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Sorption of Natural Siderophores onto Clinoptilolite-Tuff and Its Controlled-Release Characteristics

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Abstract: Iron deficiency-induced chlorosis is a widespread horti-/agricultural problem that can lead to massive crop failures, especially for plants growing on calcareous soils. The most effective agronomical practice to prevent plants from iron deficiency is to apply synthetic Fe-(chelate)-fertilizers to the soil. Because these compounds are usually not biodegradable and, therefore, may become soil contaminants, efficient and environmentally friendly solutions are needed. The present study investigates a novel approach to tackle chlorosis in plants using clinoptilolite-tuff as a carrier substrate for the natural Fe-siderophore 'coprogen'. The combination of the two substances promises economic and ecological potential to be used as a fertilizer to prevent crop failures triggered by micro-nutritional shortages. Sorption and release experiments were performed in batch and column setups in order to understand the binding characteristics; analyses were carried out using ICP-MS, HPLC, XRD, and SEM, respectively. Results show the highest sorption capacity of coprogen (92.8%) and corresponding Fe (90.2%) on clinoptilolite-tuff at pH 4 within 30 min as well as the efficient release of coprogen at pH 8, mimicking alkaline soil conditions (88% of the sorbed coprogen is released from the tuff). The gained data suggest that coprogen is bound onto the clinoptilolite-tuff through surface-mediated sorption based on electrostatic interactions.

Keywords: Fe-deficiency; sorption; clinoptilolite-tuff; siderophore; fertilizer

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1. Introduction

Iron is an essential micronutrient for all living organisms. In plants, iron (Fe) plays an essential role in a variety of metabolic processes, including respiration, nitrogen assimilation and photosynthesis [1]. It is, therefore, an inevitable component of biological, physiological, and biochemical processes within the plant [2]. Fe deficiency severely limits a plant's photosynthetic efficiency and chloroplast biology, causing insufficient chlorophyll production that is often identified by the greenish-yellow color of young leaves. This developmental disorder is referred to as Fe deficiency-induced chlorosis [3,4]. Fe chlorosis in plants is a widespread problem, causing decreases in vegetative growth and quality losses in crops [5]. Among the most affected crops are costly fruits, such as citrus, peaches, and grapes [4], but also important alimentary grains like barley, sorghum, or wheat [6,7].

Although Fe is one of the most abundant metals in the earth's crust, Fe deficiency in plants is a common phenomenon [8]. Especially crops growing on calcareous soils are affected by this nutritional disorder, a fact that results from the low solubility of Fe(III) minerals under oxic, neutral to alkaline pH conditions [5,9]. In most microbial habitats, Fe(II) is quickly oxidized to Fe(III) via reactions with, for example, molecular oxygen or enzymatically, during assimilation in organisms [10]. Under Fe-limiting conditions, natural chelates, namely siderophores, are produced by soil microorganisms, such as fungi or bacteria, and in the rhizosphere of plants. Siderophores are low-molecular-weight organic chelating agents with a high and specific affinity for Fe(III), providing an efficient Fe-acquisition system for the plant. Both Fe uptake and availability are enhanced within the

plant by forming tight and stable complexes with Fe(III), providing the metal to the cell by binding to cell receptors [10–13].

By substituting solvent water and surrounding Fe(III) in an octahedral geometry (Figure 1), Fe-siderophore complexes are formed, thus removing Fe from insoluble ferric oxide hydrate complexes ($Fe_2O_3 \cdot nH_2O$) and presenting the metal in a chelated, bioavailable form for the cellular uptake by many plants or microorganisms [10,14,15]. The siderophores' selectivity for Fe(III) is, among others, determined by the stereochemical arrangement and the number of binding units [16]. Since oxygen atoms, which represent hard Lewis bases, are mainly responsible for the coordination of Fe in the Fe-siderophore complex, the complex is kinetically stable. The high and specific affinity of the siderophore to Fe(III) is also due to additional ionic interactions between the ligand and the metal because of the ligand's Lewis acidity since Fe(II) prefers interaction with soft donor atoms, such as nitrogen or sulfur [10,16].

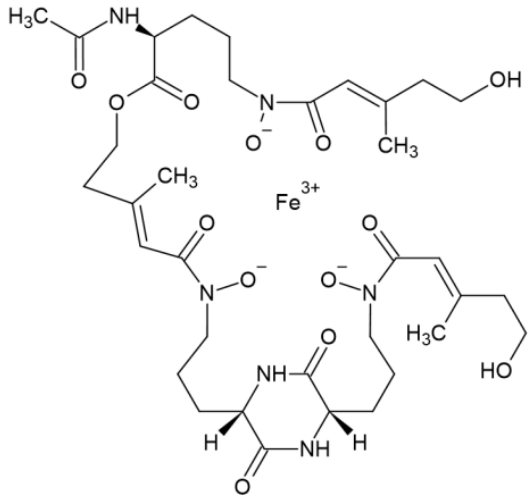


Figure 1. Molecular structure of the Fe(III) hydroxamate siderophore coprogen (adapted from EMC microcollections, Tübingen, Germany; www.microcollections.de, accessed on 23 April 2013).

These days, the application of synthetic Fe fertilizers is the most common remedy for correcting Fe deficiency chlorosis in agri- and horticulture. However, the most effective Fe correctors feature low natural degradability and thus may have negative impacts on the environment, soil and groundwater [17]; besides, their use in organic farming is highly controversial [9]. One of the most prominent chelators for Fe is ethylenediaminetetraacetic acid (EDTA), which, however, is hardly biodegradable and thus accumulates, for instance, in rivers [18]. The major environmental concern arising from high concentrations of EDTA is related to its ability to increase the solubility of highly toxic heavy metals, such as Cd and Pb, thus increasing heavy metal pollution in groundwater [19,20]. Therefore, the development of new, environmentally friendly ligands is desirable and researched intensively [9].

Various studies report on the efficiency of siderophore complexes as Fe-suppliers for plants [21,22]. However, they mainly focus on the trihydroxamate desferrioxamine (DFO) siderophores. Siebner-Freibach et al., for instance, introduced the hydroxamate siderophore ferrioxamine B (FOB) bound onto Ca-montmorillonite as an iron reservoir for peanuts to impede iron chlorosis. The study shows that iron uptake from siderophores does not require close proximity to the roots. It is therefore assumed that the superficial application of siderophore-containing fertilizers is sufficient to remediate iron chlorosis.

Because coprogen and desferrioxamine siderophores share the same fundamental structures and similar stability constants (log K_f = 30.2 and 30.5) for the Fe(III)-siderophore complex [23], the binding of coprogen onto naturally occurring zeolite tuffs is worth investigating.

Clinoptilolite-tuff, which has not been included in sorption studies of siderophores so far, is a long-time established, extensively used and cost-effective soil conditioner. Furthermore, the authors in the aforementioned studies [12,21] described cation-like sorption behavior for the organic siderophore molecules. Since clinoptilolite has shown efficient uptake of metal cations in previous studies [24,25], there is reason to deduce that clinoptilolite is an appropriate controlled-release carrier substrate for siderophores. Clinoptilolite, the major mineral constituent of clinoptilolite-tuff, is a heulandite-type zeolite mineral, a hydrated aluminosilicate that shows a unique structure due to its framework of linked SiO_4 and AlO_4 tetrahedra [26]. Because of its negatively charged crystal framework, resulting from the isomorphous exchange of Si^{4+} by Al^{3+}, mono- and divalent cations can be weakly held and exchanged by this mineral, depending on their size and charge. Because of clinoptilolite's property to bind loosely and exchange metal cations [27], the term "slow-release fertilizer" was derived, which can be understood as the result of prolonged ion exchange processes.

The objective of the present study was to examine the basic sorption characteristics of the commercial natural hydroxamate siderophore coprogen to a widely used clinoptilolite-tuff in batch- and the release from the tuff in column experiments. The given results provide fundamental information for further investigating the potential of an environmentally friendly and efficient fertilizer for plants growing under iron-deficient conditions.

2. Materials and Methods

2.1. Materials

The natural Fe(III)-hydroxamate siderophore coprogen with its formula $C_{35}H_{53}N_6O_{13}Fe$, containing Fe in its +3 oxidative state and a molar weight of 821.7 g/mol (Figure 1), isolated from *Neurospora crassa* [15], together with the corresponding high-performance liquid chromatography (HPLC) calibration kit, was purchased from EMC microcollections GmbH (Tübingen, Germany) and stored at −20 °C. The HPLC Kit contains lyophilized siderophores in a total amount of 1 mg.

For the performed experiments, natural clinoptilolite-tuff from the open-pit mine in Nižný Hrabovec (NE-Slovak Republic) was used, provided by the company ZEOCEM a.s. (Bystré, Slovak Republic). The Miocene volcanogenic-sedimentary material is composed of mainly authigenic clinoptilolite, small amounts of cristobalite, plagioclases, K-feldspar and accessory biotite and quartz. The clinoptilolite deposit at Nižný Hrabovec as well as the principal constituents of the volcanic-sedimentary rock, have been thoroughly investigated and described by Reháková et al. [28] and Tschegg et al. [29].

Its whole-rock major- and trace-element composition (SiO_2: 74.3–77.6 wt.%, Na_2O+K_2O: 4.1–5.5 wt.%) largely resembles the high-K calc-alkaline rhyolitic nature of the parental tuff [26]. The only zeolite phase present in the tuff is a heulandite-type Ca-clinoptilolite (Si/Al: 4.5–5.1) that formed as a result of dissolution-(transport)-precipitation reactions from acidic volcanic (glassy) ash under an alkaline fluid influence and slightly elevated pressure and temperature conditions [29]. The crystal-chemical formula of the natural clinoptilolite mineral is $(Ca_{1.51}K_{1.39}Mg_{0.37}Na_{0.15})[Al_{5.64}Si_{26.36}O_{72}]\cdot 11.77\ H_2O$ [26]. Partial, isomorphic substitution of Si^{4+} by Al^{3+} leads to a net negative charge in the crystal lattice, which is balanced by mono- and divalent cations. This accounts for the clinoptilolite-tuff high sorption, cation exchange capacity (of 0.97 mol/kg), and molecular sieve properties [27]. The pore volume and effective pore diameter of the tuff are documented to be 24–32% and 0.4 nm, respectively [26]. Analyzing powdered clinoptilolite-tuff of the Slovakian clinoptilolite-tuff (0.2–400 µm), Pabiś-Mazgaj et al. (2021) report a specific surface area of 29.91 m^2/g [30].

For more details about the formation of the clinoptilolite and its present appearance, as well as the evolution and composition of the clinoptilolite-tuff of Nižný Hrabovec, the reader is referred to the studies of Tschegg et al. [26] and Tschegg et al. [29]. The size-fraction of 25–45 µm was achieved through sieving with a vibratory sieve shaker (Retsch AS 200, Haan, Germany) from the original grain-size fraction of 0–500 µm zeolite-tuff.

2.2. Analytical Techniques

For determining the amount of coprogen adsorbed onto the clinoptilolite-tuff, analyses were performed with inductively-coupled plasma mass spectrometry (ICP-MS), as well as high-performance liquid chromatography (HPLC). The blank solution (without clinoptilolite), as well as the sample solutions (with 250 mg clinoptilolite and 9.12 µmol/L coprogen), were analyzed; the combination of the 2 methods allows us to follow and ensure the sorption of both the coprogen molecule as well as the included Fe.

2.2.1. ICP-MS

The total Fe content, which is dependent on the amount of present coprogen in the aqueous solutions, with and without the tuff, was measured using inductively coupled plasma mass spectrometry (ICP-MS; Elan 9000 DRC, Perkin Elmer, Waltham, MA, USA). 1 mL was withdrawn from the sample, filtered, and diluted with 1% nitric acid (HNO_3; Merck, Darmstadt, Germany). The measurements were carried out in triplicates, and the average values were determined and demonstrated. Quality checks were run at intervals with the certified reference material NCS DC 70316; precision and accuracy of the measurements were determined by comparing the reference value of the certificate (Fe = 33,640 µg/g) with our results (Fe = 32,777 with an RSD of 0.8% at n = 12).

2.2.2. HPLC

Measurements of the high-performance liquid chromatography (HPLC) calibration kit coprogen (EMC microcollections, Tübingen, Germany) and the coprogen samples were established on a Dionex HPLC system equipped with an ASI-100 autosampler, a photodiode array detector PDA Photodiode (Waters, Eschborn, Germany) and a photometer Specord 210 (Analytik Jena, Jena, Germany). HPLC separation was performed on a C18 reversed-phase column (4 × 250 mm, Nucleosil, 5 µm) using a gradient elution of water/acetonitrile (+0.1% TFA, Sigma-Aldrich, St. Louis, MO, USA) 6–10% (10 min), 10–20% (15 min) and 60% (25 min) with a flow rate of 1 mL/min and detector wavelengths of 220 nm and 435 nm, respectively. The coprogen amounts were measured with the standard addition method and calculated via absorption coefficients [31].

2.2.3. XRD

Powder XRD (X-ray diffraction) patterns of air-dried clinoptilolite-tuff samples with and without sorbed coprogen were recorded.

Oriented powder X-ray diffraction was done using a Bruker D-8 Advance diffractometer equipped with a Cu source and an X-ray generator operated at 40 kV voltage and 40 mA current (Billerica, MA, USA). During measurements, which were carried out on micronized specimens in top-loaded sample holders using a theta-2 theta configuration, an angular range from 6° to 70° and 0.01° step size at 0.5 s/step were considered. For the evaluation of the phases, the Bruker software DIFFRAC.EVA (release 2016) was used.

2.2.4. SEM

For investigations with the scanning electron microscope (SEM), samples were air- and vacuum-dried for 30 min (<1 × 10^3 mbar), sputter-coated with graphite for 1–2 s and examined with an FEI Inspect S50 SEM system (Hillsboro, OR, USA). The operating conditions were 10–15 kV and 5 µm spot size.

Another SEM campaign, focusing on the chemical composition of the samples in contact with coprogen, was done on a JEOL JSM-IT800 SEM (Tokyo, Japan) operated at 15 kV. The samples were air-dried and sputter-coated with Au/Pd for 20 s before analysis.

2.2.5. TOC

To investigate the total organic carbon content (TOC) of the tuff raw material, the vario TOC Cube (Elementar, Langenselbold, Germany) was used. The incubated material was

oven-dried at 60 °C for 4 h and stored under room-temperature conditions, whereas the unreacted material was air-dried at room temperature.

Approximately 50–80 g of both materials and a blank material (fine quartz-sand) were weighted in pre-folded tin foils and pressed into pellets. All samples were then combusted at 850 °C with oxygen as a catalyst, and the signal was detected via infrared (IR) directly. The analyzed samples were measured 3 times, the blanks 2 times.

2.3. Sorption and Release Experiments

For the sorption and release experiments, lyophilized coprogen was dissolved in aqua purificata (AP; 0.9 mS/cm) to obtain a concentration of 9 mmol/L; experiments were then performed with this particular stock solution at room temperature (20 ± 2 °C) in 25 mL centrifuge tubes with defined zeolite-tuff/coprogen fractions. The zeolite-tuff (in the fraction 25–45 µm) was flushed multiple times with aqua purificata and air dried at room temperature until a constant weight was achieved before being used. All experiments were run 3-fold, and 2 samples were taken from each solution for analysis via ICP-MS and HPLC. For interpretation, mean, SD and RSD from the gained data points were calculated.

Throughout this study, the term "sorption" is used to describe any process resulting in the loss of a sorbate from a solution (i.e., coprogen).

2.3.1. Sorption Kinetics

Three different solutions were prepared for triple-determination with ICP-MS and HPLC at zeolite-tuff/aqua purificata/coprogen ratios: Zeolite-blind: 5 mL AP + 250 mg zeolite-tuff; coprogen-blind: 5 mL AP + 0.0375 mg coprogen; and the test solution: 5 mL AP + 0.0375 mg coprogen + 250 mg zeolite-tuff. The test solution was prepared 4 times, 1 for each separate batch reactor with the same experimental condition at a certain time interval, providing a discontinuous batch study; after 30, 60, 120 and 240 min, sample amounts of 1 mL and 500 µL were taken for ICP-MS and HPLC analyses, respectively, and filtered through 0.45 µm, 25 mm diameter syringe-filters. pH values were stable during the experiments at 6.5. The coprogen-blank solution was taken for point t = 0 min in order to calculate the sorbed amount of Fe and coprogen, respectively.

2.3.2. pH-Dependent Sorption

A coprogen-blank solution was prepared (5 mL AP + 0.0375 mg coprogen), and 7 test solutions (each 5 mL AP + 0.0375 mg coprogen + 250 mg zeolite-tuff), both from stock solution, from pH 4 to pH 10, adjusted and stabilized with 0.1 M sodium hydroxide (NaOH; 50%) and diluted acetic acid (96%). After 30 min under constant stirring, 1 mL and 500 µL of the samples were taken for ICP-MS and HPLC analyses, respectively, and filtered through 0.45 µm syringe filters. The coprogen-blank solution was used to calculate the sorbed amount of Fe and coprogen, respectively.

An additional stock solution was prepared with a 5-times higher concentration of coprogen, compared to the other experiments, in order to gain rough information about the maximum sorption capacity of the zeolite-tuff. Also performed in triplicate, a comparison of the coprogen-blank solution with the solution incubated with zeolite-tuff after 30 min at pH 4 was performed.

2.3.3. Release Kinetics

The clinoptilolite-tuff was incubated, according to the clinoptilolite-tuff/coprogen fractions in experiments outlined before, for 30 min at pH 4, which turned out to be optimal sorption conditions. Before rinsing the samples under controlled conditions, 3 coprogen-blank samples, as well as 3 samples of the incubated tuff, were taken and analyzed via ICP-MS to guarantee that the incubation was efficient to determine the Fe-concentration of the leaching solution.

Leaching experiments were performed in 25 mL columns, with rates of 25 mL leaching solution per hour over 6 hours, controlled with a peristaltic pump. The leaching

solution was aqua purificata at pH 8, stabilized with 0.1 M sodium hydroxide (NaOH). pH 8 was chosen first as previous experiments of this study showed reduced sorption capacity of clinoptilolite-tuff towards coprogen at higher pH; second, this pH range worked as a rough analog to water pH in calcareous soils. After each extraction of 25 mL, the leachates were collected and saved for analysis. Parallel to these release experiments, a blank solution without coprogen was run through the tubes, with the same amount and type of rinse solution as well as zeolite-tuff, to control potential Fe-leaching from the tuff itself. After analysis, the resulting concentrations of blank solutions were subtracted from the sample concentrations.

3. Results

3.1. Sorption Kinetics

Under the experimental conditions (at pH 6.5), a maximum percentage uptake of 78% of Fe from the aqueous solution was exhibited after 30 min incubation time with the tuff. After 60 min and 240 min, 76% and 70%, respectively, of the total amount of 360 ng/g of Fe contained in the solution were bound onto the tuff, revealed by ICP-MS analysis.

The sorption of coprogen determined via HPLC ranged from 65–67% between 30 min and 240 min incubation time with the tuff (Figure 2, Table 1).

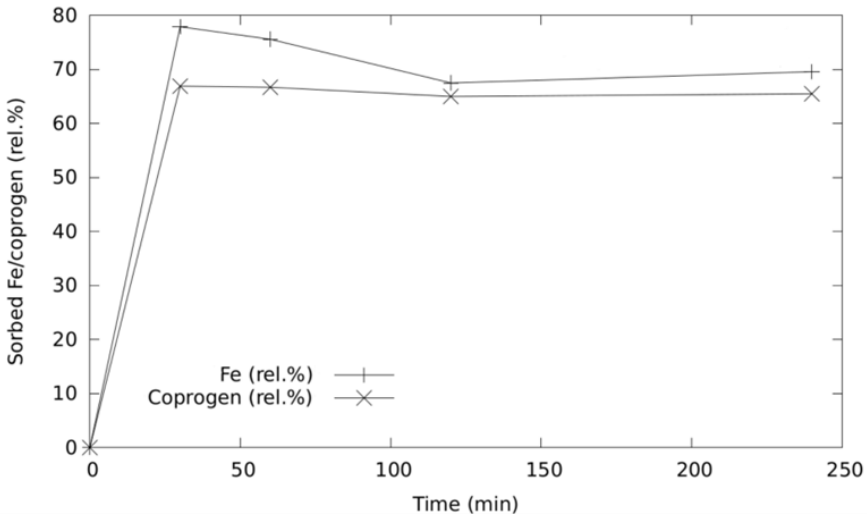


Figure 2. Sorption kinetics of coprogen/Fe onto clinoptilolite-tuff from aqueous solutions at pH 6.5.

Table 1. Sorption kinetics of coprogen/Fe. (SD = standard deviation, RSD = relative standard deviation; rel.% = $(ct - c0)/c0 \times -100$).

	ICP-MS				HPLC		
Minutes	**Fe Mean (ng/g)**	**SD (ng/g)**	**RSD (%)**	**Fe (Rel.%) ICP-MS**	**Coprogen Mean (µg/g)**	**SD (µg/g)**	**Coprogen (rel.%) HPLC**
0	360	3	0.7	0	68.4	2.8	0
30	79	15	19	77.9	22.6	0.2	66.9
60	88	12	13	75.6	22.8	0.8	66.7
120	117	7	6	67.5	24.0	0.2	65.0
240	109	4	4	69.6	23.6	0.4	65.5
Zeolite blank	0.67	0.4	63				

3.2. pH-Dependent Sorption

In order to study the sorption and release characteristics of coprogen onto clinoptilolite at different pH values, the tuff was incubated in aqueous solutions with pH values ranging from 4 to 10, and the sorption characteristics were determined (Figure 3, Table 2). From the

results obtained, it is evident that the clinoptilolite-tuff exhibited the strongest adsorption at low pH (pH 4). A total of 90.2% of the Fe content of the stock solution was bound onto the tuff (shown by ICP-MS data). With increasing pH, the sorption capacity was reduced, and thus the detectable amount of molecular coprogen and Fe in the solution was enhanced. A change in pH towards more basic solutions resulted in a decrease in the binding capacity. At pH 10, 60% were still bound onto the tuff, 30% less than at pH 4.

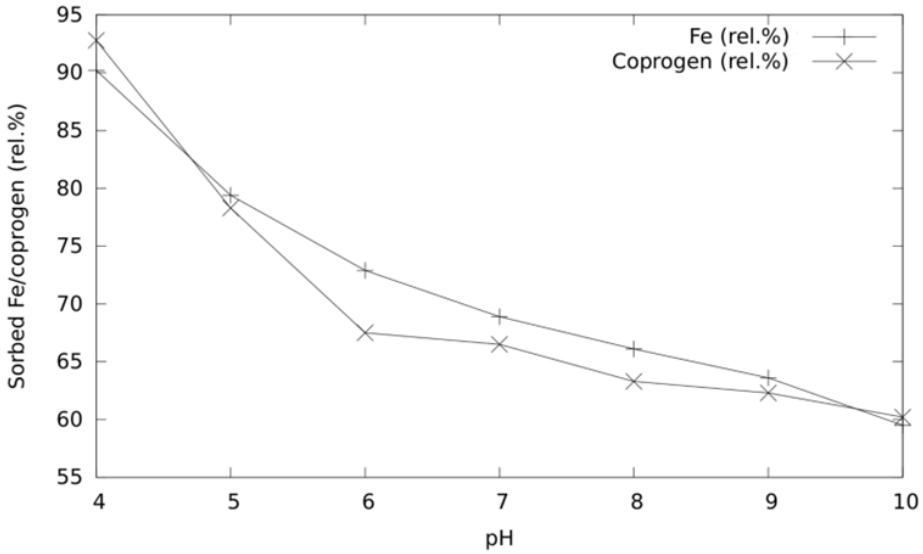


Figure 3. pH-dependent sorption of coprogen/Fe onto the clinoptilolite-tuff.

Table 2. pH-dependent sorption of coprogen/Fe. (SD = standard deviation, RSD = relative standard deviation; rel.% = $(cpH - cblank)/cblank \times -100$).

	ICP-MS				HPLC		
pH Value	**Fe Mean (ng/g)**	**SD (ng/g)**	**RSD (%)**	**Fe (Rel.%) ICP-MS**	**Coprogen Mean (µg/g)**	**SD (µg/g)**	**Coprogen (rel.%) HPLC**
4	38	2.8	7.6	90.2	6.1	0.1	92.8
5	79	9.2	11.6	79.4	18.3	0.3	78.3
6	115	18.8	16.4	72.9	27.4	0.1	67.5
7	120	10.2	8.5	68.9	28.2	0.6	66.5
8	131	7.3	5.6	66.1	31.0	0.2	63.3
9	140	19.0	13.5	63.6	31.8	0.6	62.3
10	156	10.1	6.5	59.5	21.9	0.6	60.2
Coprogen blank	385	2.7	0.7		84.4	0.2	

HPLC measurements also revealed the highest uptake at pH 4 and decreasing sorption capacities towards more basic pH values. From pH 5 to pH 10, the adsorption capacity decreased from 80% to 60%.

Samples of the clinoptilolite-tuff used in the study were analyzed with XRD (Figure 4) and SEM (Figure 5) before and after being incubated with coprogen under optimal conditions (at pH 4 and 30 min). However, although performing multiple analyses with diverse scan parameters and modi, these two methods were not capable of showing significant differences in the studied materials, neither mineralogically nor optically or in-situ chemically (with energy-dispersive X-ray spectroscopy, EDS, and element mapping techniques).

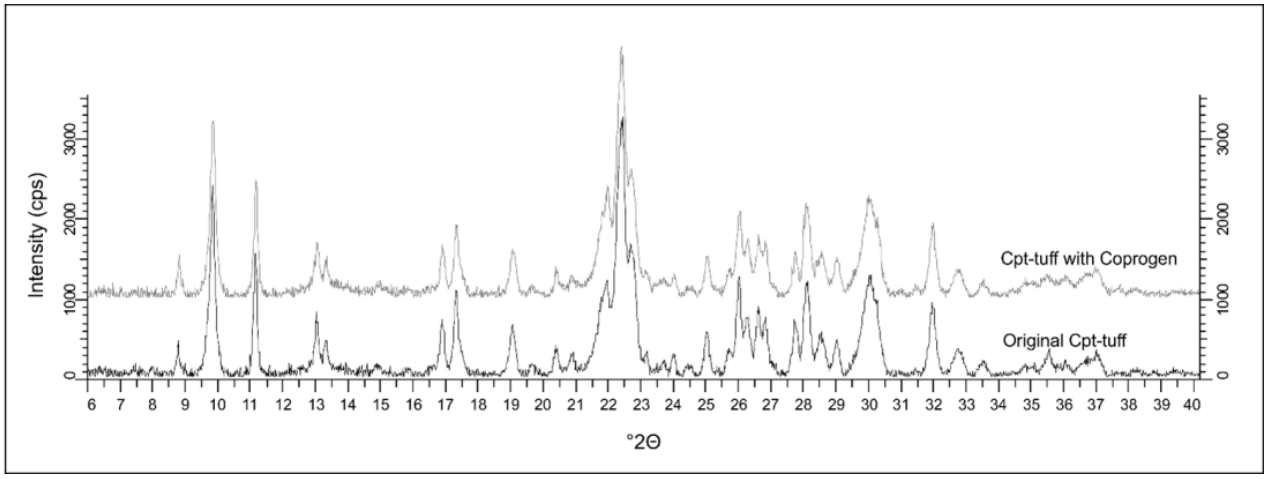


Figure 4. XRD patterns illustrating the whole-rock mineral compositions of the clinoptilolite-tuff from Nižný Hrabovec (NE-Slovak Republic) before (Original Cpt-tuff) and after incubation (Cpt-tuff with Coprogen) with the natural hydroxamate siderophore coprogen. (Cpt = clinoptilolite; cps = counts per second).

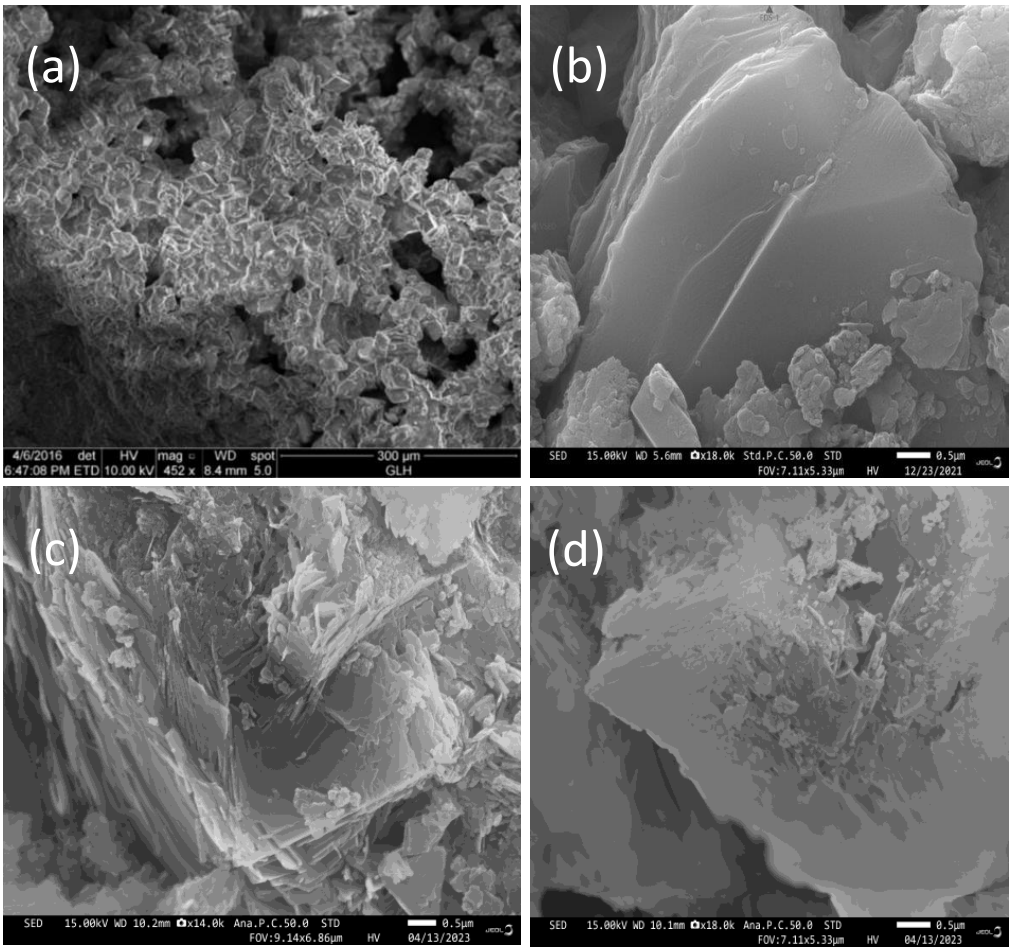


Figure 5. Representative SEM images of the clinoptilolite-tuff from Nižný Hrabovec (NE-Slovak Republic). Micrograph (**a**) shows an overview and (**b**) a detail of the original tuff paragenesis. Micrographs (**c**,**d**) show details of the mineral assemblage after sorption with coprogen. In all images, growth (micro-)structures of clinoptilolite are visible.

Furthermore, the total organic carbon (TOC) content of the samples was determined. While for samples of the tuff with sorbed coprogen, an average of 0.182 ± 0.000816% TOC, which was already close to the limit of quantification, was observed, the original tuff samples (without sorbed coprogen) showed contents below the limit of quantification of 0.04% (Table 3).

Table 3. TOC content of original clinoptilolite-tuff and after sorption (with sorbed coprogen), respectively. Values for the unreacted tuff were below the limit of quantification (LOQ = 0.04%).

	TOC (Rel.%)	TOC (Rel.%) Average	TOC (Rel.%) SD
Original tuff	<LOQ <LOQ <LOQ	<LOQ	<LOQ
After sorption	0.183 0.182 0.181	0.182	0.000816

3.3. Release Kinetics

Column experiments were carried out to analyze the potential of releasing coprogen/Fe from the tuff by rinsing the column with water. The release of coprogen from the tuff with the leaching solution (at pH 8) indicates that the coprogen content decreased steadily with each leaching step. The coprogen-containing fractions were collected, summed up, and analyzed using ICP-MS. The experimental results revealed that after only 50 mL leaching solution, about 40% of the coprogen content was removed from the tuff. After 100 mL leaching solution, only about 21 ng/g, i.e., 12% of coprogen previously bound onto the tuff, still remained adsorbed (Figure 6, Table 4).

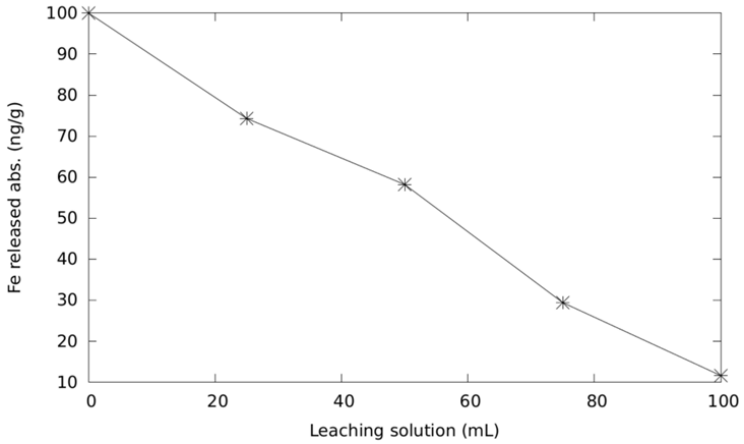


Figure 6. Release kinetics of coprogen into solution after rinsing with the leaching solution at pH 8 (Fe analyzed via ICP-MS).

Table 4. Release of Fe by rinsing with the leaching solution.

Leaching Solution (mL)	Fe Blank (ng/g)	Fe Blank SD (ng/g)	Fe Blank RSD (%)	Fe Mean (ng/g)	Fe Mean SD (ng/g)	Fe RSD (%)	Fe Summed (ng/g)	Fe Summed (rel.%)
0	29.3	2.2	7.5	29.3	2.2	7.5	177	100
25	51.5	3.1	5.9	97.0	14.8	15.2	132	74.3
50	47.7	3.2	6.7	76.2	8.9	11.7	103	58.2
75	44.0	1.8	4.2	49.6	9.5	19.2	52.1	29.4
100	43.9	2.8	6.3	46.9	1.3	2.8	20.6	11.6

4. Discussion

Healthy plant development strongly depends on the bioavailability of the micronutrient Fe. Even though Fe is a ubiquitous component in the environment, Fe-deficiency-triggered chlorosis is a widespread problem, especially for plants cultivated on calcareous soils [32,33]. Affecting horti- and agriculture, chlorosis can lead to serious economical threats and supply security problems, depending on the kind and extent of crop failures. The standard agrotechnical practice to impede and/or fight chlorosis, which may seriously affect the plant's health and growth, is to apply synthetic fertilizers (Fe chelates) in an extensive manner. However, concerns were raised regarding the environmental sustainability of such applications due to their low degradability and the presence of toxic metals in many of them [32,34]. Natural iron chelates, being microbial and non-toxic to soil biota, may outclass conventional synthetic fertilizers.

Batch experiments were set up to study the sorption kinetics of siderophores onto the Slovak clinoptilolite-tuff at pH 6.5. The resulting data show the highest binding capacity (65–67% uptake) after already 30 min, with no further increase of Fe sorption (analyzed by ICP-MS) or coprogen sorption (analyzed via HPLC), respectively, after longer incubation time (Figure 2, Table 1). The remarkably fast sorption character of the clinoptilolite-tuff was reported earlier by Haemmerle et al. [24] for the adsorption of various heavy metals and Samekova et al. [25] for the removal of toxic lead in a clinical trial. Sarmah et al. [34] described the sorption of herbicides from aquatic solution onto zeolite within 10 min; Ranftler et al. [35] documented the adsorption of gluten onto a purified version of the Slovak clinoptilolite-tuff even within 4 min.

Since electrostatic interactions are believed to play a significant role during sorption, i.e., non-covalent binding, solution pH was expected to be of great importance. The net negative charge on the surface of clinoptilolite results from the isomorphic substitution of Si^{4+} by Al^{3+} within the mineral structure and variable charge due to protonation/deprotonation. Therefore, it is the variable charge that triggers pH dependence, as the mineral structure remains constant under experimental conditions [26,36].

The dependency of binding characteristics on pH was investigated from pH 4 to pH 10, indicating the highest sorption potential at pH 4, having 90.2% of Fe and 92.8% of coprogen, respectively (Table 2). The binding capacity constantly decreases over pH 5 to pH 10, showing only 59.9% of Fe being sorbed and 60.2% of coprogen, respectively, at a pH of 10. Rapid sorption of herbicides on zeolite was demonstrated at acidic conditions (pH 5) by Sarmah et al. [34]. Furthermore, a study carried out by Cheng et al. [36] investigated the binding of s-triazine-containing herbicides onto, among other sorbents, clinoptilolite in batch sorption experiments at pH 4.5. It is reported that at these pH values, there is no influence on the sorption process expected. Interpreting the gained data of sorption kinetics and pH dependency experiments of this study (Tables 1 and 2), it becomes obvious that the measured relative amounts of Fe acquired by ICP-MS and coprogen by HPLC hardly vary. This indicates that no disintegration of the Fe-bearing molecule took place during sorption, which is of crucial importance as Fe(III) is bioavailable only when chelated by coprogen.

XRD and SEM (with coupled EDS) were both not capable of documenting any significant difference of the sorbent before and after the experiments with the natural siderophore (Figures 4 and 5). No detectable crystallographic changes in the material might be interpreted as the adsorption of coprogen onto the surface of clinoptilolite rather than extensive ion exchange or absorption processes, which may rather lead to changes in peak shapes or peak positions. However, mainly due to coprogen's organic nature and the low concentration of coprogen within the samples, the XRD technique, in this case, probably reached its limits of detection. Regarding SEM, surficially distributed coprogen is not visible via secondary electron (SE) nor backscattered-electron (BSE) detection mode. As the clinoptilolite-tuff itself contains iron, the expected slight enrichment of Fe on the surface of the tuff through coprogen sorption is not distinguishable from the background signal via EDS or elemental mapping. TOC analyses showed that the amounts of coprogen bound onto the tuff were rather low and close to the limit of quantification, whereas, for the

unreacted tuff, only values below the LOQ (0.04%) were determined. Cheng et al. [36] report a TOC content of clinoptilolite of about 0.01%.

Three major, non-covalent binding mechanisms are expected to be of significance for the sorption on the clinoptilolite-tuff: van der Waals interactions, hydrogen bonding, and electron-donor interactions [36]. The unique sorption capacity of organic compounds onto zeolite is therefore ascribed to electrostatic and hydrophobic interactions. Kraemer et al. [12] describe cation-like adsorption behavior for the binding of DFO-B siderophores onto clays by concluding that electrostatics control the observed interaction processes. These studies conclude that minerals with higher hydrophobic surfaces display higher sorption capacities. Further mechanisms that may contribute to the sorption of organic molecules onto zeolites comprise site-specific interactions between polar functional groups of the sorbed molecule and water molecules surrounding interlayer cations in the mineral structure [37], as well as cooperative effects between neighboring atoms adsorbed on the surface of the zeolite [22]. It is worth noting that coprogen, as well as DFO-B studied by Neubauer et al. [38], are relatively hydrophobic and might thus tend to precipitate with increased concentration. It is, therefore, likely that the adsorption of Fe-loaded siderophores onto clinoptilolite-tuff lies below saturation, as found by Hepinstall et al. [11] for DFO-B onto kaolinite. Furthermore, competitive sorption of co-existing metal cations, such as Na^+ or Mg^{2+}, could potentially influence the sorption of organic substances.

Regarding the sorption characteristics documented in this study, a mainly electrostatically-triggered sorption mechanism is suggested. This is deduced from the large molecular size of the coprogen impeding absorption into the cage structures of the clinoptilolite mineral and the rapid sorption time, which corresponds to well-documented adsorption behavior. As siderophores are capable of having various hydrophobic interactions, influences as such can, however, not be excluded [39].

Characteristics of sorption/release of hydroxamate siderophores have been studied for clay minerals as well; experiments with smectite and kaolinite have been carried out by Haack et al. [40] and Rosenberg et al. [41]. Smectite, an important group of clay minerals with montmorillonite as a prominent member, reportedly shows a high affinity for organic cations, which are incorporated into the smectite interlayer [40]. The results of the aforementioned studies show that the binding of siderophores occurs on the surface of clay minerals in dependence on solution pH, further suggesting that electrostatics play a leading role in the process. Compared to most clay minerals, clinoptilolite has a higher cation-exchange capacity (CEC) [42]; the most striking difference in this context, however, is the documented release mechanism of siderophores at high pH and the established high acceptance of clinoptilolite-tuff in agri- and horticultural application. Based on earlier works [21,22] on the sorption of siderophores on clay surfaces, it was assumed that the siderophore coprogen would also adsorb to clinoptilolite surfaces. Clinoptilolite is one of the most abundantly occurring zeolite minerals, which is widely accepted and used for agricultural purposes. Therefore, it is particularly interesting for investigations as a potential fertilizer carrier substrate. The use of zeolites in various technical, environmental, agricultural, nutritional, biological, and medical field applications has been massively extended over the last decades. Due to their unique crystal structures with interconnected channels and chambers, zeolites, beyond all other applications, serve as potential sorbents for plant nutrients [43]. In agriculture, clinoptilolite-tuff has been used as a soil amendment since the 1960s, especially because of its high cation exchange capacity, ion selectivity, and porosity. Another considerable advantage of zeolite tuffs, especially for cultivation in areas with water shortages, such as the Mediterranean region, is their ability to store large amounts of water, increasing water retention rates [43]. From the above-mentioned observations and considerations, it can be concluded that the combination of clinoptilolite-tuff, as a carrier-substance with controlled-release characteristics, and siderophores, as natural suppliers for bioavailable Fe, offers the potential for being used as an organic fertilizer with the advantage of providing an efficient Fe-source over prolonged periods [14,16], applicable for sustainable farming by simultaneously improving soil properties.

The nutrient will be released gradually and not be leached out of the rhizosphere easily so that it remains available for continuous need of the plants [21]. The pH at which the highest release of coprogen from the tuff was observed in this study coincides with the pH in natural calcareous soils, in which chlorosis most commonly occurs.

5. Conclusions

(1) Kinetic and pH-dependent batch experiments indicate the strongest sorption of natural siderophores (Fe and coprogen; 90.2% and 92.8%) onto the East-Slovak clinoptilolite-tuff at pH 4 after 30 min. In the presented experimental setup, sorption is interpreted as being related to surficial adsorption through mainly electrostatic, less hydrophobic interactions;

(2) Column experiments of the bound coprogen show release characteristics within the alkaline pH range. The majority of Fe-containing coprogen (88%) was released from the tuff after rinsing with a leaching solution at pH 8; the highest sorption capacity, however, was observed at low pH;

(3) Apart from being a controlled-release fertilizer of micro-nutrients, the zeolite-tuff, which remains in the soil, acts as a soil-conditioner with its already well-established mechanisms (e.g., water retention, soil aeration, etc.);

(4) Together, the results point out that the clinoptilolite-tuff represents a suitable and environmentally friendly carrier for natural siderophores, which, when combined, acts as a controlled-release fertilizer in organic and sustained farming. However, greenhouse and field trials on selected chlorosis-endangered plants (especially when grown on calcareous soils) need to be performed in the future to show how the concept of this study can be transferred into agronomical practice.

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