the extractants DTPA and Mehlich I (Figures 3.1 and 3.2) extracted the entire extractable fraction almost instantaneously. This means that for the F_{8N} soil, if these extractants have a good correlation with plant extraction kinetics, just one extraction should be enough to acquire the necessary information to take decisions about the soil pollution and risks assessment.

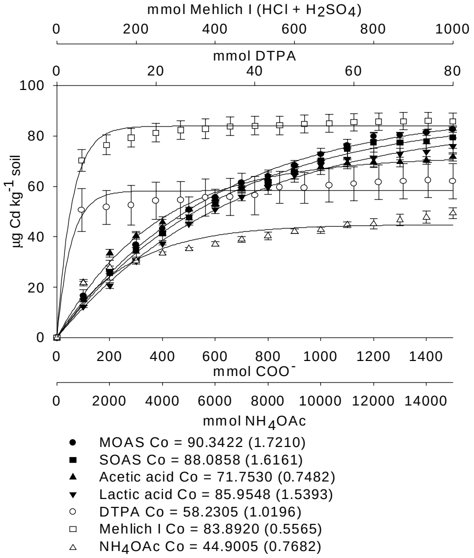

FIGURE 3.1 Kinetics of Cd extraction from the F_{8N} soil in response to the organic acid mixtures, DTPA, Mehlich I, and NH_4OAc. Values between parentheses are the standard error (n = 3).

For Cd, the acetic acid extractant solution seems to be of great importance in the simplified organic acid mixture when compared to the other organic acids employed (Table 3.4). The Cd extraction kinetics by acetic acid (Figure 3.1) show that more Cd was extracted by this solution in the beginning (from the first to the sixth point). Then, the last points (from the seventh to the fifteenth) the acetic acid solution extracted, comparatively, a little bit less than the other organic acid extractants. This singular behavior can be extremely important for acetate as a potential extractant to this element as there is a possibility it can better represent the Cd uptake by plants. For Zn, Ni, Cr, and Cu the acetic acid extractant doesn't seem to be so important (Table 3.4, figures 3.2 to 3.5).

Kuo and Mikkelsen (1980) believe the recrystallization of adsorbed Zn in the soil, which subsequently increased the bonding strength of adsorbed zinc, could be responsible for the reduction of zinc desorption by DTPA. Thus the DTPA extraction (figure 3.2) takes three extractions to get almost the entire extractable fraction.

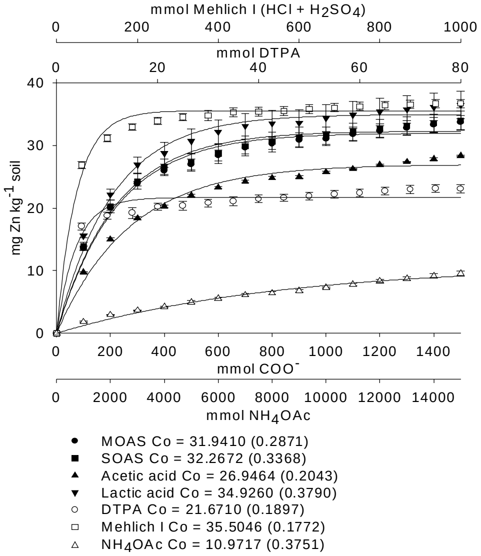

FIGURE 3.2 Kinetics of Zn extraction from the F_{8N} soil in response to the organic acid mixtures, DTPA, Mehlich I, and NH_4Oac. Values between parentheses are the standard error (n = 3).

It is interesting to observe that for Ni, Cr and Cu (Tables 3.3 to 3.5), the single DTPA and Mehlich I extraction kinetics (first point, figures 3.1 to 3.5) do not reach the equilibrium in the very first extraction as for Cd and Zn. Thus the extraction of these trace elements by DTPA and Mehlich I seems to be difficult to correlate to plant extractability in a single extraction procedure. This could explain why some authors (Singh and Narwal, 1984) found significant results for Cd and Zn extracted by a single extraction with DTPA when compared to the plant uptake, which was not true for other trace elements studied by them.

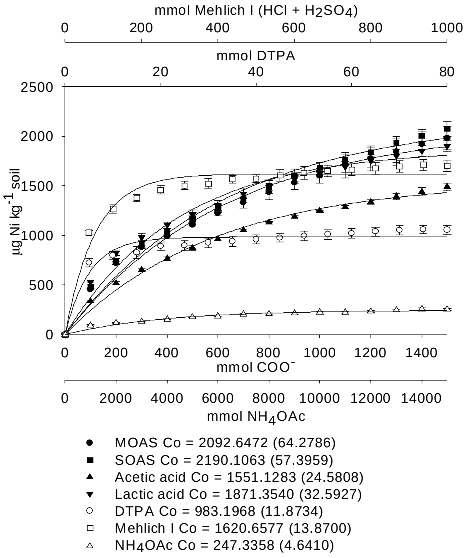

FIGURE 3.3 Kinetics of Ni extraction from the F_{8N} soil in response to the organic acid mixtures, DTPA, Mehlich I, and NH_4Oac. Values between parentheses are the standard error (n = 3).

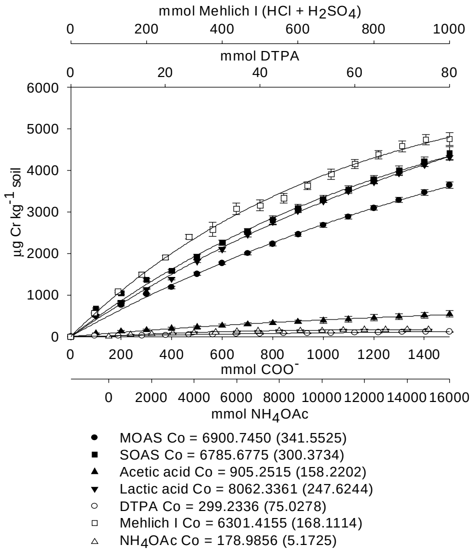

FIGURE 3.4 Kinetics of Cr extraction from the F_{8N} soil in response to organic acid mixtures, DTPA, Mehlich I, and NH_4Oac. Values between parentheses are the standard error (n = 3).

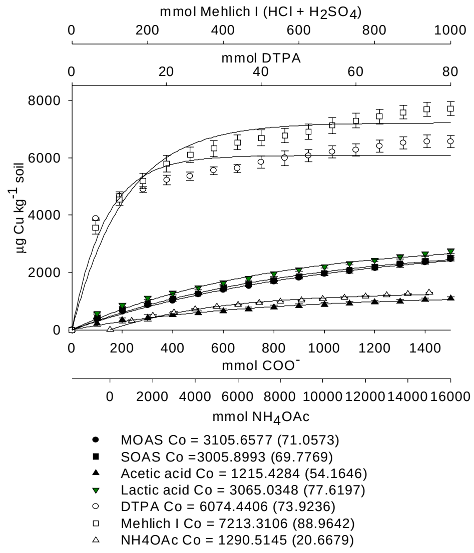

FIGURE 3.5 Kinetics of Cu extraction from the F_{8N} soil by the organic acid mixtures, DTPA, Mehlich I, and NH_4OAc. Values between parentheses are the standard error (n = 3).

The Cu extraction by DTPA and Mehlich I (Figure 3.5) was higher than for all other extractants. It is possible that the Cu extracted by the organic extractants would be re-precipitating or being resorbed to the surface sites from the soil, while DTPA and Mehlich I would be maintaining the Cu released into solution. The Mehlich I dissolves most of the labile Cu-containing organic matter that could represent a great source of Cu into the solution. The DTPA retains Cu by its high complex stability with that element while the Mehlich I, as a result of its low pH, in variable charge soils, block the free negative soil surface sites in variable charge soils. However, Lee and Zheng (1993) suggest that strong acid extractants may change the soil to conditions greatly different from those where plants are grown and cause soil minerals to dissolve. Thus, the amounts of trace elements extracted under such circumstances may have little relationship to what is available to plants.

The NH_4OAc extracted much less than all the other extractants for all trace elements (Figures 3.1 to 3.5). Despite the fact that Ure et al. (1993) describe NH_4OAc as minimizing any precipitation danger, this extractant has a high concentration, and thus the trace elements could be at low activity into solution. The NH_4^+ concentration effect in the exchange of trace elements from the soil surface must work, but the overall extraction fails due to the low trace elements activity, low complexation strength of acetate and trace elements into solution and by its high pH, which increases the affinity of the trace elements to the surface sites.

For soils not treated with sewage sludges, a sequential extraction (Gomes et al., 1997) reveals a high percentage of Cd presented in the soluble/exchangeable fraction and little in the oxidizable fraction. In the present experiment, with sewage sludge-treated soils (figure 3.6), the highest Cd percentage was found in the oxidizable fraction. This result means that, probably, the Cd content from the soluble/exchangeable fraction is bound to the organic material from the sewage sludge. This Cd distribution, mostly retained in the oxidizable fraction, is responsible for the decrease in Cd availability to plants (Shuman et al., 2002). Naturally, as showed by Oliveira et al. (2003) and Mench et al. (1994) it is expected that, given a certain time, the organic material from the sewage sludge material will decay and part of the Cd content from this compartment (oxidizable fraction) will move to the soluble/exchangeable fraction and from there to the reducible and residual fractions when the soil properties becomes more important than sewage sludge properties on the trace element dynamics.

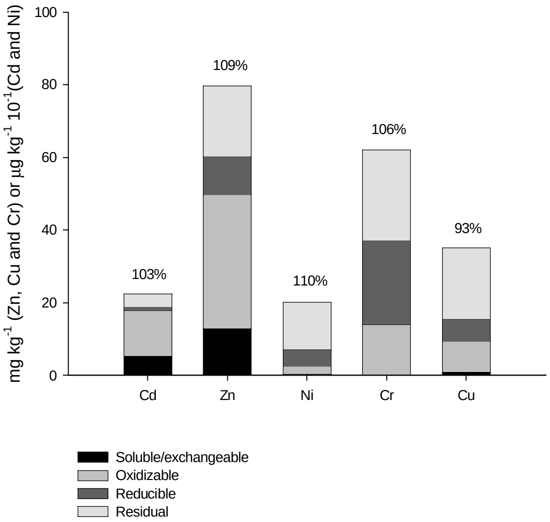

FIGURE 3.6 Sequential extraction from the F_{8N} soil. The percentage above the columns are the trace element amount recovered from the total amount.

The organic fraction in the sewage sludge is the chemically active portion that accounts for the accumulation of Zn, possibly by formation of complex compounds (Tan et al., 1971). André et al. (2003) correlated the soluble/exchangeable and oxidizable Zn fractions to the plant uptake showing, therefore, that these fractions could be used to represent the Zn phytoavailability. However, root excreted organic acids can result in rapid dissolution of the solid phases $Fe(OH)_3$ (Jones et al., 1996) and $Al(OH)_3$ (Jones and Kochian, 1996), leading to the formation of stable Fe- and Al-organic acid complexes in solution. In spite of the small trace element amounts extracted by the organic acid solutions, all of them extracted some trace elements from the reducible fraction (figures 3.7, 3.9 and 3.10), suggesting some $Fe(OH)_3$ dissolution. The solution pH and concentration certainly has increased this dissolution and, as suggested by Jones et al. (1996), the dissolution rate constants highly correlate to the concentration of organic acids into solution.

The Ni distribution was identical of that found by Gomes et al. (1997), revealing, for this soil that the background level of Ni in the soil is important. Then, the higher amounts of Ni are bound to the reducible and residual fractions rather than on the oxidizable fraction (Wang et al., 1997).

The percentage of Cr on the oxidizable, reducible, and residual fractions was higher in this study, when compared with that of Gomes et al. (1997). There is a small quantity of Cr in this soil bound to the organic matter and practically nothing is present in the soluble/exchangeable fraction.

There was a low Cu amount found in the reducible fraction in the present experiment when compared to that of Gomes et al. (1997). This can be explained because Cu shows a high affinity for the organic matter and did not move from the sewage sludge added to the soil to other soil compartments. Thus, Cu is practically immobile in soils because it forms strong complexes with organic matter (Matos et al., 1996). This fact is important because it shows that Cu must be displaced from the oxidizable fraction to be available to plant uptake as the soluble/exchangeable fraction bears a very low Cu content.

The soluble/exchangeable fraction, assessed by $BaCl_2$ relies on the trace element binding strength of chloride to hold them into solution and Ba^{2+} to displace the easily exchangeable fraction. The chloride is likely, under natural and polluted aquatic conditions, the most efficient inorganic complex former for solubilizing trace elements (Bourg, 1995). The Cl^- ion is also a complexant ligand for many metallic cations and its overall reaction with a metal can be described by:

$$M^{2+} + rCl \Leftrightarrow M\ Cl^{2-r} \qquad (3.1)$$

Cadmium, by instance, forms a variety of stable complexes with inorganic compounds (Smith et al., 1992). As found by Young et al. (2000), probably the Cd fraction extracted by the $BaCl_2$ solution (soluble/exchangeable) was greater relatively to the other trace elements studied because of the relative values of the 1:1 metal-chloride complex stability constants (log K_1): log K_1 = 1.98 for $CdCl^+$, but only 0.46, -0.43, -1.0, and 0.30, for the $ZnCl^+$, $NiCl^+$, $CrCl^+$, and $CuCl^+$ respectively. There is a possibility, however, that the soluble/exchangeable results for Zn, Ni, Cr, and Cu can be underestimated. Even though, the results concerning the trace elements extraction kinetics by organic acids (Figure 3.7 to 3.10) showed that these acids extract trace elements mostly from the soluble/exchangeable and oxidizable fractions. However, the organic acids and NH_4OAc, as found by Bermond et al. (1998), are necessarily non-specific reagents since they can extract trace elements from several soil compartments (Figures 3.6 to 3.10). Therefore, measurements of trace elements extracted at equilibrium cannot be related to their speciation.

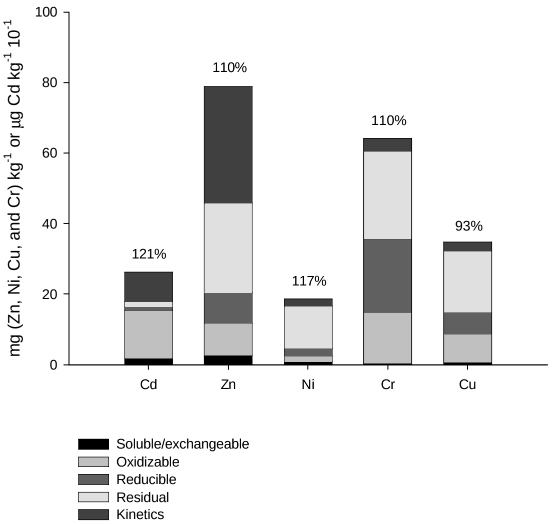

FIGURE 3.7 Sequential extraction from the F_{8N} soil after the trace elements extraction kinetics by the modified mixture as extractant. The percentage above the columns are the trace element amount recovered from the total amount.

Oliveira et al. (1999) suggested that most routine analysis extractants (HCl, Mehlich I and II, and DTPA) extract mainly the exchangeable fraction. In spite of that, when we got a sewage sludge treated soil, most of the Zn is present in the oxidizable fraction. Nascimento et al. (2002) showed that a great portion of the total Zn retained on the organic matter is present in the labile form. Thus the Zn extraction kinetics extracted a great extent of the soluble/exchangeable and the oxidizable fractions. After the soluble/exchangeable fraction is depleted by the kinetics, some part of oxidizable portion may become available to extraction by the $BaCl_2$ solution. This fraction couldn’t even be called soluble as it was extracted long ago by the kinetics.

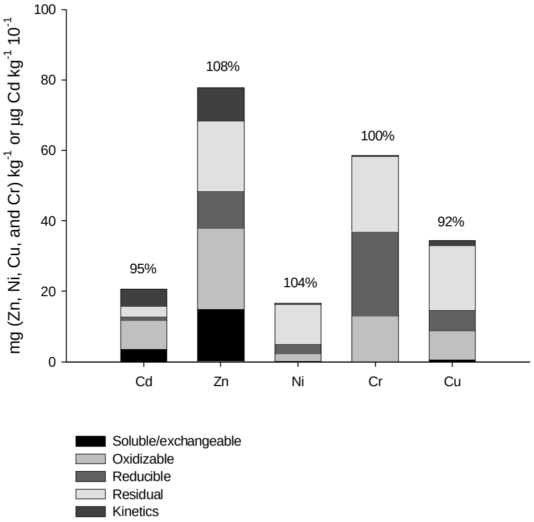

FIGURE 3.8 Sequential extraction from the F_{8N} soil after the kinetics using the NH_4OAc (pH 7.0) as extractant. The percentage above the columns are the trace element amount recovered from the total amount.

Ammonium acetate solutions do not act merely as an ion exchanger but also promote dissolution of solid phases, although in much less extent than the more aggressive media (Castilho and Rix, 1993). Great part of the Cd and Zn extracted by the NH_4OAc kinetics was from the oxidizable fraction. The pH of this extractant could increase the rate of organic matter dissolution from the sludge.

The NH_4OAc kinetics extracted a smaller amount of trace elements from the soluble/exchangeable fraction than the organic acids do. This extractant is extremely concentrated (1 mol L^{-1}) and, by this reason, may cause a decrease on the trace elements activity, resulting in a smaller extraction from the soluble/exchangeable pool. Tan et al. (1971) suggest that a higher extractant pH also causes the stability of Zn-organic compounds to increase, thus decreasing its extractability.

The acetic acid kinetics has proven to be a weaker trace element extractant than the simplified organic acid mixture for all trace elements (Figures 3.1 to 3.5). This is because acetic acid has a smaller capacity to keep the extracted trace elements into solution than the other organic acids also presented in great extension in the simplified organic acid mixture, as lactic and tartaric.

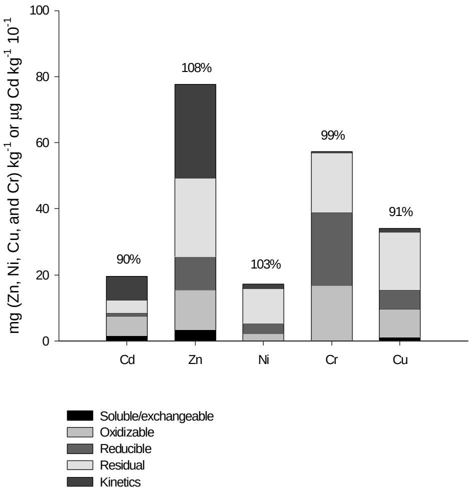

FIGURE 3.9 Sequential extraction from the F_{8N} soil after the kinetics using the acetic acid as extractant. The percentage above the columns are the trace element amount recovered from the total amount.

The trace element extraction kinetics by lactic acid was almost identical to that of the simplified organic acid mixture (Figures 3.1 to 3.5). The sequential extraction procedure shows practically the same results for the amounts extracted from the fractions by these two organic acids (Figures 3.7 and 3.10). In fact, lactic acid is responsible by great part of the trace elements complexation strength in the simplified organic acid mixture (Tables 3.3 and 3.5).

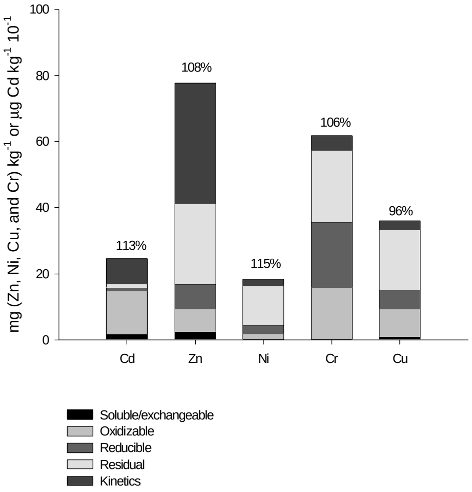

FIGURE 3.10 Sequential extraction from the F_{8N} soil after the kinetics using the lactic acid as extractant. The percentage above the columns are the trace element amount recovered from the total amount.

The soluble/exchangeable Cu represents a very small amount when related to the total Cu in the soil as for Ni and Cr (figure 3.6). Therefore, the Cu extracted by the organic acids kinetics removed a very small amount of the total. This is because copper forms high-energy bonds with both organic and layer silicate surfaces (Wu et al., 1999) that plants may not be able to uptake, but may be assessed by DTPA and Mehlich I (Figure 3.5).

Considering the results obtained with the organic acid mixtures, the use of kinetics data gives a more precise estimate of the amount of extracted trace metals than what could be obtained by conventional measurements made when equilibria are reached, owing to the refixation phenomena.

4 CONCLUSIONS

The kinetics results showed that all organic extractants extracted a similar amount of Cd, but for Zn, Ni, Cr, and Cu, the acetic acid extracted smaller amounts than the other organic acids.

For Cd and Zn, the extractants DTPA and Mehlich I extracted almost the entire extractable fraction in the first extraction. For Ni, Cr and Cu, the single DTPA and Mehlich I extraction do not reach the equilibrium in the very first extraction as for Cd and Zn.

The NH_4OAc extracted much less than all the other extractants for all trace elements.

After the kinetic, the highest Cd and Zn percentage was found to be in the oxidizable fraction. The highest amount of Ni was found to be bound to the reducible and residual fractions after the kinetics. For Cu, the kinetics extracted just a small portion due to the high Cu affinity to the oxidizable fraction.

Assuming that the modified organic acid solution (MOAS) mimics the rhizosphere and that the C_o obtained by kinetics represents the fraction of trace elements that could be available to plants and considering that the MOAS extraction is a time consuming procedure, this study suggests that Mehlich I could be used as a suitable method for assessing Zn and Cd availability in this soil, as it extracted Zn and Cd amounts similar to the C_o MOAS values but with only one extraction.

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ANNEXES

LIST OF TABLES

TABLE 1A First order equation[1] coefficients used to describe the cadmium release from the F_{8N} soil by the complete organic acids mixture.

mmol L^{-1}	C_o (std. error)[2]	k[3]	C_t [CL][4]	r^2
1	49.75 (2.93)	0.1732	40.94 [39.19;42.70]	0.95
10	64.63 (4.21)	0.0165	52.20 [49.87;54.53]	0.95
100	75.54 (3.87)	0.0020	65.39 [62.51;68.27]	0.95

[1] $C_t = C_o (1 – e^{-kt})$
[2] C_o = μg kg^{-1}
[3] k = ln (μg kg^{-1})t^{-1}
[4] C_t = μg kg^{-1}; [CL] = Confidence Limits

TABLE 2A First order equation[1] coefficients used to describe the zinc release from the F_{8N} soil by the complete organic acids mixture.

mmol L^{-1}	C_o (std. error)[2]	k[3]	C_t [CL][4]	r^2
1	36.04 (15.64)	0.0318	9.82 [9.38;10.26]	0.96
10	31.87 (0.75)	0.0131	23.30 [23.01;23.59]	0.99
100	36.43 (0.41)	0.0037	35.52 [34.89;36.15]	0.98

[1] $C_t = C_o (1 – e^{-kt})$
[2] C_o = mg kg^{-1}
[3] k = ln (mg kg^{-1})t^{-1}
[4] C_t = mg kg^{-1}; [CL] = Confidence Limits

TABLE 3A First order equation[1] coefficients used to describe the cadmium release from the F_{8N} soil by the modified organic acids mixture.

mmol L^{-1}/h	C_o (std. error)[2]	k[3]	C_t [CL][4]	r^2
50/24	43.29 (0.42)	0.0037	41.05 [40.54;41.57]	0.99
100/24	49.67 (0.55)	0.0021	48.02 [47.24;48.80]	0.98
50/48	49.88 (0.99)	0.0032	45.94 [44.93;46.95]	0.97
100/48	56.46 (0.67)	0.0018	53.19 [52.40;53.97]	0.98

[1] $C_t = C_o (1 – e^{-kt})$
[2] C_o = μg kg^{-1}
[3] k = ln (μg kg^{-1})t^{-1}
[4] C_t = μg kg^{-1}; [CL] = Confidence Limits

TABLE 4A First order equation[1] coefficients used to describe the zinc release from the F_{8N} soil by the modified organic acids mixture.

mmol L^{-1}/h	C_o (std. error)[2]	k[3]	C_t [CL][4]	r^2
50/24	29.80 (0.28)	0.0052	29.34 [28.88;29.81]	0.98
100/24	32.60 (0.27)	0.0033	32.44 [31.93;32.96]	0.97
50/48	33.74 (0.42)	0.0053	33.26 [32.56;33.96]	0.96
100/48	35.90 (0.25)	0.0036	35.78 [35.31;36.25]	0.98

[1] $C_t = C_o (1 - e^{-kt})$

[2] C_o = mg kg^{-1}

[3] $k = \ln (\text{mg kg}^{-1})t^{-1}$

[4] C_t = mg kg^{-1}; [CL] = Confidence Limits

TABLE 5A First order equation[1] coefficients used to describe the nickel release from the F_{8N} soil by the modified organic acids mixture.

mmol L^{-1}/h	C_o (std. error)[2]	k[3]	C_t [CL][4]	r^2
50/24	1659.99 (22.73)	0.0052	1633.94 [1596.92;1670.96]	0.96
100/24	2369.92 (22.50)	0.0031	354.51 [2313.97;2395.05]	0.97
50/48	1612.92 (18.10)	0.0068	1605.89 [1572.31;1639.47]	0.96
100/48	2148.82 (26.28)	0.0036	2141.66 [2092.23;2191.09]	0.95

[1] $C_t = C_o (1 - e^{-kt})$

[2] C_o = µg kg^{-1}

[3] $k = \ln (\mu\text{g kg}^{-1})t^{-1}$

[4] C_t = µg kg^{-1}; [CL] = Confidence Limits

TABLE 6A First order equation[1] coefficients used to describe the chromium release from the F_{8N} soil by the modified organic acids mixture.

mmol L^{-1}/h	C_o (std. error)[2]	k[3]	C_t [CL][4]	r^2
50/24	2263.41 (100.65)	0.0020	1797.58 [1745.36;1849.80]	0.96
100/24	4156.89 (129.88)	0.0011	3495.67 [3410.97;3580.37]	0.97
50/48	3008.15 (105.85)	0.0021	2446.69 [2386.52;2506.86]	0.97
100/48	5076.33 (141.87)	0.0013	4395.64 [4290.37;4500.91]	0.97

[1] $C_t = C_o (1 - e^{-kt})$

[2] C_o = µg kg^{-1}

[3] $k = \ln (\mu\text{g kg}^{-1})t^{-1}$

[4] C_t = µg kg^{-1}; [CL] = Confidence Limits

TABLE 7A First order equation[1] coefficients used to describe the copper release from the F_{8N} soil by the modified organic acids mixture.

mmol L^{-1}/h	**C_o (std. error)[2]**	**k[3]**	**C_t [CL][4]**	**r^2**
50/24	1228.74 (50.10)	0.0024	1053.28 [1017.77;1088.78]	0.95
100/24	1744.78 (46.88)	0.0014	1562.47 [1521.56;1603.39]	0.96
50/48	864.76 (7.93)	0.0054	852.88 [839.70;866.06]	0.98
100/48	1425.94 (13.79)	0.0023	1387.08 [1366.89;1407.28]	0.98

[1] $C_t = C_o (1 - e^{-kt})$

[2] $C_o = \mu g\ kg^{-1}$

[3] $k = \ln (\mu g\ kg^{-1}) t^{-1}$

[4] $C_t = \mu g\ kg^{-1}$; [CL] = Confidence Limits

TABLE 8A First order equation[1] coefficients used to describe the cadmium release from the F_{8N} soil by different extractants.

Extractants	**C_o (std. error)[2]**	**k[3]**	**C_t [CL][4]**	**r^2**
MOAS	90.34 (1.72)	0.0016	82.69 [80.99;84.39]	0.98
SOAS	88.08 (1.61)	0.0016	80.08 [78.54;81.61]	0.98
Acetic	71.75 (0.74)	0.0027	70.50 [69.29;71.71]	0.98
Lactic	85.95 (1.53)	0.0015	76.70 [75.37;78.02]	0.99
DTPA	58.23 (1.01)	0.3537	58.23 [56.18;60.27]	0.81
Mehlich I	83.89 (0.55)	0.0267	83.89 [82.77;85.01]	0.96
NH_4OAc	44.90 (0.76)	0.0004	44.76 [43.30;46.22]	0.92

[1] $C_t = C_o (1 - e^{-kt})$

[2] $C_o = \mu g\ kg^{-1}$

[3] $k = \ln (\mu g\ kg^{-1}) t^{-1}$

[4] $C_t = \mu g\ kg^{-1}$; [CL] = Confidence Limits

TABLE 10A First order equation[1] coefficients used to describe the zinc release from the F_{8N} soil by different extractants.

Extractants	**C_o (std. error)[2]**	**k[3]**	**C_t [CL][4]**	**r^2**
MOAS	31.94 (0.28)	0.0045	31.90 [31.33;32.46]	0.97
SOAS	32.26 (0.33)	0.0045	32.23 [31.56;32.89]	0.96
Acetic	26.94 (0.20)	0.0037	26.83 [26.45;27.22]	0.98
Lactic	34.92 (0.37)	0.0048	34.90 [34.15;35.65]	0.96
DTPA	21.67 (0.18)	0.2595	21.67 [21.28;22.05]	0.94
Mehlich I	35.50 (0.17)	0.0201	35.50 [35.14;35.86]	0.98
NH_4OAc	10.97 (0.37)	0.0001	9.13 [8.90;9.37]	0.98

[1] $C_t = C_o (1 - e^{-kt})$

[2] $C_o = mg\ kg^{-1}$

[3] $k = \ln(mg\ kg^{-1}) t^{-1}$

[4] $C_t = mg\ kg^{-1}$; [CL] = Confidence Limits

TABLE 11A First order equation[1] coefficients used to describe the nickel release from the F_{8N} soil by different extractants.

Extractants	**C_o (std. error)**[2]	**k**[3]	**C_t [CL]**[4]	**r^2**
MOAS	2092.64 (64.27)	0.0016	1895.64 [1835.63;1955.65]	0.96
SOAS	2190.10 (57.39)	0.0016	1980.32 [1927.25;2033.39]	0.97
Acetic	1551.12 (24.58)	0.0017	1431.07 [1405.68;1456.46]	0.98
Lactic	1871.35 (32.59)	0.0022	1806.82 [1761.62;1852.01]	0.97
DTPA	983.19 (11.87)	0.1982	983.19 [959.33;1007.05]	0.91
Mehlich I	1620.65 (13.87)	0.0126	1620.65 [1592.78;1648.52]	0.96
NH_4OAc	247.33 (4.64)	0.0003	242.36 [235.06;249.66]	0.95

[1] $C_t = C_o (1 - e^{-kt})$

[2] $C_o = \mu g\ kg^{-1}$

[3] $k = \ln (\mu g\ kg^{-1}) t^{-1}$

[4] $C_t = \mu g\ kg^{-1}$; [CL] = Confidence Limits

TABLE 12A First order equation[1] coefficients used to describe the chromium release from the F_{8N} soil by different extractants.

Extractants	**C_o (std. error)**[2]	**k**[3]	**C_t [CL]**[4]	**r^2**
MOAS	6900.74 (341.55)	0.0005	3619.99 [3571.67;3668.31]	0.99
SOAS	6785.67 (300.37)	0.0007	4343.15 [4267.96;4418.35]	0.99
Acetic	905.25 (158.22)	0.0006	526.94 [496.86;557.03]	0.93
Lactic	8062.33 (247.62)	0.0005	4336.00 [4298.53;4373.47]	0.99
DTPA	299.23 (75.02)	0.0064	120.40 [114.99;125.81]	0.95
Mehlich I	6301.41 (168.11)	0.0014	4799.63 [4725.09;4874.17]	0.99
NH_4OAc	178.98 (5.17)	0.0002	169.97 [163.59;176.35]	0.94

[1] $C_t = C_o (1 - e^{-kt})$

[2] $C_o = \mu g\ kg^{-1}$

[3] $k = \ln (\mu g\ kg^{-1}) t^{-1}$

[4] $C_t = \mu g\ kg^{-1}$; [CL] = Confidence Limits

TABLE 13A First order equation[1] coefficients used to describe the copper release from the F_{8N} soil by different extractants.

Extractants	**C_o (std. error)[2]**	**k[3]**	**C_t [CL][4]**	**r^2**
MOAS	3105.65 (71.05)	0.0010	2403.39 [2369.82;2436.96]	0.99
SOAS	3005.89 (69.77)	0.0011	2442.73 [2402.98; 2482.48]	0.99
Acetic	1215.42 (54.16)	0.0014	1056.71 [1015.47; 1097.95]	0.95
Lactic	3065.03 (77.61)	0.0013	2648.05 [2590.63;2705.47]	0.98
DTPA	6074.44 (73.92)	0.1411	6074.36 [5925.87;6222.85]	0.92
Mehlich I	7213.31 (88.96)	0.0072	7207.70 [7032.06;7383.34]	0.94
NH_4OAc	1290.51 (20.66)	0.0002	1223.98 [1198.71;1249.25]	0.98

[1] $C_t = C_o (1 – e^{-kt})$

[2] $C_o = \mu g\ kg^{-1}$

[3] $k = \ln (\mu g\ kg^{-1})t^{-1}$

[4] $C_t = \mu g\ kg^{-1}$; [CL] = Confidence Limits