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“The influence of geochemical parameters on metal mobilization from a calcareous soil by the phytosiderophore DMA”

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Preface:

The data and the corresponding interpretation in this work were collected in five experiments, which were done among other experiments from the beginning of October 2013 to the beginning of March 2014. All experiments were done by the author, with occasional help from my supervisor Dr. Schenkeveld. The drafts of the experiment setups were provided by Dr. Schenkeveld. The data analysis and writing of this master thesis has been done between March and the end of April 2014.

Introduction:

State of knowledge:

Micronutrients and their importance for plants:

This research focuses on metal ions, such as Fe, Mn, Zn, Cu and Ni, which are essential for plants. They are required in smaller quantities compared to macronutrients, such as N, K, Ca, Mg, P and S, and are therefore considered as micronutrients (Scheffer & Schachtschabel, 2010). Plants contain minerals to a

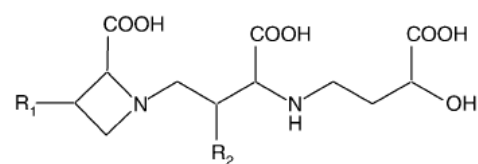
content of 3-8 % (of the dry weight) (Munk K., 2009), they are needed as chemical constituents and thus deficiency of those micronutrients leads to severe physiological damages (Nultsch, 1991) (Schenkeveld W.D.C., 2010). Beside its catalytic role in the synthesis of chlorophyll, Fe ions are parts of heme groups, which can be found within cytochromes, which are responsible for ATP production, but are also constituents of proteins with no heme groups attached, such as ferredoxin (Nultsch, 1991) (Scheffer & Schachtschabel, 2010). Under oxic conditions, the iron concentration within the soil solutions is $>0,02-0,3$ mg/l (Scheffer & Schachtschabel, 2010). Iron deficiency, especially chlorosis effects (where the chlorophyll production in leaves is insufficient), happens in calcareous and limed soils with high pH values (7,0-8,2), in subtropic and tropic soils under arid conditions where Fe is mainly bound to stable hematite or goethite minerals, or in moor environments and starts occurring at $< 50-80$ mg/kg in plant shoots (Scheffer & Schachtschabel, 2010). Plants need Cu for several enzymes; it can be found in oxidase enzymes (Nultsch, 1991), but is also essential for photosynthesis and protein- and chlorophyll-synthesis. The Cu concentration in agricultural used soils is $> 0,03$ to $0,3$ mg/l (Scheffer & Schachtschabel, 2010), Cu deficiency occurs at an amount of $< 2-5$ mg/kg within leaves (Scheffer & Schachtschabel, 2010). Zinc is a part of many enzymes, e.g. in phosphatases and proteinases (Scheffer & Schachtschabel, 2010), but it is also an important cofactor (Nultsch, 1991). Manganese is important for the oxygen production during photosynthesis (Nultsch, 1991), but also activates enzymes of chlorophyll and protein-synthesis (Scheffer & Schachtschabel, 2010). Although Ni is a recognized essential mineral nutrient element for higher plants, its agricultural and biological significance is poorly understood (Bai et al., 2006). Only low levels are needed by plants (about 1–100 ng of dry weight), it is thought that Ni has a key metabolic role and is a substituent in many proteins (Bai et al., 2006). Ni stress in heavy metal contaminated soils however significantly decreases photosynthesis (Velikova et al, 2011). Cobalt is not considered as a micronutrient for higher plants, but is essential for microorganism and therefore essential for the natural nitrogen cycle (Scheffer & Schachtschabel, 2010). For Fe and Cu the adequate mineral content of plant tissue (dry weight) ranges from 30-500 mg/kg for iron and 2-20 mg/kg for Cu respectively (Scheffer & Schachtschabel, 2010). Beside micronutrient deficiency symptoms, plants also suffer from elevated concentrations of metals within soil environments, a phenomenon which is called phytotoxicity (Reichman and Parker, 2005). An excessive amount of metals is harmful in biology due to redox reactivity and the promiscuous binding of transition metals to thiol, thioether, imidazole, and carboxylate ligands (Merchant S.S., 2010). For example, Cu containing plant protection products in wine and hop agricultures led to Cu contaminated soils and an enrichment of Cu in leaves above the toxic level of 20-30 mg/kg, which caused serious Fe, Zn and Mo deficiency (Scheffer & Schachtschabel, 2010). Macro and micronutrients acquired by autotrophic organisms (e.g. plants) are further acquired by heterotrophic organisms of higher trophic levels (Munk K., 2009).

Fe and metal acquisition by plants:

Iron is the second most abundant metal (4,2%) within the continental crust, after aluminum, and varies in soils from 0,2 to 5 % (Scheffer/Schachtschabel, 2010). Under oxic conditions, organo-metallic compounds and low grade crystalline ferrihydrite (an iron-oxyhydroxide) are the main sources of iron for plants, whereas iron in well crystalline Fe(III) oxides, such as hematite and goethite, is more or less inaccessible due to the high crystal stability of these minerals (Scheffer & Schachtschabel, 2010). In many soils, especially in alkaline soils, which cover about one-third of the world's land surface (Namba et

al., 2007), iron acquisition is limited by low solubilities and slow dissolution rates of iron bearing minerals (Kraemer et al, 2006), thus making special iron acquisition strategies necessary. Therefore, plants developed two strategies to acquire iron in soils with low solubility, especially in carbonaceous soils: Strategy I plants (most non-graminaceous plants) acidify the rhizosphere and release organic acids enhancing iron solubility and express membrane bound reductases to reductively release iron from soluble complexes followed by Fe(II) uptake (Roemheld V. and Marschner H., 1986) (Kraemer et al., 2006). Strategy II plants (grasses) release phytosiderophores within the rhizosphere and a highly specific uptake system for Fe(III)phytosiderophores (Roemheld V. and Marschner H., 1986) (Scheffer & Schachtschabel, 2010) (Kraemer et al., 2006). Barley, wheat and rye are typical examples of chlorosis resistant strategy II graminacea, which release high amounts of phytosiderophores to satisfy their Fe demand for optimal growth conditions after consideration of microbial degradation (Römhheld V., 1991).

Phytosiderophores, with a focus on DMA:



Mugineic acid (MA): $R_1 = H$, $R_2 = \alpha OH$

Deoxymugineic acid (DMA): $R_1 = H$, $R_2 = H$

3-Epihydroxymugineic acid
(3-epiHMA): $R_1 = \alpha OH$, $R_2 = \alpha OH$

Hydroxymugineic acid (HMA): $R_1 = R_2 = \alpha OH$

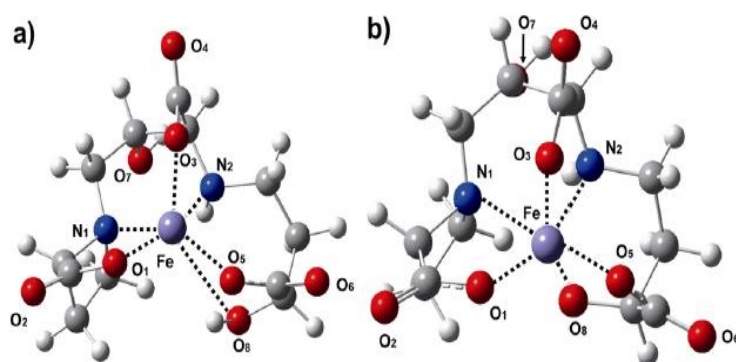
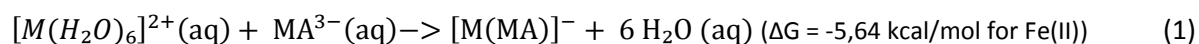


Fig. 1: Mugeneic acid and its derivatives (Kraemer S.M., 2004), on the left optimized gas-phase geometry for $[Fe(HMA)]^-$ (a) and $[Fe(Ma)]^-$ (b) (Kato M., Izuka S., Fujihara T., Nagasawa A., Kawai S. and Tanaka T., 2011).

Siderophores are biogenic chelators with both a high affinity and a degree of specificity for iron (Kraemer S.M., 2004). Many organisms, such as higher plants, bacteria and fungi, use ligand induced dissolution of minerals (siderophores or low molecular organic acids) and consequently metal complexation to maintain their nutritional demand (Scheffer & Schachtschabel, 2010). There are about 500 known siderophore structures, the best studied is DFO-B because of its commercial availability (Kraemer S.M., 2004). Siderophores exuded within the rhizosphere by plants are called phytosiderophores. Phytosiderophores comprise a relatively small group of ligands: mugineic acid and its derivatives (Dell'mour et al, 2010), that form hexadentate complexes with metals through binding to aminocarboxylate and hydroxycarboxylate functional groups (Kraemer S.M., 2004). The first proof of iron solubilizing compounds in the rhizosphere (of rice and oats) were found by Takagi in 1976 (Takagi S., 1976). Not only Fe ions are mobilized as a result of exudation of phytosiderophores within the rhizosphere; also Zn, Mn, Cu and Co are mobilized in calcareous soils (Zhang F. S., 1993). Moreover, phytosiderophores are also exuded in environments where Zn deficiency (Römhheld V., 1991) and Cu deficiency (Gries et al., 1998) occurs. Among the chelators EDTA (Ethylenediaminetetraacetic acid), DTPA (diethylene triamine pentaacetic acid), deferriferrioxamine B (FOB), mugineic acid (MA), and 2'-

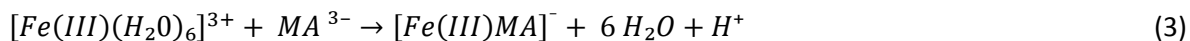
deoxymugineic acid (DMA), MA and DMA exhibit the highest efficiency of Fe extraction, especially at alkaline pH values (Takagi et al., 1988). In calcareous soils, various chelators (synthetic chelators such as EDDHA (ethylenediamine-N,N'-bis(2-hydroxyphenylacetic acid) and DTPA, but also microbial siderophores such as Desferal) mobilize metals in the following order: Cu < Fe < Zn < Mn, whereas phytosiderophores isolated from root exudates mobilize similar Cu and Zn amounts, but somewhat more Fe and considerably more Mn (Trebby et al., 1989). Also heavy metals such as Ni and Cd, are taken up via reabsorption of phytosiderophores upon their exudation, particularly in contaminated soils (Römhild V. and Awad F., 2000). The divalent metal ions (Cu, Ni, Co, Zn and Mn) and the trivalent Fe are complexed (in an aqueous solution) by the mugineic acid. This is represented by the following reaction equation, in which $MA^{3-}(aq)$ denotes the mugineic acid ligand, with all three carboxylic groups deprotonated (after Kato et al, 2011);



Combining the dissolution reaction of Fe-(oxy)hydroxides and the corresponding solubility product with the complexation reaction of iron and its corresponding stability constant, increased iron oxide solubility by formation of soluble iron complexes can be expressed as follows (Kraemer S.M., 2004):



In equation (2), it is shown that the iron bound to the oxyhydroxide is in its oxidized, Fe(III) state. Thus, equation (1) needs to be adjusted: Trivalent ions are complexed by the mugineic acid upon deprotonation in the $[Fe(MA)]^{-}$ complex, as the following equation by Kato et al. (Kato et al., 2011) shows:



Murakami et al. (1989) determined stability-constants for several $[M(II)MA]^{-}$ complexes and the $[Fe(III)MA]^{-}$ complex: For the mugineic acid and two of its derivatives (epi-HMA, MA and DMA), the order of stability for divalent ions is $Ca^{2+} < Mn^{2+} < Fe^{2+} < Ni^{2+} < Cu^{2+} > Zn^{2+}$, moreover the stability seems to increase as the ligand basicity increases (epi-HMA < MA < DMA). In the case of DMA, the stability constant of Cu(II) is slightly higher than the stability constant of Fe(III) (Murakami et al., 1989). This observations fit to the Irving-Williams series, which states that the stability of the complexes formed by the divalent transition metals in the first row with many kinds of ligands follows the order $Mn^{2+} < Fe^{2+} < Co^{2+} < Ni^{2+} < Cu^{2+} > Zn^{2+}$ (Irving and William, 1953). In order to enhance the perception on mugineic acid complexation and the results discussed later, the stability of the metal-complexes should be compared with the free energy of complex formation. Kato et al. (2011) calculated them for several bivalent metal ions (analogously to equation 1) and Fe(III) (equation 3), providing an order of increasing free energy of complexation for $[M(II)HMA]^{-}$ as follows: $Ca^{2+} < Mn^{2+} < Zn^{2+} < Fe^{2+} < Cu^{2+} < Fe^{3+}$ (Kato et al., 2011). There is no free energy of complexation available for DMA within Kato et al. (2011). Moreover, there are no stability constants and free energies of complexation on cobalt published yet. Reichmann and Parker (2005) examined the pH dependency of stability of metalDMA complexes. Fe(III) is able to effectively compete with Zn, Cd, Cu or Ni at low pH and complex the majority of the DMA present. At higher pH

values, the other metals predominate DMA complexation (Reichman S.M. and Parker D.R., 2005). Especially for environmental considerations, the soil type is of importance. Although the FeDMA complexes has the highest stability constant (see above), in calcareous soils, Fe can be outcompeted by other metals due to its very low activity. Iron deficiency in plants grown on more acidic soils is improbable (Schenkeveld et al., 2014 (accepted)). According to Schenkeveld et al., 2014 (accepted), CuDMA and NiDMA are the quantitatively most important species under equilibrium conditions in soils with a circum-neutral pH. The comparison of stability constants, the free energy of complexation, the pH effect on metalDMA complexes and the activity within the soil profile yields insights in the competitive complexation between the metals mentioned above in time and abundance. Especially considering equilibrium between metalDMA complexes and the soil, that is reached after different times for different soil types (Schenkeveld et al, 2014 (accepted)).

Following Roemheld & Marschner (1986), reduction from Fe(III) to Fe(II) is not involved during the iron uptake of phytosiderophores (Roemheld and Marschner, 1986). Inside the plant, the ferric iron complex is readily reducible by physiological available reductants such as NAD(P)H, thus the mechanism of iron transport in graminaceous plants probably includes iron reduction from the thermodynamically stable ferric mugineic acid complex (Sugiura et al., 1981). From the overall point of view, the release of phytosiderophores within Strategy II plants seems to have several ecological advantages over the release of protons within Strategy I plants, such as less inhibition by high pH values and solubilization of sparingly soluble inorganic Fe(III) compounds in the rhizosphere (Roemheld V. and Marschner H., 1986). Considering the phytosiderophore DMA, which was the only derivate of the mugineic acid used in this master thesis, and summing up the facts mentioned above, it can be concluded from a thermodynamic perspective that DMA is not an Fe specific chelating agent in a soil environment and equilibrium chemistry is unable to explain its role as an Fe carrier in calcareous soils (Schenkeveld et al., 2014 (accepted)).

Knowledge Gaps:

There are several knowledge gaps concerning environmental aspects that influence the phytosiderophore biogeochemistry within the rhizosphere. The influence of certain physicochemical and geochemical aspects on metal mobilization by phytosiderophores in calcareous soils is still unknown. In this master thesis the effect of physicochemical parameters, such as temperature, ionic strengths and moisture regime on metal mobilization by strategy II plants are addressed. Different temperature, ionic strengths and moisture regimes in soils can be related with different environments all over the world, which are determined by the local climate and geological setting (Walter, 1990). High electrolyte concentrations resulting in a high ionic strength occur naturally near tidal flats and in salty soils within arid environments, such as Solonchaks and Solonetz (Scheffer & Schachtschabel, 2010). Anthropogenic rises in soil salinity may happen due to the usage of irrigation in arid environments, fertilizers and road salt (Scheffer & Schachtschabel, 2010). Increasing CaCO₃ content in calcareous soils minimizes the different growth of wheat cultivars (Awad et al, 1999). In this master thesis, the effect of different electrolyte species and background concentrations in a soil solution on Fe and metal acquisition by DMA was examined. Different temperatures in soils may occur due to seasonal changes and geographical differences in latitude and altitude. The optimum soil temperature for cultivated plants in middle

Europe lies between 15 and 25 °C, above the optimum there is lesser root growth until severe damage occurs above 40 to 45 °C of soil temperature (Scheffer & Schachtschabel, 2010). The lower limit in soil temperature during late frost events in spring is – 8°C for summer corn and – 3°C for potatoes (Scheffer & Schachtschabel, 2010). To sum up, iron acquisition happens over a certain range of soil temperature within different climatic zones. Hence, differences of iron and metal acquisition by the phytosiderophore DMA were assessed in this thesis in order to quantify differences in metal acquisition at different latitudes and altitudes. Furthermore, the effect changing moisture regimes within an environment has on the functionality of phytosiderophores is also unknown. The water content of soils varies between different seasons and geographic localities due to different meteorological and climatic conditions, but is also dependent on the hydrogeological setting and different soil properties, such as grain size distribution and shape, field capacity and porosity (Scheffer & Schachtschabel, 2010) (Hölting & Coldewey, 2008). Therefore, the difference in phytosiderophore induced metal acquisition in already wetted soil samples (corresponds to regular precipitation and high field capacities) is compared to the mobilization of metals in recently wetted soil samples (corresponds to recently wetted, dried soils), representing different moisture regimes in soils. The concentration of phytosiderophores within the rhizosphere varies and can go up to 1mM (Römheld V., 1991). Plants exude different amounts of phytosiderophores (Römheld V., 1991), thus providing different concentrations of phytosiderophores in the rhizosphere. In this master thesis, different DMA concentrations in calcareous soils and their corresponding metal mobilization is investigated. Finally, the adsorption of different metalDMA species onto soil particles has never been examined. Beside other processes, adsorption behavior of metalDMA species determines the amount of metalDMA complexes finally being able to be reabsorbed by plants within the rhizosphere. Furthermore, the temperature and ionic strengths dependency on metalDMA adsorption is examined in this master thesis.

Practical Implications:

Beside the ecological and botanical relevance of a profound understanding on how the phytosiderophore DMA works within calcareous soil, this assessment of phytosiderophore induced metal mobilization under different temperature conditions, moisture regimes and electrolyte concentrations in the soil solution may have some practical implications on agricultural uses of soils in different (climatic) environments. Moreover, the work presented in this master thesis demonstrates the potential effect anthropogenic influences have on iron acquisition in plants, such as increased soil salinity due to irrigation or road salt input, which may influence phytosiderophore induced metal mobilization in strategy II plants. Furthermore, creating an enhanced understanding of the way the phytosiderophore DMA works over geochemical and physicochemical varying parameters may reveal practical inputs for the research field of environmental geosciences, especially considering phyto-remediation applications. The acquisition of heavy metals, such as Cd and Ni, but especially the enhanced Fe acquisition of strategy II plants may have practical and costly implications on remediation practices of sites contaminated with dispersed asbestos and/or fine dust.

Hypothesizes and goals of the master thesis:

The goal of this master thesis is to gain an understanding of how metal mobilization from soil by the phytosiderophore DMA is affected by the geochemical parameters temperature, ionic strength and the

moisture regime, as well as by DMA concentration. This was approached by formulating a series of hypotheses and by designing five experiments testing these hypotheses:

Hypothesis 1: Metal-DMA complexes adsorb onto soil particles, causing a lower bioavailability.

Different species of metalDMA complexes adsorb differently onto soil particles over a certain concentration range. FeDMA, CuDMA, NiDMA and ZnDMA may have different negative charges (for FeDMA and ZnDMA that has already been published (von Wiren et al., 2000)), resulting in a different amount of electrostatic repulsion between adsorbate and adsorbent, thus making adsorption of metalDMA complexes species dependent.

Hypothesis 2: Higher electrolyte background concentrations increase the adsorption potential of metalDMA complexes. Increased salt concentration reduces electrostatic repulsion between similarly charged adsorbate and adsorbent materials, which may influence adsorption (Jones K.L. and O'Melia C.R., 2000).

Hypothesis 3: Temperatures affect the amount of metalDMA adsorption onto soil particles. Elevated Brownian Motion of molecules at higher temperature may decrease their adsorption potential.

Hypothesis 4: Different concentrations of total DMA added to the soil create different metal mobilization patterns. At high DMA concentration added, free DMA will coexist longer at soil dependent complexation rates. A high amount of free DMA will eliminate the reason of competition effects, thus keeping weak metals (Fe, Mn and Zn) in respect to competition complexed for a longer time.

Hypothesis 5: Different moisture regimes in soils cause different rates of metal mobilization upon exudation of phytosiderophores by strategy II plants. Dried soils may contain high amounts of organic matter and microorganisms, which lost their molecular integrity upon drying out. Fe and other metals may only be loosely bounded to this kind of organic matter, thus the amounts of metals being available for complexation may increase. Hence, DMA induced metal mobilization after periodic precipitation events in arid environments (unprewetted samples within the experiment) may be higher than in humid environments with regular precipitation input (prewetted samples within the experiment). This would guarantee a higher metal acquisition potential for plants at shorter time intervals in ecosystems where a regular water supply is not given.

Hypothesis 6: Metal complexation with free DMA ligands in a soil environment depends on the electrolyte background concentration of the soil solution and thus on the local ionic strength. I assume that, at lower ionic strengths, higher ligand induced metal mobilization takes place rather than at high electrolyte concentrations. This hypothesis may be linked to hypothesis two: Higher adsorption of metalDMA complexes onto soil particles at higher background electrolyte concentrations would decrease the amount of metalDMA complexes in solution.

Hypothesis 7: Different background electrolytes (CaCl₂ and NaCl) have different effects on metal mobilization in soil environments. Ca²⁺ and Na⁺ ions may have different effects on the electrostatic repulsion between negatively charged soil surfaces and the metalDMA complexes.

Hypothesis 8: Temperatures affect metalDMA complexation in soils. At higher temperatures, complexation of different metals occurs more rapidly than at low temperatures due to higher reaction kinetics. Moreover, different temperatures influence the competitive behavior of metalDMA complexes, where higher temperatures favor a faster formation of metalDMA complexes with higher stability constants due to a faster reaction kinetic. A quicker complex formation will decrease the amount of free DMA to a faster extent; hence all metals weak in respect to competition (especially Fe and Zn) may be outcompeted faster at higher temperatures

A number of experiments were designed to test the hypotheses underlying this research work:

- Experiment one, addressing hypotheses 1, 2, and 3: Adsorption isotherms of metalDMA complexes (FeDMA, CuDMA, NiDMA and ZnDMA) at different ionic strength and temperatures.** Within Experiment one (*“Adsorption Isotherm Experiment”*), the adsorption potential of FeDMA, CuDMA, NiDMA and ZnDMA complexes onto soil particles has been conducted at 20 °C and an electrolyte background concentration of 10 mmol/l CaCl₂. MetalDMA solutions with different concentrations (from 100 µmol/l to 0,25 µmol/l) were added to the soil, afterwards the metalDMA concentration in the supernatant was measured. Furthermore, the adsorption potential of FeDMA and CuDMA was examined at different electrolyte background concentrations (0, 2, 10 and 100 mmol/l CaCl₂ and 300 mmol/l NaCl) and different temperatures (8°C, 20 °C and 35°C). *Hypothesis 1* will be falsified if there is no difference in the adsorption potential of the examined metalDMA complexes measurable. If there is a difference in metalDMA measured, *hypothesis 2* is falsified if different electrolyte background concentrations do not affect the adsorption potential of the examined metalDMA complexes. *Hypothesis 3* is falsified if different temperatures do not affect the adsorption potential of the examined metalDMA complexes.
- Experiment two, addressing hypothesis 4: Metal complexation at different DMA concentrations:** Experiment two (*“Concentration Experiment”*) was designed as follows: Metal mobilization by DMA has been examined at different amounts of total DMA concentrations (30, 100 and 1000 µmol/l) added to the soil batches at 20°C and a CaCl₂ background concentration of 10mmol/l. *Hypothesis 4* will be falsified if there are no different metal mobilization patterns determined at different amounts of total DMA added to the soil.
- Experiment three, addressing hypothesis 5: Metal complexation at different moisture regimes:** Within experiment three (*“Prewetting Experiment”*), the difference in metal mobilization between prewetted (addition of the background solution to the sample two days in advance) and unpwetted (more or less instantaneous addition of both the ligand and the background solution) samples by a total DMA concentration of 100 µmol/l was examined. The experiment was conducted at 20°C and a CaCl₂ background concentration of 10 mmol/l. *Hypothesis 5* will be falsified if there are no substantial differences in metal mobilization by DMA between prewetted and unpwetted samples measurable.
- Experiment four, addressing hypothesis 6 and 7: Metal complexation at different ionic strengths background concentrations:** Within experiment four (*“Ionic Strength Experiment”*), metal mobilization at different electrolyte background concentrations and species (0, 2, 10 and

100 mmol/l CaCl_2 and 300 mmol/l NaCl) was examined at 20°C and 100 $\mu\text{mol/l}$ of total DMA added to the batches. *Hypothesis 6* will be falsified if there is no substantial difference in metal mobilization measurable between samples with low and high electrolyte background concentration. However, if this hypothesis is verified, *hypothesis 7* can be falsified if ionic strength dependent metal mobilization is not dependent on the electrolyte species, but on the overall ionic strength of the soil solution.

- **Experiment five, addressing hypothesis 8: Metal complexation at different temperatures:** Experiment five (*“Temperature Experiment”*) was conducted at different temperatures (8°C, 20°C, 35°C and 60°C), a constant CaCl_2 background concentration of 10 mmol/l and a total DMA concentration of 100 $\mu\text{mol/l}$ in order to quantify metal mobilization from a calcareous soil by DMA at different temperatures. *Hypothesis 8* will be falsified if there is no temperature effect on metal mobilization measurable. If the temperature dependency of metal mobilization by DMA is however verified, the (sub)hypothesis made above are falsified if metalDMA complexation happens more slowly at higher temperature. Thus competition effects between the examined metals are not speeded up at higher temperatures.

Materials and Methods:

Materials:

The Soil:

The calcareous Santomera soil that was used throughout all experiments was collected in the Murcia region of Spain and has an agricultural origin. Some soil characteristics can be found in the following table, determined by Schenkeveld et al., 2014 (accepted):

pH (CaCl_2)	SOM cont. [g/kg]	Clay cont. [g/kg]	CaCO_3 cont. [g/kg]	Crystalline Fe(hydr)oxide cont. [g/kg]	Amorphous Fe(hydr)oxide cont. [g/kg]	DTPA extractable Cu [mg/kg]	Ni [mg/kg]	Zn [mg/kg]	Mn [mg/kg]	Co [mg/kg]	Fe [mg/kg]
7,5	15	300	499	15,4	0,9	1,6	0,3	0,5	3,1	0,0	4,9

Table 1: Soil properties of the Santomera Soil of Spain. Values taken from Schenkeveld et al., 2014 (accepted).

Chlorosis, and thus Fe deficiency, was already found in the Santomera soil (Schenkeveld et al., 2008). The Santomera soil was air dried and manually sieved with a 2 mm sieve.

The Ligand:

The same manufactured DMA ligand was used as within Schenkeveld et al. 2014 (accepted) throughout all experiments. *The synthetic 2'-deoxymugineic acid phytosiderophore (DMA) was synthesized as ammonium-DMA salt in accordance with Namba et al. (2007). The compound was characterized by LC-ESI-TOF-MS (Agilent Time-of-Flight LC/MS 6220 system) with a specified mass accuracy of ≤ 2 ppm that allows for compound confirmation via sum formula determination. Purity was determined to be higher than 95% by H-NMR.*

Other chemicals:

Calcium-chloride (CaCl_2), sodium-azide (NaN_3), copper-chloride dihydrate ($\text{CuCl}_2 \cdot 2\text{H}_2\text{O}$), zinc-chloride (ZnCl_2), sodium-hydroxide (NaOH), nickel-chloride ($\text{NiCl}_2 \cdot 6\text{H}_2\text{O}$) and iron-chloride hexahydrate ($\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$) were delivered by Merck KGaA, all in pro analysis quality. Sodium chloride was purchased from Carl Roth GmbH (with a purity $\geq 99,5\%$), Bronopol (2-bromo-2-nitropropane-1,3-diol, $\text{C}_3\text{H}_6\text{BrNO}_4$) was purchased from Acros Organics.

General working steps:

Beside the *Adsorption Isotherm Experiment*, the remaining four experiments described in this thesis were done in more or less equal patterns: Every single treatment was done in duplicates. At first, $10 \pm 0,02$ g of Santomere soil were weighted-in in 50 ml polypropylene centrifugation tubes. Depending on the treatment, 9 ml of different background solutions were added two days in advance ("*prewetted*") or right before the start of the experiment ("*not prewetted*"). At the start of the actual experiment, 1 ml of DMA solution was added, finally making the soil solution ratio in every single described experiment 1:1. For most of the experiments, a 10 mmol/l CaCl_2 electrolyte background and a ligand concentration of 100 $\mu\text{mol/l}$ were chosen. In order to get sterile conditions for certain treatments, the sterilizing agents sodium-azide (2 g/l; NaN_3), or Bronopol (0,2 g/l; $\text{C}_3\text{H}_6\text{BrNO}_4$), were used and prepared together with the CaCl_2 electrolyte solution. After adding 1 ml of DMA solution, the tubes remained in an overhead (Heidolph REAX 20, 18 rotations per minute) or horizontal (Excella E24 Incubator/New Brunswick Scientific or KS 501 digital/IKA Werke, 100 rotations per minute) shaker for 0,15; 0,5; 1; 2; 4; 8; 24; 48; 96 and 168 hours intervals. Samples without an added ligand (blanks) were additionally added to the 1 hour, 8 hour, 24 hour and 168 hour sample series in order to determine the background metal concentration in solution at these times. After the corresponding time for each different treatment, the duplicates were centrifuged three (*temperature-, concentration- and adsorption- experiment*) to five minutes (*prewetting-, ionic strengths- experiment*) at 4500 rounds per minute in a Jouan CR 422 Centrifuge, whereupon the solution of each sample was separated from the soil and filtered (Whatmann 0,45 μm filters) through syringes (1x100 Norm-Ject syringes/Henke-Sass-Wolf). The pH of the supernatant was measured for controlling purposes. All samples throughout all experiments had pH values between 7,1 and 8,2. Subsequently, the solution of each sample was split into 1 ml for ICP-MS measurements and 2 ml for ICP-OES measurements (except for the *Ionic Strengths Experiment* due to high background electrolyte concentrations at 100 mmol/l CaCl_2 and 300 mmol/l NaCl, where only 0,5 ml of the solution were used for the ICP-MS measurement). After acidifying every sample with 1 ml of 1,4 mol/l suprapure nitric acid (HNO_3), every sample was made up with ultra pure water until a final volume of 10 ml.

Later the samples were measured both with an inductively coupled plasma mass spectrometer (ICP-MS) from PE SCIEX (ELAN 6100) and an inductively coupled plasma optical emission spectrometer (ICP-OES). For the ICP-OES measurements, Fe, Cu, Mn, Zn, Co and Ni were measured, whereas Cu, Mn, Zn, Co, Ni, U, V and Cd were measured with the ICP-MS. Cd, V and U showed no statistical significant DMA induced metal mobilization, thus they are not included in the diagrams of this master thesis.

During interaction time, all samples remained in a temperature controlled room with 20 °C except for the *temperature experiment*, where temperature controlled shakers were used. If not specially denoted, all reactions in the experiments thus occurred at 20 °C. During interaction time, all samples remained in a closed, dark room with no light source switched on. However, during the several working described above, they were exposed to light.

Data Interpretation:

The OES and MS counts-data were re-calculated to $\mu\text{mol/l}$. The blank series were used to subtract the background metal concentrations from the ligand related metal mobilization in the solution. Therefore, the 1 hour blank series was subtracted from the 0,25; 0,5; 1 and 2 hour series, the 8 hour blank series from the 4 and 8 hour series, the 24 hour blank series from the 24 and 48 hour series and finally the 168 hour blank series from the 96 and 168 hour series. Especially for manganese, this method provided some difficulties for the 48 and 96 hour series in respect to the background blank concentration since the ligand correlated metal mobilization was lower after 96 hours and higher after 48 hours of interaction time. In these cases, the blank concentration was calculated for the problematic time series as follows: The Mn background concentrations of the four blank series (1 h, 8 h, 24 h and 168 h) were plotted, afterwards the background concentration of other time series (96 and 48 hours) were calculated with the linear equation of the plot. The values in the following plots are mean values of the duplicates. Error bars indicate standard deviations. In certain cases (especially concerning the temperature experiment at high temperatures), only one sample out of the duplicates was used due to occasional evaporation losses or other mistakes. Those samples are indicated with a single directed error bar, which shows the wrong sample of the duplicates within the plot.

All values plotted in the following diagrams in this work were taken from the calculated ICP-OES data because they included Fe and were in general more reliable. Nevertheless, all Cu, Ni, Co, Zn and Mn data were compared with the calculated ICP-MS data. That was especially important for Ni and Co within the 60°C temperature experiment due to large error bars. Concerning the 60°C treatment within the temperature experiment, there are also two ICP-MS plots in this work to compare them with the ICP-OES data.

The experiments in detail:

Adsorption Isotherm experiment

Adsorption isotherm experiments were done in order to quantify the amount of metalDMA complexes adsorbing onto soil particles what thus reduces their availability for plants. Also the dependency of metalDMA adsorption on geochemical parameters such as temperature and ionic strengths was examined. Before the actual experiments, several test experiments were made in order to assess which treatments gave the smallest spread in the metal mobilization by free DMA from soil samples compared to the metal mobilization of free DMA with FeDMA or ZnDMA added to the solution. Finally, prewetting and no sterilizing agent were chosen as best treatments to establish adsorption experiments. The free DMA content which was added to the FeDMA or ZnDMA complex solutions prevents them from getting out-competed within the two hours reaction time by divalent metals in the soil, such as Cu, Ni or Co. As long as there is a substantial amount of free DMA in the system left, there is no reason for competition

within the metalDMA complexes. Another test experiment focused on NiDMA. It was examined that there is no need to add free DMA to the NiDMA solution since it is not outcompeted by other metals. The same thing applies on CuDMA. MetalDMA complexes (FeDMA, CuDMA, ZnDMA and NiDMA) were prepared in advance to the actual experiment. At first, 1000 $\mu\text{mol/l}$ Fe and Zn-chloride solutions and 1020 $\mu\text{mol/l}$ Cu and Ni-chloride solutions (2 % excess of the metal ions) were prepared and acidified with 1,4 mol/l suprapure HNO_3 (at all $1/50^{\text{th}}$ of the overall volume of the metal-chloride solution). Afterwards, the metalDMA solutions were prepared by adding free DMA solutions with different concentrations to the metal chloride solutions. Finally, two 1000 $\mu\text{mol/l}$ (CuDMA and NiDMA) solutions without an excess of free DMA and two 1000 $\mu\text{mol/l}$ (FeDMA and ZnDMA) solutions with an excess of 1500 $\mu\text{mol/l}$ of free DMA were prepared. The amount of free DMA (1500 $\mu\text{mol/l}$) guarantees that the weaker FeDMA and ZnDMA complexes remain complexed during the competition with Cu, Ni and other metals (further explanation within the results section). Lower concentrations of FeDMA, ZnDMA, CuDMA and NiDMA were prepared by dilution steps. The 1500 $\mu\text{mol/l}$ free DMA excess was nevertheless kept constant for all FeDMA and ZnDMA concentrations by re-adding the corresponding free DMA amount. The pH of the solutions was adjusted with single 5-10 μl drops of a 1 mol/l NaOH solution, until it went up to a pH more or less comparable to the Santomara soil (a pH range from 5 to 10 was accepted). In conclusion, 100 $\mu\text{mol/l}$; 50 $\mu\text{mol/l}$; 25 $\mu\text{mol/l}$; 10 $\mu\text{mol/l}$; 5 $\mu\text{mol/l}$; 2,5 $\mu\text{mol/l}$; 1 $\mu\text{mol/l}$; 0,5 $\mu\text{mol/l}$; 0,25 $\mu\text{mol/l}$ and 0 $\mu\text{mol/l}$ metalDMA solutions were added to the soil in the batches. After the metalDMA solutions were added to the prewetted soil batches, they remained for two hours in an overhead shaker and were afterwards treated as described above. The same solutions were frozen and again used (after proper thawing) for adsorption experiments among ionic strengths and temperature variations.

The adsorption behavior of 100 $\mu\text{mol/l}$, 25 $\mu\text{mol/l}$, 5 $\mu\text{mol/l}$ and 1 $\mu\text{mol/l}$ FeDMA and CuDMA solutions was investigated at 0 mmol/l, 2 mmol/l, 100 mmol/l CaCl_2 and 300 mmol/l NaCl electrolyte background concentrations. All samples remained two hours in an overhead shaker.

Finally, the temperature dependency of the metalDMA adsorption was examined at 8°C; 20 °C and 35 °C in analogy to the *Temperature Experiment*. 100 $\mu\text{mol/l}$, 25 $\mu\text{mol/l}$, 5 $\mu\text{mol/l}$ and 1 $\mu\text{mol/l}$ of FeDMA and CuDMA solutions were added to the soil batches and kept two hours in a horizontal shaker (20°C) or in a temperature controlled horizontal shaker (8°C and 35°C).

Concentration experiment:

In this experiment, the mobilization of iron and other metals by DMA was examined as a function of DMA concentration added to the soil. Three different ligand concentrations were added to the already prewetted samples, 30 $\mu\text{mol/l}$, 100 $\mu\text{mol/l}$ and 1000 $\mu\text{mol/l}$, which afterwards remained inside an overhead shaker for the corresponding time intervals described above. Half of the samples were sterilized with sodium azide.

Prewetting experiment:

The difference in ligand induced metal mobilization for soils with changing moisture regimes was examined in this experiment. The term “*prewetted*” refers to the addition of the background solution to the sample two days in advance to the actual addition of the ligand, whereas the more or less

instantaneous addition of both the ligand and the background solution refers to “*not prewetted*”. All the samples remained in an overhead shaker for the time intervals mentioned above. Samples from both treatments, “*prewetted*” and “*not prewetted*”, were themselves split into sterile samples with added sodium azide and samples without a sterilant.

Ionic Strengths experiment:

The mobilization of iron and other metals by DMA was examined for different background electrolyte species and concentrations. In order to avoid a contribution from the sterilant sodium-azide to the ionic strengths, Bronopol was used as a sterilizing agent throughout the whole experiment because it does not dissociate upon dissolution. Metal mobilization was examined at 0 mmol/l, 2 mmol/l, 10 mmol/l and 100 mmol/l CaCl_2 background concentration as well as for a 300 mmol/l NaCl background. The prewetted samples were treated with a DMA concentration of 100 $\mu\text{mol/l}$. Directly afterwards they remained in an overhead shaker for the time intervals described above. Due to the high NaCl and CaCl_2 background concentration within the 100 mmol/l CaCl_2 and 300 mmol/l NaCl series, just 0,5 ml of solution were used for the ICP-MS analysis.

Temperature experiment:

The effect temperature has on DMA related metal mobilization was examined in this experiment. Therefore, four equal experiment series were done at different temperatures. For all four experiments, horizontal shakers were used. The 8 °C, 35 °C and 60 °C experiments were done in an Excella E24 Incubator whereas the 20°C experiment was done in a KS 501digital shaker inside a temperature controlled room. Concerning the 8 °C experiment, the incubator was not able to provide constant temperatures, leading to a temperature spread from 6,4 as a minimum up to 9,1. The mean temperature for all samples in this sub-experiment is around 8 °C. All samples of the four experiments were prewetted at the corresponding temperature and remained in the shaker after adding the free DMA ligand for the usual time intervals mentioned above. The temperature of the centrifuge was also adjusted to the corresponding experiment. Concerning the 60°C experiment, the highest temperature the machine provided was 40 °C. Sodium azide was used as a sterilizing agent on duplicates for each temperature and time series.

Outcome of the Experiments/ Interpretation:

Adsorption of metalDMA complexes (FeDMA, CuDMA, NiDMA and ZnDMA):

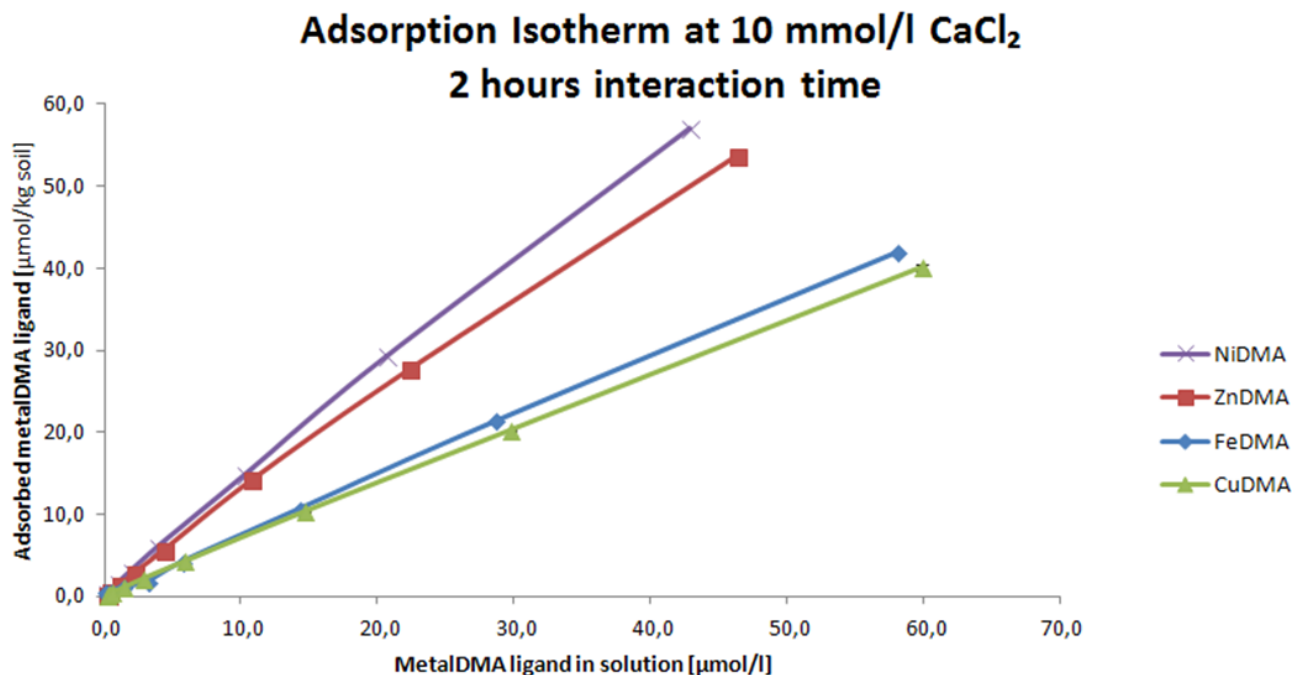


Fig.2: Adsorption Isotherms of FeDMA, CuDMA, NiDMA and ZnDMA. All samples were prewetted, no sterilant was used.

The following chapter addresses the results of the *Adsorption Isotherm Experiments*. There was no sterilizing agent added to the samples, thus biodegradation affects the results of certain metals (Mn and Co) to a certain degree. Nevertheless, concerning the metals looked in this experiment (Fe, Cu, Ni and Zn), other experiments showed that biodegradation is negligible for two hours of interaction time. All samples were prewetted two days in advance with a constant background electrolyte concentration of 10 mmol/l CaCl_2 .

Figure 2 shows four adsorption isotherms determined in the experiment. At the first glance, two different adsorption behavior patterns of metalDMA complexes can be recognized. ZnDMA and NiDMA show higher adsorption onto soil particles than FeDMA and CuDMA do at the same interaction time and electrolyte background concentration. As an example, CuDMA adsorbs to a lesser extent onto soil particles as NiDMA does (about 33 %). Both, NiDMA/ZnDMA and FeDMA/CuDMA show similar adsorption behaviors. After two hours of interaction time, more than fifty percent of the added ZnDMA and NiDMA complexes were already adsorbed onto soil particles, FeDMA and CuDMA were adsorbed to an extent of about forty percent. Although there were no other time series made within this experiment, it is assumed that adsorption equilibrium has already been reached after two hours of interaction time. More adsorption experiments with a broader focus on time intervals would verify that. All curves show a more or less linear shape, thus adsorption occurs linearly over the examined concentration range of metalDMA complexes added to the soil. **Table 2** shows the ratio between

adsorbed metalDMA and the total amount of added metalDMA. All nine concentrations of metalDMA species have more a less the same ratio:

<i>Adsorbed Total amount added</i>	FeDMA	CuDMA	ZnDMA	NiDMA
Mean	0,44	0,42	0,56	0,61
StDEV	0,060	0,030	0,019	0,036

Table 2: The ratio between adsorbed metalDMA and the total amount added for four metalDMA species after 2 hours of interaction time. All nine metalDMA concentrations (from 0,25 $\mu\text{mol/l}$ to 100 $\mu\text{mol/l}$) were used for the calculation of CuDMA, ZnDMA and NiDMA. For FeDMA, 7 concentrations (from 1 $\mu\text{mol/l}$ to 100 $\mu\text{mol/l}$) were used for calculation due to high fluctuatiosn in the low concentration range.

The soil solution ration will also have significant effects on metalDMA adsorptions onto soil particles: In an actual soil with a soil solution ratio of 1:6 (as an example), adsorption might be even higher due to a higher ratio between adsorbate and adsorbent.

The different adsorption behavior of the different metalDMA species may depend on the net charge of each examined metalDMA complex. At pH 7, FeDMA complexes have a negative net charge (von Wiren et al., 2000). There are no data on CuDMA, ZnDMA and NiDMA net charges available, but they are most probably also negative, but to a different extent as FeDMA. Negative net charges for Zn(II) and Fe(III) complexes have been determined for MA and HMA, where Zn(II) complexes have smaller negative net

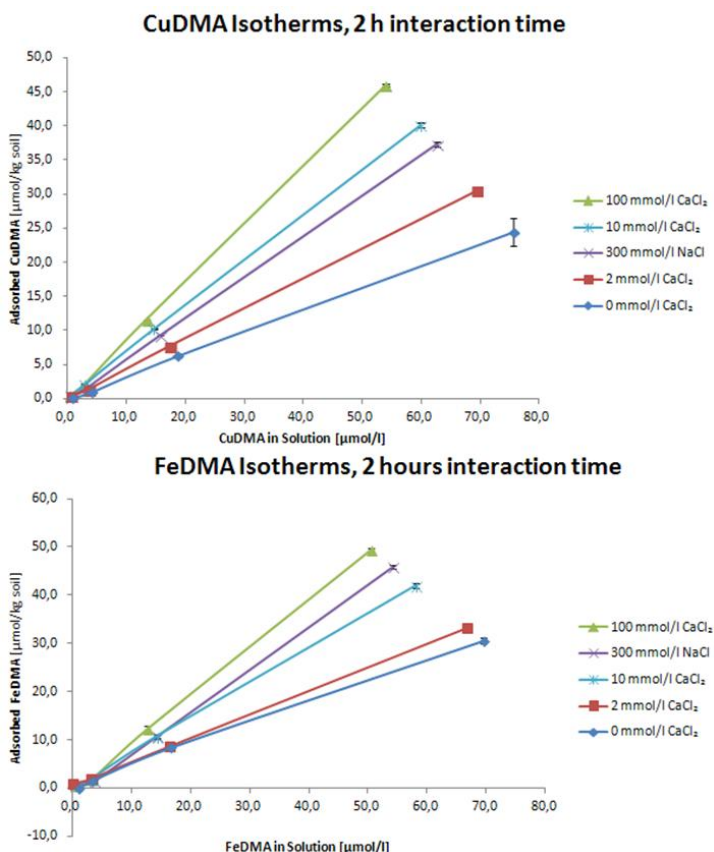


Fig. 3: CuDMA and FeDMA Adsorption Isotherms at different electrolyte background concentrations. All samples were prewetted, no sterilant was used.

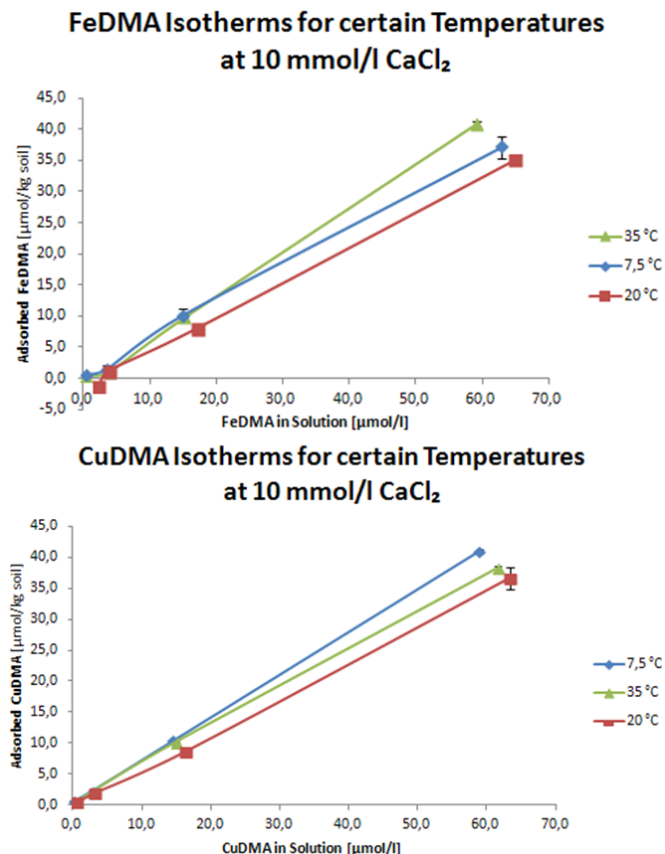


Fig.4: FeDMA and CuDMA Adsorption Isotherms for different temperatures. All samples were prewetted, no sterilant was used.

charges than Fe(III) complexes (von Wiren et al., 2000). 2:1 (Illite, Smectite or Vermiculite) or 2:1:1 (Chlorite) clay minerals and organic matter provide negatively charged surfaces within a soil (Scheffer & Schachtschabel, 2010) and are common adsorbents. Lower negative net charges of metalDMA complexes would probably result in a higher adsorption potential due to the fact that the electrostatic repulsion between adsorbate and adsorbent gets lower. Therefore, CuDMA and FeDMA should have higher negative net charges compared to the net charges of NiDMA and ZnDMA, resulting in a higher repulsion and thus lower adsorption. Assuming that DMA complexes have similar net charges to MA and HMA complexes, this assumption would be well in accordance to the higher net negative charges of Fe(III) in respect to the lower values of Zn(II) already determined by von Wiren et al, (2000).

Figure 3 shows the dependency of FeDMA and CuDMA adsorption on electrolyte background concentrations. In the case of CuDMA, there is nearly twice as much (from 24,4 $\mu\text{mol/l}$ with a significant error bar to 46,0 $\mu\text{mol/l}$) adsorption onto soil particles at high CaCl_2 concentrations (100 mmol/l) in comparison to 0 mmol/l CaCl_2 in the background solution. For FeDMA, this effect is also recognizable, but the spread in adsorption occurs to a lesser extent as seen for CuDMA (at 0 mmol/l CaCl_2 , there is 30,5 $\mu\text{mol/kg}$ FeDMA adsorbed onto soil particles, whereas 100 mmol/l CaCl_2 shows an adsorption of 49,5 $\mu\text{mol/kg}$). For means of comparison, a treatment with a 300 mmol/l NaCl background electrolyte concentration was chosen. Both, the 100 mmol/l CaCl and the 300 mmol/l NaCl electrolyte background have the same ionic strength (more details within *the Metal complexation at different ionic strengths background concentrations* chapter), but show different effects on metalDMA adsorption behavior. Thus adsorption is electrolyte-specific. In the case of CuDMA, there is even less CuDMA adsorption onto soil particles at 100 mmol/l NaCl than at 10 mmol/l CaCl_2 electrolyte background concentration.

As already mentioned within the hypothesis section, increased salt concentration reduces electrostatic repulsion between similarly charged adsorbate and adsorbent materials, hence increasing adsorption (Jones and O'Melia., 2000). Thus, increasing the electrolyte background concentration would decrease electrostatic repulsion between the negatively charged metalDMA complexes and the negatively charged surfaces within the soil, hence increasing adsorption of metalDMA complexes onto soil particles. As already mentioned above, adsorption of metalDMA complexes onto soil particles is electrolyte specific, and not dependant on the overall ionic strength of the system. The lesser adsorption observed during 300 mmol/l NaCl treatments compared to 100 mmol/l CaCl_2 treatments may be explained by the valences of the examined ions, which contribute to the ionic strength. NaCl dissociates to Na^+ and Cl^- ions, both monovalent, whereas CaCl_2 dissociates to 2 Cl^- and Ca^{2+} , the latter being divalent. Ca^{2+} ions probably have a higher affinity to chemically interact with negatively charged surfaces of particles than Na^+ ions have due to a higher charge and a shorter atomic radius. Thus there may be a higher charge neutralization of negatively charged surfaces at high Ca^{2+} compared to high Na^+ concentrations. Charge neutralization would increase the adsorption potential of metalDMA complexes since electrostatic repulsion between adsorbent and adsorbate gets lower. CuDMA is even more adsorbed to soil particles at 10 mmol/l CaCl_2 than at a 300 mmol/l NaCl electrolyte background concentrations, FeDMA at the contrary not. This difference in adsorption behavior might be explained with a higher negative net charge of CuDMA in respect to FeDMA. This would explain the higher spread of CuDMA in adsorption over different electrolyte background concentrations in respect to FeDMA.

Figure 4 shows the adsorption behavior of FeDMA and CuDMA at different temperatures (8 °C, 20 °C and 35 °C). At the first glance the effect different electrolyte background concentrations do on adsorption is much bigger than the temperature dependency on adsorption. For both complexes, the least adsorption takes place at 20 °C, whereas the highest adsorption occurs at 7,5 °C for CuDMA and at 35 °C for FeDMA.

The data on the temperature dependency of metalDMA adsorption onto soil particles determined in this experiment does not fit to the hypothesis made above. It was hypothesized that due a higher Brownian Motion at higher temperatures, metalDMA adsorption occurs to a lesser extent. The sample handling may be responsible for this discrepancy. All samples were at least ten minutes exposed to different temperatures before the separation from the soil occurred, from the time they were taken out the temperature controlled shaker until the time the solution was separated from the soil upon filtration through syringes and filters. Further experiments, where all working steps are done at the same temperature, might provide better results.

Metal complexation at different DMA concentrations:

The next chapter addresses the results of the *Concentration Experiment*. Sodium azide was used for half of the samples as a sterilizing agent, thus biodegradation is negligible in them. All samples were prewetted two days in advance with a constant background electrolyte concentration of 10 mmol/l CaCl_2 .

Figure 5 shows metal mobilization patterns at different added free DMA concentrations (30 $\mu\text{mol/l}$, 100 $\mu\text{mol/l}$ and 1000 $\mu\text{mol/l}$), both with sodium azide and without. All samples treated with sodium azide show a characteristic metal mobilization pattern over time: Starting with the 100 $\mu\text{mol/l}$ DMA treatment, which is the reference DMA treatment in this thesis and also was used within other experiments, there are two distinct mobilization behaviors. Fe and Zn mobilization have mobilization maxima at 24 h (Fe) and 48 h (Zn) interaction time (same pattern as in the prewetting and ionic strength experiment), whereas Cu, Ni and Co have a steady increase in mobilization until the end of the investigated interaction time (168 h). At 168 h, Cu is probably close to its inflection point, whereas the slope of Ni and Co still increases. However, Ni shows a lower slope after 168 hours interaction time compared to Co. At lower DMA concentrations (30 $\mu\text{mol/l}$), there is a shift of horizontal time values in the Fe and Zn maxima towards shorter interaction time intervals. The maximum Fe mobilization is reached after 2 hours, the Zn maximum after 0,5 hours. Furthermore, the maximum Cu mobilization was reached after 48 hours and Ni probably passed its inflection point between 48 and 96 hours. Only the slopes of the Co curve remain increasing after 168 hours of interaction time. At high DMA concentrations (1000 $\mu\text{mol/l}$), none of the metals mentioned above reached its mobilization maximum. Obviously there is a shift of each mobilization maximum in horizontal time values towards longer interaction time intervals. At a DMA concentration of 1000 $\mu\text{mol/l}$ DMA, a substantial amount of Mn gets mobilized, whereas at all lower concentrations and same physicochemical conditions investigated in this master thesis, its mobilization is more or less negligible. Within the first 8 hours of interaction time, it is even the most mobilized metal, probably having its mobilization maximum at 168 hours of interaction time.

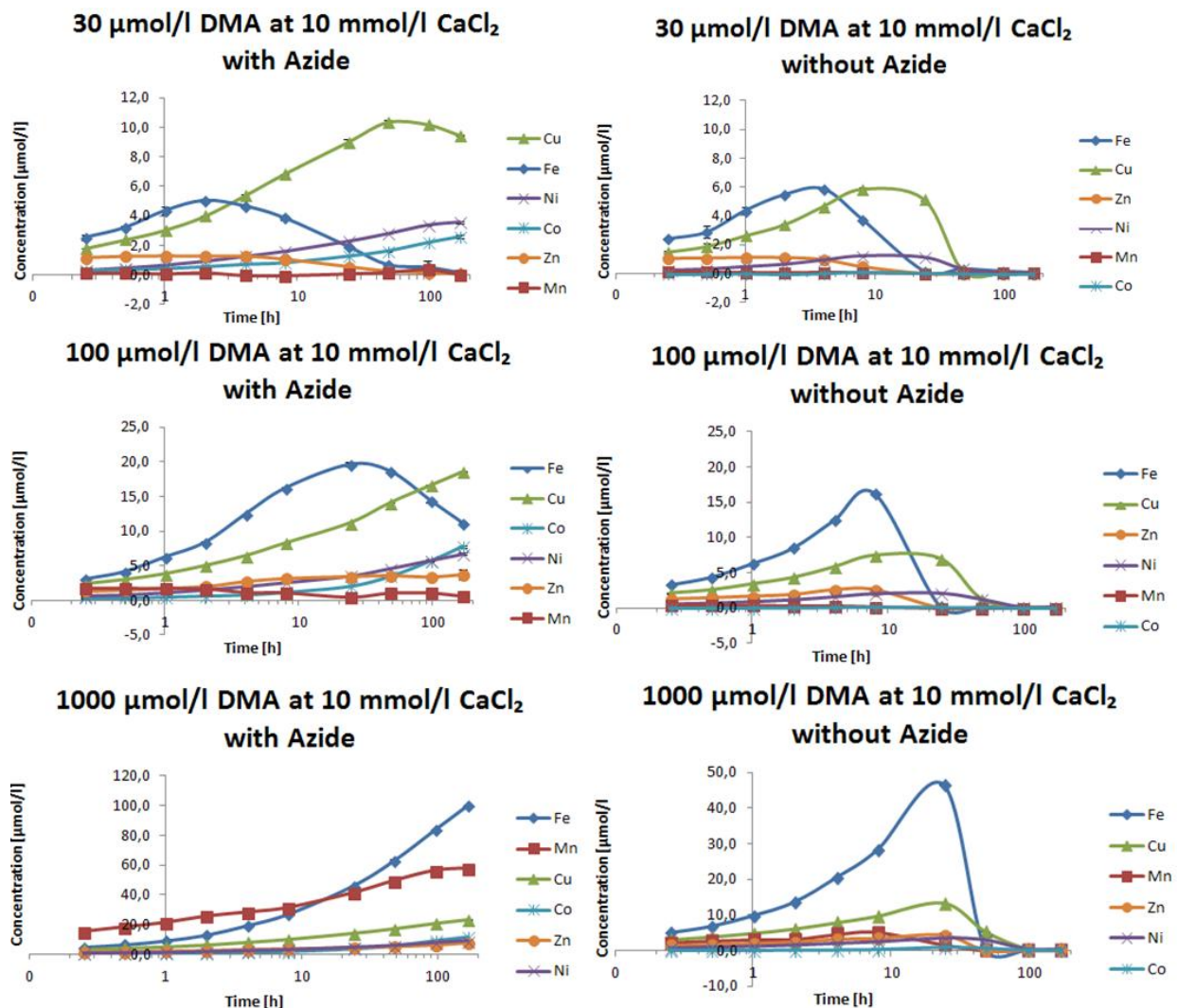


Fig.5: Metal complexation at different DMA concentrations. All samples were

All samples without an added sterilizing agent (sodium azide) show a rapid decline in metalDMA complex concentration in solution after 8-24 hours of interaction time due to biodegradation. After that time, the metalDMA concentration in solution approaches and later finally equals the background metal concentration within the solution. For all DMA concentrations (30, 100 and 1000 µmol/l), the shapes of the metal mobilization curves within the first hours of interaction time equal the shapes of the metal mobilization curves within the sodium azide treatment, before substantial biodegradation occurs. However, at high concentrations this does not apply for Mn, which is not as substantially mobilized as it is the case within the corresponding sodium azide treatment. The decline of metalDMA complexes does not take place at the same time at different DMA amounts added to the soil, what is well visible in the shift of the Fe mobilization maxima in abscissa time values. This may be explained with a combination of biodegradation and the competition effect. For both sterilized and unsterilized treatments it is noteworthy that within short interaction times, the total amount of free DMA ligand added to the soil does not make as substantial differences in metal mobilization as for longer interaction times. Within short interaction times, all unsterilized samples show a higher sum of metal mobilization as it is the case within the sterilized samples. This is most probably caused by the contribution sodium azide does to the ionic strength (more explanations within the “Other outcomes of the Experiments” section).

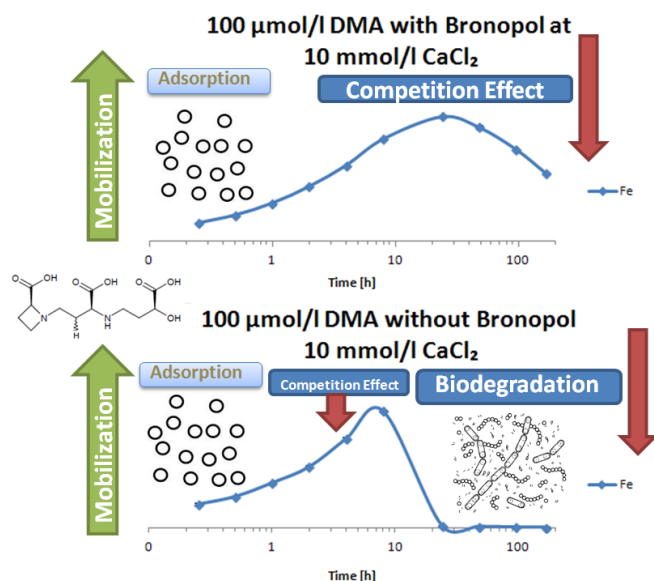


Fig.6: Factors which determine the shape of metal mobilization curves. Biodegradation largely influence unsterilized mobilization curves after 8-24 h and causes a decrease of metalDMA concentration. Adsorption of metalDMA complexes occurs fast and reduces the bioavailability. Competition Effects influence especially sterilized mobilization curves, where no biodegradation occurs. Weak metals (Fe, Zn) are outcompeted by Cu, Ni or Co.

There are several parameters which determine the outcome of this experiment. At first, the shapes of the Fe and Zn mobilization curves are to a certain degree determined by competition effects with divalent metals, especially Cu (and Mn at high DMA concentrations). Although Fe is the dominant metal species at short interaction times, it is outcompeted by other metals (except for Zn) due to its low activity in soils (Schenkeveld et al., 2014 (accepted)), even though it has the second highest stability constant after Cu (Murakami et al., 1989). The reason why it is the dominant metal species at short interaction times might be the high free energy of complex formation (there are data on HMA³⁻ ligands available (Kato et al., 2011), but unfortunately not on DMA). Competition between the examined divalent metals and Fe(III) determines the shape of the mobilization curves of Fe, especially after it passed its inflection point and mobilization maximum. Including the adsorption data

from the first experiment, the shape of the metal mobilization curves may be further explained. After two hours interaction time, there was already substantial adsorption of metalDMA complexes onto soil particles examined (read above). The Adsorption Ratio ($\frac{\text{Adsorbed}}{\text{Total amount added}}$) remained the same over different concentrations of metalDMA added to the soil (look above). Hence, the shape of the metal mobilization curves, and thus the time depending metalDMA concentrations within the system, should not be affected by adsorption processes after the adsorption equilibrium has been reached. Before reaching the adsorption equilibrium, the shape of the curves might be affected. It is however not possible to determine the effect of a non adsorption equilibrium on the mobilization curves with the dataset available in this thesis, because it is uncertain when the equilibrium is achieved. The start of the biodegradation after a certain interaction time (4-8 hours) of metalDMA complexes in solution may be best explained with the lag phase of bacterial substrate consumption correlated to the input of a new and easy biodegradable compound (the metal DMA complexes). After a certain amount of time, bacterial consumption of metalDMA complexes reaches a certain extent. Hence all metalDMA complexes can be biodegraded rapidly after sufficient interaction times. Within the sodium azide treatments, a shift of the Fe maxima in time abscissa values can be observed. Thus, Fe at higher DMA concentrations is not as rapidly outcompeted by other metals as it is at lower DMA concentrations. At high DMA concentrations, this may be due to the fact that free DMA remains in the system for a longer

time as for lower concentrations (assuming a constant complexation rate for DMA), thus preventing FeDMA to be outcompeted because there is still possibility for binding left which eliminates the reasons of competition. At a certain ratio (free DMA : complexed DMA), the out-competition of FeDMA by CuDMA takes overhand. This time point may be correlated with the inflection point of the iron mobilization curve, where the slope of the iron mobilization curve decreases for the first time within the interaction time. An even lower free DMA : complexed DMA ratio probably causes CuDMA to be out-competed by CoDMA and NiDMA after a longer interaction time. However, after long interaction times (168 hours) within the sterilized, 30 $\mu\text{mol/l}$ treatment, all slopes of the metal mobilization curves decline, except for Co. There is a statistical significant decrease of total DMA from 16,8 $\mu\text{mol/l}$ after 98 hours to 15,9 $\mu\text{mol/l}$ after 168 hours of interaction time. Hence, this effect cannot be explained with competition effects. It may be explained with the total complexation of free DMA within a system. At low concentration, this occurs faster than in all other examined treatments assuming a constant complexation rate of DMA (for more explanations read the *“Other outcomes of the experiments”* chapter). The relative shift of the iron mobilization curve within the unsterilized treatments may be explained with both competition and biodegradation effects. When looking at the Fe mobilization curve of the 30 $\mu\text{mol/l}$ treatment (without sodium azide), the first decline of the slope of the curve after 1-2 hours may be due to the competition effect (which is accelerated due to the low free DMA content), whereas the total decline of the curve after 8 hours is probably caused by biodegradation. On the contrary, the Fe mobilization curve of the 1000 $\mu\text{mol/l}$ treatment (without sodium azide) shows no competition effect, because there is not sufficient time before biodegradation starts due to the high free DMA concentration. Another observation deals with the high amount of Mn mobilized at high DMA concentrations. For 1000 $\mu\text{mol/l}$ DMA treatment with sodium azide, there is a more or less steady rise in MnDMA throughout the observed time, probably lowering the mobilization of other bivalent metals. Fe however seems to be unaffected by the high Mn concentration. Comparing the high Mn concentrations with the corresponding treatment without added sodium azide added, MnDMA is probably pretty vulnerable to biodegradation. Thus it is not to a substantial amount mobilized, but still more than at lower DMA concentrations.

Beside the differences in mobilization patterns described above, the question remains if an increase in total DMA added to the system results in a linear increase in metal mobilization. After 24 hours of interaction time within the unsterilized treatments, there is about 17 $\mu\text{mol/l}$ Fe mobilization at 100 $\mu\text{mol/l}$ DMA, but only about 49 $\mu\text{mol/l}$ Fe mobilization at 1000 $\mu\text{mol/l}$ DMA, representing a ligand concentration ten times higher. This fact may be explained by a higher stimulation of bacterial activity due to a higher substrate availability and thus higher biodegradation rates. At high concentrations (1000 $\mu\text{mol/l}$) within the sterilized treatment, it is hard to make statements on that because no element reached its mobilization maximum within the examined interaction time. For lower concentrations however (30 and 100 $\mu\text{mol/l}$ DMA added, a decrease in concentration of the factor of 3,33), this does not apply. For the sterilized 30 and 100 $\mu\text{mol/l}$ treatments, the iron mobilization maximum after 24 hours of interaction time shows a corresponding linear decrease in mobilization around the factor of 3,33. The contribution sodium azide does on the ionic strength has to be included to these comparisons (more explanations within the *“Other outcomes of the Experiments”* section).

Metal complexation at different moisture regimes:

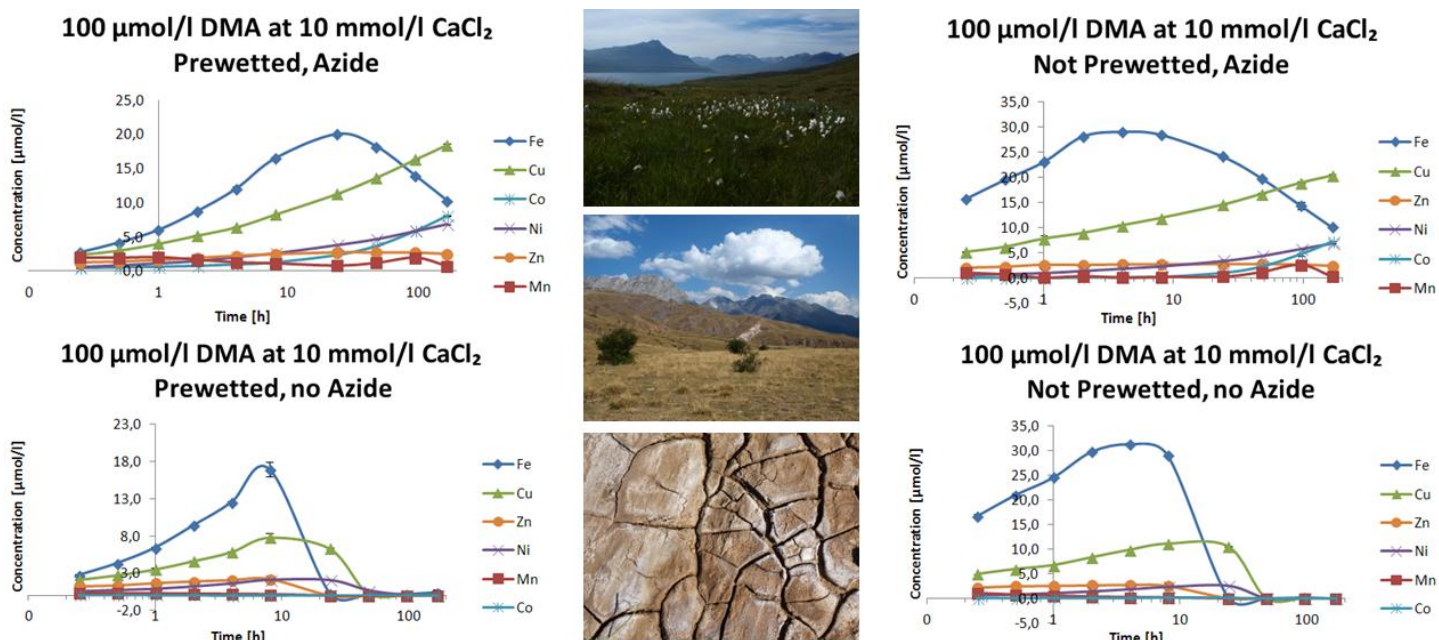


Fig. 7: Metal mobilization at different moisture regimes. The pictures denote different environments with different moisture regimes: Upper picture: Eastern fjords, Iceland humid,. Middle Picture: Pyrenees in Spain, semiarid. Lower Picture: Dry Cracks near Lake Eyasi, Tanzania, arid.

The third chapter addresses the results of the *Prewetting Experiment*. Sodium azide was used for half of the samples as a sterilizing agent, thus biodegradation is negligible in them. Half of the samples were prewetted two days in advance. All samples have a constant background electrolyte concentration of 10 mmol/l CaCl_2 .

Figure 7 shows the four different treatments of the *Prewetting Experiment*. Both sterilized and unsterilized, prewetted treatments can be analogously explained as already done above in chapter two: *Metal complexation at different DMA concentrations*. Samples which were not prewetted show however a higher metal mobilization than samples which were prewetted two days in advance. For both treatments (with and without sterilizing agent), this difference in Fe mobilization between prewetted and unpwetted samples occurs approximately by a factor of about 3,5 after 1 hour of interaction time. For longer interaction times (before biodegradation starts), this difference decreases. When looking at the Fe mobilization maxima within the sterilized treatments, it is noteworthy that the iron mobilization maximum within the unpwetted treatment occurs at shorter interaction times. **Figure 8** shows different mobilization patterns for Fe, Cu, Ni, Co and Zn under different treatments. The mobilization of Ni does not seem to be affected if the samples are prewetted or not. Cu and Zn are affected by prewetting, but to a lesser extent as Fe. Noteworthy mobilization of Co only occurs in sample were sodium azide was added. Thus it is well visible that it probably is rather vulnerable to biodegradation.

Neither biodegradation of metalDMA complexes nor adsorption of them onto soil particles are able to explain the difference in metal mobilization between prewetted and unpwetted samples. If

biodegradation was lower in unpretreated samples due to the absence of water before the system got wetted, which could result in a narrower bacterial growth curve upon adding the free DMA ligand, there would be a difference in mobilization between the unsterilized samples, but not a different amount of mobilization for both sodium azide treatments. Biodegradation occurs within both unsterilized treatments between 8 and 24 hours. Furthermore adsorption does not influence the different amount of mobilization. Within a test experiment, that has been done prior to the adsorption experiment described above, the difference of adsorption between pretreated and unpretreated samples was examined. The ICP-MS data of table 2 show that there is no substantial difference in CuDMA adsorption onto soil particles after two hours interaction time for both pretreated and unpretreated samples.

Adsorption of CuDMA	Sodium Azide [$\mu\text{mol/l}$]	Bronopol [$\mu\text{mol/l}$]	No Sterilant [$\mu\text{mol/l}$]
Pretreated	19,20 (1,18)	19,15 (0,45)	19,08 (0,35)
Not Pretreated	19,39 (0,25)	20,72 (0,80)	20,13 (0,58)

Table 3: ICP-MS data of CuDMA adsorption onto soil particles after two hours of interaction time and with different sterilizing treatments. 30 $\mu\text{mol/l}$ CuDMA was added to the samples. The values in the table represent the measured CuDMA concentration in solution. Within the braces, the Standard Deviations are denoted.

When soil dries out, OM and microorganisms lose their molecular integrity. OM shrinks whereas microorganisms die due to lysis. Upon rewetting the dried soil, the damaged OM builds a pool of relatively weak bonded macro and micronutrients. If the recently wetted soil stays wet, a new equilibrium will be achieved, and weak bonded metals will precipitate or be adsorbed to soil particles and thus be less available. This process probably also occurs during metal acquisition by phytosiderophores. Upon rewetting the soil (unpretreated samples), metals are more easily complexed by phytosiderophores than when this equilibrium is already reached after the soil has been wetted for a sufficient time (pretreated samples). This explanation may be further confirmed by the fact that big differences in mobilization between pretreated and unpretreated samples only occur for elements which are to a substantial amount present in organic matter. For the examined metals in this work, their abundance in organic matter declines as follows: Fe > Mn > Zn > Cu and Ni (Scheffer & Schachtschabel, 2010). This is well in accordance with the difference of mobilization of single elements between pretreated and unpretreated samples in **Figure 8**. The higher mobilization of Co within the pretreated, sterilized treatment however does at the first glance not fit to that explanation. Co is obviously more mobilized under pretreated conditions, which is only visible under sterilized conditions due to the very weakness of CoDMA in respect to biodegradation. This might be explained with the fact that Co is considered as a useful element rather than a micronutrient. (Scheffer & Schachtschabel, 2010) The latter is necessary for survival, whereas the presence of useful elements only promote growth of certain plants (Scheffer & Schachtschabel, 2010). Thus organic matter is probably not an important source of Co, hence there is no substantial Co mobilization within unpretreated samples. Dry and wet cycles may also play an important role on aggregate stability, and thus on different amount of metal mobilization. According to Cosentino et al., (2006), aggregation depends on dry-wet cycles, microbial activity and the content of organic matter. Formation of aggregates may decrease the surfaces where DMA

complexation potentially occurs, thus decreasing metal mobilization within all prewetted samples. The data from the *prewetting experiment* also allow a more profound insight on how biodegradation of metalDMA complexes works. The rapid consumption of metalDMA complexes within unsterilized treatments may be due to a lag phase like bacterial growth or substrate consumption upon adding the ligand to the soil. Due to the fact that there were no substantial differences in biodegradation examined between prewetted and unprewetted samples, a lag phase like bacterial growth upon adding the free DMA may be falsified because bacterial growth would certainly be favored within the prewetted samples. Excluding the lag phase like bacterial growth as the main reason for biodegradation, a lag phase like adaptation of microorganisms on DMA as a substrate seems to be a more reliable explanation.

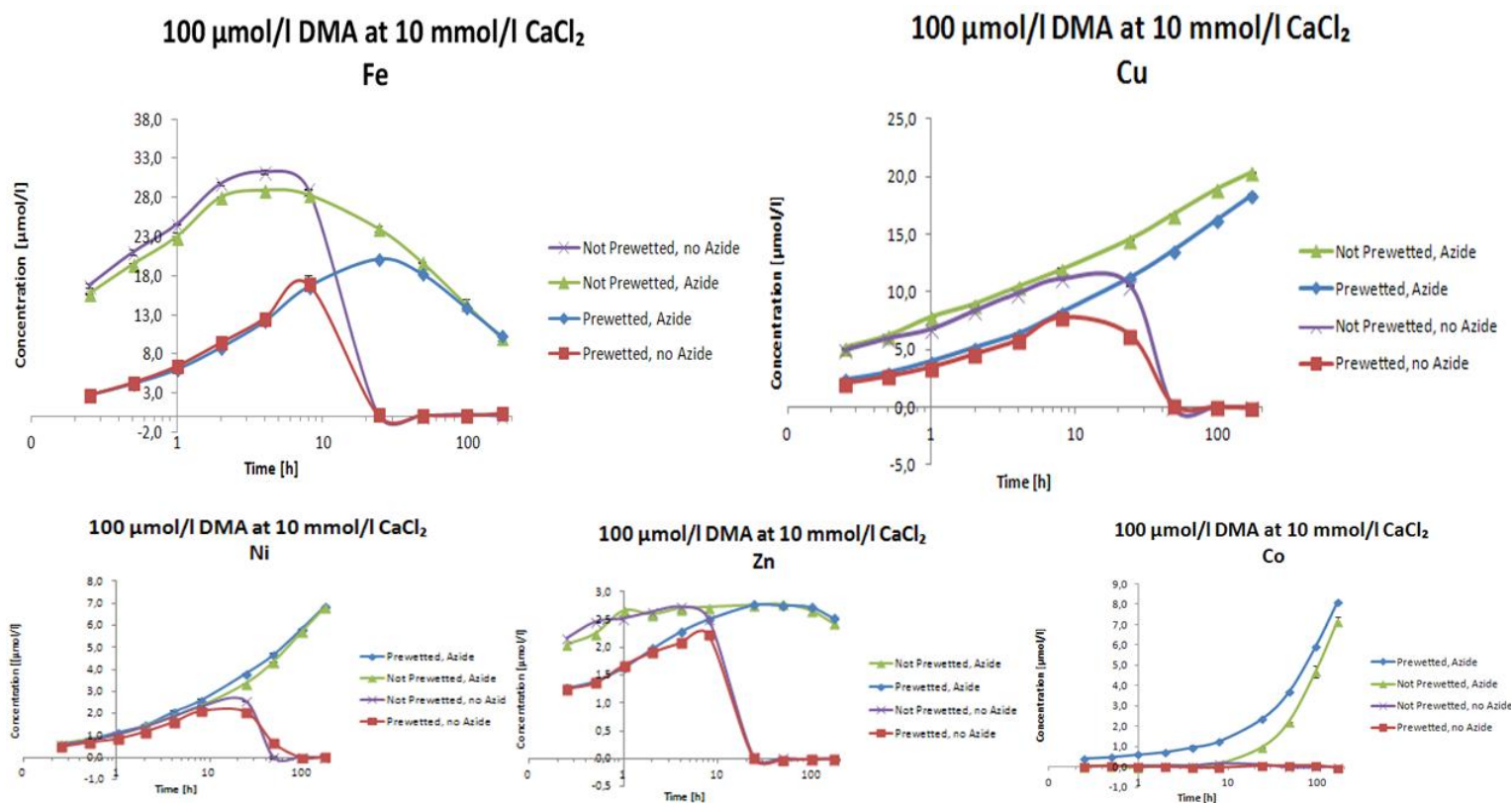


Fig. 8: Different electrolyte background concentrations for single elements. All samples were treated with 100 µmol/l DMA and 10 mmol/l CaCl₂

Metal complexation at different ionic strength background electrolyte concentrations:

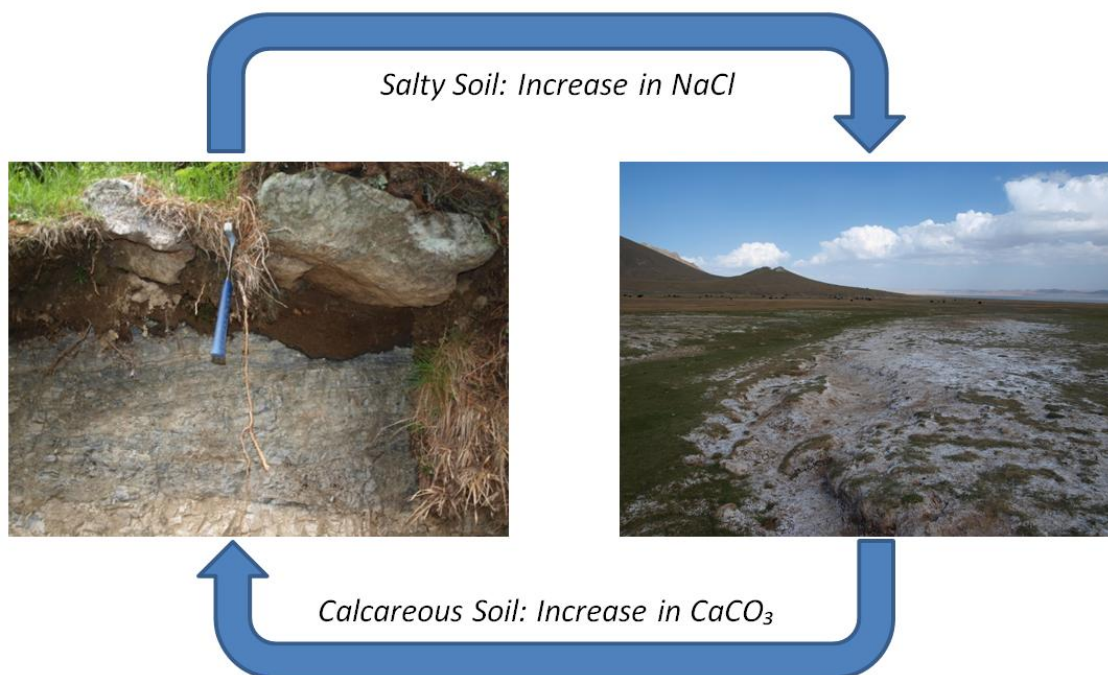


Fig. 9: This chapter focuses on iron and metal acquisition and its inhibition in calcareous soils with a high CaCO_3 content, and salty soils with a high NaCl content. The left picture demonstrates a calcareous soil, Nockalmberge, Austria. The right picture demonstrates a salty soil near Lake Chatyr Kol, Kyrgyzstan.

The following chapter addresses the results of the Ionic Strengths Experiment. Bronopol was added to all samples, thus biodegradation does not occur (or is at least negligible) and therefore does not affect the results. All samples were prewetted two days in advance.

Figure 10 shows DMA induced metal mobilization at different background electrolyte concentrations. Although all different treatments were done at different ionic strength background concentrations, all of the six examined metals (Fe, Cu, Ni, Co, Zn and Mn) show the same pattern of metal mobilization over time. The patterns of the mobilization curves can be analogously explained as done in chapter two: "Metal complexation at different DMA concentrations". Beside the elements mentioned above, there is no noteworthy Mn mobilization pattern. Although the patterns of the mobilization curves do not change with varying electrolyte background concentrations in their horizontal time values, the

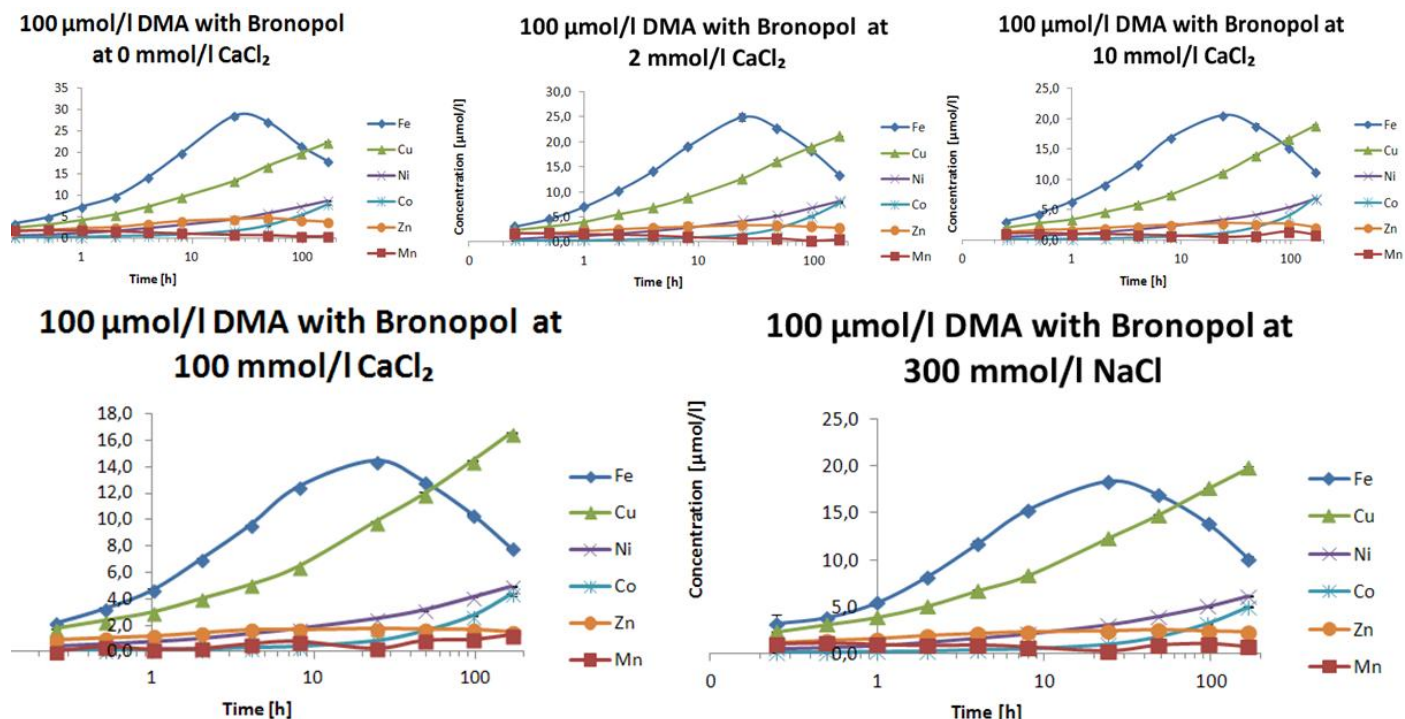


Fig. 10: Metal mobilization at different background concentrations. All samples were prewetted.

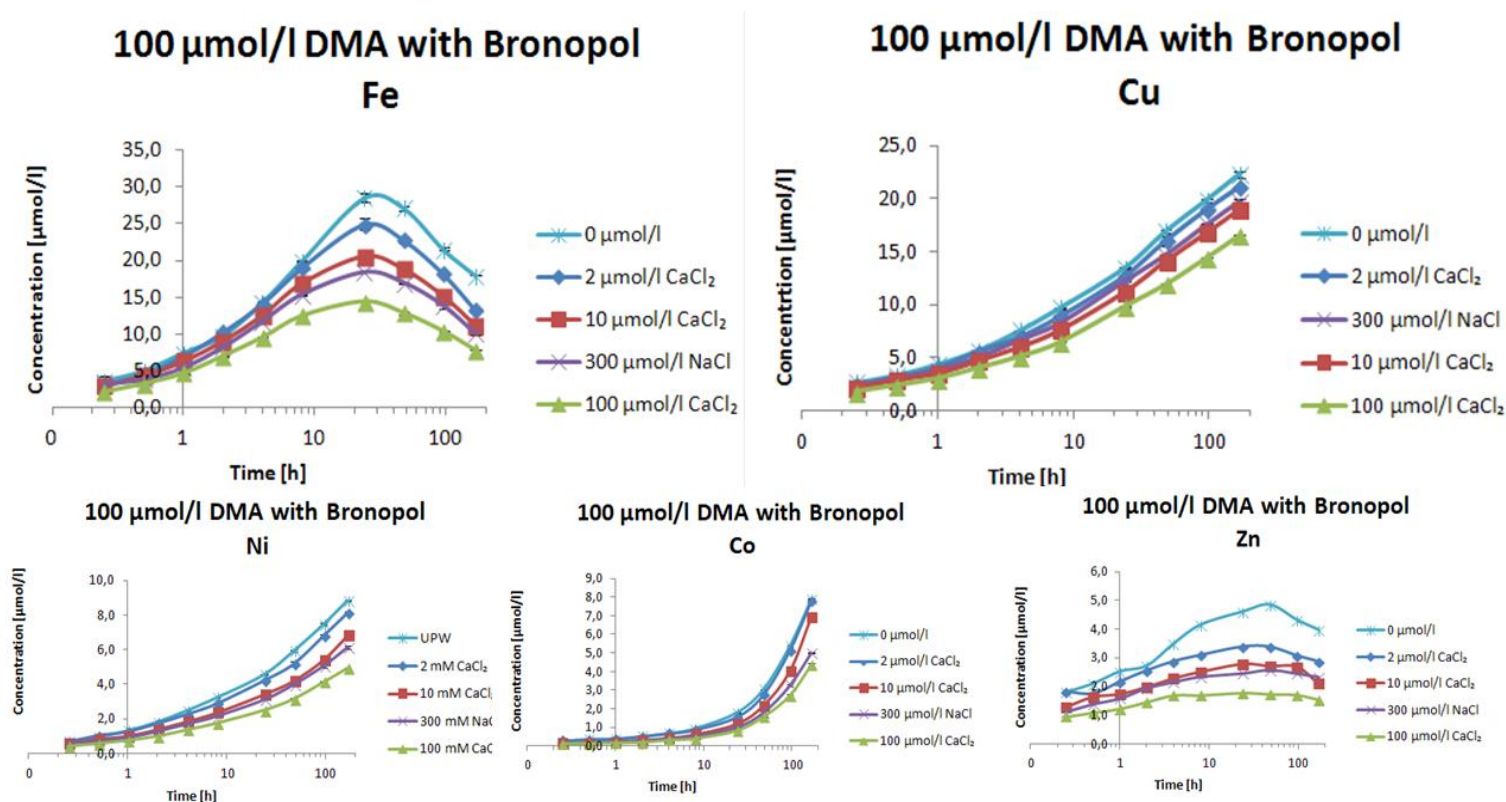


Fig. 11: Element mobilization at different background electrolyte concentrations. All samples were prewetted.

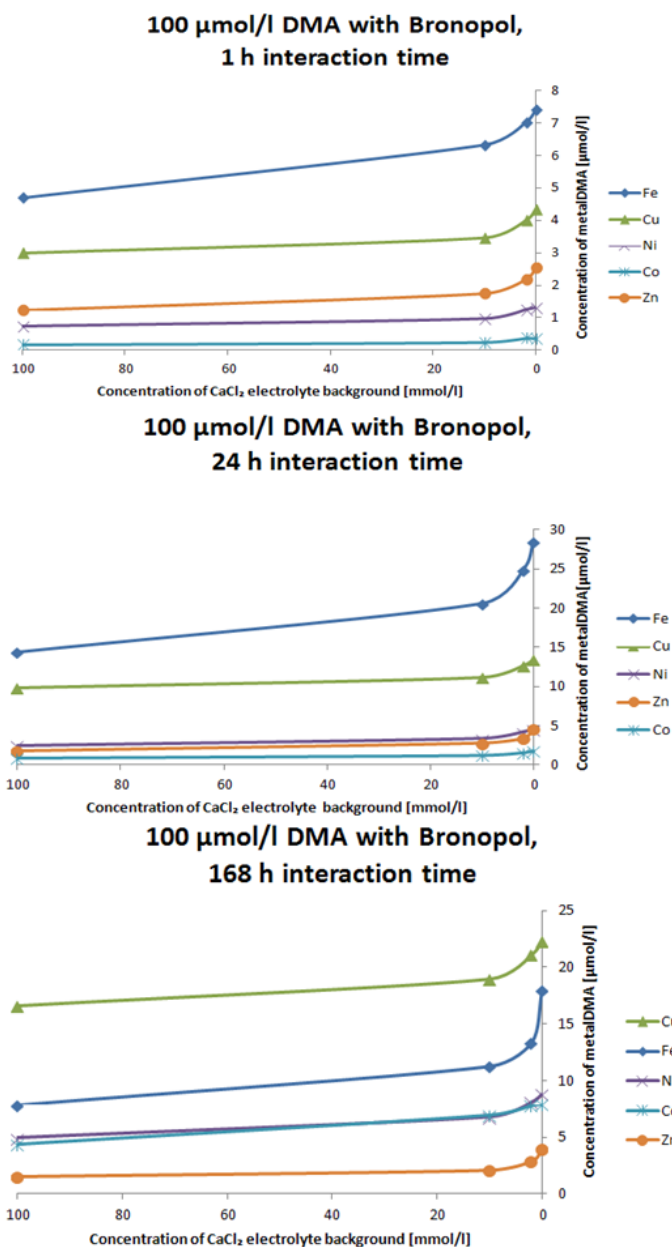


Figure 12: Different amount of metal mobilization at different CaCl_2 background concentrations for three different interaction times (1h, 24 h and 168 h).

time for plants at which major exudation amount occur, the time between one daily circle (1h to 24 h interaction time), all metals apart from Fe are more or less mobilized to a same extent for a CaCl_2 background electrolyte concentration of 10 mmol/l and 100 mmol/l. At lower (< 10 mmol/l) CaCl_2 background concentrations, the slope of the metal mobilization curves increases exponentially. At long interaction times (168 hours), the difference in mobilization for the other examined metals (Cu, Ni, Zn and Co) between 10 mmol/l and 100 mmol/l CaCl_2 background concentration gets higher. Fe shows a

amount of each metal mobilized by DMA changes. When looking at the Fe ordinate values of the diagrams in [figure 10](#), there is nearly twice as much mobilization of Fe after 24 h of interaction time at 0 mmol/l CaCl_2 in comparison to 100 mmol/l CaCl_2 electrolyte concentration. [Figure 11](#) shows mobilization of single elements by DMA plotted over different electrolyte background concentrations. Beside Fe, the mobilization of other metals (Cu, Ni, Co and Zn) is also dependant on the ionic strength background concentration. In the case of Ni and Co, DMA induced mobilization at a high background CaCl_2 concentration is lesser by a factor of approximately two after about 24 to 48 h of interaction time, thus they are well comparable to Fe. Although there was just a reasonably good Zn signal measured, it denotes that there is even a factor in mobilization of approximately 4 between 0 mmol/l CaCl_2 and 100 mmol/l CaCl_2 background concentrations after 168 h interaction time. Cu shows a less extreme spread between 0 mmol/l CaCl_2 and 100 mmol/l CaCl_2 background concentrations, there is a factor of approximately 1,4 after 168 h of interaction time. [Figure 12](#) illustrates the different amount of metal mobilization at different CaCl_2 background concentrations for three different interaction times (1h, 24 h and 168 h).

Considering the important interaction

different pattern: For all three interaction times, there is a noteworthy difference in DMA induced Fe mobilization between 10 mmol/l and 100 mmol/l CaCl_2 background concentration. Moreover, the slopes of the iron curves at lower (< 10 mmol/l) CaCl_2 background concentrations are higher than for all other examined metals at 24 and 168 hours of interaction time.

For means of comparison, a second, monovalent electrolyte treatment was chosen. According to the formula of the ionic strength:

$$I = \frac{1}{2} \sum_{i=1}^n c_i z_i^2 \quad (4)$$

a 300 mmol/l NaCl background concentration was chosen for another treatment, thus having the same ionic strengths as samples treated with a 100 mmol/l CaCl_2 background electrolyte concentration. The comparison between high CaCl_2 and NaCl electrolyte background concentrations shows that more metal mobilization occurs at high NaCl rather than at high CaCl_2 concentrations. Except for Cu, the NaCl curve of all elements plots between the 10 and 100 mmol/l CaCl_2 curve. In the case of Cu, there even is slightly more Cu mobilization at 300 mmol/l NaCl background concentration compared to a 10 mmol/l CaCl_2 background. The same trend is also visible in the adsorption isotherm of CuDMA in [figure 3](#).

The different amounts of metal mobilization at different electrolyte background concentrations may be best explained with the results of the adsorption isotherm experiment within the chapter “*Adsorption of metalDMA complexes (FeDMA, CuDMA, NiDMA and ZnDMA)*”: Adsorption behavior of FeDMA and CuDMA was examined at corresponding electrolyte background concentrations. The highest metalDMA adsorption onto soil particles occurred at high electrolyte background concentrations. Thus, metal mobilization during this experiment is well in accordance with the results determined within the adsorption experiment. As already examined within the “*Adsorption of metalDMA complexes (FeDMA, CuDMA, NiDMA and ZnDMA)*” chapter, CuDMA adsorbs to a higher extent onto soil particles than FeDMA does over a range of different electrolyte background concentrations. This observation may be used to analyze the data in this section. The data on CuDMA and FeDMA mobilization in [Fig 11](#) is well in accordance with the observations made in the *adsorption isotherm experiments*: CuDMA adsorbs to a higher extent than FeDMA does, thus lower mobilization was measured within this experiment.

Metal complexation at different temperatures:

This chapter addresses the results of the Temperature Experiment. Sodium azide was added to half of the samples, thus biodegradation does not occur in them (or is at least negligible) and therefore does not affect the results. All samples were prewetted two days in advance. For this experiment, there were some difficulties at higher temperatures (evaporation losses etc.), thus the 35°C and 60°C curves have some significant error bars which denote the standard deviation. They are longer than in the other experiments described previously. The plotted ICP-OES data were compared to calculated ICP-MS data and are in spite of the big error bars more or less identical.

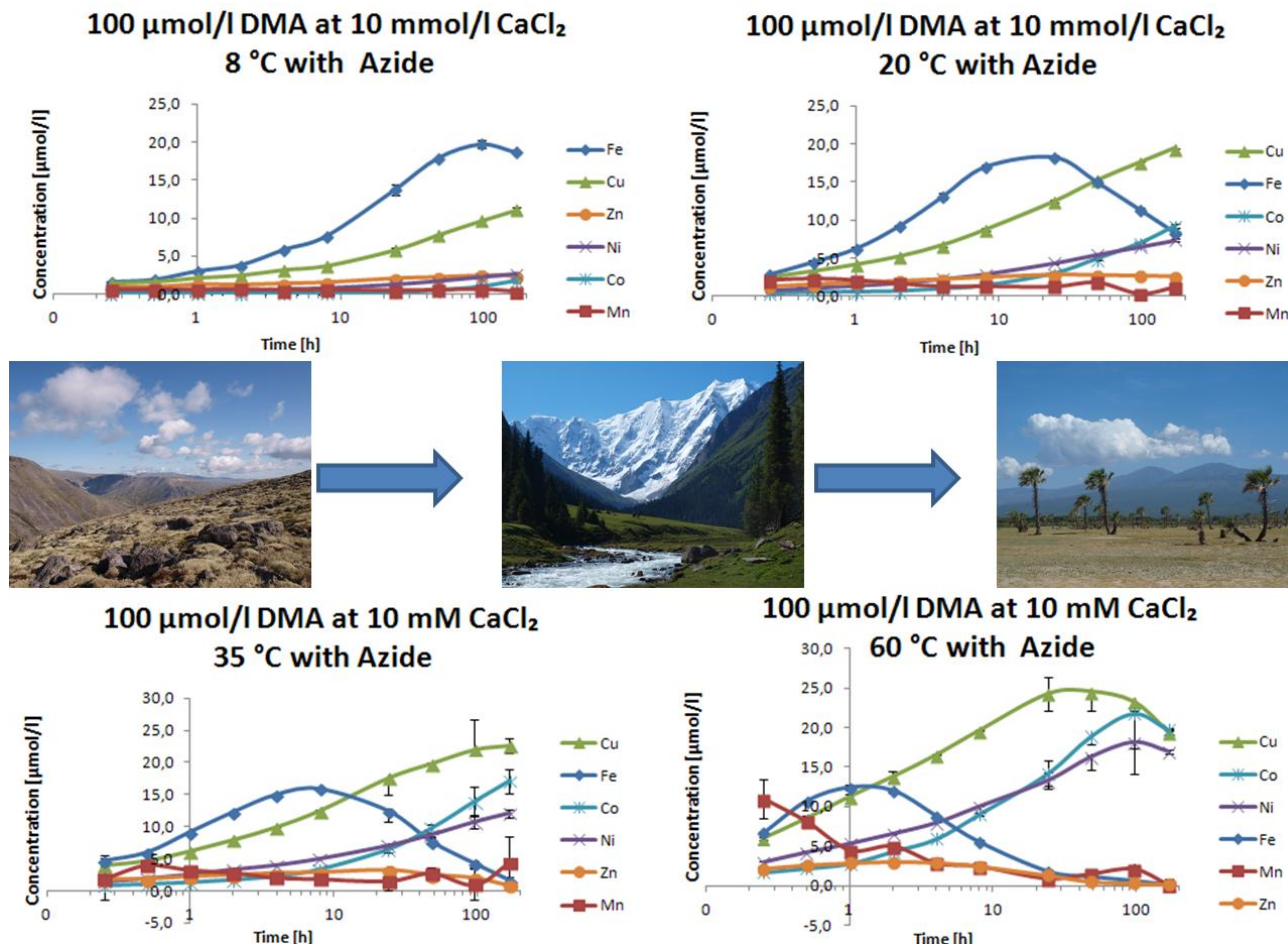


Fig. 13: Metal mobilization under different temperature regimes. All samples were prewetted and treated with sodium azide. The pictures denote the more or less corresponding environments in different climate zones. Left picture: Khibiny at Kola Peninsula, Russia. Middle picture: Jeti Oguz Valley, Kyrgyzstan. Right picture: Nearby Lake Eyasi, Tanzania.

Figure 13 shows the metal mobilization curves determined from all samples of the sodium azide treatments. The shapes of the curves can be analogously explained as done above. Within the four examined temperatures, two shifts in the metal mobilization curves deserve mention. The first shift concerns the reaction time, at which the mobilization maximum of the Fe and Zn curves occurs. At lower temperatures (8°C), the Fe mobilization peak occurs after a long interaction time (approximately after

96 hours). At 20 °C, this peak occurs after 24 hours, at 35 °C after 8 hours, and finally, at 60°C, after 1 hour. The occurrence of the maximum mobilizing Fe peak in time is thus strongly dependant on the surrounding temperature. Another shift can be recognized. The second shift of the mobilization curve concerns the ordinate concentration values. It is well visible that the peak of the iron mobilization curves moves towards lower concentrations with increasing temperatures. The divalent metals however behave differently, showing an increase in mobilization towards higher temperatures (at long interaction times). At 35°C, Cu already passed its inflection point. At 60°C, Cu reaches a mobilization maximum after about 24-48 hours of interaction time, thus being comparable in shape to the Fe mobilization curve. Moreover, at 60 °C, Co and Ni also show a mobilization maximum at long interaction times, both after 96 hours. In the case of Ni, it is unfortunately not feasible to exactly determine the shape of the curve after long interaction times due to the error bars. Beside the mobilization maximum, a continuous rise in mobilization also lies in the boundaries of the error bars of Ni. Concerning the Ni curve of the 60°C, unsterilized treatment, a mobilization maximum is visible. This would suggest that, due to the probable absence of biodegradation within this treatment (explanation later), the mobilization curve of Ni also shows a maximum within the sterilized treatment. Comparing the ICP-OES data plotted in this work with the ICP-MS data, there is however no doubt that the NiDMA concentration goes down after 168 hours interaction time ([figure 16](#) shows plots of the ICP-MS data). As seen for low DMA concentrations (30 µmol/l), the total metalDMA concentration at 60° and 100 µmol/l goes down. There are statistical lower Co and Ni values at 168 hours of interaction time than at 98 hours. [Figure 14](#) shows the temperature dependant mobilization for all unsterilized samples. The shapes of the mobilization curves can also be analogously explained as already done above. The shifts of the mobilization maxima in interaction time and concentration can also be observed for the unsterilized treatments. However, there is one major difference comparing the 60°C, unsterilized treatment with all of the other unsterilized treatments previously described in this work. Comparing both 60°C treatments in this experiment (sterilized and unsterilized), it is well visible that there are no major differences in the shape of the mobilization curves and even in the ordinate concentration values. Mn mobilization occurs to a higher extent at high temperatures (60 °C). Within the sterilized samples, a continuous rise in Mn mobilization from 8°C to 60°C is observable. There is also substantial Mn mobilization within the 60°C, unsterilized treatment. [Figure 15](#) shows the temperature dependant mobilization of each element (except for Mn and Zn, which only delivered reasonable satisfying signals). For all treatments with sodium azide added, the shifts of the mobilization maxima in concentration and time described above can be seen. All unsterilized samples can also be described with the two shifts mentioned above. However, the difference in metal mobilization between low (35°C, 20°C and 8°C) and high (60°C) temperatures is well visible, especially in case of Ni and Co.

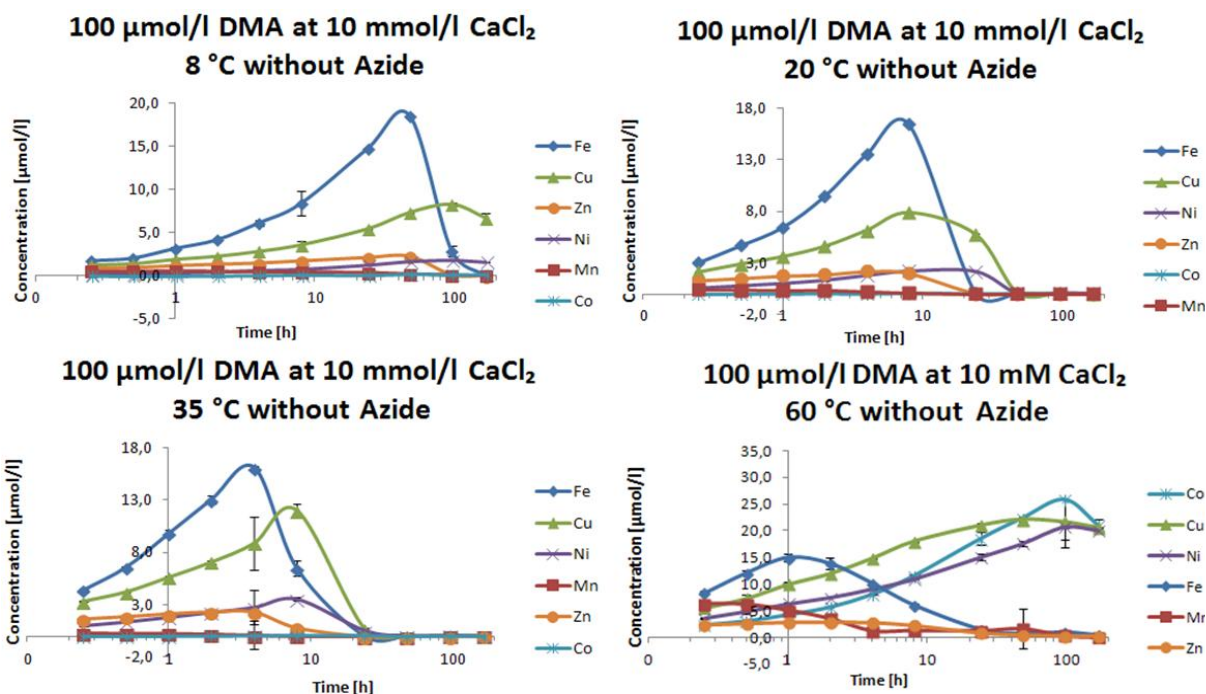


Fig. 14: Metal mobilization under different temperature regimes without a sterilant used. All samples are prewetted.

This difference in mobilization occurs most probably due to the absence of microorganisms and thus biodegradation of metalDMA complexes at high temperatures (60°). Especially for MnDMA and CoDMA, which were already identified in this thesis as particularly vulnerable to biodegradation, there is only substantial mobilization at high temperatures due to low microbial activity. The observed mobilization maximum of Cu may be best explained with Cu being outcompeted by Ni and Co. However, the fact that under sterile conditions and high temperatures (60°C) all metal concentrations go down, cannot be only described with competition effects. Hence, at high temperatures there has to be another effect responsible for decreasing the metalDMA concentration in solution. This observation has already been reported within the “*Metal complexation at different DMA concentrations*” chapter. It is supposed that higher temperatures accelerate this effect. It may be correlated to the complete complexation of the free DMA concentration within the system, which decreases faster at higher temperatures and lower concentrations (for further discussion see the chapter: “Other outcomes of the experiments”). Furthermore, the absence of biodegradation explains the more or less equal shape of the 60° mobilization curves in both sterilized and unsterilized treatments. The 60°C mobilization curves within the unsterilized treatment show higher concentration values than the corresponding mobilization curves within the sodium azide treatments. This might be due to the contribution of sodium azide to the ionic strength, which results in lower mobilization (further explanations later). The Fe and Cu curves of the unsterilized treatments denote that biodegradation happens to the highest extent within the 35°C samples, followed by the 20°C and 8°C samples. This is well in accordance with the optimum growth temperature of most bacteria. At 60°C, as already mentioned above, bacterial growth is negligible.

The shift in horizontal reaction time values and ordinate concentration values, as described above, might be explained as follows: The shift of the mobilization maxima of the examined metals in reaction time may be best explained with accelerated reaction kinetics, speeded up by higher temperatures within the system. However, the shift in ordinate concentration values might also be explained with accelerated reaction kinetics. When looking at Fe mobilization curves, the highest mobilization occurs at low temperatures (8°C). At higher temperatures (60°C, 35°C), the amount of free DMA probably decreases faster. Hence, competition effects start occurring at earlier interaction times (for explanation look above), thus lowering the concentrations of metals with a higher out-competition potential, such as Fe and later Cu.

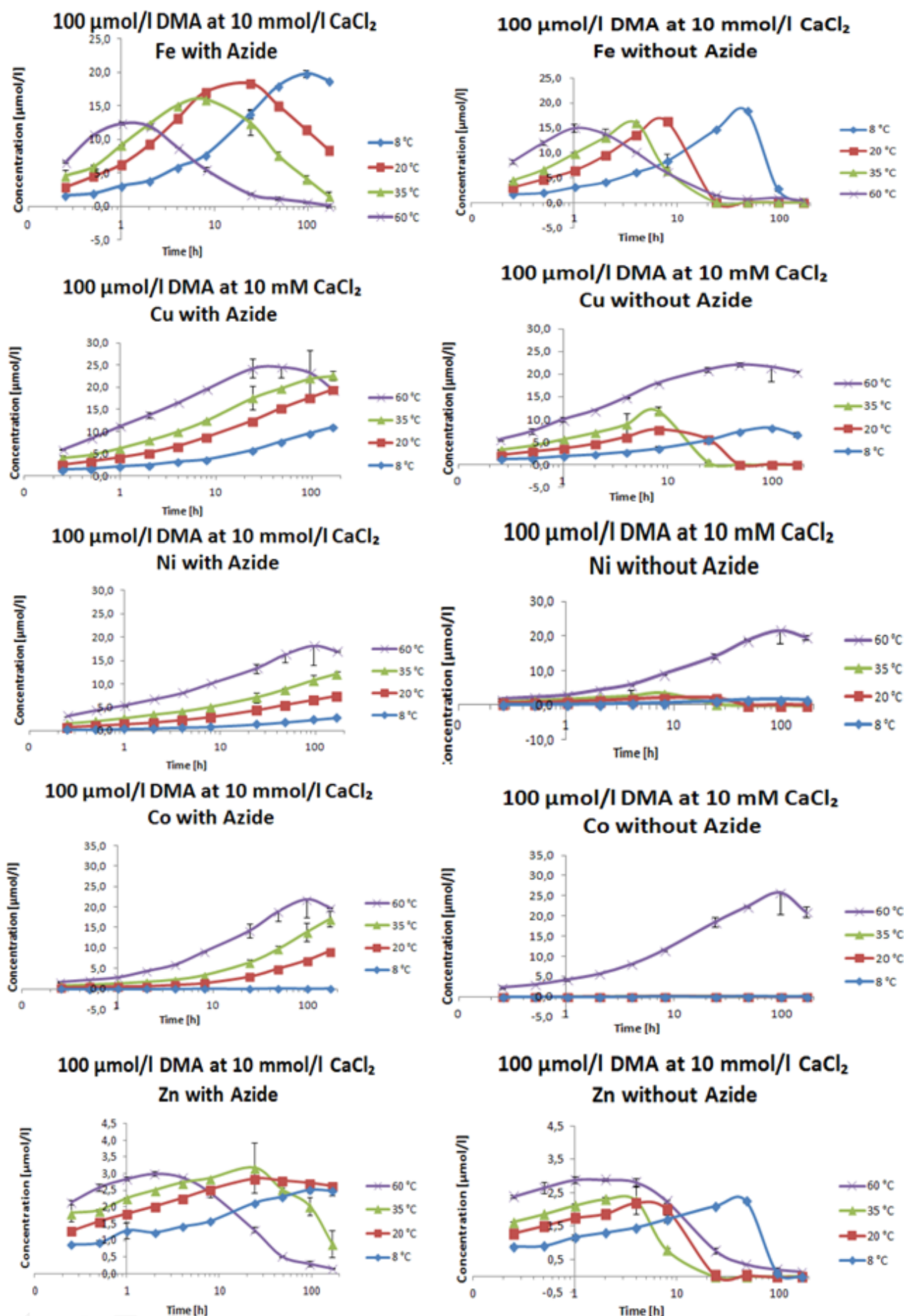


Fig. 15: Element mobilization by 100 $\mu\text{mol/l}$ DMA under different temperatures. All samples were prewetted.

Other outcomes of the experiments:

Decrease of total metalDMA concentration after long interaction times at low DMA concentrations and high temperatures:

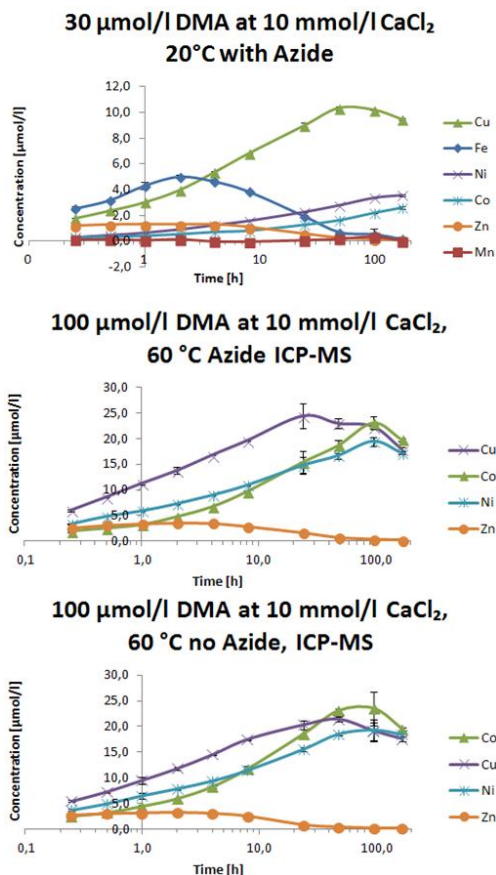


Fig. 16: Three treatments, where the overall metalDMA concentration goes down after long interaction times. The high temperature plots are taken from ICP-MS data.

After long interaction times (168 hours) within the sterilized, 30 µmol/l treatment, all metal mobilization curves decline, except for Co and Ni, where at least a decline in their slopes is observable. The same observation can be made at high temperatures (60°C) and even higher DMA concentrations (100 µmol/l) for both sterilized and unsterilized treatments within the *Temperature Experiment*. These observations, plotted in [figure 16](#), cannot be explained with the competition effect. High temperatures probably accelerate the overall decline of metalDMA complexes, thus there has to be a common effect. Schenkeveld et al. (Schenkeveld et al., 2014 (accepted)) calculated free DMA concentrations in the same Santomera soil used for this master thesis. For a 100 µmol/l DMA treatment, the free DMA content steadily decreased towards longer interaction times. At lower DMA concentrations (30 µmol/l) or accelerated reaction kinetics due to higher temperatures (60 °C), the amount of free DMA might be substantially lower or even negligible after long interaction times (168 hours). Following this assumption, the overall decline of all metal mobilization curves may be related to the total complexation of free DMA. Thus, metalDMA complexes would only be stable if there is free DMA in the system left. As already mentioned above, the

amount of free DMA probably controls the competition effect (as seen for high DMA concentrations within the “*Concentration Experiment*”). At a certain free DMA : complexed DMA ratio, FeDMA is outcompeted by CuDMA, and further on CuDMA by NiDMA and/or CoDMA. No chemical reason could be found within this thesis to prove the assumption that a total complexation of free DMA forces the overall metalDMA concentration to go down. The following assumptions can be falsified and excluded as a reason for the decrease of metalDMA concentration within a system without free DMA:

The absence of free DMA may result in a decline of the observed metals due to a further competition effect with an unexamined metal ion in this master thesis. This seems pretty improbable due to the low stability constants of other bivalent cations, such as Ca. Another possible explanation would be that CoDMA and NiDMA complexes are generally metastable, or at least metastable for the examined

Santomera soil and its chemical properties. It may be possible that CoDMA and NiDMA get oxidized after they were built by out-competition of Cu. That's however also improbable for Ni because it is not very redox sensitive. However, Co is a redox sensitive element. Hence, redox processes among CoDMA might happen, especially at high temperatures (60°C) and long interaction times, where the O₂ content within the batch might decrease (considering it a closed system). A rapid decay of NiDMA and CoDMA within the short light exposure during sample handling can also be excluded due to the results a NiDMA test experiment brought in advance to the Adsorption Isotherm Experiments. In this experiment, it was examined if NiDMA complexes are outcompeted by other metals within an interaction time of two hours. It was shown that no additional free DMA needed to be added, as done for Fe and Zn. However, NiDMA remained stable within a prepared NiDMA solution, what also eliminates the possibility of an oxidation correlated decrease of NiDMA. As already determined within the “*Adsorption of metalDMA complexes (FeDMA, CuDMA, NiDMA and ZnDMA)*” chapter, NiDMA has the highest adsorption of all examined metalDMA species (there were no adsorption experiments done on CoDMA). The decrease in NiDMA might be correlated with a higher adsorption rate upon the out-competition of CuDMA. That however would make sense for the total DMA concentration (which actually decreases), but not specifically for the NiDMA concentration. Metastability and the high adsorption potential of NiDMA would also occur at lower interaction times before the Cu mobilization curve reaches its inflection point. This might be a strong hint that the overall decline of metalDMA after long interaction times can be correlated with the consumption of free DMA. Another reason of the decrease of total metalDMA at long interaction times may be biodegradation. As already discussed, biodegradation was supposed to be

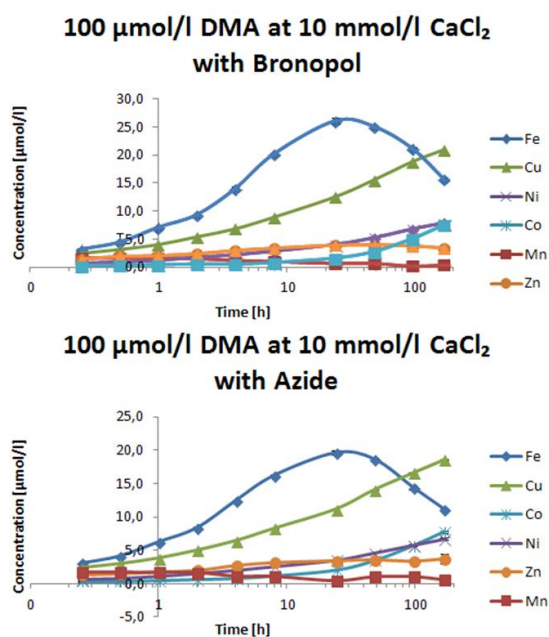


Fig. 17: Different amount of metal mobilization during the usage of different sterilizing agents

negligible at 60 °C and not existing within the sodium azide (2 g/l) treatments. For high temperature treatments this might however not be the case, although there are no signs that sodium azide fails as a sterilizing agent in all of the other samples discussed in this master thesis. Beside biodegradation, metalDMA complexes might be consumed by other processes within the batches at high temperatures (60°C). Chemical degradation of metalDMA complexes may be catalytically triggered by soil surfaces, such as clay minerals.

However the reason might be, there is no doubt that within the two treatments plotted in [figure 16](#), a decrease in the total metalDMA concentration was examined. It cannot be determined at this point why this decrease happens. Further experiments with longer interaction time would yield a deeper insight into this observation.

Different metal mobilization during the usage of Sodium-Azide and Bronopol:

It can be observed that there is less metal mobilization for samples treated with Sodium Azide as within samples treated with Bronopol. **Figure 17** illustrates this effect for two equal treatments, but different sterilizing agents. For Fe, the Fe mobilization within the Bronopol samples at the mobilization peak is about 20 % higher, going from about 20 $\mu\text{mol/l}$ up to approximately 25 $\mu\text{mol/l}$. This observation is based on the fact that sodium azide contributes to the ionic strength of the system, thus decreasing metal mobilization. Bronopol at the contrary does not contribute to the ionic strength because it does not dissociate when it is dissolved. This observation is well in accordance with the observations made within the “Metal complexation at different ionic strengths background electrolyte concentrations” chapter.

Metal mobilization at different shaking styles:

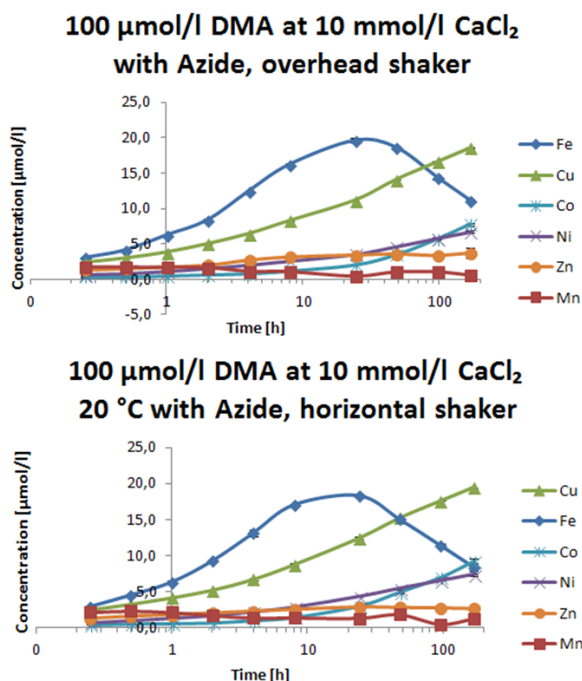


Fig. 18: Different amounts of metal mobilization for two different shaking styles.

As already mentioned in the materials and method section, two different shakers were used for the experiments done in this master thesis. A horizontal shaker was used within the temperature experiment, whereas an overhead shaker was used for the other ones. Comparing same treatments, but different shaking styles, at the first glance there are no major differences within the metal mobilization patterns already described above. There are however small differences in metal mobilization. Within the samples treated with an overhead shaker, there is slightly more metal mobilization mentionable (**Figure 18**). When looking at the Fe mobilization curve, there is higher mobilization especially at longer interaction times (> 2 hours). Before samples were put into the shaker, they were shaken by hand. This might be the reason why there is more or less equal metal mobilization at short interaction times. At longer interaction

times, the higher metal mobilization within an overhead shaker might be due to the fact that the overhead shaker probably achieved a higher mixing and stirring performance than the horizontal one did.

Mobilization of Mn by the phytosiderophore DMA:

Mobilization of Mn by the phytosiderophore DMA does occur to a substantial amount only at special conditions. Within the *Concentration Experiment*, high amounts of Mn were mobilized at high DMA concentrations added to the soil (1000 $\mu\text{mol/l}$). Within the *Temperature Experiment*, higher mobilization of Mn was observed at higher temperatures. For both experiments, Mn was only mobilized to a substantial amount under sterile conditions because, as already mentioned above, it is pretty vulnerable to biodegradation. For both treatments, Mn was substantially mobilized at “unusual” conditions, but

within them it is well visible that it is the first element of the examined ones in this master thesis (Fe, Cu, Zn, Ni, Co and Mn) which passes, or in the case of the 1000 $\mu\text{mol/l}$ DMA treatment approaches, its mobilization maximum. At “normal” conditions, Mn is rapidly outcompeted by Fe, but at high DMA concentrations and high temperatures, the out-competition of Mn takes longer. This might be explained by faster reaction kinetics of MnDMA-complexation at higher temperatures and a higher complexation kinetic of MnDMA at high free DMA concentrations. For “normal” conditions, it is however still visible, that beside the background fluctuations of Mn at longer interaction time, Mn shows the highest DMA correlated mobilization at the very first measured interaction time interval (after 15 minutes interaction time).

Conclusion

Research questions:

The data and their corresponding interpretation help to enhance the understanding on how the phytosiderophore DMA works over geochemical and physicochemical varying parameters. The eight main hypothesis made above can therefore be verified or falsified as follows:

- **Experiment one, addressing hypothesis 1, 2 and 3: Adsorption of metalDMA complexes (FeDMA, CuDMA, NiDMA and ZnDMA):** A substantial amount of adsorption of metalDMA complexes onto soil particles was determined and quantified within this master thesis. Adsorption behavior was shown to be dependent on the central atom within the DMA complex (Fe(III), Cu, Ni and Zn) and on the electrolyte background concentration within the soil solution. These results verify *hypothesis 1 and 2* made at the beginning of the master thesis. However, it was not possible to directly correlate the adsorption behavior of CuDMA and FeDMA with an increase or decrease of the temperature, therefore *hypothesis 3*, that hypothesizes that an increase in temperature results in a decrease in adsorption due to a higher Brownian Motion, can be falsified, at least with the data determined in the *Adsorption Isotherm Experiment* done within this master thesis.
- **Experiment two, addressing hypothesis 4: Metal complexation at different DMA concentrations:** It was shown that different concentrations of DMA added to the soil create different metal mobilization patterns. This may be strongly correlated to the amount of free DMA within the soil, which remained longer in the system at higher total DMA concentrations added. A high amount of free DMA within the soil retards the usual metal mobilization patterns in time due to the fact that it is slowing down competition effects between the examined metals. These observations can be used to verify *hypothesis 4* made at the beginning of the thesis.
- **Experiment three, addressing hypothesis 5: Metal complexation at different moisture regimes:** It was examined that there is a substantial difference in metal mobilization within prewetted and unpretreated soil samples. Within unpretreated soil samples, there is more metal mobilization observable as in prewetted soil samples. All metals which are concerned by this enhancement in mobilization occur in organic matter to a substantial amount, whereas Ni and

especially Co show no major difference in mobilization in unpretreated soils due to their low abundance in organic matter. *Hypothesis 5* made above can therefore be verified for Fe, Zn and to a lesser extent for Cu, but not for Ni and Co.

- **Experiment four, addressing hypothesis 6 and 7: Metal complexation at different ionic strengths background concentrations:** It has been shown that the electrolyte background concentration has a major influence on DMA induced metal mobilization. At low ionic strength background concentrations, there is substantially more mobilization of all of the examined metals within this master thesis. Furthermore it was examined that metal mobilization is electrolyte specific (NaCl and CaCl₂), it does not correspond with the overall ionic strength of the soil solution. Concluding the statements made above, *hypothesis 6* and *7* concerning the effect different electrolyte background concentrations and species have on metal mobilization by the phytosiderophore DMA can be verified.
- **Experiment five, addressing hypothesis 8: Metal complexation at different temperatures:** Metal complexation was shown to be temperature dependent. At higher temperatures, metal complexation goes faster due to a higher reaction kinetics. Moreover, competition effects were demonstrated to occur more rapidly at higher temperatures, thus resulting in lower complexation amounts of weak metals in regard to competition (such as Fe and Zn). To conclude, *hypothesis 8* on temperature dependency made above can be verified with the data interpretation in this master thesis.

Implications:

In this research work it was demonstrated how key soil-geochemical parameters enhance or limit DMA induced metal complexation in a calcareous soil. It was necessary to investigate these processes in controlled experiments in order to avoid the complexity of the rhizosphere (including time-dependent plant and microbial control of this environment). However, in order to gain insight into the impact of the observed effects, in the following section the implication of the results of this work for natural, agricultural or polluted environments will be discussed.

Implications on natural and agricultural environments:

The data analyzed above yield insight into ecological aspects of iron and metal mobilization of plants within different environments and ecosystems. The Adsorption Ratio of metalDMA complexes ($\frac{\text{Adsorbed}}{\text{Total amount added}}$) onto soil particles stays more or less the same over a concentration range from 0,25 to 100 $\mu\text{mol/l}$ of metalDMA in solution. Adsorption of metalDMA complexes occurs to a substantial amount (**table 2**) within the calcareous Santomera soil. It lowers bioavailability of metalDMA complexes equally at different DMA exudation concentrations within the soil. Beside adsorption, biodegradation of metalDMA complexes, starting to occur significantly after 4-8 hours of reaction time, decreases the availability of metalDMA complexes even further. The concentration of the total DMA ligands exuded within the rhizosphere does not change the adsorption ratio and the rapid biodegradation after a sufficient interaction time, but it changes the metal mobilization patterns due to the variable amount of free DMA within the system, which determines the competition effect between the examined metals. At higher total DMA concentrations exuded within the rhizosphere, Mn is as an example substantially mobilized, whereas at low concentrations there is hardly any Mn mobilization. Hydrological changes in

an environment may also contribute to the amount of metal mobilization between arid and humid climates. Irregular precipitation events or dry seasons may cause organic matter to dry out. Upon rewetting the soil, loosely bonded metals are mobilized by a substantially higher amount than under already wetted conditions. Concerning arid environments, this would result in a higher metal mobilization and thus a higher fertility of calcareous soils upon rewetting the soil after a dry season. In the case of iron, there is a higher mobilization by a factor of about 3,5 in unrewetted samples after 1 hour of interaction time, what is a substantial increase in Fe availability for plants. The electrolyte background concentration of the soil solution was examined to determine metalDMA mobilization due to its contribution to the adsorption potential of the complexes. It can be concluded that metal acquisition poses higher problems for plants in calcareous soils than in salty soils with the same ionic strength due to the fact that the adsorption potential of metalDMA complexes was shown to be electrolyte specific. However, within calcareous soils it was observed (*figure 11*) that the effect CaCl_2 does on metalDMA adsorption is more significant at low CaCl_2 concentrations than at higher ones (for Fe this effect is not as significant as for all other examined metals). This suggests that CaCl_2 background concentration fluctuations in calcareous soils with an elevated standard CaCl_2 background concentration do not affect metal acquisition by plants by the same extent as CaCl_2 fluctuations in soils with low CaCl_2 background concentrations do. Moreover, (soil) temperature affects metal mobilization and thus metal acquisitions by plants within habitats in different climatic zones. At higher temperatures, complexation occurs faster. When looking at the iron mobilization at short interaction times (1 hour), it is substantially more mobilized at higher temperatures. Hence plants would face an enhanced Fe mobilization within warmer climatic zones than in colder ones. At longer interaction times (8 hours) however, more Fe mobilization happens towards lower concentrations due to the fact that competition effects occur faster at higher temperatures.

Implications on anthropogenic influences on soil environments:

Anthropogenic influences on soil environments may also concern metal mobilization by plants. Increased soil salinity due to irrigation in (semi)arid environments or road salt input may reduce availability of micronutrients for plants due to a higher adsorption of metalDMA complexes at higher background electrolyte concentrations. However, it is not possible to make statements within this master thesis if limited micronutrient availability is harmful for plants beside osmotic problems in plants in soils with high salinity.

Picture credits:

All pictures in this master thesis were used to illustrate different temperature and hydrological environments and salty or calcareous soils and were made by the author.

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Appendix:

Zusammenfassung (Conclusion in German):

Im Rahmen dieser Masterarbeit wurde die Abhängigkeit der Funktionalität des Phytosiderophoren DMA (*2'-deoxymugineic acid*) von geochemischen und physikochemischen Parametern in Bodenproben eines kalkreichen, spanischen Bodens untersucht. Die folgenden Metallionen wurden zur Untersuchung herangezogen: Fe(III), Cu, Mn, Ni, Co und Zn. Für Cd, V und U konnte kein DMA spezifisches und signifikantes Mobilisierungsverhalten festgestellt werden. In fünf Experimenten wurde die Funktionalität von DMA in Hinsicht auf Variationen in der Ionenstärke, der Temperatur, der hydrologischen Rahmenbedingungen und der exudierten Konzentration des Liganden untersucht. Ferner wurde das Adsorptionsverhalten von komplexierten DMA Komplexen untersucht. Bezüglich des Adsorptionspotentials der verschiedenen komplexierten Metall-DMA Spezies konnte festgestellt werden, dass FeDMA und CuDMA geringer an Oberflächen des beprobten Bodens adsorbieren als ZnDMA und NiDMA. Dies wird mit den verschiedenen negativen Nettoladung der Metall-DMA Komplexe in Zusammenhang gebracht, wobei eine höhere negative Nettoladung im Falle des FeDMA und CuDMA ein geringeres Adsorptionspotential bewirken. Ferner konnte eine direkte Proportionalität zwischen der Elektrolytkonzentration der Bodenlösung und dem Adsorptionspotential der Metall-DMA Komplexe festgestellt werden. Je höher die Elektrolytkonzentrationen gewählt wurden, je höher fiel die Adsorption von DMA Komplexen an Bodenpartikel-Oberflächen aus. Dieses Verhalten wird mit einer Abnahme der elektrostatischen Wechselwirkung zwischen Adsorbens und Adsorbat bei höherer Ionenstärke in Zusammenhang gebracht. Eine eindeutige Temperaturabhängigkeit der Metall-DMA Komplexe konnte mit den im Zuge dieser Masterarbeit gewonnenen Daten nicht bewiesen werden. In einem weiteren Experiment wurden verschiedene DMA Konzentrationen zum beprobten Boden hinzugefügt. Es stellte sich heraus, dass verschiedene DMA Konzentrationen verschiedene zeitliche DMA Mobilisierungsmuster der gemessenen Elementen erzeugt. Fe und Zn Mobilisierungsmaxima ereignen sich nach kürzeren Interaktionszeiten bei geringeren, hinzugefügten DMA Konzentrationen. Auch Cu zeigt bei geringen hinzugefügten DMA Konzentrationen ein Mobilisierungsmaximum. Diese Beobachtung wird mit einer zeitlich längeren Stabilität von freien DMA im System bei höheren Konzentrationen an totaler, hinzugefügter DMA erklärt, da bei geringeren Konzentrationen freie DMA Liganden rascher komplexiert werden. Bleibt eine hohe Konzentration an freien DMA Liganden zeitlich länger im System unkomplexiert, gibt es weniger Wettbewerbsdruck zwischen den beobachteten Metallen. In einem dritten Experiment wurde die verschiedene Mobilisierung von Metallen zwischen vorgefeuchten „*prewetted*“ und nicht-vorgefeuchten „*unprewetted*“ Proben verglichen. Dabei stellte sich heraus, dass bei nicht vorgefeuchten Proben höhere Konzentrationen an Metallen mobilisiert werden. Diese Beobachtung wurde mit der Tatsache in Zusammenhang gebracht, dass ausgetrocknetes organisches Material einen Pool an schwach gebundenen Metallen darstellt, welche rasch komplexiert werden können. Bei vorgefeuchten Proben (es wurde 2 Tage vor dem Experiment vorgefeuchtet) wurde eine geringere Mobilisierung von Metallen beobachtet, was für ein schnelles Erreichen eines neuen Gleichgewichtes unter feuchten Bedingungen spricht, unter welchem die zumal schwach gebundenen Metalle unlöslicher gebunden sein müssen (in Form von unlöslichen Präzipitaten etc.). Dieses Experiment zielte darauf ab, Metallmobilisierung von DMA aus einem kalkreichen Boden innerhalb eines

(semi)ariden Gebietes, in dem Trockenzeiten und/oder periodische Niederschlagsereignisse vorkommen, zu quantifizieren. In einem weiteren Experiment zur Ionenstärke wurde bestimmt, dass sich das von der Elektrolytkonzentration abhängige Adsorptionspotential von Metall-DMA Komplexen in Korrelation dazu auch auf die Konzentration von Metall-DMA Komplexen in der Bodenlösung auswirkt. Ferner wurde bestimmt, dass Mobilisierung (und Adsorption) von Metall-DMA Komplexen auch bei gleicher Ionenstärke elektrolytspezifisch ist, wobei CaCl_2 eine höhere Adsorption (und geringere Mobilisierung) bewirkt als NaCl . Abschließend wurde noch ein Experiment zur Temperaturabhängigkeit der Komplexbildung von Metallen von DMA durchgeführt. Es stellte sich heraus, dass die Komplexbildung von Metallen durch DMA bei höheren Temperaturen schneller abläuft als bei niedrigeren, was auf eine durch erhöhte Temperatur beschleunigte Reaktionskinetik schließen lässt. Weiters stellt sich bei höheren Temperaturen der Wettbewerbseffekt mit anderen Metallen schneller ein, weshalb Metalle wie Fe, Zn und Mn schneller von Cu und anschließend Ni und Co als Zentralatom abgelöst werden. Es konnte ferner beobachtet werden, dass bei hohen Temperaturen (60°C) und langen Interaktionszeiten (168 Stunden) die Konzentration der totalen, komplexierten DMA sinkt. Im Rahmen dieser Masterarbeit konnte für diese Beobachtung neben einigen Erklärungsvorschlägen jedoch keine endgültige Erklärung gemacht werden. Zusammenfassend kann gesagt werden, dass physikochemische und geochemische Parameter wie unter anderen Temperatur und Ionenstärke die Funktionalität des bedeutenden Phytosiderophoren DMA in einen kalkreichen Boden signifikant beeinflussen und deshalb von ökologischer Bedeutung in Hinblick auf Mikronährstoffe, wie zum Beispiel Fe, Cu und Zn, sind.

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