University of São Paulo "Luiz de Queiroz" College of Agriculture

Chemical interaction mechanisms of metals, inorganics anions, organic acids and protein on purified material, artificial soil and cropland soil

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Thesis presented to obtain the degree of Doctor in Science. Area: Soil and Plant Nutrition

Piracicaba 2019 Matheus Sampaio Carneiro Barreto Agronomist

Chemical interaction mechanisms of metals, inorganics anions, organic acids and protein on purified material, artificial soil and cropland soil versão revisada de acordo com a resolução CoPGr 6018 de 2011

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To Brazilian society who paid my journey, I dedicate

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"To myself I am only a child playing on the beach, while vast oceans of truth lie undiscovered before me."

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"If I have the gift of prophecy and can fathom all mysteries and all knowledge, and if I have a faith that can move mountains, but do not have love, I am nothing."

1 Cor 13:2

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#### **RESUMO**

# Mecanismos de interação química de metais, ânions inorgânicos, ácidos orgânicos e proteína em materiais purificados, solo artificial e solo cultivado

O solo é um complexo sistema com variadas fases que alojam reações de interfaces que determinam a mobilidade/disponibilidade de nutrientes e contaminantes no ambiente. O entendimento profundo dos mecanismos que regem as reações é um desafio, sendo muitas vezes necessária a utilização de modelos com número reduzido de componentes e de alta pureza, a fim de que se isolem os efeitos de variáveis ambientais como tipo de minerias, pH e tempo. Podem também ser aplicadas sofisticadas técnicas analíticas para elucidar os mecanismos envolvidos. Nesse trabalho, procurou-se avaliar mecanismos de algumas reações químicas que envolvem os nutrientes P e Zn, macromoléculas como proteína e substâncias húmicas, bem como os contaminantes Cd e As; todos eles em matrizes puras ou em amostras de terra de áreas cultivadas e sob vegetação nativa (VN). As principais conclusões foram: (i) o complexo de superfície Phematita em configuração bidentada teve adsorção mais rápida do que a monodentada, sendo a bidentada preferencialmente desorvida pelo ânion citrato, cuja adsorção anterior ao fosfato diminuiu a adsorção de P na superfície da hematita; (ii) o As(V) é adsorvido em complexos de esfera interna e externa nas superfícies de hematita e de hausmanita, sendo maior a adsorção na hausmanita, que se mostrou uma opção plausível de filtro para As(V); (iii) o processo de adsorção da proteína BSA em hematita teve forte dependência do pH do meio, sendo o pH próximo ao ponto isoelétrico da proteína o que maximizou a adsorção, sendo que houve considerável mudança conformacional nas estruturas secundáriasda BSA após adsorção, o que a tornou menos "empacotada" e mais "desdobrada", (iv) o Ca aumentou a adsorção de substancias húmicas nos minerais estudados por meio de complexos ternários "mineral-Ca-substâncias húmicas", e houve preferência de adsorção nas superfícies dos minerais presentes em solos intemperizados, por moléculas orgânicas menos humificadas e/ou de menor peso molecular; (v) em pH 4,5 houve menor adsorção de Zn, sendo esse retido preferencialmente em minerais alumino-silicatados, enquanto em valores de pH 6,5 as espécies de Zn ligadas a óxido de Fe e aquele precipitado com P também foram identificadas. Contudo, após seis meses de incubação, essas espécies diminuíram ou desapareceram, sendo as espécies de Zn adsorvidas aos minerais alumino-silicatados dominantes após longo tempo de incubação; (vi) Cd e Zn foram adsorvidos rapidamente em solos sob VN ou solos agrícolas altamente fertilizados, sendo que solos agrícolas adsorveram mais esses metais do que o solo sob VN. Porém, apesar da maior adsorção, esses metais ainda foram dessorvíveis por EDTA; (vii) a fração de Zn adsorvido à caulinita esteve presente em todas as amostras de solo, independentemente da origem e do manejo, mas o manejo agrícola e o tempo de incubação aumentaram a quantidade de Zn associado a frações de menor mobilidade. Além disso, a espécie de Zn associado ao fosfato esteve presente em solos altamente fertilizados, e a composição mineralógica do solo determinou a distribuição das frações de Zn adsorvido no solo.

Palavras-chave: Fenômenos de superfície, Sistema coloidal, Adsorção, Espectroscopia de absorção de raios-X, Espectroscopia de infravermelho, Mineralogia do solo

# ABSTRACT

# Chemical interaction mechanisms of metals, inorganic anions, organic acids and protein on purified material, artificial soil and cropland soil

The soil is a complex system with several phases, where abundant interfaces phenomena happen, which determines the mobility/availability of nutrients and contaminants into the environment. The deep understanding of these chemical mechanisms is a challenge, and it is often necessary to use models with reduced numbers of components and high purity, in order to isolate the effects of environmental variables such as minerals type, pH and time. Also, sophisticated analytical techniques may be used to elucidate the mechanisms involved at atomic and molecular scales. Here, mechanisms of some chemical reactions for some nutrients such as P and Zn, macromolecules such as protein and humic substances, as well as a contaminant such as Cd and As were evaluated, using pure systems, artificial soil or soil samples from cultivated areas. The main conclusions were: (i) the previous adsorption with citrate decreased P adsorption on hematite surface. The P-hematite surface complex in bidentate configuration had a faster absorption than the monodentate, and the bidentate was preferentially desorbed by citrate; (ii) As(V) was adsorbed on inner and outersphere complexes on the hematite and hausmannite surfaces, hausmannite showing a higher adsorption than hematite. The transformation from hausmannite to manganite should increase As(V) adsorption an its stability, thus hausmannite could be a good option as As(V) filter; (iii) the adsorption on hematite surface promoted a prominent conformational change in the secondary structures of BSA protein, toward a less "packed" and more "unfolded" protein forms, and the pH near the isoelectric point maximized its adsorption; (iv) Ca increased the adsorption of humic substances in the minerals present in weathered soils. Ternary complexes "mineral-Ca-humic substances" were responsible for this increment; and organic molecules less humified and/or with lower molecular weight were preferentially adsorbed on minerals' surfaces; (v) less Zn was adsorbed at pH 4.5, which was preferentially retained in aluminosilicate minerals, while at pH 6.5, more Zn was adsorbed and Zn sorbed on Fe oxide, and Zn-P precipitates were identified. However, after 6 months, these ones decreased or disappeared, and the fraction of Zn sorbed to aluminosilicate minerals incresed; (vi) Cd and Zn are rapidly adsorbed on soils under native vegetation or highly fertilized agricultural soils, and agricultural soils adsorb more these metals than soil under native vegetation. These metals, however, were easily desorbable by EDTA; (vii) the soil mineralogy determined the main Zn fractions adsorbed on the soil, the fraction of Zn adsorbed to kaolinite was present in all soil samples, regardless of origin and management; agricultural management and time increased the amount of Zn in less mobile fraction, and Zn associated with P was present in highly fertilized soils.

# Keywords: Surface phenomena, Colloidal system, Adsorption, X-ray absorption spectroscopy, Infrared spectroscopy, Soil mineralogy

# **1. GENERAL INTRODUCTION**

The soil system is composed of several phases, like each mineral surface, organic compound, water, and gaseous phase. All these individual systems have a singular interfacial region where adsorptions processes occur. The adsorption mechanisms can be investigated from atomic, molecular to macroscopic scale, as well as in-situ process, because of the evolution of instrumental techniques and strategies for individualizing soil players. Hence, these reactions could be analyzed by traditional methods and/or sophisticated technologies such as isothermal calorimetry titration, microscopies (e.g. Force Atomic, Transmission or Scanning Electron) or spectroscopies (e.g. Xray Absorption, Xray diffraction, and Infrared) techniques in combination with interdisciplinary research of biological, chemical, and physical interfaces (Kruse et al., 2015; Lombi and Susini, 2009; Sarret et al., 2013; Sparks, 2006).

Some elements and minerals and organic components have a singular behavior and deserve attention for scientific research. Hematite is an abundant iron oxide in soils and sediments (Schwertmann, 2008), which suggest that it as an appropriated mineral surface to represent highly weathered soils. A significant feature of iron oxides is the high adsorption capacity of oxyanions as phosphate (P), which promotes the low bioavailability of P in some tropical soils (Roy et al. 2016). On the other hand, this high affinity allows the hematite uses for arsenic (As) filter employed in water treatment (Mohan and Pittman, 2007). The mineral hausmannite, as the hematite, also has high As adsorption capacity, but it is metastable at surface environmental condition and could improve As adsorption stability more than hematite-As complexes.

The organic compounds also have impacts in soil process. Citrate has been found in high concentrations in soil porewaters (Clarholm et al., 2015; Nambu et al., 2008). Rhizosphere exudates promote oxyanions' desorption (e.g. P and As), which makes it a model of organic acid in soil. The behavior of humic acids in soils has attracted attention because of climate change mitigation practices related to C soil storage mechanisms (Six et al., 2002). Likewise, some metallic cations have many functions in the soil ecosystem. Calcium (Ca) is a plant/animal nutrient and can enhance organic compounds adsorption on minerals (Sowers et al., 2018). Zinc (Zn) is a nutrient as well, however could be toxic in high concentration, whereas cadmium (Cd) is a harmful metal even in low concentration in soil (Bradl, 2004).

In this study, the chemical interaction mechanisms among metals, inorganic anions, organic acids and biological molecule on purified material, artificial soil, and cropland soil

were investigated by multi-scales spectroscopic techniques. The use of purified material or artificial soil allow as to isolate effects of some environmental variables as pH, ionic strength, surface loading, and aging on specific reactions, for example, the phosphate adsorption on hematite surface or metal fate on soil system after initial adsorption. This approach allows a more detailed and clear description of chemical process. Therefore, we carried out studies from pure materials and artificial soil to real soil sample of cropland and natural areas.

In this study, the hypothesis were: (i) citrate desorbs specific phosphate-adsorbed species, (ii) hausmannite could adsorb higher amount of arsenate and immobilize it in less desorbable forms than hematite, (iii) protein change secondary conformation after adsorbed on hematite, (iv) the presence of phosphate in minerals system drives an increase Zn adsorption as Zn-phosphate phases, (v) calcium improves humic acid adsorption on soil, (vi) cropland soils adsorbs more cadmium and zinc than soils under native vegetation, (vii) agriculture land use promotes different Zn species compared to natural soil, as Zn-phosphate and Zn-carbonate.

The objectives in this study were to evaluate: (i) phosphate and citrate adsorption and competition on hematite surface, (ii) arsenate and citrate adsorption and desorption on hausmannite and hematite surface, (iii) protein adsorption and structural conformation on hematite surface as a function of changes in pH, ionic strength and protein concentration, (iv) the effects of phosphate and pH on Zn adsorption over aging time on artificial soil "clay" fraction, (v) humic acids adsorption improvement due to calcium, on artificial soil "clay" fraction, (vi) adsorption-desorption process of Cd and Zn in soils under native vegetation and in cropland soils, and (vii) zinc chemical fractionation and speciation after adsorption in these soil in soils under native vegetation and in croplands.

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# 2. EFFECTS OF CITRATE ON PHOSPHATE ADSORPTION AND DESORPTION ON HEMATITE

# ABSTRACT

Plant strategies to grow in soils with low nutrient availability include modification of the rhizosphere by root exudates capable of increasing the bioavailability of nutrients. In-situ Attenuated Total Reflectance Fourier Transform Infrared (ATR-FTIR) spectroscopy was used to evaluate the dynamics and mechanisms of adsorption of orthophosphate and citric acid onto hematite in binary and ternary systems at near neutral to slightly acidic pH. The pH, carboxylic acids and reaction time were important factors in regulating orthophosphate solubility and speciation in soils and rhizospheres. In binary systems, phosphate adsorbed as a mixture of two inner-sphere species assigned as monodentate and bridging bidentate complexes, with the fraction of bidentate complexes increasing with decreasing pH. Citrate adsorbed predominantly as nonprotonated complexes. The carboxylate groups of adsorbed citrate consisted of a mixture of inner-sphere and both non-coordinated or outer-sphere complexes, and the inner-sphere complexation was more pronounced at low pH. Introduction of citrate into phosphate-hematite systems induced phosphate desorption at an extent that increased with decreasing pH. Citrate more effectively competed with the bidentate than the monodentate phosphate surface complexes, and consequently enhanced the fraction of monodentate phosphate species. Kinetic ATR-FTIR data tracking phosphate adsorption and speciation during the first 3.5 h of interaction with hematite, point out that the bidentate phosphate surface complexes had a faster sorption rate than the monodentate complexes, causing a shift of the phosphate surface speciation over the course of equilibration.

Keywords: Colloidal surface; Rhizosphere; In-situ Infrared spectroscopy; adsorption; Anion competition; Iron-oxide



# **Graphical Abstract**

#### 2.1. Introduction

Phosphorus (P) is the most limiting nutrient limiting in highly weathered tropical soils, and a large amount of fertilizer is commonly applied every year to guarantee sufficient availability of this element for crop growth (Lopes and Guilherme, 2016). The extent of P retention in soils is positively correlated to amounts of amorphous and crystalline Fe- and Aloxyhydroxides, that are important sinks of soil P (Parfitt and Atikinson, 1976; Abdala et al., 2014; Chacon et al., 2006; Lü et al., 2017; Santos et al., 2017).

Highly weathered tropical soils have considerable P-fixing capability caused by transformation of inorganic P into low soluble and low bioavailable chemical forms (Arai and Sparks, 2007; Barrow, 2015; McLaughlin et al., 2011; Sanyal and De Datta, 1991). Roy et al. (2016) estimated that 1-4 Tg P yr<sup>-1</sup> is fixed in soil systems worldwide. In Brazil, decades of high fertilizer addition have increased substantially the level of total contents of P in the soils, but soil P is poorly accessible to plants owing to fixation (Rodrigues et al., 2016; Withers et al., 2018).

Plant strategies to grow in soils with low nutrient availability include modification of the rhizosphere by root exudates capable of increasing the bioavailability of nutrients (Dakora and Phillips, 2002). Exudation of small organic acids such as citrate, oxalate and malate may increase the concentration of these acids to levels exceeding 50 mM in the rhizosphere of plants with cluster roots, but are less than 1 mM in most other plants (Jones et al., 1996). The organic anions may displace inorganic nutrient anions ( $HPO_4^{2-}$ ,  $MoO_4^{2-}$ ) strongly adsorbed at the surface of Fe- and Al-oxides, or dissolve mineral sorbent through ligand-promoted dissolution, thereby releasing bound nutrients while limiting reprecipitation of Fe-P or Al-P minerals (Wang et al., 2015).

Citrate ( $C_6H_5O_7^{-3}$ ) has been studied in some detail in relation to soil phosphate mobilization, because this tricarboxylic anion desorbs P from soil minerals (Dakora and Phillips, 2002; Hu et al., 2001; Jones et al., 1996; Lindegren and Persson, 2009; Mikutta et al., 2006; Oburger et al., 2011; Santos et al., 2017; Yan et al., 2015) Citrate has been found in high concentrations in soil porewaters (Clarholm et al., 2015; Nambu et al., 2008) and rhizosphere exudates (Corrales et al., 2007; Giles, et al., 2012; Silva et al., 2001). Souza et al. (2014) showed that citrate effectively desorbed P and decreased total P adsorption in highly weathered Brazilians soils, whereas Santos et al. (2017) found that leaching of P-amended Ferralsols (18 mg P kg<sup>-1</sup>) with 1 mM citrate (56 pore volumes) did not induce sufficient P desorption to satisfy plant requirements.

The processes involved in P retention and mobilization in soils are best understood when the molecular-level sorption reactions at soil mineral surfaces which control P solubility are evaluated. Attenuated Total Reflectance Fourier Transform Infrared (ATR-FTIR) spectroscopy is an in situ technique that permits the study of interfacial processes at the molecular scale in aqueous systems (Lefèvre, 2004). The technique has been successfully applied to study the interaction of Fe-oxide minerals with inorganic oxyanions such as phosphate (Anderson et al., 1985; Arai and Sparks, 2001; Barja et al., 1999; Elzinga and Kretzschmar, 2013; Elzinga and Sparks, 2007; Geelhoed et al., 1998; Persson et al., 1996), arsenate (Brechbühl et al., 2012; Jia et al., 2007; Sabur and Al-Abadleh, 2015; Vansant and Van Der Veken, 1973), sulfate (Peak et al., 1999) and borate (Peak et al., 2003), as well as with organic acids (Noerpel and Lenhart, 2015; Yang et al., 2014; Yeasmin et al., 2014).

Phosphate adsorption at goethite and hematite surfaces yields a mixture of innersphere complexes whose proportion varies with pH, surface loading, ionic strength and time (Arai and Sparks, 2001; Kubicki et al., 2012; Parfitt and Atikinson, 1976) (Barja et al., 1999; Elzinga and Kretzschmar, 2013; Elzinga and Sparks, 2007; Luengo et al., 2006). The speciation of adsorbed carboxylic acids such as citrate at goethite and hematite surfaces is complex as well, involving a mixture of inner- (high adsorption energy) and outer-sphere (low energy adsorption) complexes with variable proportions depending on pH and surface loading (Borer and Hug, 2014; Lindegren et al., 2009; Lindegren and Persson, 2009; Noerpel and Lenhart, 2015; Situm et al., 2016). The mechanistic complexity of phosphate and citrate adsorption makes it difficult to predict the behavior of these species in environments where both are present, highlighting the need for mechanistic studies of dual-sorbate systems.

Our aim in the present study was to evaluate the interactions between phosphate and citric acid at the hematite-water interface with ATR-FTIR spectroscopy. Hematite was chosen as a sorbent because it is a common Fe-oxide in P-deficient tropical soils (Fink et al., 2016; Fontes and Weed, 1996), while citrate is a widespread component of rhizosphere exudates in soils with limited P availability (Clarholm et al., 2015; Kidd et al., 2018; Lipton et al., 1987).

#### 2.2. Material and Methods

# 2.2.1. Hematite Sorbent

Hematite was synthesized based on the procedure of Sugimoto et al. (1993) and was used previously in the IR studies of Brechbühl et al. (2012) and Elzinga and Kretzschmar (2013). X-ray diffraction analysis confirmed that the material was hematite with no evidence for the presence of other crystalline phases. The N<sub>2</sub>-BET specific surface area of the hematite was 24 m<sup>2</sup> g<sup>-1</sup>, and the zero point of charge (PZC) was 9.3. A detailed description of the material is provided in Brechbühl et al. (2012).

# 2.2.2. ATR-FTIR experiments

The experimental IR setup is very similar to that used by (Elzinga and Kretzschmar, 2013). Data acquisition was performed with a Perkin–Elmer Spectrum One spectrometer equipped with a Balston-Parker purge gas generator and a liquid N<sub>2</sub>-cooled mercury– cadmium–telluride (MCT-A) detector. The sorption experiments were conducted with an ATR-FTIR flow-cell containing a horizontal ZnSe crystal (10 internal reflections; 45° angle of incidence). The crystal was coated with a hematite film (625  $\mu$ g) by drying 125  $\mu$ L of a 5 g L<sup>-1</sup> hematite suspension spread evenly across the surface, producing a stable and homogeneous deposit. A new deposit was prepared for each experiment.

The coated crystal was installed in the flow cell inside the IR spectrometer and connected to a vessel containing 500 mL of 0.1 M NaCl electrolyte which was magnetically

stirred and set to the desired pH. A peristaltic pump was used to flow solute from the reaction vessel through the cell at a rate of 2 mL min<sup>-1</sup>. The outlet solution from flow cell was reintroduced into the reaction vessel, so that the system was closed. The solution pH in the vessel was monitored throughout the experiment and readjusted or changed as necessary by addition of negligible (relative to the reactor volume) aliquots of 0.1 M NaOH or 0.1 M HCl. The hematite deposit was equilibrated with the background solution for 2 h. A final background spectrum, containing the absorbances of the ZnSe crystal, the hematite deposit and the background electrolyte, was then collected as the average of 200 scans at a resolution of 4 cm<sup>-1</sup>. Next, the vessel was spiked with aqueous phosphate or citrate to start the adsorption experiment. The spectra of adsorbed phosphate and citrate were collected by ratio-ing the IR spectra of the hematite film against the background spectrum collected at the end of the pre-equilibration step.

Three main types of adsorption experiments were carried out, each aimed at characterizing a specific aspect of phosphate and citrate interaction with the hematite surface. The first aimed to characterize the pH dependency of phosphate and citrate adsorption processes at the hematite surface. To this end, we carried out "pH envelope" experiments where adsorption was studied as a function of pH as the main variable. Pre-equilibration was performed at pH 8.5, and after collection of the background spectrum a 125  $\mu$ L aliquot of 0.1 M phosphate (as Na<sub>2</sub>HPO<sub>4</sub>) or citric acid stock was injected into the main vessel to achieve an orthophosphate or citrate solution concentration of 25  $\mu$ M. The pH was re-adjusted to pH 8.5, and the hematite deposit was allowed to achieve adsorption equilibrium with the phosphate or citrate solute, as monitored by regular scanning. The final scan was then collected as the average of 200 scans at a 4 cm<sup>-1</sup> resolution over the 4000-600 cm<sup>-1</sup> wavenumber range and the pH was lowered to pH 8.0, where a new adsorption equilibrium was established after ~20 min. This step-wise lowering of pH and re-equilibration was repeated at pH 7.5, 6.5, 5.5 and 4.5.

A second set of experiments aimed to characterize dynamic changes in the phosphate surface speciation during phosphate equilibration. These experiments used the same protocols as described previously. After pre-equilibration of the hematite deposit with background electrolyte, phosphate was added at a concentration of 25  $\mu$ M, and its adsorption onto the hematite deposit was tracked by regular collection of spectra over the course of 3.5 h. The kinetic spectra were recorded as the average of 20 scans at a 4 cm<sup>-1</sup> resolution, requiring  $\approx 18$  s per spectrum.

In a final series of experiments, the effects of citrate on the extent, dynamics, and mechanisms of P retention at the hematite surface were investigated. Hematite deposit was equilibrated with phosphate for 3.5 h, after which time citrate was added to the reaction solute at levels increasing stepwise from 10  $\mu$ M to 250  $\mu$ M. At each level of citrate addition, the system was left to reach a new sorption equilibrium (which typically required ~15 min) before the final spectrum was collected. These experiments were performed at pH 6.5, 5.5, and 4.5 to cover a range of pH values pertinent to soil rhizosphere environments. In a related set of experiments, the hematite deposit was first equilibrated with 25  $\mu$ M citric acid for ~1 h, and then phosphate was introduced at a level of 25  $\mu$ M, with spectra were collected as described above to evaluate the dynamics of phosphate adsorption.

Aqueous spectra were collected by scanning solutions of 50 mM of phosphate or citrate adjusted to pH values in the range 4.5-8.5 using 0.1M NaOH or HCl. Two milliliters of 0.1 M NaCl electrolyte were deposited on Ge ATR crystal, then after 5 min of initial interaction, an ATR-FTIR spectrum was collected as a background spectrum (200 scans) for the subsequent calculation of the absorbance spectra of the phosphate and citrate species (200 scans) without the contribution of water, 0.1 M NaCl electrolyte and Ge crystal.

Data processing involved baseline correction of the spectra collected for the phosphate and citrate surface complexes. The baseline was fitted as a straight line between 1240 to 840 cm<sup>-1</sup> for phosphate, and as a parabolic line between 1770 to 1230 cm<sup>-1</sup> for citrate. These corrections were carried with the software package MagicPlot ver. 2.7.2 (Magic Plot Systems, LLC, Saint Petersburg, Russia). The aqueous speciation was estimated using VisualMinteq software (Gustafsson, 2016). A semiquantitative estimate of phosphate adsorption was obtained by integration of the area under the phosphate IR peaks of the baseline-corrected spectra using the software package Origin 2017 (OriginLab Corporation, Northampton, Massachusetts, USA).

# 2.3. Results and Discussion

# 2.3.1. Phosphate and citrate adsorption in binary systems

The spectra of adsorbed phosphate (Fig. 1B) were considerably different from the spectra of aqueous phosphate at all pH values (Fig. 1A), indicating strong interactions of phosphate with hematite. The intensity of the IR bands of adsorbed phosphate increased with decreasing pH (Fig. 1B), demonstrating that P adsorption was enhanced at lower pH, as is expected (e.g. Hingston et al., 1967; Kingston et al., 1972; Geelhoed et al., 1998; Arai and

Sparks, 2007). This may be due to electrostatic effects, as anionic P species are dominant in solution ( $H_2PO_4^-$  and  $HPO_4^{-2-}$ ) while the hematite surface (with a PZC of 9.3) becomes more positively charged as pH is lowered, promoting sorbate-sorbent interactions. In addition, protonation of surface sites at lower pH makes ligand exchange reactions between phosphate and reactive surface hydroxyl sites ( $\equiv$ Fe-OH) more favorable, enhancing inner-sphere complexation at acidic pH.



**Figure 1**. (A) ATR-FTIR of aqueous phosphate in the experimental pH range collected for 50 mM phosphate solutions. (B) ATR-FTIR spectra of orthophosphate surface complexes on hematite in the pH range 8.5 to 4.5; (C) The difference spectrum between the spectra obtained at pH 4.5 and 8.5, which isolates the orthophosphate surface complex that grows in with decreasing pH. The sorption spectra were collected in 0.1 M NaCl background electrolyte and at a total orthophosphate solution concentration of 25  $\mu$ M. Absorbance is given in arbitrary units.

Table 1 summarizes the main possible configurations of the phosphate-hematite surface complexes based on assignments of IR data made in previous studies. For a detailed discussion of phosphate surface complex designation based on ATR-FTIR data see (Barja et al., 1999; Elzinga and Sparks, 2007; Luengo et al., 2006; Tejedor-Tejedor and Anderson, 1990). The spectrum of phosphate adsorbed at pH 8.5 contains two broad IR peaks near 1050 cm<sup>-1</sup> and 975 cm<sup>-1</sup>; the asymmetry of the 1050 cm<sup>-1</sup> vibration indicates the presence of another band near 1090 cm<sup>-1</sup> (Fig. 1B). This spectrum is similar to those reported by (Elzinga and Kretzschmar, 2013; Elzinga and Sparks, 2007) for phosphate adsorbed on hematite at alkaline pH. Using symmetry arguments and results of D<sub>2</sub>O experiments, Elzinga and Sparks, (2007) assigned this spectrum to a monoprotonated monodentate phosphate surface complex (Table 1).

The relatively broad IR bands suggest a higher degree of structural variation within the population of monodentate phosphate surface complexes as compared to earlier studies in which the bands were more clearly resolved. Although subtle, this difference was somewhat surprising because the hematite sorbent and experimental conditions used here were identical to those in Elzinga and Kretzschmar (2013), except that the data were collected on different instruments.

Phosphate complex		Band assignments	IR absorption $(cm^{-1})$			
Туре І	Protonated	v(P=O)	1108			
	monodontato	v(P-OFe)	992			
	monouentate	υ(P-OH)	974			
		v(P=O)	1095			
Type II	Bridging bidentate	v(P-OFe)	1011			
		v(P-OH)	983			
	Monodontata	asymmetric PO <sub>2</sub> stretches	1080			
At high pH	monoperatoriated	symmetric PO <sub>2</sub> stretches	1050			
	monoprotonated	v(P–OFe) or v(P–OH)	950			
Band assignments for goethite from (Barja et al., 1999)						
At high pH		$v(PO_2)$	1080			
	Monodentate monoprotonated	$v_{\rm es}(\rm PO_2)$	1050			
		$v(P-OH)$ or $v(P-(OFe)_2)$	950			
			1100			
		v(P=O)	1120			
At low pH	Monoprotonated	$v_{as}(P-(OFe)_2)$	1008			
1	bridging bidentate	v(P-OH)	980			
	N	$v_{\rm s}({\rm P-}({\rm OFe})_2)$	925			
At high pH or	Non-protonated		1075			
higher P surface	monodentate inner-		990			
coverage	spnere					
Band assignments for hematite (Elzinga and Kretzschmar, 2013; Elzinga and Sparks, 2007)						
Citrate comple	ex E	Band assignments	IR absorption (cm <sup>-1</sup> )			
		v(C=O)	1724.9			
рН 1.5		$v_{as}(COO^{-})$	-			
1		v <sub>sym</sub> (COO <sup>-</sup> )	1398.7			
		v(C=O)	1723.2			
рН 3.5		v <sub>sym</sub> (COO <sup>-</sup> )	1578.8			
		$v_{as}(COO^{-})$	1397.5			
		v(C=O)	1725.9			
рН 5.6		v <sub>sym</sub> (COO <sup>-</sup> )	1569.7			
		$v_{as}(COO^{-})$	1390.3			
		v(C=O)	-			
pH 8.7		v <sub>sym</sub> (COO <sup>-</sup> )	1567.3			
		$v_{as}(COO^{-})$	1390.1			
Band assignments for hematite (Noerpel and Lenhart, 2015)						
		v(C=O)	1713			
A. J 1 . 1		v <sub>svm</sub> (COO <sup>-</sup> )	1454,1404,1365			
Adsorbed citrate of	n	$v_{as}(COO^{-})$	1558.1535			
hematite	v(	$CO_2H$ + $\delta(CO_2H)$	1238			
		v(CO <sub>2</sub> Fe)	1616,1581.1420			
Band assignments for hematite from (Situm et al., 2016)						
		p(C=0)	1710			
Adsorbed citrate of	n	$\mathbf{p}_{\rm max}(\mathbf{CO})$	1610 1400			
goethite		n (CO)	1565			
Socuric	Fe(III)-coor	rdinated carboxylate groups	1626 1565			
Band assignments for goethite from (Lindegren et al. 2009)						
Dune assignments for goodine from (Lindogren et u., 2007)						

 Table 1. Assignments of IR bands and configuration of phosphate and citrate surface complexes formed on Fe-oxides surfaces.

Lowering of pH not only increased the intensity of the IR spectra of adsorbed phosphate (due to increased adsorption), but also caused systematic shifting of the main peak from 1050 to 1105 cm<sup>-1</sup> indicating changes in the phosphate surface speciation (Fig. 1B and S4). To isolate the spectral changes resulting from lowering pH, the difference spectrum of the spectra collected at pH 4.5 and pH 8.5 was calculated. The result is presented in Figure 1C and shows a spectrum with absorbances at ~1116, ~1000 and ~890 cm<sup>-1</sup> (Fig. 1C). Elzinga and Sparks (2007) assigned this spectrum to a monoprotonated bidentate phosphate surface complex based on symmetry arguments and results from D<sub>2</sub>O experiments, and demonstrated that this surface species becomes increasingly prominent with decreasing pH and increasing surface loading (Table 1). Overall, our IR results indicate the simultaneous presence of at least two phosphate species at the hematite surface that change proportion with pH, consistent with earlier studies (Tejedor-Tejedor and Anderson, 1990; Barja et al., 1999; Elzinga and Kretzschmar, 2013; Elzinga and Sparks, 2007; Luengo et al., 2006; Persson et al., 1996; Rahnemaie et al., 2007).

Figure 2 presents the IR spectra of aqueous citrate references (Figure 2A) and of the pH edge measurements of citrate adsorption (Figure 2B). Most aqueous spectra contained prominent peaks at ~1570 cm<sup>-1</sup> and ~1390 cm<sup>-1</sup>, which were the asymmetric and symmetric C-O stretching vibrations ( $v_{as}(COO^{-})$  and  $v_{s}(COO^{-})$ , respectively) of deprotonated-carboxyl groups (Table 1). At low pH, the aqueous spectra additionally contained a peak at ~1720 cm<sup>-1</sup> (Fig. 2A) which is the carbonyl stretch ( $v_{C=O}$ ) of protonated carboxyl groups (Borer et al., 2007; Kubicki et al., 1999; Lackovic et al., 2003; Lindegren et al., 2009; Mudunkotuwa and Grassian, 2010; Noerpel and Lenhart, 2015; Situm et al., 2016). The triple feature at 1230~1320 cm<sup>-1</sup> consists of C-OH bending of the hydroxyl group (at 1280 cm<sup>-1</sup>) and of C-H rocking of the CH<sub>2</sub> groups of the citrate skeleton (1255 and 1295 cm<sup>-1</sup>; (Noerpel and Lenhart, 2015; Situm et al., 2016). The  $v_{as}(COO^{-})$  and  $v_{s}(COO^{-})$  stretching bands dominate the spectra of adsorbed citrate (Fig. 2B). The intensity of these bands increases with decreasing pH and indicates that citrate adsorption is enhanced by lower pH values, in line with macroscopic results (Lackovic et al., 2003; Lindegren et al., 2009; Noerpel and Lenhart, 2015).



**Figure 2.** (A) ATR-FTIR spectra of aqueous citrate at pH 4.5-8.5; (B) ATR-FTIR spectra of citrate surface complexes on hematite surface at pH 4.5-8.5 collected in flow cell experiments using a 25  $\mu$ M citrate solution concentration. (C) The difference spectrum between the spectra obtained at pH 4.5 and 8.5. Noteworthy, the negative area around 1470 and 1358 cm<sup>-1</sup> (dashed line, y = 0) are due to carbonate desorption by pH increasing (Brechbühl et al., 2012). All spectra were collected in 0.1M NaCl background electrolyte. Absorbance is in arbitrary units.

Comparison of the ATR-FTIR spectra of adsorbed and dissolved citrate is a useful starting point for assessing the protonation state and surface coordination of adsorbed citrate, as demonstrated in previous IR studies of citrate adsorption onto Fe-oxide surfaces (Table 1). The C=O stretching band at 1720 cm<sup>-1</sup> is absent in the spectra of adsorbed citrate at pH 6.5 and small at pH 5.5 and 4.5 (Figure 2B), suggesting that the carboxylate groups of adsorbed citrate are non-protonated at pH > 5.5 and partially protonated at pH 5.5 and 4.5 (Hwang et al., 2007; Lindegren et al., 2009; Mudunkotuwa and Grassian, 2010; Noerpel and Lenhart, 2015). The lack of protonation at pH 6.5 is noteworthy, since dissolved citrate contains

protonated carboxylate groups at these pH values (Figure 2A). Surface-promoted deprotonation may be the result of repulsive electrostatic interactions between protons and the positively charged hematite surface, or increased polarization of C-O-H bonds due to inner-sphere coordination of the C=O moiety (Hwang et al., 2007; Mudunkotuwa and Grassian, 2010).

The spectra of adsorbed citrate are overall similar to that of fully deprotonated dissolved citrate (Figure 2), but several key differences revealed interactions with the hematite surface. The C-O stretching bands of adsorbed citrate are located at similar positions but are broader than for aqueous citrate, which has been interpreted in previous studies to indicate outer-sphere (electrostatic) interactions as the main mechanism of citrate adsorption (Gao et al., 2017; Noerpel and Lenhart, 2015; Roddick-Lanzilotta and McQuillan, 2000). Apparent splitting of the symmetric C-O stretch into peaks at ~1395 and 1435 cm<sup>-1</sup> may, however, indicate inner-sphere complexation, although the band at 1435 cm<sup>-1</sup> alternatively may be due to -CH<sub>2</sub>- bending (Mudunkotuwa and Grassian, 2010). Other than activation of the  $v_{C=O}$  stretch at pH 4.5, the spectra obtained at pH 4.5, 5.5 and 6.5 are very similar (Figure 2B), which indicates limited variation in the mechanisms of citrate adsorption under the conditions of this study.

Despite the overall similarity of the spectra of adsorbed citrate at the three pH values, the difference spectrum of the spectra obtained at pH 6.5 and 4.5 suggests that pH-dependent changes in the citrate surface speciation do occur (Figure 2B). The difference spectrum is distorted by negative absorbances from carbonate desorption resulting from lowering pH from 6.5 to 4.5 (Figure 2C and S2), but nevertheless reveals increased absorbances of a  $v_{as}$  C-O stretch at 1590 cm<sup>-1</sup> and of  $v_s$  C-O band at 1405 cm<sup>-1</sup> at lower pH (Figure 2B). The difference in position of the  $v_{as}$  band relative to  $v_{as}$ (COO-) of aqueous citrate indicates that these peaks are associated with carboxylate groups coordinated in an inner-sphere fashion at the hematite surface (Lindegren and Persson, 2010; Mudunkotuwa and Grassian, 2010; Situm et al., 2017) (Table 1). The IR results therefore suggest enhanced inner-sphere coordination of citrate carboxylate groups with decreasing pH, as also concluded in previous studies (Lackovic et al., 2003; Noerpel and Lenhart, 2015; Situm et al., 2016; Yeasmin et al., 2014).

The asymmetric and symmetric C-O peaks in the spectra of adsorbed citrate presented in Figure 2B consisted of overlapping bands associated with different carboxylate species. If these different C-O bands are associated with inner- and outer-sphere citrate complexes, then the IR results reflect the simultaneous presence of inner- and outer-sphere citrate species at the hematite surface (Noerpel and Lenhart, 2015; Yeasmin et al., 2014), and

show that the inner-sphere complexes become more prominent as pH is lowered. If, on the other hand, the overlap is between contributions from non-coordinated and inner-sphere carboxyl groups of adsorbed citrate (Lindegren et al., 2009; Situm et al., 2016), then the IR results indicate an increase in the number of inner-sphere carboxylate groups through which citrate associates with the surface at lower pH. Distinction between these options is not possible based on the IR results of Figure 2 alone. However, results from the ternary citrate-phosphate-hematite experiments, presented in the next section, suggest that purely outer-sphere citrate complexes are not a major species under the experimental conditions of this study.

Additional information of the citrate surface speciation is provided by features other than the C-O stretch bands. The IR spectra of adsorbed citrate lack the C-OH bending mode at 1280 cm<sup>-1</sup> present in the aqueous spectra (Figure 2), which may indicate inner-sphere interaction of the citrate hydroxyl group with the hematite surface (Lindegren et al., 2009; Noerpel and Lenhart, 2015). Protonation of carboxylate groups occurs at acidic pH, as evident from activation of the  $v_{C=O}$  stretch (1720 cm<sup>-1</sup>) at pH 4.5, and was ascribed in previous studies to protonation of outer-sphere citrate complexes (Lindegren et al., 2009; Situm et al., 2016).

Overall, our results are consistent with previous IR studies of citrate interaction with Fe-oxides, in that at least two citrate surface species are present that vary with pH. The citrate surface complexes are predominantly deprotonated, and lowering pH promotes inner-sphere interactions between citrate carboxylate groups and the hematite surface. However, given the ambiguity in the interpretation of ATR-FTIR data of adsorbed citrate, no conclusive assignments regarding the number of surface complexes and their configurations can be made based on the current results.

# 2.3.2. Phosphate and citrate adsorption and desorption in ternary systems

Figure 3 presents the results of the IR flow experiments where the hematite deposit was first equilibrated with phosphate (25  $\mu$ M) and subsequently exposed to aqueous citrate (10-250  $\mu$ M). The addition of citric acid induces phosphate desorption, as observed from decreasing absorbance in the 900-1200 cm<sup>-1</sup> range, concurrent with accumulation of citrate at the hematite surface, as evidenced by increased absorbance at 1200-1600 cm<sup>-1</sup> (Fig. 3). This competitive behavior of citric acid towards adsorbed phosphate is consistent with macroscopic studies demonstrating that citrate is capable of displacing phosphate surface

complexes from Fe-oxides and soils (Barrow et al., 2018; Geelhoed et al., 1998; Gerke, 1994; Shields et al., 2018; Souza et al., 2014; Wang, et al., 2013; Yan et al., 2015).



**Figure 3**. Left-hand panels: *In situ* ATR-FTIR spectra collected during desorption of orthophosphate from hematite by citrate after 210 min of initial contact, at pH 6.5, 5.5 and 4.5 (from top to bottom) as a function of the concentration of citric acid (CA). The right-hand panels display the difference spectra between the spectra collected after citrate addition and the spectrum collected at the end of pre-equilibration with phosphate (210 min). The arrows in the lower right panel indicate the frequency changes resulting from citric acid addition. Negative absorbances in the left-hand panels around 1500 to 1250 cm<sup>-1</sup> are due to carbonate desorption by phosphate (Brechbühl et al., 2012), while the negative absorbances at ~1200-900 cm<sup>-1</sup> in the right hand panels are due to phosphate desorption by citrate. All spectra were collected in 0.1 M NaCl background electrolyte and at a total orthophosphate solution concentration of 25  $\mu$ M. Absorbance is given in arbitrary units.

The spectral region containing the citrate bands in these experiments is influenced by negative absorbances resulting from carbonate desorption during the initial equilibration with phosphate (Brechbühl et al., 2012; Elzinga and Kretzschmar, 2013) distorting the citrate IR bands. To eliminate this effect, the difference spectra between the spectra collected before and after citrate introduction were calculated. The results are presented in the right-hand panels in Figure 3 and show the growth of C-O stretching bands from adsorbed citrate with increasing

concentration, concurrent with increasingly negative P-O bands resulting from phosphate desorption from the hematite surface.

Comparison of the experiments conducted at different pH values shows that citrateinduced desorption of phosphate is less pronounced at pH 6.5 than at pH 4.5 and 5.5 (Figure 3), demonstrating that the competitive effect is pH-dependent consistent with macroscopic results (Barrow et al., 2018; Geelhoed et al., 1998; Lindegren and Persson, 2009). A semiquantitative analysis of the amount of phosphate displaced was obtained by comparing the area under the phosphate IR peaks before and after citrate addition. Citrate induced desorption of approximately 11%, 18%, and 24% of adsorbed phosphate at pH 6.5, 5.5, and 4.5, respectively, and we attribute the increased efficiency of citrate competition at lower pH to the increased propensity of citrate to form inner-sphere complexes as pH decreases, as discussed in section 3.1.

The negative absorbances at 1200-900 cm<sup>-1</sup> in the difference spectra of Figure 3 are of interest, because these characterize the phosphate complexes displaced by citrate competition. The spectra were inversed and normalized, and are compared in Figure 4 to the normalized spectra of adsorbed phosphate before citrate addition, and the difference spectrum of adsorbed phosphate at pH 8.5 and pH 4.5 from Figure 1C assigned to bidentate phosphate surface complexes (Elzinga and Sparks 2007). The spectra of desorbed phosphate are dissimilar from the adsorption spectra collected prior to citrate addition, particularly at pH 6.5 and 5.5 (Figure 4). This demonstrates that citrate does not equally compete with the entire set of P surface complexes present, but selectively displaces specific P species.

The main peak in each desorption spectrum is shifted to higher wavenumbers compared to the corresponding spectrum of adsorbed phosphate at the same pH (Figure 4). This suggests that citrate more effectively competes with the bidentate phosphate surface complexes than with the monodentate species. This selective desorption is pronounced at higher pH 6.5 and 5.5, while the desorption and adsorption spectra are quite similar at pH 4.5 (Figure 4). This pH dependence may be attributed to the overall higher fraction of bidentate phosphate complexes present at the hematite surface at lower pH (Figure 1). An important implication of the selective desorption of phosphate by citrate is that it modifies the surface speciation of phosphate.



**Figure 4**. Normalized ATR-FTIR spectra of adsorbed phosphate after 210 min of interaction with at pH 4.5, 5.5 and 6.5 (solid lines) compared to the normalized spectra of phosphate desorbed from the hematite surface by 250  $\mu$ M citrate (dashed lines). The spectra of desorbed phosphate were obtained by inverting and normalizing the negative absorbances at 1250-875 cm<sup>-1</sup> shown in Figure 3. The bottom spectrum is the same as shown in Figure 1C, and represents the bidentate bridging phosphate surface complex per the assignment of Elzinga and Sparks (2007) (Table 1).

Effects of phosphate on the surface speciation of citrate were studied in a series of IR flow cell experiments where the addition sequence of phosphate and citrate was reversed, i.e. hematite was first equilibrated with citrate (25  $\mu$ M) and subsequently with phosphate (25  $\mu$ M). The results are presented in the Supporting Information, with Figure S1 displaying the spectra of adsorbed citrate and phosphate; Figure S2 presenting the corresponding difference spectra of the 1770-1370 cm<sup>-1</sup> region (containing the asymmetric and symmetric C-O stretching bands) before and after phosphate addition; and Figure S3 comparing the normalized spectra of adsorbed citrate before and after phosphate addition.

A complication in these experiments was desorption of carbonate by adsorbing phosphate, causing negative absorbances at 1550-1300 cm<sup>-1</sup> (Brechbühl et al., 2012; Elzinga et al., 2012) that distorted the citrate C-O stretching bands and prevented detailed analyses of the citrate speciation. These distortions were particularly strong at pH 6.5, and the results of

these experiments are therefore not included. A further complication was the appearance of a water band at  $\sim 1630 \text{ cm}^{-1}$  that overlapped with the vas C-O stretch (Figures S1 and S2), an effect also seen in the citrate spectra presented in Figure 3. We attributed this to slight erosion of the hematite film over the course of the experiments.

Despite the experimental complications, the results are informative in that they demonstrate the limited response of citrate to the introduction of phosphate. Phosphate addition causes a general decrease in absorbance at 1600-1350 cm<sup>-1</sup> due to citrate and carbonate desorption (Figure S1). The negative absorbances, however, are small relative to the total absorbance of adsorbed citrate (Figures S1 and S2). This implies that the retention of citrate at the hematite surface is relatively insensitive to changes in surface charge induced by phosphate in these systems.

Electrophoretic mobility studies have shown that inner-sphere phosphate surface complexes strongly reduce or even reverse the positive surface charge of hematite at the pH values applied here (Del Nero et al., 2010; Li and Stanforth, 2000). The limited response of citric acid adsorption to this change suggests that citrate retention at the hematite surface is to a significant extent controlled by inner-sphere interactions, so that electrostatics alone have limited impact on the stability of citrate surface complexes at the pH values and citrate concentrations considered here. This in turn implies that purely outer-sphere citrate surface complexes, held exclusively by electrostatic interactions with the surface, are not a major species in our experimental systems. Further work is needed to confirm this conclusion.

## 2.3.3 Time-resolved IR measurements of phosphate adsorption

Time-resolved IR spectra tracking phosphate adsorption to the hematite surface at pH 4.5, 5.5 and 6.5 are presented in Figure S4 for citrate-free systems and in Figure S5 for systems where hematite was pre-equilibrated with 25 uM citrate prior to phosphate addition. At each pH value, the IR spectra of adsorbed phosphate are different in the citrate-free and citrate-containing systems (Figures S4 and S5), and this is a further indication that citrate changes the phosphate surface speciation, as shown previously from the results presented in Figure 4 and discussed in section 3.1. Secondly, the band intensities of adsorbed phosphate in the citrate-containing systems are 2-3 times lower than in the citrate-free experiments at each pH (Figure S4 and S5), demonstrating that citrate not only changes the mechanism of phosphate adsorption but also lowers the extent of sorption. This likely is due to the direct competition between citrate and phosphate molecules for adsorption sites evident from the results presented in Figure 3, but may additionally result from surface charge effects (Xu et

al., 2003), or by impairment of phosphate diffusion to the surfaces of micropores due to pore blocking (Mikutta et al., 2006).

A third observation of the results presented in Figures S4 and S5 is that the IR spectra showed time-dependent changes not only in the overall intensity of the phosphate spectra, but also in the relative intensities of the peaks. This indicates that the relative proportions of the phosphate surface species shift over the course of equilibration. This time-dependency is well illustrated by Figure 5, in which the normalized spectra collected after 15 and 210 min in the citrate-free systems were compared. In the short-term spectra, two peaks at 1104 and 1000 cm<sup>-1</sup> are prominent in the spectrum of adsorbed phosphate, whereas after 210 min these features are less resolved because of increased absorbance at 1100-1050 cm<sup>-1</sup> and near 950 cm<sup>-1</sup> have (Figure S3).



**Figure 5.** Normalized ATR-FTIR *in situ* spectra of phosphate surface complexes on hematite after 15 and 210 min of contact time at pH 6.5, 5.5 and 4.5. The bottom spectrum is the difference spectrum between the spectra obtained at pH 4.5 minus 8.5 from Figure 1C, and represents the bidentate orthophosphate surface complex (Table 1). All spectra were collected in 0.1 M NaCl background electrolyte and a phosphate solution concentration of 25  $\mu$ M.

The time effect was observed at all pH values studied and in both the absence and presence of citrate, but is most pronounced at pH 4.5 (Figures S4, S5 and 5). The bands at 1104 and 1000 cm<sup>-1</sup> are attributed to bidentate bridging complexes, while absorbances at 1110-1050 cm<sup>-1</sup> and near 950 cm<sup>-1</sup> are assigned to monodentate complexes (Elzinga and Sparks, 2007; Figure 1; Table 1). These results therefore suggest that phosphate adsorption is dominated by bidentate complexes during the earlier sorption stages, whereas monodentate complexes become increasingly pronounced at longer sorption times. This is not a surface loading effect, because Elzinga and Sparks (2007) demonstrated that the bidentate complexes become more prominent (relative to the monodentate complexes) as phosphate surface loadings increase in response to higher phosphate solution concentrations. The effect instead appears to be kinetic, reflecting differences in the adsorption rates of bidentate and monodentate complexes, with the latter species adsorbing slower than the former. The complexity of the phosphate surface speciation and the differences in adsorption rate constants of the various phosphate surface species, as suggested by the kinetic IR data, highlight the need for additional work coupling spectroscopic and kinetic studies of phosphate interactions with Fe-oxide surfaces.

# 2.4. Conclusions and Agronomical Implications

We report here on the interactions between citric acid and phosphate at the hematite surface at pH 4.5-6.5. The results demonstrate mutual impacts on the mechanisms and extent of sorption between these two species, and thus indicate that their geochemical behavior in soils will be influenced by each other's presence. The displacement of adsorbed phosphate from the hematite surface by citrate implies that citrate exudation may effectively solubilize phosphate, particularly at lower pH. Moreover, the presence of citrate changes the phosphate surface speciation, and thus affects phosphate bioavailability and solubility even without considering effects of direct mobilization. These abiotic effects likely accompany the synergetic effects of citric acid and other root exudates on P availability through enhanced microbiological activity in rhizosphere zones Phosphorus solution levels as regulated by hematite increased with pH and were enhanced by citric acid. This suggests that agricultural management practices that include liming and maintain a steady supply of organic acids, e.g.no-till management and use of cover crops, may reduce P fixation in highly weathered tropical soils
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## **Supplementary information**



**Figure S1.** ATR-FTIR spectra collected in the citrate adsorption range during the adsorption of 25  $\mu$ M orthophosphate onto hematite, following initial equilibration of the hematite deposit with 25  $\mu$ M citric acid pH 5.5 and 4.5 as a function of time after the introduction of the phosphate solution into the flux system. Arrows locate citrate band shifts, while the spectral range shaded in orange contains negative absorbances resulting from carbonate desorption by phosphate.



**Figure S2.** Difference spectra between the spectra collected before and after phosphate addition in the experiment presented in Figure S1. Negative absorbances at 1570-1350 cm<sup>-1</sup> are due to citrate and carbonate desorption induced by phosphate. The positive band at 1630 cm<sup>-1</sup> is a water band which overlaps with the asymmetric C-O stretch.



**Figure S3.** Normalized ATR-FTIR spectra of adsorbed citrate in the presence and absence of phosphate. The red and blue spectra were obtained for phosphate containing systems, where citrate was added either before (straight line) or after (dotted line) phosphate. Phosphate and citrate concentrations were 25  $\mu$ M in all cases.



**Figure S4.** Time-resolved *in-situ* ATR-FTIR spectra of phosphate adsorption onto hematite in the absence of citrate at pH 4.5, 5.5 and 6.5. The spectra were collected over the course of 210 min following phosphate introduction into the flux system at t = 0. The experiments used a 0.1M NaCl background electrolyte, and an orthophosphate solution concentration of 25  $\mu$ M. Absorbance is given in arbitrary units.



**Figure S5.** Time-resolved *in-situ* ATR-FTIR spectra of phosphate adsorption onto hematite pre-equilibrated with citrate at pH 4.5, 5.5 and 6.5. The spectra were collected over the course of 210 min following phosphate introduction into the flux system at t = 0. The experiments used a 0.1M NaCl background electrolyte, and orthophosphate and citrate solution concentrations of 25  $\mu$ M. Absorbance is given in arbitrary units.

# 3. CITRIC ACID TO DESORB ARSENIC ADSORBED IN HAUSMANNITE AND HEMATITE

## ABSTRACT

Arsenic (As) is a toxic element that leads the list of human health threats and is one of the priority contaminants in soil and water. Oxic conditions are predominant in cultivated soils, in surface water and near-surface groundwater, and as result As(V) is the main inorganic species in these environments. In order to remove As(V) and/or reduce its mobility, filters and amendments with high affinity for As(V) adsorption are used in drinking water treatment or directly applied to the soil, thereby promoting its immobilization. Hausmannite and hematite were compared by in-situ Attenuated Total Reflection - Fourier Transform Infrared (ATR-FTIR) spectroscopy and batch experiments for evaluating As(V) adsorption and sequential desorption by citrate. The pH and contact time were used as variables. As(V) was retained on the mineral surface by the formation of simultaneously inner- and outer-sphere species. Inner-sphere bidentate complex form preferentially at high pH, early adsorption time and low surface loading, while the monodentate species should be responsible to increase total As(V) adsorption at low pH, later adsorption kinetics and higher As(V)surface loading. Citrate was effective in causing As(V) desorption at higher citric acid concentrations and higher pH values. Contact time promoted increasingly As(V) resistant against citrate desorption, even in the presence of high citric acid concentrations. Hausmannite was an efficient mineral for As(V) removal and immobilization.

Keywords: Colloidal surface; Soil and water contamination; In-situ Infrared spectroscopy; adsorption; anion competition; Iron-oxide

#### **3.1. Introduction**

Arsenic (As) is a toxic element that leads the list of human health threats and is one of the priority contaminants in soil and water listed by U.S. Environmental Protection Agency (USEPA), which has classified it as Group-A for human carcinogen (USEPA, 1996). The World Health Organization, in 1993, reduced the tolerable value of As concentration in drinking water from 50 to 10  $\mu$ M L<sup>-1</sup> (Graham, 1999). Arsenic contamination in drinking water, soils and food is of concern for human health world-wide, and consequently has received much attention from researchers during the past two decades (Bissen and Frimmel, 2003; Mandal and Suzuki, 2002; Mohan and Pittman, 2007; Smedley and Kinniburgh, 2002). Alam et al. (2002) described the well-known contamination of As groundwater and the associated impacts on human health in Bangladesh.

The arsenic species arsenite  $(AsO_3^{3-})$  and arsenate  $(AsO_4^{3-})$ , henceforth referred to as As(III) and As(V), respectively are the main species in earth surface system. The occurrence of these species depends on environmental conditions, in particular redox potential and pH value (Bissen and Frimmel, 2003; Mohan and Pittman, 2007; Saha and Ali, 2007; Smedley and Kinniburgh, 2002). Oxic conditions are predominant in cultivated soils, in surface water and near-surface groundwater. As a result, As(V) is the main inorganic species in these environments, which are closely associated with human activities (Adriano, 2001a; Bissen and Frimmel, 2003; Mohan and Pittman, 2007; Smedley and Kinniburgh, 2002). In order to remove As(V) and/or reduce its mobility, filters and amendments are used in drinking water treatment or directly applied to the soil. The filtration systems and soil amendment consist of mineral phases that have a high affinity for As(V) adsorption, thereby promoting its immobilization (Mohan and Pittman, 2007).

The mechanism of As(V) adsorption predominantly involves the formation of inner sphere complexes (Jia et al., 2007; Ladeira et al., 2001; O'Reilly et al., 2001; Yuji Arai et al., 2003; Farrell, 2017), making As(V) sequestration by the adsorbents quite stable, similar to phosphate reaction (Strawn, 2018). There is a strong influence of pH on total As(V) adsorption for a wide range of mineral including hematite (Mamindy-Pajany et al., 2009), goethite (Dixit and Hering, 2003; Grafe et al., 2001; O'Reilly et al., 2001), ferrihydrite (Jia et al., 2007; Klaus P. Raven et al., 1998), aluminum hydroxides (Anderson et al., 1976; Xu and Catalano, 2016), and clay materials (García-Sanchez et al., 2002; Mohapatra et al., 2007).

Besides pH, the efficiency of As(V) filter and soil amendments is quite dependent on others parameters, including contact time between As(V) and adsorbents. Wang et al (2015) found a decrease in As(V) availability of approximately 70-90%, extracted by 0.5M NaHCO<sub>3</sub> when they compared 1d and 30d of initial contact time between As(V) and several Chinese soils. Rahman et al. (2017) suggested that after long contact times (on the order of decades) the mineral sorbent may greatly reduce the mobility and accessibility of adsorbed As(V) by neomineral precipitation. Using As-EXAFS analysis O'Reilly et al(2001) found that after the initial fast As(V) adsorption on goethite surface by bidentate binuclear bound, the molecular environment did not change over the 43d of contact As-mineral.

Humic substances dissolved in groundwater may compete for adsorption sites with As species (Johanna Buschmann et al., 2006; Lin et al., 2004), increasing As mobility (Bauer and Blodau, 2006; Wang and Mulligan, 2006; Weng et al., 2009). Low molecular weight organic acids are found in considerable concentration in soil systems, especially in rhizosphere environments (Dakora and Phillips, 2002; Jones et al., 1996; Pavinato et al., 2008; Silva et al., 2001; Xu et al., 2003) and have been proposed as important competitors for anion adsorption sites (Grafe et al., 2001). Organic acids could improve As(V) mobility in soils, as well as in As-filters, and it decreases their efficiency in retaining As (Onireti et al., 2017; Onireti and Lin, 2016; Wang and Mulligan, 2006; Weng et al., 2009; Zhang et al., 2005).

Hausmannite  $(Mn^{2+}[Mn^{3+}]_2O_4)$  is a spinel-structured metastable manganese oxide that is a common constituent of soils and sediments where Mn redox transformations occur (Luo et al., 2018). The presence of Mn(III) in the hausmannite structure allows it to act as a reducing and oxidizing agent (Luo et al., 2018; Trouwborst et al., 2006). Under oxic conditions, incongruent dissolution induced by hydrolysis, protonation and oxidation of Mn(II) leads to conversion of hausmannite into manganite ( $\gamma$ -MnOOH) often at pH  $\geq$  4 (Kirillov et al., 2009; Luo et al., 2018; Ristić et al., 2013). Manganite is the most stable trivalent Mn hydroxide polymorph and can persist for a considerable length of time, especially at neutral and basic pH levels (Hens et al., 2018; Qin et al., 2019).

In this study we compared As(V) adsorption on hematite (Fe<sub>2</sub>O<sub>3</sub>), which is a common adsorbent used in filters, and on hausmannite. We used the in-situ ATR-FTIR spectroscopy and batch experiments for evaluating of molecular for As(V) adsorption, and desorption process using citrate anion competition, which is broadly founded on environmental system and could simulate a model organic anion substance. The pH and contact time were used as environmental variables.

## 3.2. Material and Methods

#### **3.2.1.** Minerals preparation

The hematite sorbent used for the experiments was synthesized based on the procedure of Sugimoto et al (1993) and has been used previously Elzinga and Kretzschmar

(2013). A detailed description of this material is provided in Brechbühl et al. (2012). The hausmannite was synthesized following the method of Giovannelli et al (2012). Briefly, a solution of 0.5 mol  $L^{-1}$  Mn(NO<sub>3</sub>)<sub>2</sub> was titrated by slow addition of 1 M NaOH solution at room temperature and under atmospheric conditions. The pH was adjusted to 7, causing oxidation of Mn(II) by O<sub>2</sub>(g) which resulted in the precipitation of hausmannite. The pH was maintained close to 7 during 24 h under stirring in air. X-ray diffraction analysis (Supplementary material, Figure 1SM) confirmed that hausmannite formed and showed no evidence for the presence of other crystalline phases.

#### **3.2.2. ATR-FTIR experiment**

The experimental setup followed Elzinga and Kretzschmar (2013). Briefly, the ATR-FTIR spectra were collected on a Perkin–Elmer Spectrum 100 spectrometer equipped with a Balston Parker purge gas generator and a liquid N<sub>2</sub>-cooled mercury–cadmium–telluride (MCT) detector. The experiments employed an ATR-FTIR flow-cell. A horizontal ZnSe crystal (45° angle of incidence) was coated with mineral film (625  $\mu$ g of either hematite or hausmannite) by drying 125  $\mu$ L of a 5g L<sup>-1</sup> of finely dispersed mineral suspension, evenly spread across the crystal surface and allowed to dry overnight in air at room temperature, which produced a stable and homogeneous deposit firmly adhered to the ATR crystal. A new film deposit was prepared for each experiment.

The coated crystal was installed in a flow cell inside the IR spectrometer and connected to a vessel containing 100 mL of 10 mM NaCl background electrolyte which was continuously stirred with a magnetic bar and set to the desired pH. A peristaltic pump was used to circulate solute from the reaction vessel through the flow cell at a rate of 2 mL min<sup>-1</sup>. The solution pH was monitored throughout the experiment and readjusted or changed as necessary through addition of negligible aliquots of 0.1M NaOH or 0.1M HCl. The pH electrode used was an Orion 9102BNWP combination pH electrode. The ZnSe crystal+mineral deposit was equilibrated with the background solution at desired pH for 2h, then a final background spectrum (absorptions of the ZnSe crystal + mineral deposit + electrolyte background) was collected as the average of 100 co-added scans at a 4 cm<sup>-1</sup> resolution. All subsequent spectra were ratioed to this background spectrum.

We carried out "pH envelope" to characterize the pH dependency of arsenate adsorption processes experiments. The adsorption was evaluated as a function of pH as the main variable. Hence, pre-equilibration was performed at pH 8.5, and after collection of the background spectrum 125  $\mu$ L of a 20 mM L<sup>-1</sup> As(V) stock (as Na<sub>2</sub>HAsO<sub>4</sub>, Sigma-Aldrich)

was added to the main vessel to achieve a 25  $\mu$ M L<sup>-1</sup> As(V) solution concentration. The pH was re-adjusted to pH 8.5, and the mineral deposit was allowed to achieve adsorption equilibrium with the arsenate solute, as monitored by regular scanning. The final scan was then collected as the average of 100 scans at a 4 cm<sup>-1</sup> resolution over the 4000-600 cm<sup>-1</sup> wavenumber range) and the pH was lowered to pH 8.0, where new adsorption equilibrium was established after ~20 min. This step-wise lowering of pH and re-equilibration was repeated at pH 7, 6, 5 and 4.

To evaluate the As(V) adsorption as a function time, time-resolved analyses were carried out where consecutives spectra (22 co-added scans at a 4cm<sup>-1</sup> resolution,  $\approx$ 19s per spectrum) were collected in the time range of 1-120 min following As(V) addition. To evaluate As(V) desorption by citrate, we first equilibrated the mineral deposit with 25  $\mu$ M As(V) for 2 hours. Next, citric acid (CA) (CAS No. 77-92-9, Sigma-Aldrich) was added stepwise from a stock solution (20 mM L<sup>-1</sup> CA) to reach final concentrations 10, 25, 50, 100, 150 and 250  $\mu$ M L<sup>-1</sup> citrate. At each CA concentration, the system was left to reach a new equilibrium. After stabilization (~20 min) the final spectrum was collected and then the CA solution concentration was raised to the next level. This experiment was performed at pH 7, 6, 5 and 4 for both the hematite and hausmannite mineral sorbents.

Aqueous spectra were collected by scanning solutions of 5 mM of arsenate adjusted to pH values in the range 8 to 4 using 0.1M NaOH or HCl. Two milliliters of 10 mM NaCl electrolyte were deposited on Ge ATR crystal, then after 5 min of initial interaction, an ATR-FTIR spectrum was collected as a background spectrum (200 scans) for the subsequent calculation of the absorbance spectra of arsenate species (200 scans) without the contribution of water, 10 mM NaCl electrolyte and Ge crystal.

Origin 2017 software was used for these spectral processing (OriginLab Corporation, Northampton, Massachusetts, USA). The raw spectra were smoothed using the Savitzky-Golay method (30 points of window and second polynomial order). The baseline of each spectrum was fitted using a parabolic line from 990 to 700 cm<sup>-1</sup>, which represents the main arsenate peak range, and the area under the area was determined from integration over the 990-740 cm<sup>-1</sup> range.

## 3.2.3. Batch experiment

#### 3.2.3.1 Adsorption isotherm

We measured adsorption isotherms to determine the adsorption of As(V) by hematite and hausmannite as a function of As(V) concentration at pH 6 and pH 4. We prepared 20 mL volumes of 15 g L<sup>-1</sup> suspension stock adjusted to pH 6.0 or 4.0 by negligible addition of 0.1 M HCl or NaOH. These pH values were chosen after previous evaluation of ATR-FTIR results. Then, 0.1 mL subsamples of the mineral suspension were transferred to separate centrifuge tube, and mixed with 0.1 mL of 0.15 M NaCl. After 2h, 1.3 ml of solutes with variable As(V) were added to reach final suspensions with a 1 g L<sup>-1</sup> suspension density, 10 mM NaCl and As(V) concentration of 1.0, 2.0, 3.0, 10.0, 15.0 or 30.0 mg L<sup>-1</sup>. These suspensions were shaken for 24 h on an orbital shaker (4 cycles per min).

After 24 h, the solution of the suspensions was separated from the solids by ultracentrifugation ( $\approx 6600$ g) for 5 min. The filtered supernatants were analyzed to determine the dissolved arsenic concentrations with the molybdenum blue method (Johnson, 1971), with modifications suggested by (Tsang et al., 2007), using 725 nm wavelength for quantification. The instrument used was a Carry 3 UV-Vis spectrophotometer (Varian, USA) using 1 cm quartz cell. The calibration curve was prepared at a concentrations range of 0.1-6 mg L<sup>-1</sup> (1.33-80  $\mu$ M L<sup>-1</sup>), reach R<sup>2</sup> = 0.996 for linear calibration coefficients.

The adsorbed As(V) concentrations were calculated as the difference between the initial and equilibrium solutions concentration, according to equation 1:

$$Q_e = \frac{(\text{Ci-Ce})}{M} \qquad \qquad \text{Eq. 1}$$

where Qe is the concentration of As(V) adsorbed on mineral (mg g<sup>-1</sup>), Ci and Ce are the concentration (mg L<sup>-1</sup>) of As(V) in the initial and final solution, remaining in solution after the adsorption process (equilibrium concentration) and M is the mineral concentration used (1.5 g L<sup>-1</sup>). The Langmuir isotherm was performed following the equation 2:

where Qe is the amount of As(V) adsorbed (mg g<sup>-1</sup>), C<sub>e</sub> is the solution concentration at equilibrium (mmol L<sup>-1</sup>), Q<sub>max</sub> is the maximum sorption capacity (mg g<sup>-1</sup>) and K<sub>L</sub> is the Langmuir constant (L g<sup>-1</sup>), related to the adsorption capacity and increases exponentially with sorption energy. All adsorption experiments were conducted in duplicate at room temperature (22±2 °C). Origin 2017 software was used to apply a non-linear method for fitting the Langmuir equation to our experimental data.

## 3.2.3.2 Desorption experiment

Desorption experiments were conducted with sorbent that were loaded with the maximum As(V) surface capacity determined from the adsorption experiments described in

the previous section. The initial mineral stocks suspensions (15 g  $L^{-1}$ ) were diluted to 10 g  $L^{-1}$  with 10 mM NaCl, and As(V) was added at the required concentration, The suspensions were stirred for 24 h, with pH readjusted to 4 or 6 with small addition of 0.1M HCl or NaOH as needed after 24 h, and then left to react for another 24 h under stirring.

Next, 0.725 mL subsamples were each mineral suspension were distributed over Eppendorf centrifuge tubes, and amended with addition of a 0.725 mL of desorption solute. This desorption solute consisted of 10 mM NaCl and citrate in variable concentration, previously adjusted to pH 6 or 4. The final suspension in each tube had a particle loading of 5 g  $L^{-1}$  in 10 mM NaCl background electrolyte, and citric acid (CA) concentrations at 0, 0.2, 0.4, 1, 2, 4 and 10x equivalent (Mol:Mol) As(V). The solutions were shaken for 2h on an orbital shaker and then ultracentrifuged for analysis of As(V) concentrations in supernatant as described above (section 3.2.3.1).

Possible interference of citrate on color development of arsenomolybdate-blue complex was previously assessed, and found to be negligible (data not showed), consistent with the results of Grafe et al (2001). This is likely due to the low total citrate concentration in the samples (maximum concentration was for hausmannite at pH 4, approximately 2.47 mM), which was lower than the 3 mM suggested by He et al (1998) and and Wei et al (2009) as the threshold concentration of citrate interference in the molybdate blue colorimetric method that is used for analysis of chemically similar P.

To evaluate the effect of "aging" on the stability of As(V) surface complexes towards desorption, we repeated the same procedure after 40d of initial arsenate mineral contact. The data were presented relating the amount initially added to the minerals (100%) with amount desorbed at each citrate concentration.

#### **3.3. Results and Discussion**

## **3.3.1. ATR-FTIR experiments**

The spectra of aqueous arsenate at all pH values (Fig. 1A) were considerably different from the spectra of adsorbed arsenate (Fig. 1B), indicating strong interactions of arsenate with hausmannite and hematite. However, the low total arsenate interaction with hematite produced lower Infrared (IR) absorbance signal and high noised spectra. The negligible As(V) signal was detected at pH 8 and 7 (data not showed) for hematite.



**Figure 1.** (A) ATR-FTIR spectra of 5 mM As(V) aqueous species at pH 4-8. The species composition of aqueous arsenate was estimated using VisualMinteq software for each pH. The spectra were stacked to enable comparison. (B) ATR-FTIR spectra of arsenate surface complexes on hausmannite and hematite in the pH range 4-8 at an arsenate solution concentration of 25  $\mu$ M. The difference specta isolates the arsenate surface complexes that form with decreasing pH. The sorption spectra were collected in 10 mM NaCl background electrolyte.

The intensity of the IR bands of adsorbed arsenate increased with decreasing pH (Fig. 1B), demonstrating that As(V) adsorption was enhanced at lower pH, as expected. The pKa<sub>1</sub> (2.22) and pKa<sub>2</sub> (6.94) As(V) result in  $H_2AsO_4^-$  and  $H_2AsO_4^{-2}$  being the dominant As(V) aqueous anions in most environmental solutes. Lowering pH leads to increases the in the adsorption of these anions at lower pH values.

This may be due to electrostatic effects, as anionic As species are dominant in solution ( $H_2AsO_4^-$  and  $HAsO_4^{2^-}$ ) while the hausmannite (zero point of charge - ZPC ~10) and hematite surface (ZPC = 9.3) becomes more positively charged as pH is lowered, promoting sorbate-sorbent interactions. In addition, protonation of surface sites at lower pH makes ligand exchange reactions between arsenate and reactive surface hydroxyl sites ( $\equiv$ Mn–OH or  $\equiv$ Fe–OH) more favorable, enhancing inner-sphere complexation at acidic pH.

Lowering of pH not only increased the intensity of the IR spectra of adsorbed arsenate due to increased adsorption, but also caused spectral changes, especially for hausmannite. To isolate the spectral changes resulting from lowering pH, the difference spectrum of the spectra collected at low and high was calculated, which shows shifting of the main peak from  $\sim$ 850 to  $\sim$ 880 cm<sup>-1</sup> in the arsenate surface speciation (Fig. 1B).

Table 1 summarizes the possible configurations suggested by (Brechbühl et al., 2012) of the arsenate-hematite surface complexes based on IR data, which were based on previous studies. Detailed discussions of arsenate surface complex designation based on IR data are provided by previous studies (Di Iorio et al., 2018; Farrell, 2017; Goldberg and Johnston, 2001; Lumsdon et al., 1984; Myneni et al., 1998; O'Reilly et al., 2001; Voegelin and Hug, 2003). Noteworthy, there are a higher proportion of bands at higher IR energy (lower wavenumber) in hematite, which suggested a higher proportion of bidentate As(V)-complex for hematite than hausmannite.

**Table 1.** The Gaussians peaks positions used to fit the experimental spectra of As(V) adsorption on hematite used for (Brechbühl et al., 2012).

Peak position (cm <sup>-1</sup> )	Assigned vibrational mode	Assigned complex
700	v <sub>sym</sub> As1-OH	IS <sub>prot</sub> , H <sub>br,prot</sub> , OS <sub>prot</sub>
802	v <sub>sym</sub> As2-OFe	<b>IS</b> <sub>deprot</sub>
834	v <sub>sym</sub> As1-OFe	IS <sub>prot</sub>
847	v <sub>asym</sub> As2-OFe	IS <sub>deprot</sub>
875	v <sub>asym</sub> As-O(uncomplexed)	$IS_{prot}, H_{br}, OS(H_2AsO^{-})$
908	v <sub>asym</sub> As-O	$H_{br}$ , OS( $H_2$ AsO <sub>4</sub> )
938	v <sub>asym</sub> As1-OFe	<b>IS</b> <sub>prot</sub>

The complexes were assigned based on previous studies from (Harrison and Berkheiser, 1982; Lumsdon et al., 1984; Myneni et al., 1998; Vansant and Van Der Veken, 1973) .The symbol v refers to symmetric (sym) and asymmetric (asym) stretching vibrations. Inner- sphere (IS), outer-sphere (OS), and hydrogen-bridged (Hbr) complexes were distinguished, in each case protonated (prot) or deprotonated (deprot). As1 and As2 represents two different arsenates complexes.

Our IR results suggest the simultaneous presence of at least two arsenate species at the mineral surface that change proportion with pH, from inner-sphere deprotonated (~850 cm<sup>-1</sup>) at high pH to outer-sphere and/or hydrogen-bridged (~880 cm<sup>-1</sup>) at low pH (Figure 1 and 2). Under acidic conditions, the growth of positive charge is expected due to protonation of  $O^{2-}$  ions bridging surface Fe<sup>3+</sup> (Roddick-Lanzilotta et al., 2002) or Mn cations. Thus, the weakly adsorbed arsenate may be bound to protonated bridging oxide ions. Of note, it should be carefully accepted to hausmannite, because these species assignments were designated to hematite.



**Figure 2.** Upper spectra are ATR-FTIR spectra collected during the adsorption of As(V) onto hausmannite (left) and hematite (right) surface at pH4 as a function of time after the introduction of the As(V) solution into the flux system. Absorbance is given in arbitrary units. Noteworthy observe the different y-scale. All spectra were collected in 10 mM NaCl background electrolyte and at a total As(V) solution concentration of 25  $\mu$ M. Bottom figures represents the As(V) adsorbed complex peak area over the kinetic time. Area is given in arbitrary units.

Time-resolved IR spectra tracking arsenate adsorption onto minerals surface at pH 4 showed the remarkable difference between minerals of arsenate surface complexes (Figure 2). After 120 min, the As(V)-hematite spectrum showed with main centers at ~874 cm<sup>-1</sup> and ~846 cm<sup>-1</sup> while As(V)-hausmannite had a centered peak at 876 cm<sup>-1</sup> (Figure 2, upper). Relatively broad IR bands suggest a higher degree of structural variation for As(V) surface complexes on as compared to hausmannite. Although at early adsorption times, at 30 min for example, there are additional peaks around ~784 cm<sup>-1</sup> for hematite and ~825 cm<sup>-1</sup> for hausmannite, both bands assigned as inner-sphere complex. The similar time-dependence for As-adsorbed complex is less pronounced in others pH values.

As(V) adsorption was lower on hematite than on hausmannite, as observed from the difference in y-scaling of the IR absorbance signals (Figure 2). A semi-quantitative analysis of this difference is provided by the arsenate peak areas, which are plotted against time in Figure 2 (bottom). The majority of adsorption occurs within 30 min, except at lower pH in the case of hausmannite. The As(V) adsorption on mineral surface over the time could follow different mechanisms, likely begin as bidentate in early kinetic times and changes to monodentate. Both species could reduce the positive surface charge of the minerals, which could explain the observed changes in arsenate peak symmetry (Figure 2, upper) and kinetic rate (Figure 2, bottom).

The efficiency of citrate in causing arsenate desorption is dependent on pH, the citrate concentration, and mineral sorbent type. Spectral analyses were not possible at pH 7, as low As(V) sorption levels at this pH value led to very noisy spectra, particularly after citrate addition (data not shown). The absence of significant arsenate peaks at this pH suggested total As(V) desorption. The desorption efficiency of citrate increased with increasing concentration and increasing pH. At citrate concentration levels that exceeded the As(V) concentration by a factor 10 (250  $\mu$ M) citrate was able to desorb ~40%, 60% and 67% of sorbed As(V) from hausmannite at pH 4, 5, and 6, respectively (Figure 3). For hematite As(V) desorption levels of ~17%, 46% and 71% were observed pH 4, 5, and 6, respectively (Figure 3). The effect of pH is difficult to ascribe to a single factor, since lowering pH intensifies not only citrate adsorption (Lindegren et al., 2009; Noerpel and Lenhart, 2015) but also arsenate adsorption (Brechbühl et al., 2012; Shi et al., 2009).



**Figure 3.** Decrease in peak area of adsorbed As(V) as function of citrate concentration at different pH values. The As(V) peak area after 120 of contact time at pH 4 was considered 100% for each mineral.

There are several interacting effects that cumulatively lead to the observed trend. Catalano et al. (2008) revealed the unexpected but considerable presence of outer-sphere As(V) complexes adsorbed on hematite. This complex is easily overlooked when inner-sphere species are also present. Catalano et al. (2008) argue that the lack of a strong connection between As(V) and mineral surface suggests that such species could have faster desorption rates than inner-sphere species under conditions where desorption of both is favorable, as in presence of citric acid. As desorption was higher for hausmannite than hematite, indicating that, despite having a lower As adsorption capacity, hematite forms a more stable As(V) bond with As(V). This is consistent with the information about more of bands at higher IR energy (lower wavenumber) in hematite than hausmannite presented in Figures 1 and 2. However, the spectra of desorbed arsenate complex (Figure 4SM) shift the center to right (lower IR bands) follow the increment the pH, mainly to hausmannite. That suggested preferentiality for desorption of inner-sphere As-complex (bi- or mono-dentate species) by citrate anions. This

conclusion is opposite to Catalano et al. (2008) conclusion, but follows similar behavior for phosphate studied in chapter 1).

## **3.3.2. Bacth experiment**

The isotherm of arsenate onto hematite and hausmannite at pH 6 and 4 are well-fitted with the Langmuir equation (Figure 3SM); the fit parameters are summarized in Table 2. The estimated maximum adsorption capacity for hausmannite was, on average, three times higher than for hematite. This is consistent with the semi-quantitative FTIR data shown in Figure 2. Solution pH impacts the extent of As(V) adsorption, especially for hausmannite (Table 2). The higher  $K_L$  value for hematite agrees with a relatively strongly hold As(V) surface complexes despite lower total adsorption amounts than for hausmannite (Table 2).

Tuble 2. Langman parameters used to moder sorption of his ( ) on minerals.				
Mineral	pH	$\begin{array}{c} Q_{max} \\ (mg As(V) g^{-1}) \end{array}$	K <sub>L</sub>	$R^2$
Hausmannite	6	3.06	0.64	0.90
	4	3.69	0.73	0.89
Hematite	6	0.96	6.59	0.98
	4	1.10	1.99	0.90

Table 2. Langmuir parameters used to model sorption of As(V) on minerals

Where  $Q_{max}$  is the estimative of maximum As(V) adsorbed; K<sub>L</sub> Langmuir constant and R<sup>2</sup> is the coefficient of determination.

Citrate was more efficiently desorbed As(V) at pH 6 than at pH 4, and that increasing citrate concentration increase the As(V) release (Figure 4). These conclusions agree with the ATR-FTIR data (Figure 3). However, after two days, the higher stability of As(V)-hematite complex was not observed, with release of ~35-40% of initial arsenate even at low citrate concentration. When citrate reaches ten times the concentration level of initial arsenate, hematite releases ~60-65% against ~20% and ~40% of hausmannite at pH 4 and 6, respectively. Some divergence between batch and FTIR results about As(V) desorption by citric acid are expected due to higher contact time for batch (days) than FTIR (hours); and solution concentration magnitude from  $\mu$ M into FTIR setup to mM of batch experiment. Regardless of these differences, the results of both experiments generally agree well with each other.



Figure 4. Desorption of As(V) over the citrate concentrations. As(V) was previously adsorbed for 2d and 40d in amount enough to saturation the surface adsorption capacity estimated by Langmuir isotherm equation.

Aging affects the stability of adsorbed As(V) for both minerals regardless of pH. After 40 d, the As(V) released at low citrate concentration for both pH for hausmannite and hematite at pH 4 had a considerable reduction reaching just ~10% of initial arsenate adsorbed. Hematite at pH 6 also showed strong decrease from ~60 to ~30% of release. As(V) desorption reduction was even more remarkable at higher citrate concentrations. Arsenate desorption decrease from ~65, ~60, ~40, ~20% to ~40, ~20, ~18, ~10% for hematite and hausmannite at pH 6 and 4, respectively.

The mechanisms behind increased stability of As(V) surface complexes with contact time (the so-called "aging effect") possibly originated from changes in the As surface speciation, such as a rearrangement of surface complexes as observed in a study by (Arai and Sparks, 2002), who found a slight increment of radial distances of As-Al with increasing contact time from XAS results. Abdala et al (2015b) used phosphate-EXAFS, a close chemical analogue of arsenate, and showed that aging over the course of several weeks slightly shortens phosphate bond lengths to goethite surface groups. Structural changes of As(V)-mineral complexes due the formation of surface precipitates (Jia et al., 2006) or due to intraparticle diffusion (Swedlund et al., 2014) also may be involved. On the other hand, O'Reilly et al (2001) using As-EXAFS data from goethite incubated for long-time periods, reported that the As molecular environment did not change over time, and found no measurable effect of aging or residence time on the desorption of arsenate in the presence of phosphate at concentration three times higher than initial arsenate at pH 6 and 4.

We are not able to identify the exact cause of the strong effect of contact time leading to strongly increased stability of adsorbed As(V). This considerable ageing effect suggests a significant change in As molecular environment, due to arsenic specie and/or mineral surface modifications. Additional analyses, e.g. by XAS spectroscopy, are needed to address the mechanisms of this effect.

## 3.3.3. Hausmanite aging

We reanalyzed hausmannite and hematite samples after 40 d incubation by FTIR and XRD. None considerable changes were visualized for hematite (data not showed), which is expected because of high structural stability. For hausmannite, however, we observed a phase transformation to manganite (Figure 5), as previously seen (Kirillov et al., 2009; Luo et al., 2018; Ristić et al., 2013). The presence of arsenate did not affect this phase transformation. This differs from the findings of Das et al (2011), who observed that arsenate retard ferrihydrite dissolution and reprecipitation into goethite, and concluded that the arsenate adsorbed onto ferrihydrite during the transformation to hematite does not merely remain adsorbed on the surface, but is incorporated into the hematite structure. Further studies should focus to describe the impacts of presence of environmental ions (e.g. organic acids, Ca, phosphate) to hausmannite phase changes and ions adsorption efficiency.



**Figure 5.** The aged hausmannite after 40d of incubation of As(V) (in 10 mM L<sup>-1</sup> NaCl) showed new peaks in (A) FTIR spectra (1150, 1116, and 1082 cm<sup>-1</sup>) and in (full (B) and detail (C) range) XRD spectrum (d-spacing (Å):  $\sim 3.40^{\circ}$ ;  $\sim 2.65^{\circ}$ ;  $\sim 2.27^{\circ}$  compared with original fresh hausmannite. These spectroscopic evidences confirm the presence of manganite as a new mineral phase.

## 3.4. Final Remaks and Conclusions

Hausmannite seems to be an efficient mineral substrate for As(V) removal and immobilization. This mineral adsorbed considerable amount of arsenate. There was a mineralogical shift from hausmannite to manganite over time, which allowed a high retention of As(V) and decreased its desorption by citrate. So, hausmannite may be considered an excellent As(V)-filter. Other advantage is that hausmannite could be quickly synthesized using few reagents and a pressure microwave (Bousquet-Berthelin and Stuerga, 2005).

In general, As(V) was retained on the mineral surface by the formation of simultaneously inner- and outer-sphere species. Differences in As(V) surface speciation occurred as a function of on mineral type, pH, and contact time. Lower total adsorption occurred at pH 7-8, basically by inner-sphere bidentate complex, while at pH 4-6 the monodantate As(V)-adsorbed species should be responsible to increase the total adsorption at higher As(V) surface loading. Citric acid more effectively caused As(V) desorption at higher citric acid concentrations and higher pH values. Contact time had a considerable effect on

As(V) adsorption stability, with As(V) surface complexes becoming increasingly resistant towards citrate desorption over time even in the presence of high citric acid concentrations.

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Figure 1SM. X-Ray diffraction pattern of minerals used. Other crystalline phases were not detected.

#### **Deconvolution process**

The spectrum obtained at equilibrium (120 min) at pH 4 and 6 and after desorption by citric acid at higher concentration (120 min + 250  $\mu$ M CA) in pH4, after smoothing and baseline correction, were deconvoluted. For deconvolution process, the amount and peaks positions followed ones founded by (Brechbühl et al., 2012) due to similarity of experimental conditions used, such as the same hematite sample and electrolyte background (10 mM NaCl). Second-derivative spectra were calculated to determine the position of overlapped and hidden peaks in spectrum deconvolution for our data (not shown) for hematite, as well as hausmannite, suggesting the similar number of peaks and centroid positions (variation about  $\pm$ 7cm<sup>-1</sup>) of contributing bands for both minerals.

The height and width of Gaussian peak curves were visually adjusted as an initial estimative of arsenate peak, using fixed centroid positions. The peaks were fitted to minimize residual sum square of estimated spectrum from Gaussians curves sum to experimental

spectra, using the Levenberg–Marquardt algorithm. A new fitting was executed without the initial constrains (peak position). We used the Magicplot software (Magic Plot Systems, LLC, Saint Petersburg, Russia) for deconvolution process. The coefficient of determination ( $R^2$ ) >0.992 was reached for all adjustment. The adjustment could be visualized in the figure 2SM.



**Figure 2SM.** Normalized ATR-FTIR spectra of As(V) adsorbed onto hausmannite and hematite (solid black line) at different pH values in equilibrium with a 250  $\mu$ M citrate. The total arsenate concentration was 25  $\mu$ M. The thin lines represent the set of Gaussians used to fit the spectra. The total fit is represented by the dashed pink line.

deconvolution process illustrated in Figure 2SM.								
IR spectra deconvolution								
	Hausmannite			Hematite				
	pH 4		рН б	pH 4	pH 4 after 250 μM of citrate	pH 6		
Peak position (cm <sup>-1</sup> )		Relative area (%)						
700	700 2 -		2	1	-	-		
802	15	25	18	34	32	16		
834	10	18	12	19	26	6		
847	12	-	18	1	-	-		
875	30	30	31	33	32	70		
908	19	17	12	9	10	8		
938	11	10	6	5	-	-		

Table 1SM. IR spectra deconvolution for As(V) adsorbed on minerals surfaces obtained from

3.5 3.0 Ð As(V) sorbed (mg  $g^{-1}$ ) 2.5 Hausmannite pH6 0 Hausmannite pH 4 2.0 Hematite pH6  $\wedge$ Hematite pH4 1.5 1.0 Δ 0.5 0.0 0 5 10 20 25 15 30 35 As(V) in equilibrium solution (mg  $L^{-1}$ )

**Figure 3SM**. Isotherm adsorption of As(V) onto minerals at pH 6 and 4. The Langmuir equation fitting is represented by dashed black line. The parameters are presented in table 2 in main text.



pH 4 pH 6 pH 5 0.000 -0.006 120 min+10µM CA 120 min+25µM CA IR Absorbance 120 min+50µM CA 120 min+100µM CA 120 min+150µM CA 120 min+250µM CA -0.012 В С Α 0.0000 -0.0015 -0.0030 F D Ε 990 940 890 840 790 990 940 890 840 790 990 940 890 840 790

## wavenumber (cm<sup>-1</sup>)

**Figure 4SM**. ATR-FTIR *in situ* spectra were collected during the As(V) desorption onto mineral surface at pH values as a function of concentration of citric acid into the flux system. The spectra represented the difference spectrum obtained after citric acid addition minus the spectrum at 120 min (last time of kinetic studies without citric acid addition) for hausmannite (A, B, C) and hematite (D, E and F). All spectra were collected in 0.01M NaCl background electrolyte and at a total As(V) solution concentration of 25  $\mu$ M. Absorbance is given in arbitrary units.

#### **4.** PROTEIN ADSORPTION KINETICS ON THE HEMATITE SURFACE

#### ABSTRACT

Proteins are of ubiquitous interest in life sciences. Interest in the geosciences regarding protein behavior is to a large extent the result of the role of these compounds in the biogeochemical cycling of trace and nutrient elements. Structural changes of an adsorbed protein may alter protein biological function and other environmental interactions. Iron oxides are major sinks to several environmental elements including organic compounds. In this study, the adsorption of a broadly studied model protein (BSA), onto the hematite mineral surface as a function of pH, ionic strength, and BSA concentration was evaluated by Attenuated Total Reflection Fourier Transform Infrared spectroscopy (ATR-FTIR). BSA underwent conformational changes in the secondary structure during adsorption on hematite as a function of pH. The pH value close to BSA isoelectric point (~pH5) maximized the total BSA adsorption, while BSA adsorption decreased either at pH 4 or pH 7. The increment of ionic strength decreased the total BSA adsorption. The higher BSA concentration enhances BSA adsorption by protein-protein interaction at the mineral surface, duo to capacity for compacting the structural arrangement and promoting multilayers/aggregation formation

# Keywords: Bovine serum albumin; pH; Ionic strength; Colloidal surface; Spectroscopy

#### 4.1. Introduction

Proteins are of ubiquitous interest in life sciences. Their behavior is relevant to a several scientific fields including the biochemistry of early life (Nisbet and Sleep, 2001; York, 2018), pathogen-host interaction (Chattopadhyay et al., 2018; Davis et al., 2007), food chemistry (Böcker et al., 2017), human health (Bouhekka and Bürgi, 2012; Cohen, 2001; Mavropoulos et al., 2011; Ribeiro et al., 2016; Rual et al., 2005), industrial reactions (Bernal et al., 2018; Choi et al., 2015; Mateo et al., 2007), and drugs development (Estey et al., 2006; Huang et al., 2015; Hwang et al., 2015), microbial biofilm (Cai et al., 2018; Donlan, 2002; Hori and Matsumoto, 2010; Liu et al., 2015; Lorite et al., 2013). They are transferred into the environment during cellular lysis or biological activity (Mudunkotuwa and Grassian, 2015).

Interest in the geosciences regarding protein behavior is the result of its role in the biogeochemical cycling of trace and nutrient elements. Enzymes are of paramount influence

to plant nutrients such as N (Bowles et al., 2014; Fatemi et al., 2016; Jian et al., 2016), P (Fate-mi et al., 2016; Jarosch et al., 2015), and S (Saha et al., 2018) availability and C mineralization (Bowles et al., 2014; Jian et al., 2016; Trivedi et al., 2016). Many studies provide evidence for the importance of microbial-derived soil organic matter in soils (Kallenbach et al., 2016; Newcomb et al., 2017), with the proteinaceous compounds being major contributors to soil organic matter accumulation and persistence. N-containing compounds can adsorb directly onto mineral surfaces which should decrease degradation action by others catalytic enzymes (Moon et al., 2018; Newcomb et al., 2017).

Proteins in soil environment, as enzymes, are subject to adsorption process on the mineral fraction. This should affect the protein's biological functions (Huang et al., 2005; Nannipieri et al., 2012; Tietjen and G. Wetzel, 2003), as its functionality is determined by protein chemical and physical properties, which in turn are determined by the interactions between its amino acid residues in three dimensional space (Vogler, 2012). The adsorption of proteins at solid surfaces was described by Nakanishi et al (2001) as "a common but very complicated phenomenon". Protein adsorption is driven by multiple forces, including cation exchange, van der Waals and electrostatic interactions, hydrogen bonding, and hydrophobic interactions.

Environmental variables such as the nature of the adsorbent surface (e.g. hydrophobicity) and protein properties (e.g. molecular weight, amino-acids composition and sequence), medium pH, ionic strength, others ions/compounds in to the system are understood to influence these protein adsorption forces, which guides to a complex organo-mineral system with simultaneously kinetic, structural, and thermodynamic controls. An extensive discussion on this topic is presented in some valorous works (Kim and Cremer, 2001; Limo et al., 2018; Lyklema, 1984; McClellan and Franses, 2005; Paul Roach et al., 2005; Rabe et al., 2011; Sanfeld et al., 2015; Topală et al., 2014; Vogler, 2012).

Iron oxides are abundant as mineral constituents of soils, where they occur as the products of weathering and bacterial processes, and act as a source of the nutrient iron (Navrotsky et al., 2008). During rock weathering, Fe is released and subsequently precipitated as ferric oxides and hydroxides (Schwertmann, 2008a). Iron oxides are major sinks to several environmental compounds including organic (Rufus et al., 2019) and inorganic pollutants (Sherman and Randall, 2003) and control the availability of plant nutrients in soils (Fontes and Weed, 1996).

Hematite ( $\alpha$ -Fe<sub>2</sub>O<sub>3</sub>) is the most thermodynamically stable and often the most abundant iron oxide in soils and sediments among a number of other polymorphs of iron oxides and oxyhydroxides (Navrotsky et al., 2008; Schwertmann, 2008b). It has the corundum crystal structure, with layers of distorted hexagonally close-packed oxygen atoms separated by an iron double layer with Fe<sup>3+</sup> occupying two-thirds of the octahedral sites in a stacking sequence of -(Fe-O<sub>3</sub>-Fe)- along the c axis (Mazeina and Navrotsky, 2007). Its surface has an elevated free energy of formation and surface enthalpy, which promotes strong water adsorption and interaction with (Mazeina and Navrotsky, 2007; Navrotsky et al., 2008). Although it is less common in soils than goethite ( $\alpha$ -FeOOH), water adsorption experiments have shown that hydrated hematite surfaces behave thermodynamically as goethite surfaces (Mazeina and Navrotsky, 2007).

Bovine serum albumin (BSA) is suitable for adsorption studies because of its high stability, availability, purity its low cost (Kubiak-Ossowska et al., 2017; Phan et al., 2015; Schmidt and Martínez, 2016). This protein is a widely used as a model protein in studies of protein-surfaces interactions due to the synthesis of very thin protein layers on solid substrates (Scarangella et al., 2018). BSA and human serum albumins exhibit similar folding, a well-recognized primary structure, and they have been associated with the binding of many categories of small molecules (Bouhekka and Bürgi, 2012; Kubiak-Ossowska et al., 2017; Topală et al., 2014). The geomedical approach had used biological fluids, counting BSA, to assess bioavailability of nutrients and contaminants of soil and sediments (Dean and Ma, 2007; Yu et al., 2013; Yu and Yang, 2019).

Once a protein is associated with a surface, processes such as protein reorientation can induce conformational changes accompanied by protein unfolding, lateral protein-protein interactions, and desorption (Phan et al., 2015; Rabe et al., 2011; Vogler, 2012). Structural changes of an adsorbed protein may alter protein biological function and others environmental interactions (Bernal et al., 2018; Mateo et al., 2007). Because of its ability to observe of structural changes in adsorbed protein by Amide I band analysis (Yang et al., 2015), Fourier Transform Infrared (FTIR) is a useful tool for determining the secondary structure of proteins. Attenuated Total Reflectance FTIR (ATR-FTIR) spectroscopy is an in situ technique that permits the study of interfacial processes at the molecular scale in aqueous systems in real time (Lefèvre, 2004). The technique has been successfully applied to study the interaction of Fe-oxide with inorganic oxyanions such as phosphate (Elzinga and Sparks, 2007) and recently for protein adsorption on clay material (Schmidt and Martínez, 2016).

Our goal for this study was to provide new insights into the adsorption of a broadly studied model protein (BSA), onto the hematite mineral surface as a function of pH, ionic strength, and BSA concentration.

#### 4.2. Material and Methods

#### 4.2.1. Materials

The hematite sorbent used for the experiments was synthesized based on the procedure of Sugimoto et al (1993) and it has already been used by Elzinga and Kretzschmar (2013). A detailed description of this material is provided in Brechbühl et al (2012). X-ray diffraction analysis confirmed that the material does not contain crystalline phases other than hematite. The N<sub>2</sub>-BET specific surface area and the zero point of charge (ZPC) were 24 m<sup>2</sup> g<sup>-1</sup> and 9.3, respectively.

The protein used here was the bovine serum albumin (BSA) (fraction V, purchased by Sigma-Aldrich), which has an isoelectric point at pH 4.9-5.1 (Jachimska and Pajor, 2012; Schmidt and Martínez, 2016) and a molecular weight of  $\approx 66.5$  kDa. All chemical reagents were of analytical grade, and Milli-Q water (18.2 M $\Omega$ .cm, Millipore®) was used throughout the experiment.

#### **4.2.2. ATR-FTIR experiment**

The experimental IR setup is very similar to that used by (Elzinga and Kretzschmar, 2013). Data acquisition was performed with a Perkin–Elmer Spectrum 100 spectrometer equipped with a Balston-Parker purge gas generator and a liquid N<sub>2</sub>-cooled mercury– cadmium–telluride (MCT-A) detector. ATR-FTIR flow-cell containing a horizontal ZnSe crystal (10 internal reflections; 45° angle of incidence). The crystal was installed in the flow cell inside the IR spectrometer and connected by Teflon tubes to a vessel containing 100 mL of 10 mM NaCl electrolyte which was magnetically stirred and set to the desired pH. A peristaltic pump was used to flow solute from the reaction vessel through the cell at a rate of 2 mL min<sup>-1</sup>. All experiment described below were carried out at  $22\pm2$  °C.

Aqueous free-BSA spectra were collected using ATR-FTIR flow-cell crystal without hematite coating. The flow-cell was equilibrated (20 min) with 10 mM NaCl and ATR-FTIR spectrum was collected as a background spectrum (100 scans). Then, BSA was added in the vessel to reach 0.15 mM L<sup>-1</sup> of BSA concentration ( $\approx$ 10 g L<sup>-1</sup>). The initial spectrum (100 scans) was collected at pH 8.7. To each progressive addition of negligible amounts of 0.1M HCl, the pH was measured and a new spectrum was collected until we reach pH 4.

The sorption experiments were conducted with the ATR-FTIR flow-cell coated with hematite. The mineral film was made applying 125  $\mu$ L of a 5 g L<sup>-1</sup> hematite suspension plus ~1 ml water and spread evenly across the crystal surface and left drying in room temperature

overnight, which produced a stable and homogeneous deposit. Ending each experimental step, hematite on flow-cell was removed using soft paper with detergent by gentle scrubbing the crystal surface under plenty water, until last pure water rising. Other components flow setup (tubes and vessel) were cleaning using detergent solution followed water rising. A new hematite deposit was prepared for each experiment.

For BSA adsorption experiment, the hematite deposit was equilibrated with the background solution (~2 h). A final background spectrum, containing the absorbance of the ZnSe crystal, the hematite deposit and the background electrolyte, was then collected as the average of 100 scans at a resolution of 4 cm<sup>-1</sup>. Next, the vessel was spiked with aqueous BSA to start the adsorption experiment. The spectra of adsorbed BSA were collected by ratio-ing the IR spectra of the hematite film against the background spectrum collected at the end of the pre-equilibration step. The solution pH in the vessel was monitored throughout the experiment and readjusted or changed as necessary by addition of negligible aliquots of 0.1 M NaOH or 0.1 M HCl.

Three main types of adsorption experiments were carried out, each aimed at characterizing a specific aspect of BSA interaction with the hematite surface. First we tested the BSA conformation and loading on hematite surface varying the BSA concentration in equilibrium solution at pH 5 followed (Schmidt and Martínez, 2016). After pre-equilibration of the hematite deposit with background electrolyte, BSA was added to reach 0.2  $\mu$ M L<sup>-1</sup>, and then it was allowed to achieve adsorption equilibrium, which typically required 20 min. This step-wise increasing of BSA concentration and re-equilibration was repeated at [BSA] = 0.2 to 1.5  $\mu$ M L<sup>-1</sup>.

Similar to the first experiment, we tested the ionic strength for BSA conformation and loading on hematite surface. Thus, after of the hematite deposit was previously preequilibrated at pH 5 and 1 mM L<sup>-1</sup> NaCl, we added BSA to reach 1  $\mu$ M L<sup>-1</sup>. Then, it was allowed to achieve adsorption equilibrium (20 min) and this step-wise increasing of NaCl concentration and re-equilibration was repeated at [NaCl] = 1 to 100 mM L<sup>-1</sup>.

After previous analysis for BSA concentration effects assessment in the previous experiments, we aimed to characterize kinetic adsorption and dynamic changes in the BSA conformation during it adsorption dependent on pH. Then, after pre-equilibration of the hematite deposit with background electrolyte (~2h), BSA was added to reach a concentration of 1  $\mu$ M L<sup>-1</sup> (66.5 mg L<sup>-1</sup>), and its adsorption onto the hematite deposit was tracked by regular collection of spectra over the course of 120 min.

The kinetic spectra were recorded as the average of 20 scans at a 4 cm<sup>-1</sup> resolution, requiring  $\approx$  18 seconds per spectrum. To this end, we simulated the BSA desorption using a new solution of background electrolyte (10 mM L<sup>-1</sup> NaCl) at same pH, however without BSA. The new solution got into the system then was wasted after desorption process, no recirculate as in the adsorption experiment. After 30 min, the residual adsorbed-BSA spectrum was collected. To evaluate the pH dependency of BSA adsorption/desorption processes, we carried out this experiment at pH 7, 6, 5, and 4.

#### 4.2.3. Data Processing

#### 4.2.3.1 Quantitative analysis

The main data process pathway is described in figure 1. A semi-quantitatively BSA adsorption loading and kinetic was obtained by integration of the area under the Amide II vibrational band (1600-1480 cm<sup>-1</sup>) (Figure 1C). This band was chosen for semi-quantitative analyses because, compared to the amide I band vibration (1700-1600 cm<sup>-1</sup>), it is less impacted by interference of the water bending mode at ~1645 cm<sup>-1</sup> and less affected by conformational changes related to the adsorption process (Schmidt and Martínez, 2016; Yang et al., 2015). The baseline was fitted as a straight line between 1600 to 1480 cm<sup>-1</sup> and the area under the peak determined by integration using the software package MagicPlot ver. 2.7.2 (Magic Plot Systems, LLC, Saint Petersburg, Russia). Data processing of the kinetic and isotherm IR data process was carried with the software package Origin 2017 (OriginLab Corporation, Northampton, Massachusetts, USA).



**Figure 1**. Process of kinetic data analysis: (A) Raw data of BSA adsorption kinetic experiment collected between 1 min (bottom black line) and 120 min (top red line) of reaction time. (B) Individual spectrum of the 120 min spectrum. (C) Double baseline fit of the Amide I and II band in the 120 min spectrum. The Amide II band area was integrated and is presented in Figure 4 (D) The Amide I band was deconvoluted into Gaussian curves at peak position determined by the 2<sup>st</sup> derivative spectrum of the original data, which is shown in panel (E).

#### 4.2.3.2 Protein conformational structure

The IR data of adsorbed BSA were analyzed quantitatively to determine secondary structure following the method described by Yang et al. (2015). The method is illustrated in Figure 1. Briefly, we used the software package MagicPlot to first fit a linear baseline across the Amide I band (1700–1600 cm<sup>-1</sup>), and then the second derivative spectrum was determined to find the position and number of structural component bands for subsequent peak fitting. Once the component positions were identified and the baseline established, Gaussian peaks were added to the Amide I band according to position suggested by the second derivative spectrum (Figure 1D). This procedure was used for the aqueous spectra and for the kinetic spectra obtained after 120 min for each pH.

In the peak deconvolution process, the peak height and width were initially manually adjusted to obtain a "visually" good fitting result. Next, we applied the Levenberg–Marquardt

algorithm to minimize the residual sum square of the fitted and experimental spectra, with peaks fitted with Gaussians curves fixed at the peak position determined by the second derivative. In a second fitting procedure, we followed the same method but allowed peak positions to shift. The resulting peak positions were generally within  $\approx 3 \text{ cm}^{-1}$  among aqueous and adsorbed BSA, regardless of pH value, and within  $\approx 5 \text{ cm}^{-1}$  of the position determined by the second derivative. The coefficient of determination (R<sup>2</sup>) was  $\geq 0.995$  all fits. The secondary structural assignments are described in the table 1.

Table 1. Vibrational frequencies of the absorption bands associated with secondary structure conformation of BSA in the Amide I region used for curve fitting.

Secondary structure assignment -	Vibrational frequency range						
Secondary structure assignment	(cm <sup>-1</sup> )						
	(Givens et al., 2017)	$\begin{array}{c} (\text{Lu et al.}, \\ 2015)^1 \end{array}$	(Schmidt and Martínez, 2016)	Peak center found in this work			
β-sheets/turns	1685-1663	1690-1680	1690-1660	1676			
β-sheets/turns	-	1670-1662		1662			
α-helix	1655-1650	1655-1650	1660-1650	1652			
Random Coin	1648-1644	1645-1642	1648-1644	1644			
Extended chains/ $\beta$ -sheets / Short- segment chains connecting the $\alpha$ - helical segment <sup>2</sup>	-	1638-1632	1640-1630	1637			
Extended chains/β-sheets, Aggregated strands	1639-1621	1620-1610	1630-1620	1628			
Side chain moieties	1616-1600	-	1620-1600	1612			

<sup>1</sup>The authors used D<sub>2</sub>O solution, <sup>2</sup> (Gilmanshin et al., 1997; Lu et al., 2015)

#### 4.3. Results

#### 4.3.1. Aqueous BSA

Acquisition of ATR-FTIR data of proteins in aqueous solution is not straightforward due to experimental challenges. The first is that relatively high concentrations are needed to obtain spectra of reasonable quality. This is likely due to low the affinity between protein and the ATR crystal as the result of electrostatic repulsion between BSA and ZnSe, and the weakly hydrophobic nature of the ZnSe surface (Jeon et al., 1994). From pH 8.4 to 5, the negative charge of both ZnSe (isoelectric point = 4.9, (Gao and Chorover, 2010) surface and BSA (isoelectric point = 4.9-5.1) results in a strong electrostatic repulsion.

Protein accumulation in the surface region above the ZnSe crystal probed by the IR beam may be driven by hydrophobic effects at these pH values. The hydrophobic effect could be suggested by preferential desorption from ZnSe crystal interfacial of "ice-like" water (3400 cm<sup>-1</sup> peak) desorption (Figure 2A), which has a more oriented structure than bulk water (3200 cm<sup>-1</sup>) (Kim and Cremer, 2001). At lower pH, (red lines in Figure 2A), the charge densities of the protein and the ZnSe crystal surface decreased, thereby lowering both protein-protein and protein-surface repulsion. This resulted in increased BSA surface loadings (Figure 2A), although the intensities remain overall relatively low.



**Figure 2.** (A) Raw IR spectra of aqueous BSA (15 mM L<sup>-1</sup>) collected over the pH range from 8.7 (top black line) to 4 (bottom red line) in 10 mM L<sup>-1</sup> NaCl. The amide bands are located in the yellow-highlighted spectral region indicated by \* (B) Zoom-in of the spectral region containing the Amide I and II bands. Water IR absorption ( $v_2$  H<sub>2</sub>O) at 1644 cm<sup>-1</sup> overlaps with the Amide I band (1700-1600 cm<sup>-1</sup>) but not with amide II. (C) Relative band intensities as a function of pH indicate Secondary structure proportion of free aqueous-BSA at pH 7, 6, 5, and 4. See the corresponding structural assignments in relation to wavenumber peak in the table 1.

A further challenge for analysis of aqueous protein by ATR-FTIR is the interference of water bands in the IR region. As seen in figure 2B, water has a strong IR absorbance around 1640 cm<sup>-1</sup> (H-O-H bending), which overlaps with the Amide I mode of BSA (1700

cm<sup>-1</sup> and 1600 cm<sup>-1</sup>). Despite these experimental challenges, we succeeded in acquiring BSA spectra over the pH range of interest here that could be used to characterize the BSA secondary structure.

Secondary structure defines the local conformation of amino acids in the protein chain. In aqueous media, interactions of the hydrophilic side chains with water are maximized through hydrogen bonding, while interactions of hydrophobic side groups are minimized. The structural arrangement of the protein is determined by these interactions, and is termed the secondary structure, which is of great interest because it affects protein functioning. Information of protein secondary structure is available in abundance in the literature.

Secondary structure can be determined by IR spectroscopy, because structural arrangements in the protein chain are associated with specific vibrational bands (Giacomelli et al., 1997; Gilmanshin et al., 1997; Lu et al., 2015; Militello et al., 2004; Schmidt and Martínez, 2016; Vermonden et al., 2002). Besides IR, other techniques used to determine structure include X-ray crystallography (He and Carter, 1992) and UV-circular dichroism (Chi et al., 2018; Güler et al., 2016; Zhang et al., 2010). The pertinent structures observable in the amide I region of IR spectra are summarized in Table 1; these include  $\alpha$ -helix,  $\beta$ -sheet, and random coil structures, among others.

The pH and ionic strength affect BSA forms changes in secondary structure. Jachimska and Pajor (2012) and Chi et al. (2018) presented changes in BSA conformation correlated with  $\alpha$ -helix content. At pH 7 the BSA molecule has a "heart" form (an equilateral triangle with 8 nm side  $\times$  3 nm depth), while decreasing pH leads the protein to expand and adopt an extended conformation (Edri and Regev, 2004). At the isoelectric point, the BSA molecules are maximally packed. This decreases the contributions of the native main ("heart") forms and the  $\alpha$ -helix (1652 cm<sup>-1</sup>) arrangement, but enhances the  $\beta$ -forms (1662 and 1637 cm<sup>-1</sup>) (Militello et al., 2004; Vermonden et al., 2002).

The protein secondary structure of aqueous free-BSA had the  $\alpha$ -helix conformation as the prevalent form (Figure 2C), with estimated contributions of 47%, 40%, 35%, 45 % at pH 7.0, 6.0, 5.0, and 4.0 respectively. Literature values for native BSA secondary structure showed a variation around ±10% among structures distribution, depending on the experimental setup, the spectroscopic technique used for characterization, and experimental conditions such as BSA concentration and electrolyte. We found similar results of  $\alpha$ -helix content in the total secondary structure of aqueous BSA, similar to others studies (Giacomelli et al., 1997; Gilmanshin et al., 1997; Lu et al., 2015; Militello et al., 2004; Schmidt and Martínez, 2016; Vermonden et al., 2002).

#### 4.3.2. BSA adsorption onto hematite: Concentration effects

Isotherm data of BSA adsorption on hematite were derived from the Amide II areas in the IR spectra of adsorbed BSA at aqueous BSA concentrations that ranged from 0.2 to 1.5  $\mu$ M L<sup>-1</sup>. The results indicate that BSA has a strong affinity for the hematite surface (Figure 3). The isotherm data do not conform to the Langmuir model, in that no surface saturation was observed (Figure 3A). Of note is the apparent point in the isotherm at [BSA] > 1  $\mu$ M, suggestive of a change in adsorption mechanism that promotes BSA adsorption at higher concentrations. We tentatively assign this to surface clustering and aggregation of adsorbed protein molecules, leading to multilayer adsorption, as suggested by results from Meissner et al. (2015).



**Figure 3.** (A) Adsorption for BSA adsorption onto hematite over BSA concentration at pH = 5 and [NaCl] = 10 mM L<sup>-1</sup>. (B) Secondary structure proportion of adsorbed BSA on hematite. See the corresponding structural form in relation to wavenumber peak in table 1. Dotted line represents the BSA concentration used to kinetic experiment

The Langmuir isotherm assumes uniformity of adsorption sites, each position being able to hold only one molecule creating a monomolecular layer on the sorption surface. The lack of Langmuir behavior of BSA adsorption observed here differs from results of batch adsorption experiment with oxides (Alkan et al., 2006; Fukuzaki et al., 1996; Song et al., 2012) and clay materials (Andersen et al., 2016; Barral et al., 2008; Duarte-Silva et al., 2014), and *in-situ* ATR-FTIR results presented by Schmidt and Martínez (2016) characterizing BSA adsorption on montmorillonite surface with an experimental setup similar to ours. BSA multilayer adsorption is a surface specific phenomenon and requires further study.

Comparison of the IR spectra of aqueous and adsorbed BSA showed that the secondary structure of adsorbed-BSA has changed considerably compared to that of aqueous-BSA (Figure 3B). There is a loss of  $\alpha$ -helix (35 to 30%, 1652 cm<sup>-1</sup>) and short-segment chains connecting the  $\alpha$ -helical segment (17 to 8%, 1637 cm<sup>-1</sup>), concomitant with a gain of extended chains/ $\beta$ -sheets, aggregated strands (2 to 17% 1628 cm<sup>-1</sup>). These changes are consistent with an unfolding process of the protein upon its adsorption (Militello et al., 2004; Vermonden et al., 2002). These findings reinforce the arguments about no Langmuir roles acceptations, since that the Langmuir isotherm premise about "The adsorbing molecule adsorbs into an immobile state" was overlooked. Based on these isotherm measurements, the remainders of the IR experiments were conducted with a BSA aqueous concentration of 1  $\mu$ M, because at this concentration level we reach maximum BSA adsorption with minimal effects of clustering and aggregation.

#### 4.3.3. BSA adsorption onto hematite: Ionic strength effects

The influence of NaCl concentration in the background electrolyte on the Amide I band of adsorbed BSA is illustrated in Figure 4. An increase in NaCl concentration 1 to 100 mM results in a decrease of ~14% of the amide I band intensity (Figure 4A), but does not lead to any distinct changes in the secondary structure of adsorbed BSA (Figure 4B). We attributed the ionic strength effect to decreasing electrostatic attraction between the BSA molecules and the hematite surface as the result of increased charge screening (Wendorf et al., 2010), particularly for the loosely bound BSA molecules adhered in multilayers (Latour, 2015; Meissner et al., 2015). The slight increase in the contribution of the extended chains/ $\beta$ -sheets (1628 cm<sup>-1</sup>) structure with increasing ionic strength likely is due to the compression of the diffuse double-layer, which could force an increase the protein unfolding.



**Figure 4.** (A) Adsorption for BSA adsorption onto hematite over NaCl concentration at pH = 5 and [BSA] =  $1\mu M L^{-1}$ . (B) Secondary structure proportion of adsorbed BSA on hematite. See the corresponding structural form in relation to wavenumber peak in table 1. Dotted line represents the BSA concentration used to kinetic experiment.

Ionic strength effects were likely to be dependent on the background electrolyte, in particular the cations. The sodium cations used here did not interact strongly with dissolved anionic macromolecules or mineral surfaces. However, other common cations such as  $Ca^{2+}$  are more reactive, and this may lead to different ionic strength effects than observed here. For example,  $Ca^{2+}$  may promotes protein aggregation by bridging negatively charged proteins (Simons et al., 2002), which would leads to increased protein adsorption with increasing  $Ca^{2+}$  solution concentrations. The interaction between Ca and organic molecules in soil systems are well known (Rowley et al., 2018). Further study is needed to evaluate the impacts of  $Ca^{2+}$  and other common cations (e.g.  $Mg^{2+}$ ,  $K^+$ ) on protein interactions with minerals surfaces.

#### 4.3.4. BSA adsorption kinetics pH dependency

Figure 5 displays the kinetics of the BSA adsorption process as determined from changes in the Amide II area of adsorbed BSA (Figure 1) which are plotted as a function of time intervals for various pH values. These kinetic results were successfully modeled with a

pseudo-first order rate model, which yielded higher  $r^2$  and lower reduced  $\chi 2$  values (Table 2) than other model assessment (Table 1SM).



**Figure 5.** Amide II area of adsorbed-BSA over the time. Pseud-first order kinetic models for each pH are drawer by dashed lines of corresponding color. The [BSA] =  $1\mu M L^{-1}$  in electrolytic background (ionic strength) as [NaCl] =  $10mM L^{-1}$ . The equation parameters are listed in table 2.

BSA adsorption was fast, reaching ready-state after about 15 min, regardless of pH value (Figure 5; Table 2). The maximum Amide II area estimated by this model reveals a maximum BSA adsorption capacity on hematite of 3.70 at pH 5.0, with lower capacities at both lower and higher pH values (Table 2). Achievement of maximum adsorption capacity at pH 5 coincides with the pH-isoelectric point of BSA, which is one of the main factors governing the adsorptive properties of proteins rather than the nature of the adsorbent surfaces (Fukuzaki et al., 1996).

pН	AmideII <sub>max</sub>	K	_	Reduced	Desorption	
	Peak area (A.U. <sup>2</sup> )	1/min	$\mathbb{R}^2$	$\chi^2$	%	
7	2.99	0.119	0.88	0.14	1	
6	3.53	0.109	0.95	0.06	5	
5	3.70	0.114	0.96	0.07	10	
4	1.79	0.164	0.91	0.02	3	

**Table 2.** Pseudo-first order kinetic parameters for BSA adsorption and desorbed fraction for each pH value.

BSA desorption was negligible at pH 7 and 4 but observable at pH 6 and pH 5, where approximately 10% desorption occurred (Table 2). The overall low rates of desorption indicate the relatively high stability of adsorbed BSA. Slight desorption at pH 5 and 6 was attributed to the relatively high surface loadings at these pH values, maybe causing some multilayer protein-protein (Latour, 2015; Meissner et al., 2015), which could has lower bonding stability than a direct interaction protein-hematite. Besides pH, ionic strength also affects surface coverage (as discussed in section 4.3.3), and may therefore affect the stability of BSA adsorption as well.

Conformational protein analysis as a function of time during the process of BSA adsorption is presented in Figure 6. The secondary structure of BSA changes as it transfers from the aqueous phase to the hematite surface (Figure 6), which causes protein unfolding as indicated by the loss of  $\alpha$ -helix and gain of  $\beta$  and extended structures (Militello et al., 2004; Vermonden et al., 2002).The conformation changes stability around 15 min, which is the same time that there is the overall adsorption process stabilizes (Figure 5). This indicates that conformational changes are relatively fast.



**Figure 6.** Secondary structure proportion shifts of adsorbed BSA on hematite surface over the kinetic time in different pH values. At 150 min (\*) represents the secondary conformation of BSA remained after desorption process. Dotted lines of the corresponding color represent the mean secondary structural composition acquired of free aqueous-BSA in to pH variations. See details in figure 2.

For pH 7 and 6, there were fast shifts with initial increase followed decrease and stabilization of  $\alpha$ -helix (1652 cm<sup>-1</sup>); increase at  $\beta$ -sheet/turns (1676 cm<sup>-1</sup>) and decrease of short-segment chains connecting the  $\alpha$ -helical segment (1637 cm<sup>-1</sup>). At pH 5 and 4, there was in increased loss of  $\alpha$ -helix and short-segment chains connecting the  $\alpha$ -helical segment and raising of  $\beta$ -sheet/turns (1662 cm<sup>-1</sup>) and extended chains/Aggregated strands (1628 cm<sup>-1</sup>). From pH 5 to 4, a notable decrease in  $\alpha$ -helix and increase of  $\beta$ -sheet/turns and random coils (1644 cm<sup>-1</sup>) were observed. No considerable changes are observed after desorption process, which could endorse the idea of multilayers, even at low BSA concentration (1  $\mu$ M ), but the amount of desorption was too small to make a difference in the IR spectra, which not allow to confirming this assumption.

The BSA structural changes during adsorption to the hematite structure were different than structural changes due to thermal (Lu et al., 2015) or photocatalytic (Bouhekka and Bürgi, 2012) denaturation process, which beyond loss  $\alpha$ -helix, had been followed by

increase at random coil. Here, the random coil was at higher pH lower than aqueous-BSA, this structure increase at lower pH boosted by pH effect (Chi et al., 2018; Jachimska and Pajor, 2012).

#### 4.4. How much is complex the protein adsorption?

We noted the difference between our results and those of an earlier study by Schmidt and Martinez (2016) that used the same experimental ATR-FTIR setup but used montmorillonite instead of hematite as the mineral sorbent for BSA. This earlier study observed a low absorbance signal and no significant change in protein secondary structure upon BSA adsorption at pH 5 (see figure 1 and 5 of Schmidt and Martínez (2016), respectively), which is quite different than our observations (see Figure 1SM and 2). The difference in mineral type is a key factor. Montmorillonite is a 2:1 aluminosilicate clay with a permanent structural negative charge (Bolt, 1985), while hematite is a variable charge Fe(III)oxide mineral with a ZPC of 9.3. At pH 5, the BSA is slightly negatively charged as well (isoelectric point = 4.7), which results in electrostatic repulsion with the montmorillonite surface but electrostatic attraction with the hematite surface.

The hematite is positively surface charged (ZPC = 9.3) over the pH range studied, while on the other hand, at pH 7 and 6, BSA has a negative charge which will enhance BSA adsorption by electrostatic attraction. At pH 4, however, both BSA and mineral surface have a positive charge, causing electrostatic repulsion. Despite these electrostatic considerations, BSA adsorption does occur readily at pH 4, while maximum BSA adsorption occurs at pH 5, near the isoelectric point of BSA where electrostatic attraction to the surface will be limited. These results indicate that forces beyond electrostatic interactions between the surface and BSA are involved in the adsorption process.

The binding affinity of BSA to a model hydrophilic silica surface was investigated using computational simulations by Tokarczyk et al (2018), and they concluded that the protein's charge distribution is inhomogeneous and positive residues get have interaction, also a once adsorbed, the protein does not desorb, but it does freely diffuse across the surface. The authors found that the adsorption kinetics are dominated by energy barriers associated with electrostatic interactions, but once adsorbed, protein-surface interactions were dominated by short-range non-electrostatic interactions such as van der Waals forces (McUmber et al., 2015).

The adsorption of BSA onto the hematite surface produces an unfolded conformation by changes of secondary structure (Figure 6). This may expose the hydrophobic core that was previously kept isolated from water. The adsorbed molecule may therefore act as a new adsorption site for a bulk protein or other hydrophobic substances in the system. Hydrophobic forces may therefore be a major factor in the adsorption of proteins on soil mineral surfaces. The higher affinity of BSA for the hematite than the montmorillonite surface, in combination with the different structure and composition of the montmorillonite and hematite surfaces, are undoubtedly responsible for the significant differences in BSA adsorption behavior. Further experimental studies as well as theoretical work such as molecular dynamics simulations (Andersen et al., 2016) will be very useful to further resolve the interaction of mineral surfaces with protein molecules.

#### 4.5. Soil implications

The protein are recognized as a chemically labile soil compound, instead seem to turn over (on average) at rates similar to those of stabilized organic matter, on the order of years or even decades (Schmidt et al., 2011). The protein-mineral protection is a remarkable feature of protein stabilization thus; formation of soil organic carbon and nitrogen stocks (Andersen et al., 2016). Other point of view referee to enzymes (i.e. all proteins) used as catalytic apparatuses by microorganisms for the biogeochemistry process, such as decomposition of plant, xenobiotics contaminants, organic matter turnover. Extracellular enzymes had activity distinctly affected to their affinity to soil mineral surfaces (Arnosti et al., 2014).

We got described a pH dependency of total protein adsorption. Hence, agriculture practices as lime application could change protein-mineral stability. Some studies for nanoparticles (Cheng et al., 2017; Wang et al., 2011) or natural clay (Zhu et al., 2016) suggested a deactivation of enzymes proprieties duo to adsorption process. This information could be useful to development of engineering products to optimize, for example, the nitrogen fertilization using urea, through decrease at urease activity.

#### 4.6. Conclusions

BSA underwent conformational changes in secondary structure during adsorption on hematite. Protein-protein interaction at the mineral surface enhances BSA adsorption capacity by compacting the structural arrangement and promoting multilayers/aggregation formation. The detailed molecular-level observations of the configurations of BSA were enabled by the ATR-FTIR technique used here. Our results suggest a strong dependency pH value, confirming the effect of surface charge in total adsorption.

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#### **Supplementary information**

**Table 1SM.** Pseudo-second order and Elovich model parameters for BSA adsorption and desorbed

 fraction for each pH value.

Pseudo-second order				Elovich model					
	AmideII <sub>max</sub>	K							
рН	Peak area (A.U. <sup>2</sup> )	Area peak/Area hematite/min	R <sup>2</sup>	Reduced $\chi^2$	рН	α	β	R <sup>2</sup>	Reduced $\chi^2$
7	1.85	0.135	0.82	0.20	7	0.84	1.43	0.72	0.32
6	2.00	0.125	0.92	0.11	6	0.92	1.20	0.83	0.23
5	2.05	0.131	0.92	0.11	5	1.05	1.16	0.84	0.24
4	1.42	0.206	0.95	0.01	4	1.23	2.87	0.90	0.02

 $\alpha$  represents the initial adsorption rate and  $\beta$  is an empirical constant of activation energy distribution



Figure 1SM. Raw FTIR spectrum of BSA kinetics adsorption on hematite at pH 7. This same figure is presented in Figure 1A in main test. \* = baseline disturb over the adsorption time.

### 5. CALCIUM ENHANCES HUMIC ACID ADSORPTION ON SOIL MINERALS

#### ABSTRACT

Increasing organic matter storage in highly weathered soils is an important strategy to improve soil fertility and may help to mitigate rising atmospheric CO<sub>2</sub> levels resulting in climate change. High calcium (Ca) availability in soils resulting from lime and gypsum application has been shown to increase soil organic matter contents in weathered tropical soils, and this is frequently attributed to enhanced bonding between soil minerals and organic molecules. Here we aimed to study the effects of Ca on the extent and nature of organo-mineral interactions. A mix of pure mineral phases to mimic the mineralogy of the clay fraction of a weathered tropical soil was used. Sorption experiments were conducted with standard humic acid (HA from Elliot Soil, IHSS) at two pH and four Ca solution concentrations. HA adsorption increased with decreasing pH. The presence of Ca increased HA adsorption by a factor of approximately 1.4 and 0.6. UV-Vis analyses suggested the preferential adsorption of organics with humification levels and/or low molecular weight at low surface coverage. Attenuated Total Reflection-Fourier transform infrared (ATR-FTIR) spectroscopy results showed an association of Ca with organic carboxyl groups, confirming the potential of Ca act as a bridge between HA and mineral surfaces. Our results suggest that soil use and management practices that promote high Ca concentration in soil solution may improve the ability of soil to act as a sink of organic C.

Keywords: Tropical soil; Carbon storage; Organometallic complex; In-situ infrared spectroscopy; Adsorption

#### **5.1. Introduction**

Soil systems represent both a huge sink and source of atmosphere CO<sub>2</sub>. Soil organic matter (SOM) storage up to 1500 Pg C in the first 1 m of soil profiles worldwide, which is two times the combined amounts of atmospheric carbon (830 Pg C) and that allocated in plant biomass (56 Pg C) (Olson et al., 1983). Soil management practices that intensify or decrease the accumulation of SOM need to be understood in order to develop strategies to improve accumulation for mitigation of climate change process.

The mechanisms of SOM stabilization are varied and complex (Six et al., 2002). The chemical recalcitrance of organic matter inputs was long assumed to be the main factor
(Schmidt et al., 2011). However, effects of climate (Castanha et al., 2018; Moinet et al., 2018), soil mineralogy (Souza et al., 2017; Singh et al., 2017), aggregate structure (Six et al., 2002), land use (Oliveira et al., 2016) and agricultural practices (Castro Filho et al., 2002; Curtin et al., 2015) are controlling factors of SOM stabilization as well. Certainly, all these variables act concurrently (Han et al., 2016), but those which we can efficiently modify through land management measures for improving C storage are limited.

Changes in soil chemistry could contribute to SOM accumulation. In addition to the well-known interaction and positive correlation between SOM and Al-, Fe- and Mn-oxides (Souza et al., 2017; Johnson et al., 2015), high Ca availability resulting from lime and gypsum applications has shown to increase SOM in weathered tropical soils. Carmeis Filho et al. (2017) concluded that surface liming is an important tool for increasing the SOM input, which may be due to increased primary biomass production (Caires et al., 2011; Tiecher et al., 2018; Toma et al., 1999; Zoca and Penn, 2017), or by increased carbon adsorption through Ca bridging between mineral surfaces and organic compounds, as shown for ferrihydrite (Sowers et al., 2018), goethite (Weng et al., 2005), clay materials (Feng et al., 2005) and soils (Kloster and Avena, 2015; Inagaki et al., 2017). Detailed information about the impact of Ca on SOM stabilization is provided by Rowley et al. (2018). Following the research gaps pointed by Rowley et al. (2018), this research was aimed to study the Ca effects on (i) amount of organic carbon adsorption, (ii) nature of organo-mineral interactions.

#### 5.2. Material and Methods

#### 5.2.1. Organic carbon solutions

Standard Elliott Soil Humic Acid (1S102H) was obtained from the International Humic Substances Society and used without further purification. Humic acid (HA) stocks solutions of 1 g  $L^{-1}$  were prepared by suspending the HA solid into 10 mM NaOH. The stock solution was diluted to achieve a desired series of initial HA concentrations. The pH of these solutions was corrected to 6.5 and 4.5 using negligible amounts of 1M NaOH or HCl. The solutions were stored in the dark and 4 C° to prevent degradation.

#### 5.2.2. Soil Mineral Mix

We aimed to mimic the clay size-fraction of a weathered soil by mixing relevant mineral phases. The mineral types and their proportions were selected based on the "Representative Pedon and Data" for Oxisols from the Soil Taxonomy Classification (KuoLiang Chung and Shih-Tung Wu, 2005). The mixture contained 70 wt-% kaolinite, 20 wt-% gibbsite, and 10 wt-% goethite, in accordance with mineral identification and abundances in other studies of the mineralogy of weathering soils (IUSS Working Group WRB, 2014) (Fink et al., 2016).

The kaolinite used in this study was purchased from the Clay Minerals Society Source Clays Repository (Purdue University, USA) called KGa-2. It is considered relatively poorly crystallized, similar to the kaolinite present in soil samples. We used  $\gamma$ -Al<sub>2</sub>O<sub>3</sub> powder (99.99% purity, Alpha Aesar) replacing the gibbsite mineral. Although  $\gamma$  -Al<sub>2</sub>O<sub>3</sub> is not found in natural environments,  $\gamma$ -Al<sub>2</sub>O<sub>3</sub> converts into gibbsite and bayerite during hydration (Carrier et al., 2007; Lefèvre et al., 2002), and these minerals are representative of the Al-oxides in soils (Yang et al., 2013). To ensure conversion of the  $\gamma$ -Al<sub>2</sub>O<sub>3</sub>, the  $\gamma$ -Al<sub>2</sub>O<sub>3</sub> was aging in aqueous solution for 15d at 80 C° (4g in 80 ml water), and then freeze-dried.

Mineralogical transformation into gibbsite and bayerite was confirmed by XRD analysis (Supplementary material). The goethite (Gt) used in this study was synthesized based on a method described previously (Elzinga et al., 2001). Briefly, the mineral was made by a titrating of 50 mL 1 mol  $L^{-1}$  ferric nitrate solution with 450 mL of 1 mol  $L^{-1}$  KOH, and aging the resultant ferric oxide suspension for 2 weeks at 25°C. Next, the goethite suspension was centrifuged, washed with water and resuspended in 0.4 mol  $L^{-1}$  HCl for 2 h to remove any remaining amorphous iron oxides. Goethite was then centrifuged and washed with deionized water. Finally, the material was dialyzed to remove excess salts and then freeze-dried.

Mineral stock suspensions of 50 g L<sup>-1</sup> were prepared by combining 140 mg of KA-2, 40 mg of aged  $\gamma$ -Al<sub>2</sub>O<sub>3</sub>, and 20 mg Gt in a 50-mL tube. The mineral mixture was washed once with 40 mL of 0.1M KCl solution and subsequently four times with ultrapure water, and then dried for three days at 40 C°, finally, new 40 ml of ultrapure water were added. This initial suspension was left in horizontal shaker for two weeks to initial mineral interaction. This mix will be hereafter referred to as the "clay".

A modified barium method (Rhodes, 1982) was applied to determine the cation exchange capacity (CEC). Briefly, a portion of dried clay ( $\approx 2g$ ) were previous saturated by 10 mL of 0.1M BaCl<sub>2</sub> for 1h, after centrifugation the solution was discarded and the solid was washed with plenty water three times and thus dried. Then, 0.50 g was weighed and 40mL 0.1 CaCl<sub>2</sub> were added. After 1h, the solid was centrifuged and the supernatant collected for Ba measurement. The total of Ba desorbed was considered here as CEC.

The method used to determine the zero point of charge  $(pH_{zpc})$  of clay was a modified version of the solid addition method by Balistrieri and Murray (1981). Briefly, to a

series of 50 ml polyethylene tubes containing 45 ml of 0.05M KCl, different volumes of either 0.1 M HCl or 0.1 M NaOH solution were added to obtain wide ranging pH values (pH 2–10), then more 4 ml of electrolyte solution was added. The solutions were homogenized, and the pH values were measured; these initial pH values are referred to here as pH<sub>initial</sub>. Next, 1 mL of clay stock suspension (50 g L<sup>-1</sup>) was added to each tube. After 72 h of equilibration at room temperature and shaking end-over-end (100 cycles per minute), the pH values of suspension in each bottle was noted and designated as final pH or pH<sub>final</sub>. The difference between pHi and pHf ( $\Delta$ pH) was plotted against pHi. The resultant curve intersects  $\Delta$ pH = 0 at a certain value of pHi. This means that at this pH the clay does not induce the release of either H<sup>+</sup> or OH<sup>-</sup> ion in solution; i.e., the surface does not undergo or acquire any charge through acid–base dissociation, a condition for pH<sub>pzc</sub>. The CEC and ZPC of clay were 66 mmol<sub>c</sub> kg<sup>-1</sup> and 6.5, respectively.

## **5.2.3. HA adsorption to clay**

Batch adsorption experiments were performed following the method used by Chotzen et al. (2016). The clay suspension was diluted to  $3.75g L^{-1}$  and split up in two portions. The pH in one portion was set to 6.5 and that of the other to 4.5 using small amounts of 1M NaOH or HCl. The suspensions were pre-equilibrated for three days with regular readjustment of suspension pH to 6.5 and 4.5 as necessary. Next, 0.4 mL aliquots of the clay suspensions were mixed with 0.1 mL 0.15 M NaCl containing 0, 1.5, 3 or 6 mM dissolved Ca<sup>2+</sup> in 2 mL centrifuge tubes. The suspensions were equilibrated for 4 h. After this time, 1 mL aliquots of 0.15 M NaCl solutions containing variable concentrations of HA were added. The final suspensions contained 1 g L<sup>-1</sup> of clay; Ca concentrations of 0, 0.1, 0.2 or 0.4 mM; and HA concentrations ranging from 5 to 120 mg L<sup>-1</sup>. All samples were prepared in duplicate.

The samples were placed on orbital shaker for 24 h to equilibrate at room temperature (23 ± 2 °C). After equilibration, the solid phase was separated by centrifugation at 8,500 rpm for 8 min. The solution HA concentration was determined by the Carry 3 UV-Vis spectrophotometer (Varian, USA) at 280 nm using 1 cm quartz cell. Calibration curves at two working pH were constructed with HA solutions prepared from the stock solutions (pH 6.5 and 4.5) with concentration range between 1-60 mg L<sup>-1</sup> (R<sup>2</sup> ≥ 0.998 for both pH). Beyond the absorbance at 280 nm (E2), the absorbance at 465 nm (E4) and 664 nm (E6) of HA solutions were also measured to calculate E2/E4 and E4/E6.

The amount of HA adsorbed on clay was calculated using the differences in the concentrations of HA solutions before and after adsorption. Langmuir equation (Eq.1) was used to describe the adsorption process.

$$Q_e = \frac{Q_{max}k_LC_e}{1+k_LC_e} \dots Eq. 1.$$

where  $C_e$  is the equilibrium concentration of HA in solutions (mg L<sup>-1</sup>), q is the adsorption amount of HA to clay (mg g<sup>-1</sup>),  $Q_{max}$  is the maximum adsorption capacity (mg g<sup>-1</sup>) and  $K_L$  is Langmuir constant (L mg<sup>-1</sup>).

# 5.2.4. ATR-FTIR Spectroscopy

The residual solid (Clay+HA) and initial solid HA were air-dried at room temperature for 24 h. Attenuated Total Reflection–Fourier transform infrared (ATR-FTIR) spectra of clay solids were collected on a Perkin–Elmer Spectrum 100 spectrometer equipped with internal reflection element accessory from Pike Miracle (Pike Technologies, USA) and a liquid N<sub>2</sub>-cooled mercury–cadmium–telluride (MCT) detector. Spectra were collected in the 4000–600 cm<sup>-1</sup> range, with a resolution of 4 cm<sup>-1</sup>. For each sample 40 scans were collected and averaged to produce the final spectrum.

We normalized all spectra (0 to 1) and subtracted the spectrum of non-reacted clay to improve the identification of weak peaks associated with adsorbed HA (Figure S1). The spectral region 1850-1220 cm<sup>-1</sup> contained bands associated with adsorbed HA. The baseline of this region was fitted using a parabolic curve in Magicplot Student software (MagicPlot System, 2.7.2, LCC; see free version https://magicplot.com/downloads.php).

#### 5.3. Results and discussion

# 5.3.1. Adsorption of HA

The isotherm data of HA adsorption onto the clay mixture are presented in Figure 1. The data could be described well with the Langmuir equation as shown by the high model efficiency ( $R^2$ ) (Table 1). HA adsorption had strong pH dependence, with generally high adsorption at pH 4.5 than 6.5 under given conditions (Figure 1). This is reflected in the Langmuir fit parameters, returning HA adsorption maxima that are generally 25% higher at pH 4.5 than at pH 6.5 under equivalent conditions (Table 1). This pH dependence likely is the result of increased positive surface charge due to protonation of Al- and Fe oxyhydroxides

surface sites and the aluminol sites of kaolinite, which enhances the electrostatic attraction of negative charged HA functional groups, and thus promotes adsorption.

			2	
рН	Ca (mM)	$Q_{max}$ (mg HA g <sup>-1</sup> clay)	K <sub>L</sub>	$R^2$
	0	11.4	0.17	0.84
6.5	0.1	18.1	0.13	0.88
	0.2	24.7	0.10	0.94
	0.4	27.7	0.10	0.96
-	0	19.5	0.12	0.94
4.5	0.1	24.0	0.10	0.88
	0.2	25.2	0.11	0.90
	0.4	30.5	0.12	0.91

**Table 1**. Langmuir parameters used to model sorption of HA on clay

Where  $Q_{max}$  is the estimative of maximum HA adsorbed concentration;  $K_L$  Langmuir constant and  $R^2$  is the correlation coeficient



**Figura 1**. Adsorption isotherm and Langmuir equation fitting (solid line) for HA on mimic soil clay fraction in function of pH and Ca concentration in solution.

There is a notable impact of Ca with increased HA adsorption as the Ca concentration increased, particularly at higher pH (Fig. 1). The presence of Ca increased HA adsorption by a factor of approximately 1.4 and 0.6, for pH 6.5 and 4.5 respectively. These

findings are significant to soil environments, since concentration of that 0.1 and 0.2 mM  $L^{-1}$  Ca are common levels of dissolved Ca in soil solution that receive lime and gypsum applications (Mclay et al., 1994; Antonangelo et al., 2017), and may stabilize labile SOM (Carmeis Filho et al., 2017; Inagaki et al., 2017).

The mechanisms explaining the enhancing effect of Ca are described by Rowley et al. (2018). The possible increasing occlusion of HA physically into mineral aggregates are here discarded, since that we worked with a clay suspension. Of note is the maximum amount of HA adsorption observed in our experiments (around 1-3 %) is similar to the mean C content widely observed in natural humid tropical soils of around 2% in the topsoil (0–30 cm) and 0.5% in the subsoil (30–100 cm) (Gomes et al., 2019). However, the clay aggregation and posterior OC occlusion could promote OC preserving against microbial decomposition, hence improving the total C content (Lutzow et al., 2006) to 3-6 %.

A likely mechanism is the formation of mineral-Ca-OC ternary complexes, which may enhance HA sorption to Fe-oxides, Al-oxides and phyllosilicates (Weng et al., 2005; Sowers et al., 2018; Janot et al., 2012; Majzik and Tombácz, 2007; Rowley et al., 2018). A further consideration is that Ca<sup>2+</sup> bound to HA functional moieties may act as a bridge between HA molecules (Kloster et al., 2013), enabling multilayer interactions (ex. mineral-Ca-OC-Ca-OC) that would enhance HA sorption. Chen et al. (2017) conducted ATR-FTIR and calorimetry studies of HA adsorption onto kaolinite at pH 4 and 6, and concluded that HA binding occurred via OH groups on the edge faces and basal octahedral faces (both positively charged), plus some adsorption at Si-O group by ligand exchange and electrostatic binding.

#### 5.3.2. UV-Vis analyses

The HA fractionation during adsorption was evaluated by the E2:E4 and E4:E6 ratios which presented negative correlation to humification or molecular weight (Park et al., 1999; Rodríguez et al., 2016). The variations in E2:E4 and E4:E6 ratios are presented in figure 2.



**Figure 2.** E2/E4 (top) and E4/E6 (buttom) ratios of HA solution after equilibrium with clay at pH 6.5 (left) and 4.5 (right). The dotted line represents the initial value of 60 mg  $L^{-1}$  HA in each ph value.

A HA solution absorbs light in a wide range of energy contrary to inorganic elements, that almost do not absorb light above 230 nm. Thus, light absorption could be a quantitative indicator of the HA or dissolved organic carbon concentration in water. The absorption coefficient at different light energies could suggest the number of different HA fractions. The HA chromophores, identified as aromatic groups, phenols, and aromatic acids, absorb light wavelength below 400 nm (Barreto et al., 2003). Thus, the ratios between discrete wavelengths are likely to reflect the quality of HA (e.g., molecular weight, aromaticity, humification, and hydrophobicity), which could give a quality information of HA composition by UV-Vis index. More details about UV-Vis absorbance index to dissolved organic matter see Li and Hur (2017).

The UV-Vis index ratio in equilibrium solution, regardless of pH or Ca concentration, was initially lower and increase followed increases in HA initial concentration. These results suggest that at lower clay surface coverage, compounds of lower humification grade or molecular weight were preferentially adsorbed. The increment in ratios with increasing initial HA concentration is expected (Qin et al. 2015a), since that after adsorption

saturation the ratio tends to reach the one of initial solution. Nevertheless, the ratio keeps above the initial values of initial solution of HA, which supports the clay preference adsorption to low humification or molecular weight even at higher HA loading.

Hur and Schlautman (2004) found that smaller molecular weight compounds were preferentially adsorbed on hematite and kaolinite at pH <8, whereas larger molecular weight fractions were mainly adsorbed at pH >8.5. Kang and Xing (2008) concluded that relatively small molecular weight from HA fractions had a greater affinity for the goethite surface based on analyses of the high performance size exclusion chromatography results. These results are consistent with our findings. However, other studies reported in literature showed different results. Chorover and Amistadi (2001) using dissolved organic carbon from forest floor found preference to high molecular weight, aromatic constituents via ligand exchange for adsorbed on goethite; Janot et al. (2012) using  $\alpha$ -Al<sub>2</sub>O<sub>3</sub>, observed an inverse tendency of E2/E3 ratio, a similar index of index that were used here; and the same results were showed by Qin et al. (2015b) for HA adsorption on pure hematite. Maybe, differences from HA sources, solid:solution ratio, and background solution composition and concentration, should be reason for this results variation. Further studies are needed to obtain a more in-depth understanding of organic fractionation by mineral sorbents.

Calcium effects on the adsorptive fractionation of HA are limited, although the results suggest a tendency where higher Ca concentrations decrease both ratios indices (Fig. 2). This suggests that the additional HA adsorbed in the presence of Ca is dominated by compounds of lower humification grade and/or lower molecular weight. These results differ from those of Kloster et al. (2013) , who found the opposite in studies of the effects of Ca on HA aggregation. However, these authors used a different HA source and clay sample dominated by amorphous minerals, as illites and poorly crystallized smectites, which are remarkable different of minerals used for us. Both factors could impact the results observed. (Kloster et al., 2013) did not have a conclusive explanation for their results, and suggested the need for further research in this respect.

#### 5.3.3. ATR-FTIR spectroscopy

The ATR-FTIR spectra of adsorbed HA are presented in Figure 3. The low amounts of total HA adsorbed ( $\approx$ 1-2.5 %) are reflected in the relatively weak IR absorbance in the spectra. The spectra contain the absorbance bands of carboxyl groups of adsorbed HA, consisting of the C=O stretch at 1720 cm<sup>-1</sup>, the asymmetric  $-COO^-$  stretch at 1650 to 1600 cm<sup>-1</sup>, and the symmetric  $-COO^-$  stretch at 1410 to 1400 cm<sup>-1</sup>) (Chorover and Amistadi,

2001) (Heckman et al., 2011) (Sowers et al., 2018), which are similar to the strongest bands founded in pure HA spectra (Figure S2). Carboxylic acids corresponded to 18 % of HA composition (1S102H, IHSS).



**Figure 3**. Background corrected and normalized ATR-FTIR spectra for HA-bearing clay with and without calcium addition (A and B) and over HA concentration on pH 6.5 (C and D). Infrared bands absorbance 1720, 1650 to 1600 and 1410 to  $1400 \text{ cm}^{-1}$  are assignments by carboxylic groups.

Higher HA adsorption at pH 4.5 is reflected in the relatively high peak area (Fig. 3A) compared to pH 6.5 (Fig. 3B). Similarly, the increase in HA with increasing Ca concentration is reflected in increased total area (Figures 3C and 3D). At pH 6.5, HA adsorption was more sensitive to Ca (Fig. 1) and this was reflected in the IR data as well. Likely at higher pH, the presence of negative charges sites on mineral could be a suitable site for creating Ca bridges between mineral surface and deprotonated carboxylic groups, that had a notable increment in ATR-FTIR results.

The increment of Ca concentration leads a subtle increase in band ranges 1750-1700  $\text{cm}^{-1}$  (Fig 3A an 3B). This frequency is assignment as the stretching vibration of the C=O

within protonated –COOH groups (Noerpel and Lenhart, 2015) likely formatted after dehydration. This increment endorses the association between Ca and carboxylic acids from HA, as also showed by Sowers et al. (2018). These authors agree with the proposed synergistic effect of Ca on organic compounds (OC) sorption through mineral-Ca-OC ternary complex formation

#### **5.4.** Conclusions and Environmental Implications

A strong pH effect was observed, with enhanced HA adsorption at lower pH attributed to increased electrostatic forces between negatively charged HA molecules and positively charged mineral surfaces. The UV-Vis analyses suggest preferential uptake of HA compounds having low humification rate and/or molecular weight, while the FTIR data demonstrate the involvement of carboxylic acid functional units in HA adsorption. Ca enhanced the extent of OC sorption considerably, indicating that the sequestration of OC may be boosted by Ca, likely through the formation of mineral-Ca-OC ternary complexes. This has significant implications for the retention of OC in soil ecosystems, since adsorbed OC compounds are more protected from microbial degradation processes than free OC in solution. Soil amendments with Ca sources like lime and gypsum are commonly applied to weathered soils to improve nutrients availability and to moderate pH values. These Ca applications may also improve soil organic carbon stocks.

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# **Supplementary information**



**Figure S1**. (A) Raw ATR-FTIR spectra for HA-bearing clay at pH 6.5 and absence Ca. (B) Spectra region used to assessment to (C) carboxylic group adsorption region.



**Figure S2.** Background corrected and normalized ATR-FTIR spectra for original Elliott Humic Acid (Standard 1S120H) obtained from International Humic Substance Society (IHSS). See bands frequency assignment in Heckman et al. (2011).

# 6. EFFECTS OF pH, PHOSPHATE, AND TIME IN ADSORPTION OF Zn AN ARTIFICIAL SOIL

# ABSTRACT

Zn is a nutrient for plants, animals, microorganisms, and humans. The concern is that plants could bioaccumulate Zn in edible parts not only for biofortification but also in toxic concentrations for human and other animals. The solid chemical species could determinate the Zn activity in the aqueous phase. Beyond of mineral phases, pH, phosphate availability and aging affect Zn mobility and bioavailability. We evaluated the effect of pH and phosphate recovering on the long-term fate of Zn adsorbed on "mimic" clay fraction of a highly weathered soil. The macroscopic data confirmed the fast stabilization of metal adsorption and the effect of pH in Zn adsorption. After one week at pH 4.5, aluminum-silicates minerals were the main Zn-sink. At pH 6.5, other species as xZn-P (following the increment in P loading) and Zn goethite were observed. These fractions, however, decreased or disappeared after six months, concomitantly with increment in the fraction of Zn adsorbed at aluminum-silicates minerals

Keywords: Xray absorption spectroscopy; Metal adsorption; Soil mineralogy

# **6.1. Introduction**

Zn is a nutrient to plants, animals, microorganisms and humans (Kambe et al., 2015). The total concentration in the Earth's crust is approximately 10-100 mg kg<sup>-1</sup> (Mertens and Smolders, 2013). Contents of Zn in agricultural soils tend to increase over time due to inputs resulting from the addition of chemical defensives (Phinney and Bruland, 1997), irrigation water (Arora et al., 2008) and phosphate fertilizer (Cheraghi et al., 2012; De López Camelo et al., 1997; Nicholson et al., 2003). A concern is that plants may bioaccumulate Zn in edible parts at concentrations toxic for humans and animals.

The Zn chemical form is a major determinant of the concentration of Zn in the aqueous phase. For example, the solubility product (Kps) decreases in the order Zn- carbonate  $(1.4 *10^{-11}) >$ Zn-sulfide  $(2*10^{-25}) >$ Zn-phosphate  $(9*10^{-33})$  (Clever et al., 1992). It is therefore important to know the chemical speciation as well the total Zn concentration to determine environmental threats posed by Zn in soils. Environmental conditions such as pH

and the activity of other ions (e.g.  $CO_3^{2-}$  or  $PO_4^{2-}$ ) need to be considered as well (Alloway, 1995; McBride, 1994; Tan, 1998).

Adsorption processes are important in controlling Zn solubility and result from the reaction between dissolved  $Zn^{2+}$  in solution and the surfaces of soil solid phases. There have been numerous studies of  $Zn^{2+}$  adsorption mechanisms by soil solids, including Fe-oxides (EHa et al., 2009; Liu et al., 2016; Pokrovsky et al., 2005), Mn-oxides (Della Puppa et al., 2013; Li et al., 2004; Pokrovsky et al., 2005) Al-oxides (Pokrovsky et al., 2005; Ren et al., 2015; Stietiya and Wang, 2014), calcite (Elzinga and Reeder, 2002; Zachara et al., 1988), aluminum silicates (Arias and Sen, 2009; Churakov and Dähn, 2012; Malamis and Katsou, 2013) and organic matter (Li et al., 2010; Ringqvist and Öborn, 2002). Since these compounds represent the main solid-phases constituents of soil, studies that asses the reaction of Zn with these materials provide important constraints on the solubility and fate of Zn in soil systems.

Soil systems contain a broad array of minerals which may mutually interact for Feoxides and clay minerals in Brazilian Ferrasols (Fontes, 1992). These interactions bring a high complexity to metal adsorption mechanism. Chemical interactions between goethite and kaolinite yielded a mineral complex with a set of physicochemical properties that differed from the arithmetical average of constituting phases (Wei et al., 2014). This has implication for the reactivity towards trace metals and other. Nachtegaal and Sparks (2004) and Sipos et al. (2018) showed that Zn adsorption on goethite-coated kaolinite resulted in the initial adsorption of Zn on goethite, with subsequent incorporation of Zn into a secondary brucite Zn-hydroxide phases resulting from Zn interaction with kaolinite.

The agriculture practices carried out changes in soil properties, which should have an effect on Zn, as like as other micronutrients and elements potentially toxics. The humid tropical agriculture has developed on extensive areas, where weathered soils are broad recognized as acid soil pH and having low nutrients availability (Lopes and Guilherme, 2016). Often application of lime amends the availability of Ca and Mg and increase the pH to adequate value for plant growth. Phosphorus is the principal nutrient limiting for vegetal production (Barreto et al., 2018), which compels the phosphate fertilization in considerable amounts and frequency (Roy et al., 2016).

Both pH and phosphate had strong influences on Zn fate in environmental systems. Soil pH is the most important parameter influencing metal-solution and soil-surface chemistry (Bradl, 2004; McBride, 1994, 1979), because it influences variable surface charge of some soil minerals. At low pH there is a higher protonation of surface, which reduce available sites of metal adsorption; with the increase of pH value new sites are available. See detailed information about metal adsorption in Bradl (2004).

The presence of phosphate in solution will increase metal adsorption (Elzinga and Kretzschmar, 2013). Liu et al. (2016) presented adsorption increment for Zn and phosphate on ferrihydrite attributed to the formation of ternary complexes as well as electrostatic interactions, while surface precipitation could not be completely ruled out. Hence, in this chapter, we evaluated the effect of pH and phosphate recovering on long-term fate of Zn adsorbed on "mimic" clay fraction of weathered soil.

#### **6.2.** Materials and Methods

## 6.2.1. Purified Minerals Compounds and Mix

We aimed to mimic the clay size-fraction of a weathered soil by mixing relevant mineral phases. The mineral types and their proportions were selected based on the "Representative Pedon and Data" for Oxisols from the Soil Taxonomy Classification (Kuo-Liang Chung and Shih-Tung Wu, 2005). The mixture contained 70 wt-% kaolinite, 20 wt-% gibbsite, and 10 wt-% goethite, in accordance with mineral identification and abundances in other studies of the mineralogy of weathering soils (IUSS Working Group WRB, 2014) (Fink et al., 2016).

The kaolinite used in this study was purchased from the Clay Minerals Society Source Clays Repository (Purdue University, USA) called KGa-2. It is relatively poorly crystallized, similar to the kaolinite present in soil samples. We used  $\gamma$ -Al<sub>2</sub>O<sub>3</sub> powder (99.99% purity, Alpha Aesar) replacing the gibbsite mineral. Although  $\gamma$ -Al<sub>2</sub>O<sub>3</sub> is not found in natural environments,  $\gamma$ -Al<sub>2</sub>O<sub>3</sub> converts into gibbsite and bayerite during hydration (Carrier et al., 2007; Lefèvre et al., 2002), and these minerals are representative of the Al-oxides in soils (Yang et al., 2013). To ensure conversion of the  $\gamma$ -Al<sub>2</sub>O<sub>3</sub>, the  $\gamma$ -Al<sub>2</sub>O<sub>3</sub> was aging in aqueous solution for 15d at 80 C° (4g in 80 ml water), and then freeze-dried. Mineralogical transformation into gibbsite and bayerite was confirmed by XRD analysis (Supplementary material).

The goethite (Gt) used in this study was synthesized based on a method described previously Elzinga et al. (2001). Briefly, the mineral was made by a titrating of 50 mL 1 mol  $L^{-1}$  ferric nitrate solution with 450 mL of 1 mol  $L^{-1}$  KOH, and aging the resultant ferric oxide suspension for 2 weeks at 25°C. Next, the goethite suspension was centrifuged, washed with water and resuspended in 0.4 mol  $L^{-1}$  HCl for 2 h to remove any remaining amorphous iron

oxides. The goethite was then centrifuged and washed with deionized water. Finally, the material was dialyzed to remove excess salts and then freeze-dried.

Mineral stock suspensions of 50 g L<sup>-1</sup> were prepared by combining 140 mg of KGa-2, 40 mg of aged  $\gamma$ -Al<sub>2</sub>O<sub>3</sub>, and 20 mg Gt in a 50-mL tube. The mineral mixture was washed once with 40 mL of 0.1M KCl solution and subsequently four times with ultrapure water, and then dried for three days at 40 C°, finally, new 40 ml of ultrapure water were added. This initial suspension was left in horizontal shaker for two weeks to initial mineral interaction. This mix will be hereafter referred to as the "clay". The cation exchange capacity and pH<sub>ZPC</sub> of clay were 66 mmolc kg<sup>-1</sup> and pH 6.53, respectively. See methods for exchange capacity and pH<sub>ZPC</sub> determination in the supplementary material.

# 6.2.2. pH

After initial interaction, two portions of 60 ml of initial suspensions were adjusted to pH 4.5 and another to 6.5. Daily, the pH was adjusted daily by addition of small amounts of HCl or KOH (0.1 M) under magnetic stirring over the course of > 1 week.

# 6.2.3. Phosphate loading

After pH stabilized, aliquots of 15 ml of suspension from each pH were transferred to a new centrifuge tube and amended with 15 ml of 0; 10 and 100 mg L<sup>-1</sup> P (as Na<sub>2</sub>HPO<sub>4</sub>) in 0.05 mol L<sup>-1</sup> KCl and a drop of toluene to inhibit microbial activity. The suspensions were equilibration for 10d on a reciprocal shaker to facilitate reaction of added P with the minerals surface (Abdala et al., 2015a). The pH was measured daily and, if necessary, re-adjusted to 6.5 or 4.5. At the end, the mineral suspensions (30 ml of 25 g L<sup>-1</sup>) were centrifuged at 6441*g* for 10 min and the supernatant collected. Analysis of dissolved P in the supernatants showed that, on average, about 92% of the P added was adsorbed on the clay surface for both P concentrations and pH values.

After adsorption to eliminate residual P in solution, 30 ml 0.05 mol L<sup>-1</sup> KCl without P were added and solids suspended. After new centrifugation (6441x g for 10 min), the supernatant was discarded and the residual solids dried at 40 °C for 48 h. Finally, the solids were resuspended into a new 30 mL volume of 0.05 mol L<sup>-1</sup> KCl. Hence, the suspension had  $25g L^{-1}$  of minerals with approximately 0, 200, or 2000 mg kg<sup>-1</sup> P adsorbed.

# 6.2.4. Zinc addition

For the Zn sorption studies, 20 ml of the final suspensions (25 g L<sup>-1</sup> minerals) were mixed with 80 ml of 0.05 mol L<sup>-1</sup> KCl containing 6.25 mg L<sup>-1</sup> Zn (as ZnCl<sub>2</sub>). The resulting suspensions had a 5 g L<sup>-1</sup> minerals concentrations and 1000 mg kg<sup>-1</sup> Zn. After 24 h the pH was newly adjusted to 6.5 or 4.5 and readjusted when necessary. The suspensions were sampled at reaction time points of one week, one month and six months. Sampling involved centrifugation of 30 ml of suspension at 6441*g* for 10 min. The supernatants were filtered and analyzed by ICP-OES to quantify dissolved Zn, P, Al, Fe and Si. The wet mineral pastes collected after reaction times of one week and six months were stored at -20 °C until EXAFS analyses.

#### 6.2.5. XAS analysis

Synchrotron Zn *K*-edge (9659 eV) spectra were collected on beamline 12-BM of the Advanced Photon Source at Argonne National Laboratory. (APS; Argonne National Laboratory, Lemont, IL). Spectra were collected in fluorescence mode and at room temperature using a 13 element Ge solid-state detector (Canberra) covered with Al foil to suppress Fe florescence from the sample. Multiple scans (4–7) were collected for each sample to improve signal:noise. The scans were averaged for further analysis.

Data processing was performed with the program WinXAS 3.1. For normalization, a linear function was used for the pre-edge region and a second-order polynomial in the postedge region. The  $\chi(k)$  functions were extracted using a cubic spline and were Fourier transformed with  $k^3$  weighting over the approximate *k* range about 2-10 Å<sup>-1</sup>. The EXAFS spectra [(*k*)] were k<sup>3</sup>-weighted and converted to R-space by fast Fourier transform [FT( $\chi(k)$ \*k3)] over the k range of 2-10 Å<sup>-1</sup> using a Kaiser–Bessel window with the window parameter (*dk*) set to 2.5 Å<sup>-1</sup> for obtain radial structure functions (RSFs). The two major peaks in the Fourier transformed magnitude were isolated and backtransformed and these were fitted in *k* space. Structural parameters were extracted with fits to the standard EXAFS equation.

Theoretical backscattering paths were calculated with FEFF7 (Zabinsky et al., 1995) in combination with ARTEMIS (Ravel and Newville, 2005). Zn–O and Zn–Zn/Al based on the crystal structure of hemimorphite (Nachtegaal and Sparks, 2004). For fitting parameters of the first (O) and second (Si) neighbor shells, was fixed the amplitude reduction factor  $(S_0^2)$  was set at 0.85 (Starcher et al., 2017) to reduce the number of free parameters in the fitting procedure. Also, the values of Debye-Waller factors ( $\sigma^2$ ), radial distance (R(Å)) and

coordination number (CN) were initially estimated for suitable values (0.005, 2 and 6, respectively) for first shell and second shell (0.005, 3 and 4, respectively). The energy shifts ( $\Delta E_0$ ) was constrained to be equal for both shells. Error estimates are ±0.02Å for the R values of the first shells (Scheidegger et al., 1997) and ±0.05 Å for the second shell (Nachtegaal and Sparks, 2004). For CN, which is correlated to the Debye-Waller factor, the error is estimated at ±30% (Nachtegaal and Sparks, 2004).

# 6.2.6. EXAFS analysis by PCA, TT, and LCF

Principal component analysis (PCA) and target transforms (TT) were performed on the clay sorption samples and the Zn standards, respectively, using the SixPACK software (Webb, 2005). To determine the number and identity of the Zn species present in the soil sample, a dataset of spectra from the eight samples were analyzed using principal component analysis (PCA) (Beauchemin et al., 2002; Wasserman et al., 1999). The Zn standard spectra were kindly provided by Dr. Matt Siebecker and Dr. Donald Sparks (University of Delaware), and were previously reported (Nachtegaal et al., 2005; Nachtegaal and Sparks, 2004).

The PCA technique determines if the data can be described as weighted sums of a smaller number of components, which would be the case if each point in the dataset is comprised of a smaller number of distinct compounds (McNear et al., 2007). The number of components required to reproduce the entire set of spectra excluded the experimental noise was determined based on the empirical indicator function (IND) (Malinowski, 1977). The IND achieve a lowest value when part mainly components (i.e., combination of chemical species spectra) and marginal components (i.e., background signal) in PCA (Kirpichtchikova et al., 2006a).

Following this step, we applied the TT approach, which is used to determine the aptness of each reference spectra to describe the experimental data identified by PCA (Jacquat et al., 2008). The standards spectra are evaluated for their "goodness of fit" by the higher coefficient of determination ( $\mathbb{R}^2$ ) between standards and TT spectra and SPOIL value (Malinowski, 1978). SPOIL measures the degree to which replacing an abstract component with the reference would increase the fit error. This is a positive dimensionless number for which values of 1.5 are considered excellent, 1.5 to 3 are good, 3 to 4.5 are fair, 3.5 to 6 are poor and > 6 are unacceptable (Nachtegaal et al., 2005). See TT adjustment fitting in supplementary material (Figure 2SM).

The combinatorial linear combination fits (LCF) analysis were performed using the Athena software (Demeter 0.9.16) (Ravel and Newville, 2005). LCF was carried out by

calculating all possible combinations of TT-suggested standards considered until fair (SPOIL  $\leq 4.5$ ) limited to maximum four standards forcing its weights from 0 to 1 and its sum reaching 1. The goodness of fit was determined by R-factor. Fits with smallest R-factors were used. Both PCA, TT, and LCF procedures described were carried out in k<sup>3</sup>-weighted  $\chi$  functions and k(Å) range between 1.8 to 9 Å<sup>-1</sup>.

# 6.3. Results and discussion

# 6.3.1. Equilibrium solution

After six months of incubation time no suitable changes were observed in clay mineralogical features by XRD and FTIR (Figure 1), which ensure that Zn species changes were duo to mainly of pH, P loading and aging effect.



**Figure 1.** (A) XRD and (B) ATR-FTIR patterns of the clay sample after six months of equilibrium. See in the supplementary material (Figure. 1SM) for evaluation of initial clay sample.

The Zn, P, Al, Fe and Si concentrations in equilibrium solution were so lower (Table 1). Ca had similar values after 1 week, 1 month or 6 months, which should be from kaolinite impurities that desorbed after Zn adsorption. Fe started no quantifiable in first incubation time and just was quantified after 6 months. Dissolved P was observed only at the higher P loading in the clay (2000 mg kg<sup>-1</sup>), with levels approximately 2.5 times higher at pH 6.5 than pH 4.5, consistent with increased phosphate adsorption at lower pH (Antelo et al., 2005). We found higher concentration of Si and Al at lower pH likely due to acid neutralization by kaolinite dissolution (Chin and Mills, 1991) causing release of these elements. Al released tends to precipitate at pH 6.5, pH value close to the minimum Al solution activity (Gabelich et al., 2005; McBride, 1994) The increment of Si over P loading suggest a competition between phosphate and silicate.

рН	P dose	$Zn^1$	Р	Si	Fe	Al	Ca	
	mg kg <sup>-1</sup>	$mg L^{-1}$						
1 week								
4.5	0	2.07	_2	2.27	-	0.20	0.26	
4.5	200	2.08	-	2.78	-	0.23	0.26	
4.5	2000	2.00	0.75	3.80	-	0.15	0.27	
6.5	0	0.16	-	1.09	-	0.00	0.27	
6.5	200	0.23	-	1.75	-	0.00	0.26	
6.5	2000	0.23	1.43	2.90	-	0.00	0.20	
1 month								
4.5	0	1.81	-	2.77	-	0.16	0.24	
4.5	200	2.09	-	3.25	-	0.26	0.28	
4.5	2000	2.03	0.57	4.18	-	0.08	0.27	
6.5	0	0.17	-	1.02	-	0.01	0.24	
6.5	200	0.30	-	1.90	-	0.00	0.28	
6.5	2000	0.18	1.36	3.08	-	0.01	0.21	
6 month								
4.5	0	1.84	-	2.39	0.02	0.32	0.24	
4.5	200	1.89	-	2.90	0.02	0.29	0.23	
4.5	2000	1.89	0.35	4.13	0.02	0.12	0.23	
6.5	0	0.22	-	1.50	0.03	0.08	0.29	
6.5	200	0.39	-	1.97	0.02	0.05	0.26	
6.5	2000	0.32	1.27	3.20	0.03	0.07	0.22	

Table 1. Elements concentration in equilibrium solution after incubation times.

<sup>1</sup> Initial concentration was 5 mg  $L^{-1}$  Zn, equivalent to 1000 mg kg<sup>-1</sup> Zn of clay. <sup>2</sup> Lower than limit of quantification.

At higher pH we found about 95% adsorption of initial Zn addition while at lower pH we found 60% (Table 1). The Zn adsorption was strongly influenced by the pH value. The adsorption at low pH suggested strong and, likely, specifics sites to Zn adsorption because there is high positive charge coverage in clay (pH lower than  $pH_{zpc}$ ). In fact, this is an unfavorable adsorption by electrostatic reaction. At high pH, the ZnOH<sup>+</sup> species concentration increased. This monovalent hydrated ion had adsorption favored as compared to Zn<sup>2+</sup>, duo to lower electrostatic repulse from positive charge surface of clay than Zn<sup>2+</sup>. Also, there are the decreases in the competition by H<sup>+</sup> and increase in negative charge sites when pH goes up (Casagrande et al., 2008; Kim, 2014).

Results from Table 1 were added in Minteq software for chemical speciation to aqueous of elements quantified, and there was not any Zn mineral saturation in solution (data no showed). Basically,  $Zn^{2+}$  was the main specie (~96%) regardless of pH value and the presence of phosphate anion (H<sub>2</sub>PO<sub>4</sub><sup>-</sup>), even at highest P loading. The marked difference is the Al main specie between pH values. At pH 6.5, Al(OH)<sub>4</sub><sup>-</sup> was the main specie (~45%) and at pH 4.5, Al<sup>3+</sup> (83%) unrelatedly the aging effect. We should consider that in interface location, there is higher elements concentration duo to adsorption process than bulk solution. The chemical environmental of this region permits that Al(OH)<sub>4</sub><sup>-</sup> could be an important sink to Zn<sup>2+</sup> adsorption duo to high reactivity this polymeric hydroxyl-Al (Haynes, 1982) in this pH. The EXAFS data could help to elucidate this possibility.

#### 6.3.2. XAS data

The extended X-ray adsorption fine structure (EXAFS) data of Zn sorbed in clay samples are presented in Figure 2, showing the raw and LC-fitted  $\chi$  spectra (Fig. 2a), in the left panel (Fig. 2b) presents the radial structure functions (RSFs) obtained from Fourier transformation of the raw  $\chi$  data. The data fit results are summarized in table 3.



**Figure 2.**  $k^3$ -weighted Zn K-edge EXAFS (A) and Fourier transformed (B) spectra (not corrected for phase shift) data of sorption samples in corresponding radial structure functions (RSFs). The black and red lines represent raw and fitted spectra. Dotted lines locate first-shell O (blue) and second-shell Zn-Si/Al (green) atomic neighbors. The data fit results are summarized in table 3.

The main peak in all RSF spectra's observed around 2 Å represents O ligands surrounding the central Zn at in first-shell. The CN of 7.0-6.2 and inter-atomic distance (R(Å)) range from 2.10 to 1.99 (Table 2) agree with arrangement of O atoms in octahedral coordination with the central Zn atom (Roberts et al., 2003; Starcher et al., 2017). The Zn-tetrahedral (~ 4 CN and 1.97 R(Å)) has a shorter Zn-O bond length and higher bond energy than Zn-octahedral; thus, the first is regarded as more stable than last one. Even though, the presence of a mixed tetrahedral and octahedral coordination for the Zn-O shell is expected, as

observed in other studies of Zn sorption to Al (Roberts et al., 2003) and Mn oxides (Qin et al., 2018).

Atomic Sien								
Sampla	Zn-O		Zn-Al/Si			Reduced		
Sample	CN	R(Å)	$\sigma^2(\text{\AA}^2)$	CN	R(Å)	$\sigma^2(\text{\AA}^2)$	$\chi^2$	$\Delta \mathbf{L}_0$
pH 4.5 0P 1w	7.9	2.10	0.010	3.04	3.06	0.006	15409	+5.8
pH 4.5 200P 1w	6.5	2.09	0.015	2.60	3.04	0.006	67988	+5.1
pH 4.5 2000P 1w	6.2	2.10	0.015	2.18	3.08	0.004	70398	+6.2
pH 6.5 0P 1w	6.8	2.03	0.014	-	-	-	23854	+1.7
pH 6.5 200P 1w	7.3	2.02	0.015	-	-	-	139	+1.1
pH 6.5 2000P 1w	6.4	1.99	0.014	-	-	-	16523	+1.3
pH 4.5 0P 6M	7.1	2.09	0.013	2.87	3.04	0.008	61258	+5.6
pH 4.5 200P 6M	7.3	2.08	0.014	1.58	3.04	0.003	1948	+4.3
pH 4.5 2000P 6M	6.6	2.05	0.012	1.18	3.03	0.001	63348	+3.1
pH 6.5 0P 6M	6.4	2.04	0.012	-	-	-	638	+2.7
pH 6.5 200P 6M	7.4	2.03	0.014	-	-	-	450	+2.3
рН 6.5 2000Р 6М	7.3	2.00	0.015	-	-	-	1510	+1.4
Reference <sup>1</sup>								
Zn-aqueous	6	2.07	0.009	-	-	-	-	-
Zn-kaolinite	6.4	2.06	0.011	1.3	3.09	0.010	-	-
Zn-gibbsite	5.1	2.01	0.010	4.4	3.02	0.008	-	-
Zn-vermiculite	5.8	1.97	0.003	2.5	3.05	0.001	-	-
Zn- hemimorphite	3.6	1.93	0.005	3.0	3.01	0.008	-	-

Table 3. Zn *K*-edge EXAFS refinement results from first (Zn-O) and second (Zn-Si/Al) shell fitting of Zn reference compounds and soil samples after Zn adsorption.

The amplitude reduction factor  $(S_0^2)$  was fixed in 0.85; CN = coordination number; radial structure functions or R = inter-atomic distance (±0.02Å for first shell; Scheidegger et al., 1997);  $\sigma^2(Å^2)$  = Debye Waller factor;  $\Delta E_0$  = phase shift. <sup>1</sup>References values obtained from (Roberts et al., 2002) and (Nachtegaal et al., 2005).

The blue line in figure 3 point out a shift for Zn-O peak position to higher R(Å) at pH 4.5 than pH 6.5. In table 2, the R(Å) is lower at pH 6.5 than 4.5, regardless of P loading and aging. This suggests that at lower pH there is a larger fraction of octahedral Zn than at higher pH, consistent with the results of Roberts et al. (2003). The lower Zn adsorption levels at lower pH suggest that adsorption sites in acidic condition are more specific and less stable than at neutral medium (van Genuchten and Peña, 2016). Qin et al. (2018) concluded that the percentage of Zn-tetrahedral on birnessite is prominently decreasing during desorption by H<sup>+</sup> or Pb<sup>2+</sup>, and they suggested that Zn-tetrahedral will be transformed to Zn-octahedral to maintain the layer charge balance in of vacancy sites. There is a slight decrease in R(Å) value as P loading increase, but the difference is within the uncertainty of the fit (Scheidegger et al., 1997), which discourage any affirmation.

The absence of substantial spectral features and signal degeneration at higher R(Å) values indicate a lack of heavy backscattering atoms, such as Zn or Fe, close to the central Zn atom. This eliminates the formation of Zn precipitates or polynuclear complexes as a major sorption process (Vasconcelos et al., 2008). Only in the samples reacted at pH 4.5, it is possible distinguish other peak ~2.8 Å, even with the lowest Zn loading, unlike results by (Roberts et al., 2003).

The second-shell feature was fitted using Zn-Si and Zn-Al paths, which are difficult to distinguish because of the similar atomic weights of Si and Al. The distances and CN of the Zn-Si/Al paths observed from 1 week to 6 months, in average, were 3.06 and 3.04 Å, and 2.6 to 1.88. Similar values were reported by Nachtegaal and Sparks (2004) and interpreted as the formation of Zn inner-sphere complexes on aluminol groups on the edge sites of the kaolinite. Over time, the shorter Zn-Si/Al distance and lower CN proposes an increment in bidentate inner-sphere complex, considering just that bidentate complexes usually have shorter distances than monodentate complexes (Vasconcelos et al., 2008). The low Zn concentration produced a spectral with reduced quality (Figure 2), which difficult the precise Zn complexes determination.

# 6.3.3. LCF analysis

The Zn K-edge EXAFS spectra of reference compounds used here (Figure 2SM). PCA suggest that three principal components were sufficient to replicate the data sample (Table 1SM). Hence, we used the first three components to evaluate standards fitting by TT approach. Visual inspection was used as well to select potentially suitable references for fitting and TT testing (Voegelin et al., 2005).

The TT indicated seven suitable candidates, based on relatively low SPOIL values ( $\leq$  3). These were Zn adsorbed on goethite; kaolinite pH 5, gibbsite pH 7 and vermiculite, hemimorphite, hopeite, and Zn aqueous. The species adsorbed in minerals similar to ones initially mixed are expected. The potentially appropriate reference spectra suggested by TT was higher than the minimum number of principal components (#3) from PCA (Table 1SM). This was also observed by Jacquat et al. (2008) and Panfili et al. (2005), and may be the result of several two main factors: (i) species that occur at similar proportion in several samples (as the Zn-kaolinite or hemimorphite) are not identified by the IND, but are still needed to get a good LC fit of the spectra; (ii) significant similarities in the spectra of different Zn standards, as for Zn sorbed on kaolinite at pH 5 and at pH 7. For this case, we chose the last one for LCF due to better adjustment of individual "fits" for each sample (data not shown). The LCF fits are presented in Figure 2, where the fitted spectra (in red) are overlain with the raw spectra (in black); the standards proportion of LCF fit is presented in Figure 3.



**Figure 3.** Chemical species fraction of Zn adsorbed in clay samples estimated by LCF of  $k^3$ -weighted Zn K-edge EXAFS from 1.8 to 9 k(Å) range.

The major factor that drives the Zn species diversity was the pH value. The pH effect already was observed in macroscopic analyses of total Zn sorption, showed in item 3.1. This effect is due to the deprotonation-protonation of clay surface, which should become a site more or less available to metal adsorption. Additionally, the pH has strong interference in ionic speciation in solution, which toward the water hydrolysis.

At lower pH, we found Zn associated with kaolinite, vermiculite and hemimorphite, which are all aluminum-silicates minerals. There is a high specificity of this fraction because at lower pH, it has low Zn adsorption. It confirms the preference of aluminum-silicates for Zn adsorption, as suggested Churakov and Dähn (2012). Ours results followed observations by Jacquat et al. (2009), who found a substantial fraction of total Zn was contained in hydroxy-interlayered smectite with high Zn loading in contaminated soils with acidic to neutral pH. Also they found Zn was octahedrally coordinated to O at 2.06-2.08 Å and surrounded by Al atoms at 3.03-3.06 Å in the second-shell; both values similar these found our results. At pH 6.5, beyond aluminum-silicate species, we found the presence of Zn sorbed on goethite and gibbsite. Likely, the deprotonated surface of these minerals allowed the Zn adsorption.

Zn speciation was affected by aging. At lower pH, Zn-vermiculite was present at short incubation time (1 week) but disappeared after longtime incubation (6 months), with a concomitant increment in Zn-kaolinite. At pH 6.5, aging results in a remarkable decrease of Zn-goethite and Zn-vermiculite fraction concurrent with an increase in Zn-kaolinite (Figure 4). The aging affect had a diversify approaches and results on literature (Elzinga et al., 2006; Schlegel et al., 2001; Shinwoo Lee et al., 2004; Yan et al., 2018a), however using a unique mineral phase, not a mix as used here.

Among these numerous variables, the fate of Zn over the time in soil system was assessed by Voegelin et al. (2011, 2005) that showed the dissolution of ZnO and ZnS in the soil to new phases, more thermionically stable, mainly layered double hydroxides (Zn-LDH). The same species were found by Khaokaew et al. (2012) in a paddy soil, like a principal phase for Zn immobilization. Nevertheless, Sayen and Guillon (2014) showed the changes in Zn speciation on calcareous soils partitioning of Zn from organic matter to Fe oxy(hydr)oxides over time. Both studies described aforetime used the X-ray Absorption spectroscopy, which is an analytical approach that use synchrotron radiation for followed Zn that permit geoscientists to appraisal the metal speciation in soils, sediments and minerals (Gillespie et al., 2015; Kirpichtchikova et al., 2006a; Kopittke et al., 2017).

In a similar study, Nachtegaal and Sparks (2004) described that, at pH 7, with a kaolinite surface coating with goethite (~6 %), Zn was immediately assimilated into a Zn-Al

layered double hydroxide (Zn-LDH) surface precipitate. In the presence of ~10% goethite coating, similar for our system, inner-sphere sorption complex of Zn formation on the surface hydroxyl groups of goethite was the dominant mechanism. Thus, goethite has a higher affinity for Zn compared to the kaolinite clay mineral or the surface precipitate. This affirmative is plausive for our system, since that we found that 10% of goethite represented ~20% of adsorbed Zn. However, with increasing aging time, Nachtegaal and Sparks (2004) observed the Zn inclusion into a precipitate phase assumes the dominant metal sorption mechanism. They concluded that precipitate phase at the kaolinite surface is thermodynamically favored over adsorption to the goethite coating. This conclusion is strongly applicable for our study.

The Zn-LDH is point out as an important bearing for limiting metal mobility in soils and sediments (Siebecker et al., 2018), however it was absence all samples here. At pH 4.5 this species is no expected. At pH 6.5, the ratio of the formation of Zn-LDH and Zn adsorbed at aluminum-silicates, alike Zn-kaolinite, is controlled by the supply of Si and Al from the dissolution of primary and secondary soil minerals. In the soil studied by Voegelin et al. (2005) they found mica and chlorite; which suggested a high availability of Al and Si, which favors the formation of Zn-LDH. Our system had a like-gibbsite phase and kaolinite for supply Al and Si, both have low solubility, thus Zn adsorbed at aluminum-silicates should be preferentially formatted. Also, thermodynamic data approach used by Voegelin et al. (2005) would suggest that Zn-LDH would be transformed into Zn-phyllosilicate in the long term.

The synergetic interaction between phosphate and metal for improve both adsorption have been studied (Elzinga and Kretzschmar, 2013; Li et al., 2006; Ren et al., 2015a; Unuabonah et al., 2007). The previous phosphate loading had a slight effect at low pH. At pH 6.5 and highest P loading (2000 mg kg<sup>-1</sup>) we could found an appreciable amount (25%) of hopeite (Zn-P), which was stable over the incubation time. However, higher P loading allowed maintenance of Zn-goethite on for a long-time incubation. (Liu et al. (2015) showed considerable increment for total adsorption for both Zn and P on ferrihydrite surface, which was associated with ternary complexes formation in addition to electrostatic interactions, while surface precipitation could not be completely excluded.

This last option was rule out for Zn and P interaction on  $\gamma$ -Al<sub>2</sub>O<sub>3</sub> surface in Ren et al. (2015a) study. They found enhances in Zn retention on alumina in wide pH range because of the presence of phosphate and concluded that there is a mutual effect of Zn and phosphate on their retention to alumina due to the formation of ternary surface complex. For ours results, the ternary complex Zn-P-Clay (or P-Zn-Clay) has elevated stability, which delay at low P

recovery (200 mg kg<sup>-1</sup>) or inhibit at high P loading (2000 mg kg<sup>-1</sup>) Zn-goethite shift to others species. Final remakes and environmental implication

In this study, the effects of three environmental variables (pH, phosphate loading, and aging) on Zn retention in an artificial soil clay fraction with mineralogy typical of tropical fine-earth fraction were explored. XRD and FTIR data did not indicate mineralogical phase changes over the experimental time frame, indicating the stability of soil mineral compounds. Macroscopic study confirms the fast rate of metal adsorption and the importance of pH as a control of total Zn adsorption. XAS results suggested that all three variables impacted the speciation of adsorbed Zn. At initial time and pH 4.5, aluminum-silicates minerals are the main Zn-sink. At pH 6.5, we additionally observe Zn adsorbed and Zn-P secondary phases followed at high soil P loadings. However these species disappeared after six months, concomitant with increases in the fraction of Zn adsorbed at aluminum-silicates minerals.

These information checks common practices of land remediation used to Zn soil contamination, the lime and phosphate fertilizer application. For ours system, the results obtained suggests that regardless the pH and P presence, there are a Zn remobilization on mineral phase, and the final Zn should be aluminum-silicates minerals. Hence, the application of silicate minerals for Zn areas reclamations could be a suitable option. The Zn desorption from aluminum-silicates minerals should be assessment as well. Further studies could disclosure the fate of others metals and contaminants in a pure but complex system, similar to this used here

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# **Supplementary information**

A modified barium method (Rhodes, 1982) was applied to determine the cation exchange capacity (CEC). Briefly, a portion of dried clay ( $\approx 2g$ ) were previous saturated by 10 mL of 0.1M BaCl<sub>2</sub> for 1h, after centrifugation the solution was discarded and the solid was washed with plenty water three times and thus dried. Then, 0.50 g was weighed and 40mL 0.1 CaCl<sub>2</sub> were added. After 1h, the solid was centrifuged and the supernatant collected for Ba measurement. The total of Ba desorbed was considered here as CEC.

The method used to determine the  $pH_{pzc}$  of clay was a modified version of the solid addition method by (Balistrieri and Murray, 1981). Briefly, to a series of 50 ml polyethylene tubes containing 45 ml of 0.05M KCl, different volumes of either 0.1 M HCl or 0.1 M NaOH solution were added to obtain wide ranging pH values (pH 2–10), then more 4 ml of electrolyte solution was added. The solutions were homogenized, and the pH values were measured; these initial pH values are referred to here as pH<sub>initial</sub>. Next, 1 mL of clay stock suspension (50 g L<sup>-1</sup>) was added to each tube. After 72 h of equilibration at room temperature and shaking end-over-end (100 cycles per minute), the pH values of suspension in each bottle was noted and designated as final pH or pH<sub>final</sub>. The difference between pHi and pHf ( $\Delta$ pH) when plotted against pHi. The resultant curve intersects  $\Delta$ pH = 0 at a certain value of pHi. This means that at this pH the clay does not induce the release of either H<sup>+</sup> or OH<sup>-</sup> ion in solution; i.e., the surface does not undergo or acquire any charge through acid–base dissociation, a condition for pH<sub>pzc</sub>.



**Figure 1SM.** (A) XRD and (B) ATR-FTIR patterns of the original materials used to compos the mimic fine earth fraction. The dashed circle highlighted the 17.5-20.0  $^{\circ}2\theta$  range, which containing reflections indicative of gibbsite and bayerite (Bhattacharya and Elzinga, 2018).



**Figure 2SM.** Zn K-edge EXAFS spectra of selected Zn references (black lines) and target transforms (red line) calculated with the first three components obtained from PCA presented in Table 2 in main text.

Component	Eigenvalue	Variance explained	Cumulative Variance	$IND^1$
#1	72.09	0.37	0.37	0.112
#2	28.97	0.15	0.51	0.109
#3	22.55	0.11	0.63	0.107
#4	13.88	0.07	0.69	0.122
#5	11.30	0.06	0.76	0.146
#6	9.84	0.05	0.81	0.184
#7	8.79	0.04	0.85	0.243
#8	8.40	0.04	0.89	0.334
#9	6.87	0.03	0.92	0.526
#10	5.71	0.03	0.96	1.040
#11	4.42	0.02	0.98	3.883
#12	3.88	0.02	1	-

Table 1SM. Results from principal component analysis of Zn K-edge EXAFS spectra for 12 clay samples.

<sup>1</sup>(IND) Indicator Error functions proposed by (Malinowski, 1991). Bold components were selected by IND criteria. PCA was obtained of 12 EXAFS spectrums using  $k^3$  weighting over the approximate k range about 2-10 Å<sup>-1</sup>.

# 7. Cd AND Zn SORPTION AND KINTEICS IN A HIGHLY FERTILIZED TROPICAL SOIL

# ABSTRACT

Brazil has a huge area covered by highly weathered soils used for agriculture. Massive fertilization is applied to overcome soil nutrition limiting such as high acidity, low nutrient availability (i.e. Ca, Mg, K) and high adsorption capacity of nutrients as P. Especially to vegetable production, the rates of fertilizers applied are often five to 20 times higher than cereal crops. Some potentially toxic elements (PTE) present in fertilizers should increases PTE soil contents due to high frequency and quantity of fertilizer application. We evaluated the changes in Cd and Zn adsorption-desorption parameters in highly-fertilized tropical soils. The pseudo-second-order model reached better fitting for kinetics data. The soil samples had a substantial Cd and Zn adsorption capacity. The agriculture soil had higher Cd and Zn maximum adsorption capacity than a soil under native vegetation. Despite to higher amount immobilized, Cd was preferentially adsorbed to fast release compartment than Zn. The increment in the total metal adsorption presented by agricultural soils improved the fractions readily desorbable for Zn, while there was an increment in residual fraction for Cd, which is less mobile. The aging effect could convert few fractions from fast to slow desorption sites. Changes in land use promoted a new chemical environment, which enhanced Cd and Zn adsorption. This increment, however, ocurred mainly in easily desorbable metals fractions.

Keywords: Potential toxic elements; Maximum adsorption capacity; Land use change; Soil organic matter

# 7.1. Introduction

Brazil has about 71 million hectares of field crops such as soybeans (*Glycine max*), corn (*Zea mays*) coffee (*Coffea sp.*) and sugarcane (*Saccharum officinarum*), which are the main agricultural crops (IBGE, 2015). Most of these cultivated soils has a high degree of weathering, which results in some undesirable characteristics to crop production such as high acidity, low reserves of nutrients to plants and high adsorption capacity of nutrients such as P (Tan, 1998; McBride, 1994; Fink et al., 2016). Much of the daily diet of Brazilians and world population consists of vegetable crops, such as potatoes (*Solanum tuberosum*), tomatoes (*Solanum lycopersicum*), beets (*Beta vulgaris*), lettuce (*Lactuca sativa*), carrots (*Daucus*)

*carota*), garlic (*Allium sativum*) and onion (*Allium cepa*). Because of the intrinsic characteristics of the cultures and their higher economic value compared to the major cereal crops described above, nutrient availability cannot be a limiting factor for the growth and/or the quality. So, the rates of fertilizers applied are often five to 20 times higher in vegetable crops than in areas with cereal crops (Raij et al., 1996; CFSEMG, 1999; Pereira and Daniels, 2003; CQFS, 2004).

Potentially toxic elements (PTE) may be accumulated in agriculture, which could offer toxicity for plants, animals and humans (Bolan and Duraisamy, 2003; Peralta-Videa et al., 2009). One of the sources of contamination of PTE in the environment is the fertilizers used in agriculture. Among them, P sources offer the greatest risk of environmental contamination. Cd and Zn were already found in amounts ranging from 0 to 56 and 9 to 500 mg kg<sup>-1</sup>, in phosphate rocks respectively (De López Camelo et al., 1997; Cheraghi et al., 2012) or in soluble phosphate fertilizers (Nicholson et al., 2003). In some fertilizers sold in Brazil, Cd and Zn ranged from 1.5 to 43 and from 5 to 300 mg kg<sup>-1</sup>, respectively (Conceição and Bonotto, 2006; Bizarro et al., 2008). The high frequency and quantity that P fertilizers has been applied in the land crops increases the potential of contamination for these elements by P sources (Mulla et al., 1980; Jiao et al., 2012).

Others chemical changes are consequence of agricultural soil management practices such as increase of pH, exchangeable contents of Ca and Mg due to the liming and, especially, the increase in P available contents in the soil. The new chemical environmental influences the PTE dynamics. The pH is a key attribute to determinate whether the element is either as free species in the solution, or polymerized, or as a soluble ionic compound (Yang et al., 2006; Alloway, 1995). Ca and Mg compete with PTE for metallic adsorption sites (Acosta et al., 2011; Adebowale et al., 2006). Specially, the addition of large amounts of P can promote its adsorption on the surface of some colloids and increase their negative charge, as in goethite (Li and Stanforth, 2000; Li et al., 2007) and in Al-hydroxides (Yang et al., 2007; Del Nero et al., 2010). Some precipitates between P and PTEs can also be formed (Mignardi et al., 2012). These two mechanisms increase the retention of PTE when there is high availability of P in the soil (Wang and Xing, 2002; Adebowale et al., 2005; Li et al., 2007).

The organic fraction of the soil plays a special rule in the dynamics of PTEs in the soil. Low molecular weight organic acids could form organometallic complexes then increase the bioavailability and or the mobility of PTEs along the soil profile (Antoniadis and Alloway, 2002; Yang et al., 2006; Li et al., 2015). However, stabilized soil organic matter

(SOM) has the ability to complex and immobilize metal cations, as PTE (do Nascimento et al., 2006; Clarholm et al., 2015).

Adsorption studies for PTE could indicate which soil properties governing the mobility-availability of the metals and could help the evaluate remediation strategies and soil management practices to minimize contamination risks by PTE for soil and food. Thus, our objective was to evaluate the changes in Cd and Zn adsorption-desorption parameters in highly-fertilized tropical soils.

#### 7.2. Material and Methods

# 7.2.1. Study sites location and experimental design

The study sites were in four strategic and representative areas in Brazil regarding oleraceus crops (Figure 1). The soil geneses condition of the sites resulted in highly weathered soils. Soil samples were collected in croplands and under native vegetation in nearby area for guarantee that the main difference of sites should be the agriculture practices (e.g soil tillage, fertilization). The soil sample was composited by 12 subsamples, which were collected using an auger at 0-20 cm of depth. After homogenization, subsamples were air-dried and passed through a 2-mm sieve for chemical and physical analysis.



**Figure 1.** Geographic location of the study areas in Brazil. The colors represent Guarapuáva-PR (black), Taquarituba-SP (red), Rio Paranaíba-MG (blue), and Piedade-SP (green). The geografic coordanates are available in Table 1SM.

# 7.2.2. Chemical and physical attributes

For analyses of soil size fractions, the sand fraction (50  $\mu$ m–2 mm) was removed from the suspension of dispersed mineral particles by wet sieving. Then, the silt (2  $\mu$ m - 50  $\mu$ m) and clay (< 2  $\mu$ m) fractions were collected by sedimentation according to Stokes' Law. Soil organic matter was determined by dry combustion on elemental analyzer - LECO® CN-2000 (furnace at 1350 °C in pure oxygen). Phosphorus and potassium were extracted by mixed cationic and anionic exchange resin (AER) (van Raij et al. 1986) and by Mehlich - I solution (0.05 mol L<sup>-1</sup> HCl + 0.025 mol L<sup>-1</sup> H<sub>2</sub>SO<sub>4</sub>); Al, Ca and Mg were extracted by 1 mol L<sup>-1</sup> KCl and Al determined by titration with NaOH. Available S was extracted by a monocalcium phosphate solution (500 mg L<sup>-1</sup> of P in 2 mol L<sup>-1</sup> acetic acid). Potential acidity (H+Al) was extracted by 0.5 mol L<sup>-1</sup> calcium acetate at pH 7. Cation exchange capacity (CEC) was estimated by the sum of bases (Ca + Mg + K) plus potential acidity. The amounts of pseudototal metal contents were obtained by using the EPA 3051a protocol (HNO<sub>3</sub>+HCl, 3:1 (v/v)) (USEPA 2007).

# 7.2.3. Mineralogy composition

Poorly crystalline Fe, Mn and Al oxides were extracted with 0.2 mol L<sup>-1</sup> ammonium oxalate at pH 3 in the dark. These elements in high crystalline oxides were extracted by sodium dithionite–citrate–bicarbonate (DCB) at 80 °C. Both procedures followed Pansu and Gautheyrou (2007). To evaluate the main mineralogical phases component in the clay fraction, the Xray diffraction was measured with an X-ray diffractometer (Rigaku Geigerflux), uising CuK $\alpha$  radiation ( $\lambda = 1.5418$  Å) generated at 30 kV and 15 mA. The powder clay samples were analyzed using random and oriented disposal. The XRD patterns were recorded in the range of 3 to 65°2 $\theta$  with a scanning speed of 0.015°2 $\theta$  and 1s counting time per step.

Clay natural fraction was analyzed by diffuse reflectance spectroscopy (DRS) following acquisition of the 380–800 nm wavelength range in 0.5 nm steps on a Cary 5 spectrometer (Varian, USA). Spectra were smoothed to calculate the Kubelka–Munk (K-M) function. The Gt/(Gt + Hm) ratio was calculated from the amplitude of the peaks for goethite (420–450 nm) and hematite (530–570 nm) in the second derivative of the K-M function (Torrent et al., 1983; Barron and Torrent, 1986). The contents in Gt and Hm were estimated from that of Fe extracted by DCB minus the amount extracted in oxalate pH 3 (Bahia et al., 2015). To quantify kaolinite and gibbsite contents, and aliquot of clay fraction after DCB treatment was treated with sodium hypochlorite for organic matter elimination, thereafter the residue followed differential thermal analysis (DTA) and thermogravimetric analysis (TGA) with a DTG-60H-Simultaneous DTA-TG (Shimadzu, Japan) in heating and cooling cycle from room temperature to 1100 °C at a heating rate of 10 °C min<sup>-1</sup> and N<sub>2</sub> atmosphere. The results of characterization could be seen in the table 1.

#### 7.2.4. Equilibrium isotherms and Adsorption Kinetic study

The equilibrium sorption isotherms were obtained according to the procedure described by Moreira and Alleoni (2010). Briefly, 3.00 g of soil were weighed, in duplicate in a 50-mL polypropylene centrifuge tube, and 30 ml of solutions at different initial concentrations were added: 0, 0.017, 0.034, 0.085, 0.17, 0.255, 0.51 and 1.28 mmol L<sup>-1</sup> Cd or Zn, prepared in 10 mmol L<sup>-1</sup> KNO<sub>3</sub> as background electrolyte. The suspensions were shaken for 24 h (end-over-end) at 25  $\pm$  2 C° and then centrifuged at 1,606 x g for 15 min. filtered through Whatman 42 filter paper, and the pH was immediately measured.

Based on the equilibrium results, the kinetic experiment was carried out at different periods using solutions concentration of Cd or Zn equivalent to the maximum sorption capacity estimated by Langmuir equation for each treatment (Ozdes et al., 2011). Briefly, 3.00 g of soil were weighed, in duplicate in a 50-mL polypropylene centrifuge tube, and we added 30 ml Cd and Zn solutions prepared in 10 mmol  $L^{-1}$  KNO<sub>3</sub> as the background electrolyte. The tubes were shaken (end-over-end) at 25 ± 2 C° for 0.5, 2, 4, 12, 24, 96, 240 and 480 h. After that, the tubes were centrifuged at 1,606 x g for 15 min and filtered through similar Whatman 42 filter paper, and the pH was immediately measured.

Blank samples were prepared in 10 mmol  $L^{-1}$  KNO<sub>3</sub> solution with the same metal concentrations but without the addition of soil to account for the adsorption of metals onto container walls under typical experimental conditions and others possible variations.

Ca, Mg P, S and Mn concentrations in supernatant solution were determined in the filtrate by inductively coupled plasma optical emission spectrometry (ICP-OES) (iCAP 6300 Duo, Thermofisher). Also, an aliquot was passed through a nylon membrane filter (0.45 $\mu$ m) to quantify the dissolved organic carbon following Bartlett and Ross (1988) with modification suggested by Giasson et al. (2014). The calibration curve for the all elements had R<sup>2</sup>>0.995.

The adsorbed Cd or Zn concentrations were calculated as the difference between the metal concentrations in the initial and equilibrium solutions, according to equation 1:

$$Q_e = \frac{(Ci-Cf)*V}{M}$$
 .....eq. 1

where Qe is the concentration of metal adsorbed on the soil (mmol kg<sup>-1</sup>), Ci and Cf are the concentration (mmol L<sup>-1</sup>) of metals in the initial and final solution, remaining in solution after the adsorption process (equilibrium concentration), V is the volume of the solution (ml) and M is the weight of soil used (g).

# 7.2.5. Equilibrium Isotherms and kinitic fitting

We chose the Langmuir and Dubinin–Radushkevich isotherm. The Langmuir isotherm assumes uniformity of adsorption sites, each position being able to hold only one molecule creating a monomolecular layer on the sorption surface.

Langmuir isotherm, which can be represented at equation:

$$Q_e = \frac{Q_{max}k_LC_e}{1+k_LC_e} \quad \dots \qquad eq. 2$$

where Qe is the amount of adsorbed material (mmol Kg<sup>-1</sup>), C<sub>e</sub> is the solution concentration at equilibrium (mmol L<sup>-1</sup>), Q<sub>max</sub> is the maximum sorption capacity (mmol kg<sup>-1</sup>) and K<sub>L</sub> is the Langmuir constant (L mmol<sup>-1</sup>), related to the adsorption capacity and increases exponentially with sorption energy.

The Dubinin–Radushkevich (D-R) is generally applied to express the adsorption mechanism isotherm equation has been used to determine the mean free energy of sorption. The D-R equation assumes adsorption mechanism based on based on the Polanyi potential theory of adsorption assuming heterogeneous surface (Chen and Yang, 1994; (Dąbrowski, 2001). D-R isotherm, which can be represented as equation 3 (Günay et al., 2007):

$$Q_e = Q_{max} * exp\left(\frac{(RTln(1+1/C_{eq}))^2}{-2E^2}\right)$$
.....eq. 3

where Qe is the amount of adsorbed material (mmol Kg<sup>-1</sup>), C<sub>eq</sub> is the solution concentration at equilibrium (mg L<sup>-1</sup>), Q<sub>max</sub> is the maximum sorption capacity (mmol kg<sup>-1</sup>), R is the ideal gas constant, 8.314J/mol/K, and T (K) is the temperature. E (kJ/mol) is defined as the free energy change required transferring 1mol of ions from solution to the solid surfaces and the magnitude of E is useful for estimating the type of sorption reaction.

To evaluate the controlling mechanism of the adsorption process such as mass transfer, diffusion control or chemical reaction, we used the pseudo-first order (Ho, 2004) (equation. 4) and pseudo-second order models (Ho, 2006) (equation 5) and Elovich (Chien and Clayton, 1980) (equation. 6).

$\mathbf{q}_{t} = \mathbf{q}_{e}^{*}(1 - \exp^{-k_{1}t}) \dots$	eq. 4
$q_t = \frac{k_2 q_e^2 t}{1 + k_2 q_e t}$	eq. 5
$q_t = \frac{1}{b*\ln(1+a*b*t)}$	eq. 6

where qe and qt (mmol kg<sup>-1</sup>) represent the amount of metal adsorbed at equilibrium and at time t, k<sub>1</sub> (h<sup>-1</sup>) and k<sub>2</sub> (mmol kg<sup>-1</sup> h<sup>-1</sup>) are the rate constants for the pseudo-first order and pseudo-second order models, respectively. *a* and *b*, are known to represent the initial adsorption rate (mmol kg<sup>-1</sup> h<sup>-1</sup>) and the Elovich adsorption constant (kg mmol<sup>-1</sup>), respectively.

# 7.2.6. Desorption experiment

#### 7.2.6.1. Soil incubation

The amount metal equivalent the maximum capacity adsorption of Cd and Zn estimated by Langmuir equation was applied to each soil. Hence, 20.0 g of soil were weighed in polypropylene tube 400-mL and added in each tube 200 ml solutions (ratio 1/10 soil:solution) at different initials concentrations of metals (Cd or Zn), all solutions prepared in 10 mmol L<sup>-1</sup> KNO<sub>3</sub> as background electrolyte.

To investigate the aging effect in metal-soil adsorption, the suspensions were shaken for 1 or 20 days (end-over-end) at  $25 \pm 2$  C°. Finishing stirred time, the tubes were centrifuged at 4,390 x g for 10 min, filtered through similar Whatman 42 filter. The supernatant was collected for posterior Cd and Zn quantification in supernatant solution. The total metals adsorbed were calculated following equation 1.

The residual soil was twice washed with 100 ml of acetone 70% for eliminating free interstitial metals loosely held by the soils. The amount of metals desorbed in this process was neglected, however could promotes overestimated values of desorption in first times in the desorption kinetics. Then, the tubes were vigorous hand shaking, then centrifuged at 4,390 x g for 5 min. The samples were dried at 40 °C for 72 h, ground and thoroughly homogenized for the posterior desorption proceedings.

#### 7.2.6.2. EDTA extraction

EDTA removes metals organically bound, occluded in oxides, and associated with secondary clay minerals (Gleyzes et al., 2002; Labanowski et al., 2008). This compound is a well-known strong chelating agent and has been widely used in agronomy for estimating the total extractable metal pool (Meers et al., 2007; Manouchehri et al., 2006).

For desorption proposal the method proposed by Fanguero et al. (2005) was applied with modifications. Briefly, 0.50 g dried-soil of residual soil was weighed in 15-mL polypropylene centrifuge tube and then 10 ml of 10 mmol L<sup>-1</sup> EDTA (as Na<sub>2</sub>-EDTA) at pH 6 were added. The mixture was stirred with an orbital shaker (50 rpm) for 10, 20, 40, 120, 240, 480 and 1,440 min at room temperature ( $25\pm2$  °C). Then the tubes were centrifuged at 2,098 x g for 5 min, the supernatant filtered (0.45 µm) and stored at +4 °C until the metal determination in the extracts. Blank samples were prepared (10 mmol L<sup>-1</sup> Na<sub>2</sub>-EDTA solution) with the same metal concentrations but without the addition of soil as aforementioned. The concentration of Cd and Zn were determined each by atomic absorption spectrometry (AA 220FS, Agilent Technologies). The calibration curve for the all elements had R<sup>2</sup>>0.996. The desorption kinetics followed the two first-order reactions model. In this model, two different first-order reactions are attributed to discrete types of binding sites available in the sediments (Fangueiro et al., 2005) or soils (Casali et al., 2008). Basically, the model equation is (equation 7):

 $Q=Q_1(1-exp^{-k_1t})+Q_2(1-exp^{-k_2t})$  .....eq. 7

where  $Q_1 \text{ (mg dm}^{-3})$  is a fraction of metal readily extractable, associated to the rate constant  $k_1$ .  $Q_2 \text{ (mg dm}^{-3})$ , fraction of metal less extractable, associated to the rate constant  $k_2$ .  $Q_3 \text{ (mg dm}^{-3})$  is the metal fraction not extractable.  $Q_3$  was obtained by difference between the total metal adsorbed in soil through and the concentration extracted at equilibrium (equation 8):

$$Q_3 = Q_{total} - Q_1 - Q_2$$
 ..... eq. 8

The native concentrations of Zn and Cd were contempt seeing that are smaller than the amount additional. More details about this mathematical approach are found in Fangueiro et al. (2005) and Labanowski et al (2008).

#### 7.2.7. Data Analyses

The experimental equilibrium and kinetics results were adjusted to non-linear models to avoid introduces bias due to linearization processes (Foo and Hameed, 2010; Rezaei Rashti et al., 2014; Tran et al., 2017). The Origin 2017 software (OriginLab, USA) was using for estimating the parameters of each equations.

#### 7.3. Results and Discussion

#### 7.3.1. Soil properties

The granulometric fractions distribution remains stable, similarly the mineralogical compounds in fine fraction, because these attributes are derivatives of parent material and pedogenetic process. Then, hardly agriculture processes could modify these attributes (Table 1). The mineralogy composition, mostly kaolinite and gibbsite in fine ( $<2 \mu$ m), confirms the high weathering degree of these soils. The sum of minerals quantifiable reached about 83% of clay content. The other minerals that were not quantified should be oxides i.e. anatase, ferrihydrite, maghemite and aluminum silicates, which could be identified by XRD of nature clay (Figure 1 in Supplementary Material). These aluminum silicates could be identified as phyllosilicates 2:1 as micas and vermiculites, which presented a considerable capacity to adsorbed metals (Shahwan et al., 2006; Hyun and Hayes, 2015).

	ъН	P	K	Р	K	S	С	Ca <sup>2+</sup>	Mg <sup>2+</sup>	$Al^{3+}$	H+A1	SB		CEC	V	Sand		Silt	Clay
Sample ID		Resin Mehlich-I		-	a ka <sup>-1</sup>				nol dm <sup>-1</sup>	3					0/				
	$H_2O$			mg dm⁻³			g kg			1111	noi <sub>c</sub> ann						%		
Gu(v)	4.5	6	110	3.1	80	2	62	29.2	13.1	21	145	42.3	;	196	22	5		32	63
Gu(a)	5.3	30	136	15.6	135	12	38	82.1	16.2	01.9	80	98.3	;	180	55	4		27	69
Ta(v)	5.4	39	82	16	77	6	15	37.0	13.2	0.8	56	50.2	2	101	49	60		12	28
Ta(a)	6.7	123	144	47	156	8	17	66.6	21.9	0.7	14	88.5	5	103	86	58		12	30
RP(v)	5.8	24	98	4.1	86	21	30	42.3	11.8	09.5	52	55		117	47	22		22	56
RP(a)	5.9	141	117	74.3	156	35	24	56.7	9.4	0.1	58	66.1		124	53	27		26	47
Pi(v)	4.6	18	89	2.3	60	8	42	44.1	7.9	4	90	52		145	36	49		10	41
Pi(a)	6.1	123	136	37.2	139	8	21	46.3	13.6	0	35	59.9	)	95	63	53		4	43
Minerals <sup>2</sup> Oxalate pH 3																			
	Kaolini	te G	ibbsite	Goethite	Hemati	ite	Fe	Al	Mn	Р	S	Ca	Mg	Κ	Cu	Zn	Pb	Cd	Ba
			g k	kg								m	ig kg '						
Gu(v)	326		31	162	13		157	160	30	704	637	561	1061	1175	133	80	-	0.5	29
Gu(a)	371		33	158	13		187	158	17	1003	344	1357	890	1185	163	92	-	0.7	38
Ta(v)	190		18	29	32		32	41	9	112	296	946	216	390	12	26	-	0.1	9
Ta(a)	197		26	29	34		62	76	6	396	146	4010	2020	570	20	30	-	0.2	16
RP(v)	156		306	12	4		22	32	1	900	338	947	234	575	10	42	-	0.7	50
RP(a)	144		261	12	4		19	29	2	2096	414	1416	200	680	22	63	-	0.6	90
Pi(v)	251		94	34	2		32	96	6	229	316	744	272	430	5	18	-	0.2	44
Pi(a)	265		102	34	2		36	117	3	714	172	987	320	330	10	36	-	0.2	42

**Table 2.** Chemical, physical and mineralogical properties of the studied soils<sup>1</sup>.

<sup>-1</sup> Standard deviation < 5%, n = 3.

<sup>2</sup> The minerals were quantified in fraction  $< 2 \ \mu m$ .

After decades of tillage practices and high fertilizers' application, the main chemical modifications caused by the soil cultivation were the higher pH and the strong increase of nutrients' contents, mostly P, K and Ca. A decrease in total organic carbon revealed the decline in soil organic matter (SOM), as a consequence of soil tillage (Guo and Gifford, 2002).Therefore, we should expect a cation exchange capacity decrease following the SOM reduction, as showed by Curtin et al. (2015).

The pseudo total contents obtained by EPA3051A extraction (Table 2) of P and Ca increased markedly. K, Cu, Zn and Ba had a moderate increase, and Cd had a weak increase, likely consequence of the fertilized inputs. However, without surpass the environmental quality thresholds (Cetesb, 2001). Conversely, the contents of Mg and S decreased, likely associated to SOM loss and crops exportation.

# 7.3.2. Equilibrium Isotherm

The fit for isotherm model were better to Zn than Cd (Table 3). The soil samples showed a substantial Cd and Zn adsorption capacity, which was confirmed by remarkable increase in maximum adsorption capacity (mmol kg<sup>-1</sup>) coefficients of Langmuir and D-R isotherms equations. The land use effects improve increments about 50 and 42% in the maximum adsorption capacity for Cd and Zn, respectively, to the cultivated soil.

		•		Cd			
		Langmuir		Dubinin-Radushkevich			
Sample ID	Qmax <sup>(1)</sup> mmol kg <sup>-1</sup>	k <sub>L</sub> L mmol <sup>-1</sup>	$\mathbf{R}^2$	Qmax mmol kg <sup>-1</sup>	$E^{(2)}$ kJ mol <sup>-1</sup>	$R^2$	
Gu(v)	6.1	16.2	0.88	5.3	8.1	0.85	
Gu(a)	10.0	16.0	0.95	7.1	12.3	0.84	
Ta(v)	7.5	29.7	0.92	6.9	12.5	0.89	
Ta(a)	13.4	94.5	0.88	11.7	50	0.95	
RP(v)	13.0	17.2	0.97	10.6	8.8	0.90	
RP(a)	15.5	24.2	0.97	12.3	13.4	0.93	
Pi(v)	8.2	18.3	0.89	6.9	9.9	0.89	
Pi(a)	13.4	24.9	0.97	11.4	11.2	0.91	
				Zn			
Gu(v)	7.9	4.3	0.98	5.7	5.7	0.94	
Gu(a)	10.3	8.2	0.99	7.5	11.0	0.91	
Ta(v)	6.8	16.3	0.99	5.7	14.7	0.95	
Ta(a)	14.6	102.1	0.84	12.9	70.7	0.92	
RP(v)	11.4	46.3	0.96	9.2	40.8	0.89	
RP(a)	12.7	52.1	0.95	11.7	28.9	0.90	
Pi(v)	7.9	8.4	0.98	6.2	9.2	0.92	
Pi(a)	10.5	30.8	0.98	8.4	31.6	0.89	

Table 3.	Isotherm	model	parameters.
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<sup>1</sup> Coefficient of determination. <sup>(2)</sup> free energy change required transferring 1 mol of ions from solution to the solid surfaces

Soil sample from Gu had the higher SOM, CEC and clay content, which should propone higher metal adsorption capacity. But, interesting, we observed the inverse. The soil under native vegetation (Gu(v)) has an extremely acidity buffer capacity (table 2), and it disfavor hydroxides precipitation summed the  $H^+$  competition for metal adsorption sites, resulted in the lower values for metals adsorption. Also, the higher amounts to dissolved organic carbon summed to lower pH could inhibit the metals immobilization on soil surface (Fan et al., 2016). We found an increase in the metal adsorption after agriculture management in this site (Gu(a)), however, it kept lower compared to the others soil sample.

The kaolinite, which was the main constituent in the clay fraction soils, with the exception of RP sites, has low CEC and low ability to adsorb metals (Coles and Yong, 2002) and dominated adsorption process is via outer-sphere complexes at low pH (Srivastava et al., 2005). Consequently, the higher affinity between soils samples and the studied metals should be due to gibbsite and goethite components, which seem to be the main players responsible to metal adsorption (Bradl, 2004).

The physical adsorption is indicated when *E* value lies between 1 and 8 kJ mol<sup>-1</sup>. *E* values ranging from 8 to 16 kJ mol<sup>-1</sup> indicate ion exchange. However, if *E* value is between 20 and 40 kJ mol<sup>-1</sup>, chemical adsorption occurs (Chen et al., 2010; Hamayun et al., 2014; Liu et al., 2016). In our study, *E* values were, on average, 16 kJ mol<sup>-1</sup> to Cd and 27 kJ mol<sup>-1</sup> Zn. The *E* value calculated from D-R equations is useful for distinguishing the adsorption as a physical or chemical process, which suggests an increase in metal adsorption by chemical process in the agricultural soils.

The isotherms suggested that the change in land use has created new sites to metal adsorption, which are made mostly by chemical adsorption, followed *E* value interpretation obtained from D-R isotherm (Table 3). There are some causes for the adsorption sites increase in agriculture soils as: the higher pH, which increase the cation exchange capacity in variable-charge sites by deprotonation of hydroxyl groups in the surfaces of clay materials and Fe and Al oxyhydroxides (Essington, 1997; McBride, 1994) and can lead the nucleation of Cd and Zn hydroxide on clay mineral surfaces (Bradl, 2004).

Other alternative is that the higher P contents due to continuous fertilizations could promote surface potential shifts in Fe/Al oxyhydroxides, which rise negative charge and decreases the electrostatic potential close the colloid surface, which could cause increase of Cd and Zn adsorption by ternary linkage adsorption (Elzinga and Kretzschmar, 2013; Ren et al., 2015). Noteworthy that ternary linkage is more evident in simple colloidal system at higher P availability and P covering surface, hence this mechanism should be admitted with prudence in soils system (Li and Stanforth, 2000; Li et al., 2007).

# 7.3.3. Adsorption Kinetics

The Cd and Zn adsorption kinetics was well fitted to kinetics models used in this study (Table 1SM). After initial 30 min, 92 and 88 % of total Cd and Zn had been adsorbed. From a practical point of view, the time needed to obtain equilibrium conditions is more useful than the k value (Kulikowska et al., 2015). This process nearly instantaneous in pure clay (Chen et al., 2015; Starcher et al., 2017) or in soils (Chen et al., 2006; Diagboya et al., 2015). Čipáková et al (2011) observed that Cd reach more the 90 % of total adsorption after 5 min of contact with soil. See more examples in Tran et al. (2017).

The pseudo-second order model had better adjust due to higher values of determination coefficients of equation and lower differences between the estimated and the experimental value of equilibrium concentration. The pseudo-second-order model indicates that metal adsorption kinetics happens at a faster stage and then another slower (Ho et al., 1996; Ho, 2006). In theory, this is due to adsorption sites of different energies (Colombo et al., 1994) and intra-particle diffusion, responsible for the slower stadium (Barrow and Debnath, 2015).

# 7.3.4. pH and the other ions in the equilibrium solutions

The addition of amounts of metals promoted a decrease on pH value, remarkable to Cd in the agricultural soils, whose initial pH was higher (Figure 2). This is expected once that the metal can replaces H<sup>+</sup>, theretofore adsorbed on colloidal surfaces (Günay et al., 2007; Rajput et al., 2016; Melo et al., 2016). However, Gu2 and Ta1 for both metals had a slight increase in the higher metal concentrations.





Time (h)

Figure 2. pH variation in function in the isotherms (A and B) and kinetics (C and D) experiments.

Until 4 h after initial adsorption, a strong decrease in pH was observed. In a second moment, the values were stable and followed a continuum increases. Ta(a) and RP(a) had a distinct increasing. After this time the pH reached a new equilibrium, similar to pH evolution in isotherms study.

The adsorption to both metals promotes an extensive alteration in the concentration of others ions on equilibrium solution both the isotherm (Figure 3) and kinetics (Figure 4) studies. The elements release in solution after metal addition in the kinetics study was quick, as well as in isotherm equilibrium study.



**Figure 3.** Elements released from the soils in isotherm equilibrium solution in function of Cd (left) and Zn (right) concentration added, after 24 h of contact time.

Overall, there are similar results for Ca, Mg and Mn after adsorption for Cd or Zn. Assuming that once the adsorption finishes available surface sites, will be necessary to replace others cations from adsorptive surface. Thus, Ca and Mg were linearly released after metal's addition. Curiously, the higher Ca desorption after Zn addition than Cd (Figure 3 and 4), is in accordance to Ugrina et al. (2015) which used zeolites. This result proposes a preference for Zn in sites previously occupied for Ca, therefore a chemical similarity between Zn and Ca.



Figure 4. Elements released from the soils in kinetic study after addition of Cd (left) and Zn (right) solution over the contact time.

Dissolved organic carbon (DOC) in both studies had similar behavior. The slight reduction over the time maybe had followed the lower solubility of humic substances in low pH, resulting metal adsorption (Figure 2). The soil sample that had the higher DOC content also had lower maximum adsorption metal capacity, which is in agreement to results from Fan et al. (2016), which evaluated the DOC ability to complex Zn, which allows more Zn in solution, consequently increase the metal mobility.

Sulfur concentration in equilibrium solution of isotherms study had a decline, mainly in Zn adsorption. A possible explanation is the continuum association between metals, especially Zn, with thiol group in organic substances (Karlsson et al., 2007). However, in kinetic study, the sulfur concentration had a fast decrease, followed slight increase over the time, alike Mn pattern.

Phosphorus had the most complex behavior among the elements analyzed. In the isotherms data, the P concentration in equilibrium solution decreased after both metals added until  $\approx 6 \text{ mmol } \text{L}^{-1}$ . After that, P concentration has stabilized in solution. However, in the

kinetics study, P concentration linearly increased for Cd; meanwhile for Zn, the opposite behavior was found. P and S quantification showed considerable variations, and their concentrations were close to the limit of quantification.

In the chemisorption process, the adsorbate adheres on the adsorbent to form a chemical bond and occupy sites that will maximize their coordination number with the surface (Atkins, 2010). Thus, there was a pH decrease (release of  $H^+$ ) and an increase of Ca and Mg concentrations in the equilibrium solution (figure 3 and 4), because of the replacement by Cd and Zn in the adsorptive complexes after Cd and Zn adsorption (Figure 2).

In similar experiments for Zn, Casagrande et al. (2008, 2005) found that its adsorption increased with the elevation of the pH and with the decrease of the ionic strength. The pH effect, however, surpassed the effect of the ionic strength, and the authors concluded that the largest variation of adsorption with pH suggests the presence of high-affinity adsorption sites. For Cd, Soares et al. (2009) the adsorption increased drastically with increasing pH, varying from 20 to 90% in a narrow pH range.

# 7.3.5. Desorption kinetics

The results of desorption processes are presented in the figure 5, and the values are described in Table 2SM. The EDTA confirmed its strong affinity to metals studied, since it got recovery 71 at 100 % for Cd and 71-95% to Zn. Rezaei Rashti et al (2014) assessed Cd desorption in subtropical soils from Iran, and also recovered considerable amounts of Cd added using DTPA, a similar complexant agent. The faster stage represents the portion more susceptible to desorption process. In general, regardless of the contact time, Cd had a lager fast compartment (68%) than Zn (47%). Despite the slight increase in total metal adsorption between 1d and 20d, the increment in contact time contributed to a discreet decrease in the faster compartment followed the increase in slow and residual fractions, for both metals.



Figure 5. Metal compartments from EDTA desorption kinetics after 1 or 20 days of soil and solution metal contact.

Four our adsorbent material (the soils) Zn seem be preferentially adsorbed in more stable sites, less able to desorption, if comparable to Cd. Covelo et al. (2007) observed differences in the selectivity to adsorption and desorption in the same adsorbent, which suggest specialized sites for interaction in the same material for different metals. The higher amounts Cd adsorbed, a like for other metals, do not guarantee strong fixation to colloidal soil surface. We could considerer that the agriculture land use increased the adsorption sites, most probably via precipitation of Cd and Zn hydroxides (e.g Cd(OH)<sub>2</sub> and Zn(OH)<sub>2</sub>) in minerals surfaces, once the agricultural soils have higher pH, which support precipitation reaction (Bradl, 2004; Casagrande et al., 2008). This results is often observed in others adsorption studies (Labanowski et al., 2008; Acosta et al., 2011), which corroborate the strong dependence between site and species how the metal is adsorbed and posterior desorption process.

The aging affect changes soluble/mobile Zn fractions to insoluble/immobile forms also is highlights by (Kuo and Mikkelsen, 1980; Voegelin et al., 2005;Lair et al., 2007). Donner et al. (2012) evaluated zinc adsorption in six highly weathered using isotopic exchangeability, attributed the decrease in extractability (extraction 10 mM CaCl<sub>2</sub>) and Zn isotopic exchangeability (called for them as "lability") over the time to precipitation reaction

in higher pH, and interparticle and intraparticle diffusion. However, McBride and Cai (2016) showed that despite of 10 years of aging, Cu and Zn showed significant phytotoxicity, even presented a shift of the metals into less labile forms.

The increment in slow and residual fraction for Zn in some soil, give us evidences to possible change in chemical metal species as showed by Voegelin et al. (2011). The lower proportion of fast desorption sites for Zn than Cd could had relation to an irreversible nonexchangeable form of Zn associated with iron oxides (Buekers et al., 2008) and lattice entrapment in aluminum silicates (Bradl, 2004) mostly in 2:1 type. Lately, many researchers found the minerals named layered double hydroxides as a Zn stable phase in many soils systems (Voegelin et al., 2005; Khaokaew et al., 2012; Starcher et al., 2017), which is stable forms over the time, thus also is in accordance with the strong chemisorption suggested by E values from D-R isotherms model.

For Cd, we found a negligible changes in desorption compartments between the contact times studied. Jalali and Khanlari (2008) showed the tendency of high immobilization over the time, except to Cd. Recently, Colzato et al. (2017), using diverse Brazilian soils, concluded that Cd bound preferentially to organic matter after short-time contact, but also did not find changes in Cd immobilization after four months of soils incubation. These results are overlapping in part of our observations.

Some studies about Cd showed a decrease in metal availability over the long contacttime. Hamon et al.(1998) using a radioisotope dilution technique, concluded that a meaning amount of Cd added was found in a non-bioavailable pool in the soil and a model developed estimated that Cd fixation in Australian soil (clay loam, Plinthic Paleustalf) at a rate of 1-1.5% of the total added Cd per year. Grant et al.(2013) after 8 years of higher fertilization rate in Canadian soils (most Chernozem) there was not a strong cumulative effect, indicating attenuation of Cd availability over time.

Methodological approach beyond soils properties could explain some differences between laboratory and fields experiments results. Maybe the higher Cd mobility observed by Colzato et al. (2017) and here to Cd and also, in part to Zn, no represent high bioavailability in field conditions. Further research is necessary to determine the mechanisms over long contact-time between metal and soil, and its impacts to bioavailability of these elements at Brazilians field conditions.

After repeated fertilizer applications over the years, the soil chemical condition could modify the behavior of nutrients and heavy metals. Usually, the agricultural soil had high pH and P availability. Both of this new propriety allows a higher adsorption capacity, as our results highlighted. Despite the higher Cd and Zn adsorption, our results suggested that the most part of this increment in adsorbed metals are easily desorbed. New studies should be designed to understand the aging effect in metal mobility in heavily fertilized weathered soils, as which are the mineral phases formed after long time of contact and if the trend about decrease in metal mobility observed in our study is found in other environmental systems.

The desorption kinetics disclosed the following conclusions:(i) despite the higher amount immobilized, Cd is preferentially adsorbed to fast released compartment than Zn; (ii) the new adsorption sites created by agricultural practices contributed for easy desorbable Zn fractions, while there was an increase in the residual fraction for Cd, and (iii) the aging effect could converted few parts of fast desorption sites to slow desorption.

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# Supplementary information

					Cd				
	Pseudo-fir	st order kine	etic model	Pseudo-se	econd order kinetic	model	Elo	vich Model	
$q_e(exp)^{(1)}$	$q_e(est)^{(2)}$	$K_1$	$r^{2(3)}$	q <sub>e</sub> (est)	$K_2$	$r^2$	а	b	$r^2$
mmo	l kg <sup>-1</sup>	$(h^{-1})$		mmol kg <sup>-1</sup>	kg mmol <sup>-1</sup> h <sup>-1</sup>		mmol kg <sup>-1</sup> h <sup>-1</sup>	kg mmol <sup>-1</sup>	
4.43	3.95	9.71	0.93	3.99	10.62	0.94	8x10^18	12.39	0.95
7.87	8.05	7.96	0.95	8.10	4.98	0.95	1x10^25	7.80	0.96
6.66	6.52	7.88	0.98	6.57	5.62	0.98	4x10^35	13.44	0.99
15.14	14.97	92.1	0.99	15.01	9.31	0.99	1x10^44	7.12	0.99
12.62	12.04	5.81	0.95	12.15	1.92	0.99	2x10^27	5.66	0.99
16.28	15.73	5.83	0.99	15.85	1.54	0.99	3x10^30	4.78	0.99
7.54	6.79	5.81	0.95	6.88	2.61	0.96	5x10^15	6.07	0.98
13.25	12.69	8.80	0.99	12.76	4.14	0.99	1x10^41	7.86	0.99
					Zn				
	Pseudo-fir	st order kine	etic model	Pseudo-se	econd order kinetic	model	Elo	vich Model	
$q_e(exp)$	$q_e(est)$	K <sub>1</sub>	$r^2$	$q_e(est)$	$\mathbf{K}_2$	$r^2$	a	b	$r^2$
mmol	g kg⁻¹	(h <sup>-1</sup> )		mmol kg⁻¹	kg mmol <sup>-1</sup> h <sup>-1</sup>		mmol kg <sup>-1</sup> h <sup>-1</sup>	kg mmol⁻¹	
5.14	5.14	4.58	0.96	4.64	3.27	0.97	1x10^13	7.74	0.99
7.68	7.13	4.71	0.97	7.24	1.84	0.98	5x10^14	5.46	0.99
5.05	5.09	5.52	0.99	5.13	4.69	0.99	1x10^39	19.06	0.99
13.96	13.26	5.76	0.99	13.38	1.67	0.99	2x10^24	4.60	0.99
9.69	9.41	4.56	0.99	9.54	1.41	0.99	1x10^19	5.21	0.99
11.44	10.83	3.74	0.99	11.01	0.84	0.99	3x10^14	3.53	0.99
5.98	5.62	4.17	0.97	5.72	1.78	0.98	1x10^13	6.32	0.99
9.38	8.79	5.68	0.98	8.87	2.37	0.99	9x10^21	6.33	0.99
	$\begin{array}{r} q_{e} (exp)^{(1)} \\ mmo \\ 4.43 \\ 7.87 \\ 6.66 \\ 15.14 \\ 12.62 \\ 16.28 \\ 7.54 \\ 13.25 \\ \hline \\ q_{e} (exp) \\ mmol \\ 5.14 \\ 7.68 \\ 5.05 \\ 13.96 \\ 9.69 \\ 11.44 \\ 5.98 \\ 9.38 \\ \hline \end{array}$	$\begin{tabular}{ c c c c } \hline Pseudo-fin $$q_e$(exp)^{(1)}$ $$q_e$(est)^{(2)}$ $$nmol $kg^{-1}$$$$$.$$$A.55$$$$.$$$A.55$$$$$.$$$A.55$$$$$A.55$$$$$$A.55$$$$$A.55$$$$$$$$$	$\begin{tabular}{ c c c c c } \hline Pseudo-first order kinese $$ $$ $$ $$ $$ $$ $$ $$ $$ $$ $$ $$ $$$	$\begin{tabular}{ c c c c c } \hline Pseudo-first order kinetic model $$ K_1$ $$ r^{2(3)}$ $$ mmol kg^{-1}$ $$ (h^{-1})$ $$ 0.93$ $$ 7.87$ $$ 8.05$ $7.96$ $$ 0.95$ $$ 6.66$ $$ 6.52$ $$ 7.88$ $$ 0.98$ $$ 15.14$ $$ 14.97$ $$ 92.1$ $$ 0.99$ $$ 15.14$ $$ 14.97$ $$ 92.1$ $$ 0.99$ $$ 15.2$ $$ 12.04$ $$ 8.81$ $$ 0.95$ $$ 16.28$ $$ 15.73$ $$ 8.83$ $$ 0.99$ $$ 16.28$ $$ 15.73$ $$ 8.83$ $$ 0.99$ $$ 16.28$ $$ 15.73$ $$ 8.80$ $$ 0.99$ $$ 13.25$ $$ 12.69$ $$ 8.80$ $$ 0.99$ $$ 13.25$ $$ 12.69$ $$ 8.80$ $$ 0.99$ $$ 13.25$ $$ 12.69$ $$ 8.80$ $$ 0.99$ $$ 13.25$ $$ 12.69$ $$ 8.80$ $$ 0.99$ $$ 13.25$ $$ 5.14$ $$ 4.58$ $$ 0.96$ $$ 7.68$ $$ 7.13$ $$ 4.71$ $$ 0.97$ $$ 5.05$ $$ 5.09$ $$ 5.52$ $$ 0.99$ $$ 13.96$ $$ 13.26$ $$ 5.76$ $$ 0.99$ $$ 13.96$ $$ 13.26$ $$ 5.76$ $$ 0.99$ $$ 13.96$ $$ 13.26$ $$ 5.76$ $$ 0.99$ $$ 11.44$ $$ 10.83$ $$ 3.74$ $$ 0.96$ $$ 11.44$ $$ 10.83$ $$ 3.74$ $$ 0.95$ $$ 5.98$ $$ 5.62$ $$ 4.17$ $$ 0.95$ $$ 0.98$ $$$	$\begin{tabular}{ c c c c c c c c c c c c c c c c c c c$	$\begin{tabular}{ c c c c c c } \hline Pseudo-first $$$$$$$$$$$$$$$$$$$$$$$$$$$$$$$$$$$$$	$\begin{tabular}{ c c c c c c c } \hline Pseudo-first order kinetic model \\ \hline Pseudo-second order kinetic model $	$\begin{array}{c c c c c c c c c c c c c c c c c c c $	$ \begin{array}{c c c c c c c c c c c c c c c c c c c $

**Table 1SM.** Models kinetic parameters of metals adsorption in the soil samples.

Metal adsorbed amount at equilibrium obtained <sup>1</sup> experimentally and <sup>2</sup> estimated by the models. <sup>3</sup> Determination coefficient of the regressions nonlinear models.

use.				
Sample ID	Total adsorbed	Fast Desorption	Slow desorption	Residual
Cd		<i>1d</i> (mg kg	1)	
Gu(v)	439.7	387 (88) <sup>1</sup>	51.9 (12)	0.8 (≈ 0)
Gu(a)	890.3	623 (70)	100 (11)	167.3 (9)
Ta(v)	753.7	520 (69)	90 (12)	143.7 (19)
Ta(a)	1685.6	1267 (75)	52.5 (3)	366.1 (22)
RP(v)	1370.3	1063 (78)	30.8 (2)	276.5 (20)
RP(a)	1761.5	1166 (66)	203.5 (12)	392.0 (22)
Pi(v)	742.5	456 (61)	161 (22)	125.5 (17)
Pi(a)	1429.9	859.6 (60)	157 (11)	413.3 (29)
		<b>20d</b> (mg kg	-1)	
Gu(v)	498.4	380 (76)	93 (19)	25.4 (5)
Gu(a)	982.5	602.6 (61)	157 (16)	222.9 (23)
Ta(v)	748.7	523 (70)	34.44 (5)	191.2 (26)
Ta(a)	1702.4	1015 (60)	296 (17)	391.4 (23)
RP(v)	1418.1	1012 (71)	159 (11)	247.1 (17)
RP(a)	1830.0	1274 (70)	148 (8)	408.0 (22)
Pi(v)	851.5	441 (52)	234 (27)	176.5 (21)
Pi(a)	1488.9	1017 (68)	42.2 (3)	429.7 (29)
Zn		<i>ld</i> (mg kg <sup>-</sup>	1)	
Gu(v)	346	213 (61)	61 (18)	71.5 (21)
Gu(a)	456	185.3 (41)	156 (34)	115.1 (25)
Ta(v)	340	166 (49)	91 (27)	83.3 (24)
Ta(a)	862	628 (73)	108 (13)	126.4 (15)
RP(v)	621	195 (31)	317 (51)	109.1 (18)
RP(a)	710	289 (41)	311 (44)	110.4 (16)
Pi(v)	364	141 (39)	158 (43)	65.2 (18)
Pi(a)	572	380 (66)	61 (11)	131.4 (23)
		<b>20d</b> (mg kg	-1)	
Gu(v)	336.1	217 (64)	69 (21)	50.1 (15)
Gu(a)	501.8	167 (34)	167 (33)	168 (33)
Ta(v)	330.2	148 (45)	116 (35)	66.2 (20)
Ta(a)	912.4	645 (71)	156 (17)	111.4 (12)
RP(v)	633.2	481 (76)	80 (13)	72.2 (11)
RP(a)	747.6	471 (63)	180 (24)	96.6 (13)
Pi(v)	391	150 (38)	185 (47)	56 (14)
Pi(a)	612.9	277 (45)	171 (28)	165 (27)

**Table 2SM.** Partitioning metals pools of desorption kinetic (mg kg<sup>-1</sup>). Effects of aging and soil land use.

 $\frac{P_{1}(a)}{^{1}} \frac{612.9}{^{2}} \frac{277}{(45)} \frac{171}{(45)}$ 

# 8. Zn SPECIATION AND CHEMICAL FRACTIONATION IN HIGHLY FERTILIZED TROPICAL SOILS

# ABSTRACT

The increment on industrial economy may increase the exposition to potentially toxic elements (PTE) in the environment. The agriculture in weathered tropical soils often needs to surpass soil chemical limitations. The large amounts of lime and fertilizers are spread on the soils to reach a satisfactory nutrients' levels in the soil, hence assuring the biomass and food production. After a long term of agriculture practices, fertilizations had changed the chemical attributes of tropical soils. Zn is a PTE that also plays as an important micronutrient for plants and animals. In this study, we evaluated Zn speciation in tropical soils by chemical fractionation and X-ray Absorption Spectroscopy as a function of fertilization and contact time. The amount of Zn mobile fraction was lower in agricultural soils and positively correlated to the addition of phosphate via fertilizers. The soil mineralogical compounds and total organic matter content were good indications for Zn-bearing. Aluminum-silicates minerals were notable Zn-sink regardless of soil origin and land use. Zn-organic matter decreased and Zn associated with P increased after the conversion from soils under native vegetation to highly fertilized soil

Keywords: Fertilizer; Plant nutrition; Xray absorption spectroscopy; Soil mineralagy

# 8.1. Introduction

The increment on industrial economy could increase the exposition to potentially toxic elements (PTE) (e.g As, Ba, Cd, Cr, Cu, Ni, Pb, and Zn) in the environment, which could be a threat to human health and ecosystem equilibrium. An important sink for PTE is the inadequate waste disposal in soils, atmospheric deposition, industrial disasters and so on (Adriano, 2001b).

Soil ecosystem has important inputs of PTE via agriculture practices, especially by fertilizers, which may have considerable amounts of impurities (Cheraghi et al., 2012; Li et al., 2015; Nicholson et al., 2003). The agriculture in highly weathered tropical soils often need to surpass chemical limitations such as high activity of toxic aluminum in soil solution, low pH and scarcity availability of nutrients (Lopes and Guilherme, 2016). Over the last decades,

agriculture practices had overcome these chemical limitations. The large amounts of lime and fertilizers are spread on the soils to reach a satisfactory nutrient levels in soil, hence assuring the biomass and food production.

Phosphate fertilization have been required on higher amounts over the years (Rodrigues et al., 2016; Withers et al., 2018, Barreto et al., 2018) in agriculture practices. After long term of fertilizations, soil chemical attributes had changed tropical soils. The changes are especially evident for vegetable crops, which had fertilization recommendations five to twenty times higher than cereal crops (CFSEMG, 1999; CQFS, 2004; Pereira and Daniels, 2003; Raij et al., 1996; Yan et al., 2013). Nowadays, some agricultural soils have a pH about 5.5 and high P availability (Rodrigues et al., 2016).

The new chemical environment in fertilized soils could affect the dynamics of micronutrients and PTEs. Higher pH should improve the precipitation of some metals by means of metal-(OH)<sub>2</sub> formation (Bradl, 2004; Uddin, 2017). Also, the high P availability could promote the formation of ternary complex as described by (Martin et al., 2017; Elzinga and Kretzschmar, 2013; Liu et al., 2016; Ren et al., 2015a), which increase metal adsorption.

Curiously, the total amount of any elements on soil had a weak or inexistent relation to availability/mobility of each element, and good examples are Si, Al and Fe that although present in high total amounts in soils (in % scale), have just some mg per ha as a bioavailable amounts. Other ways to estimate the potential risks and availability should be applied rather than total concentration.

The chemical fractionation is a consolidated approach to detach the elements over class of solubility. Currently, we had several methods to assess the chemical species of elements. Instead of widely-used by scientific community, theses approach had some limitations and have been disapproved due to: (i) lack of uniformity in the procedures and extensive modifications; (ii) lack of selectivity of the reagents used for specific fractions; (iii) lack of quality control, which could be figure out if all labs used the same control soil sample (e.g. NIST, BCR). Valorous reviews are available (Gleyzes et al., 2002; Qian et al., 1996; Gismera et al., 2004; Rao et al., 2008), which describes the several methods of chemical fractionation and potential limitations and problems about those approaches.

Although the limitations, these studies have given us advances about geochemical origin and stability of different elements. This compartmentalization is operationally distinct as geochemical fractions, which depends on the reagents chosen to solubilize the different metal fractions. Briefly, the most common fractions are called: exchangeable; carbonates (acid-soluble); Fe and Mn oxides (reducible); organic matter (oxidisable); and, residual.

Recently, the advances of spectroscopic methods to direct speciation has been featured because they allow a direct sample analyses, without the invasive treatment as described to chemical fractionation, which is the preeminent issue the wet-chemical analyze. The speciation by X-ray Absorption spectroscopy (XAS) had has become popular in geosciences research. XAS analysis could involve the extended fine structure (EXAFS), which yields the local coordination numbers, ligand types, and interatomic distances that are difficult to obtain for noncrystalline precipitate or sorption-complex structures(Waychunas et al., 2002; Lombi and Susini, 2009; Kopittke et al., 2017; Lehmann and Solomon, 2010; Gillespie et al., 2015).

Zn is a PTE that also plays as an important micronutrient for plants and animals. It is found in concentrations ranging from 10 to 100 mg kg<sup>-1</sup> in soils around the world (Mertens and Smolders, 2013). On other hand, the environmental threshold limit established by Brazilian Environmental Agency (CONAMA) for Zn is 450 mg dm<sup>-3</sup> for implementation of remediation practices (CONAMA, 2009). However, millions of hectares of cropland are affected by Zn deficiency, thus promotes inadequate Zn intake for about one-third of the human population (Alloway, 2009). Zn behavior could be a useful proxy to evaluate others metals' PTE dynamic in highly fertilized soils, like pollutants and/or micronutrients. In this study, we evaluated Zn speciation in tropical soils by chemical fractionation and XAS, affected by fertilization and the contact time.

#### **8.2.** Material and Methods

#### 8.2.1. Soil sampling

The study sites were in located in four strategic and representative areas in Brazil regarding oleraceous crops. The soil geneses condition of the sites resulted in highly weathered soils (Figure S1). The soil samples were collected in agriculture lands (ag) and under native vegetation (nv) nearby area, for guarantee that the main difference of sites should be the agriculture practices (e.g soil tillage, fertilization). The soil sample was composited by 12 subsamples, which were collected using an auger at 0-20 cm of depth. After homogenization subsamples were air-dried and passed through a 2-mm sieve for chemical and physical analysis.

# 8.2.2. Chemical and physical attributes

For analyses of soil size fractions, the sand fraction (50  $\mu$ m–2 mm) was removed from the suspension of dispersed mineral particles by wet sieving. Then, the silt (2  $\mu$ m - 50  $\mu$ m) and clay (< 2  $\mu$ m) fractions were collected by sedimentation according to Stokes' Law.

Soil organic matter was determined by dry combustion on elemental analyzer - LECO® CN-2000 (furnace at 1350 °C in pure oxygen). Phosphorus and potassium were extracted by mixed cationic and anionic exchange resin (AER)(van Raij et al., 1986) and by Mehlich-I solution (0.05 mol L<sup>-1</sup> HCl + 0.025 mol L<sup>-1</sup> H<sub>2</sub>SO<sub>4</sub>); Al, Ca and Mg were extracted by 1 mol L<sup>-1</sup> KCl and Al determined by titration with NaOH. Available S was extracted by a monocalcium phosphate solution (500 mg L<sup>-1</sup> of P in 2 mol L<sup>-1</sup> acetic acid). Potential acidity (H+Al) was extracted by 0.5 mol L<sup>-1</sup> calcium acetate at pH 7. Cation exchange capacity (CEC) was estimated by the sum of bases (Ca + Mg + K) plus potential acidity. The amounts of semi-total metal contents were obtained by using the EPA 3051a protocol (HNO<sub>3</sub>+HCl, 3:1 (v/v)) (USEPA, 2007).

#### 8.2.3. Mineralogy composition

Poorly crystalline Fe, Mn and Al oxides were extracted with 0.2 mol L<sup>-1</sup> ammonium oxalate at pH 3 in the dark. These elements in high crystalline oxides were extracted by sodium dithionite–citrate–bicarbonate (DCB) at 80 °C. Both procedures followed Pansu and Gautheyrou (2007). To evaluate the main mineralogical phases component in the clay fraction, it was measured with an X-ray diffractometer (Rigaku Geigerflux) with CuKa radiation ( $\lambda = 1.5418$  Å) generated at 30 kV and 15 mA, it in random and oriented powder. The XRD patterns were recorded in the range of 3 to 65°20 with a scanning speed of 0.015°20 and 1s counting time per step.

Clay natural fraction was analyzed by diffuse reflectance spectroscopy (DRS) following acquisition of the 380–800 nm wavelength range in 0.5 nm steps on a Cary 5 spectrometer (Varian, USA). Spectra were smoothed to calculate the Kubelka–Munk (K-M) function. The Gt/(Gt + Hm) ratio was calculated from the amplitude of the peaks for goethite (420–450 nm) and hematite (530–570 nm) in the second derivative of the K-M function (Torrent et al., 1983; Barron and Torrent, 1986). The contents in Gt and Hm were estimated from that of Fe extracted by DCB minus the amount extracted in oxalate pH 3 (Bahia et al., 2015).

To quantify kaolinite and gibbsite contents, and aliquot of clay fraction after DCB treatment was treated with sodium hypochlorite for organic matter elimination, thereafter the

residue followed differential thermal analysis (DTA) and thermogravimetric analysis (TGA) with a DTG-60H-Simultaneous DTA-TG (Shimadzu, Japan) in heating and cooling cycle from room temperature to 1100 °C at a heating rate of 10 °C min<sup>-1</sup> and N<sub>2</sub> atmosphere. The results of characterization could be seen in the table 1.

#### 8.2.4. Zn incubation on soil

The metal spike was applied to ensure the minimal concentration of Zn for XAS analysis (~100 mg kg<sup>-1</sup>) for produce suitable data (Kirpichtchikova et al., 2006b), as performed by Voegelin et al. (2011a). An amount equivalent the maximum capacity adsorption of Zn was applied to each soil. The method to estimate the maximum adsorption capacity by Langmuir isotherm was described in the Chapter 7.

For soil incubation, 3.00 g of soil were weighed in polypropylene tube 50-mL and added in each tube 30 ml (ratio 1/10 soil:solution) of Zn solution. All solutions prepared in 10 mmol L<sup>-1</sup> KCl as background electrolyte. To investigate the aging effect in metal-soil adsorption, the suspensions were shaken for 1 or 20 days (end-over-end) at  $25 \pm 2$  C°. This experiment was carried out in triplicate.

Finishing the stirred time, the tubes were centrifuged at 4,390 x g for 10 min, filtered through similar Whatman 42 filter. The supernatant was collected and placed in bottles for posterior quantification of Zn in remaining solution; hence figure out the total Zn was adsorbed. The residual soil was washed with 10 ml of acetone 70% for eliminating free interstitial metals loosely held by the soils. Then, the tubes were vigorous hand shaking, then centrifuged at 4,390 x g for 5 min. The amount of metals desorbed in this process was neglected. The samples were dried at 45 °C for 72 h, aground and thoroughly homogenized for the posterior chemical fractionation and XAS analysis.

#### 8.2.5. Chemical fractionation procedure

In this study, a modified version of the sequential extraction method of (Tessier et al., 1979) was carefully applied. The extracting agents, as well as their operationally defined fractions, are presented in table 1SM. The modification was to use an oxalate solution for solubilizing low-crystalline Fe, Al and Mn minerals, instead of dithionite, which extracts both high and low-crystalline. The low crystalline fraction is more reactive in soil system than high-crystalline minerals due to higher specific superficial areas. Hence, oxalate extraction is more sensitive to evaluate the dynamics of metal fractions in soils, as suggested in others studies (Gleyzes et al., 2002; Qian et al., 1996; Gismera et al., 2004; Rao et al., 2008).

Briefly, 1g of air dried incubated soil sample, from each replicate plus extracting agent solution were shaken (overhead 120 rotations min<sup>-1</sup>) at  $23 \pm 3^{\circ}$  C. After each step, the samples were centrifuged for 5 min at 1053 x g and the extraction solution was collected, filtered (similar Whatman 42 filter) and standardized to 30 mL. The Zn concentration in each fraction was determined by atomic absorption spectrometry (AA 220FS, Agilent Technologies). One calibration curves was performed for each extractant solution of fractionation procedure. All of them reached R<sup>2</sup> > 0.993.

This fractionation method sets apart the metals into five target phases, namely: soluble + exchangeable fraction (F1), easily mobilizable fraction (likely sorbed and bound at Fe, Al and Ca) (F2), associated with low crystalline Al, Fe and Mn oxyhydroxides (F3), associated with organic matter fraction (F4) and residual fraction (F5). The residual fraction was obtained by differences from the total metal adsorption and the sum of previous fractions (F1 to F4) obtained in the chemical fractionation. We applied this fractionation method in the original soil sample without metals spikes (data not showed) and concluded that the initial amount of metals in each fraction was negligible.

#### 8.2.6. XAS experimental details

The powdered and dried replicates of each sample (1.00g) were mixed and homogenized to produce a composite sample for analysis by X-ray absorption spectroscopy (XAS) at the Zn *K*-edge. Due to limited beamline time for data acquisition and previously analysis of chemical fractionation, we used samples that were incubated for 20d.

The XAS spectra were recorded on 12-BM-B beamline at the Advanced Photon Source (APS; Argonne National Laboratory, Lemont, IL). Energy calibration used the corresponding Zn metal foil. Spectra were collected in fluorescence mode at the Zn K-edge (9659 eV) and at room temperature using a 13 element Ge solid-state detector (Canberra) with a Al foil in detection window to suppress the Fe florescence from the sample. Each composite sample was loaded into a sample holder with Kapton tape. Multiple scans (4–6) were collected for each sample to improve signal:noise. The scans obtained for each sample were energy calibrated and averaged. Extended X-ray absorption fine structure (EXAFS) regions were used for data analysis. The standard spectra were kindly provided by Dr. Matt Siebecker and Dr. Donald Sparks (University of Delaware), and were previously reported (Nachtegaal et al., 2005; Nachtegaal and Sparks, 2004).

#### 8.2.6.1. EXAFS Refinament

The EXAFS data refinement processing and fitting were performed using the program WinXAS 3.1. The  $\chi(k)$  functions were extracted using a cubic spline and were Fourier transformed with k<sup>3</sup>-weighting over the approximate k range about 2-10 Å<sup>-1</sup>. Theoretical backscattering paths were calculated with FEFF7 (Zabinsky et al., 1995) in combination with ARTEMIS (Ravel and Newville, 2005) based on the crystal structure of zincite (ZnO).

For fitting parameters of the first-neighbor O shells, the first fit trial was fixed the amplitude reduction factor  $(S_0^2)$  was set at 0.85 to reduce the number of free parameters in the fitting procedure. Also, the values of Debye-Waller factors ( $\sigma^2$ ), radial distance (R(Å)) and coordination number (CN) were initially estimated for suitable values (0.005, 2 and 6, respectively). Then, a new fitting was repeated using the values founded first but without to fixing the initial values of S02. Error estimates are ±0.02Å for the R values of the first shells (Scheidegger et al., 1997). For CN, which is correlated to the Debye-Waller factor, the error is estimated at ±15% for the first O shell (Elzinga et al., 2009).

# 8.2.6.2. EXAFS analysis by PCA, TT, and LCF

Principal component analysis (PCA) and target transforms (TT) were performed in soil samples and Zn standards, respectively, in k<sup>3</sup>-weighted  $\chi$  functions between k(Å) range values of 2 to 9 Å<sup>-1</sup> using the SixPACK software (Webb, 2005). Hence, to determine the species present in the soil sample, a dataset of spectra from the eight samples were analyzed using principal component analysis (PCA) (Beauchemin et al., 2002; Wasserman et al., 1999).

The PCA technique determines if the data can be described as weighted sums of a smaller number of components, which would be the case if each point in the dataset is comprised of a smaller number of distinct compounds (McNear et al., 2007). The number of components required to reproduce the entire set of spectra excluded the experimental noise was determined based on the empirical indicator function (IND) (Malinowski, 1977). The IND achieve a lowest value when part mainly components (i.e., combination of chemical species spectra) and marginal components (i.e., background signal) in PCA (Kirpichtchikova et al., 2006a).

Followed this step, we applied the TT approach, which is used to determine the aptness of each reference spectra to describe the experimental data identified by PCA (Jacquat et al., 2008). The standards spectra are evaluated for their "goodness of fit" by the higher coefficient of determination ( $R^2$ ) between standards and TT spectra and SPOIL value

(Malinowski, 1978). SPOIL measures the degree to which replacing an abstract component with the reference would increase the fit error. This is a positive dimensionless number for which values of 1.5 are considered excellent, 1.5 to 3 are good, 3 to 4.5 are fair, 3.5 to 6 are poor and > 6 are unacceptable (Nachtegaal et al., 2005). See TT adjustment fitting in supplementary material (Figure 1SM).

The combinatorial linear combination fits (LCF) analysis were performed in Athena software (Demeter 0.9.16) (Ravel and Newville, 2005). LCF was carried out by calculating all possible combinations of TT-suggested standards considered until fair (SPOIL  $\leq$  4.5), also in k<sup>3</sup>-weighted  $\chi$  functions and k(Å) range between 2 to 9 Å<sup>-1</sup>. The goodness of fit was determined by R-factor. Fits with smallest R-factors were used.

#### 8.3. RESULTS AND DISCUSSION

#### **8.3.1.** Soil Physicochemical Properties

The granulometric fractions distributions and mineralogical proportions had a distinct variation because of site collections, and then highlighted mainly the diversity of parent material for soil formation for each site collected (Table 1). The massive presence of gibbsite in clay fraction reflected the accented stage of weathering especially for RP site. Noteworthy that we just can quantify about 85% of total minerals in the clay fraction, others minerals as 2:1 clays, anatase, quartz, as suggested XRD analysis (see supplementary material, Figure 2SM), as well as low-crystalline material (e.g. ferrihydrite, amorphous aluminum oxides) should be present in minor proportion. Although the minor contribution, both no-quantified compounds in clay fraction could present high metal capacity, which will be discussed afterward.

Sampla	ъЦ	Р	K	Р	Κ	S	С	Ca <sup>2+</sup>	$Mg^{2+}$	$Al^{3+}$	H+A1	SB	CEC	V
ID	$H_2O$	Res	sin m	Me g dm <sup>-3</sup>	hlich-I		g kg <sup>-1</sup>			mme	$pl_c dm^{-3}$			- % -
Gu(nv)	4.5	6	110	3	80	2	62	29	13	21	145	45	196	23
Gu(ag)	5.3	30	136	1	135	12	38	82	16	2	80	101	180	56
Ta(nv)	5.4	39	82	16	77	6	15	37	13	1	56	52	101	52
Ta(ag)	6.7	123	144	47	156	8	17	67	22	1	14	89	103	86
RP(nv)	5.8	24	98	4	86	21	30	42	12	10	52	57	117	49
RP(ag)	5.9	141	117	74	156	35	24	57	9	0.1	58	69	124	56
Pi(nv)	4.6	18	89	2	60	8	42	44	8	4	90	54	145	37
Pi(ag)	6.1	123	136	37	139	8	21	46	14	0	35	63	95	66
	Gra	inulometri	c fraction	S		Mine	rals <sup>2</sup>		0	xalate pH 3			Zn	
	Sand	Silt	Cl	ay	Kao	Gib	Goe	Hem	Fe	Al	Mn	Original <sup>3</sup>	1d	20d
		9	6			g k	kg <sup>-1</sup>				mg ]	kg <sup>-1</sup>		
Gu(nv)	5	32	2	63	326	31	162	13	157	160	30	23	302	336
Gu(ag)	4	27	7	69	371	33	158	13	187	158	17	32	456	502
Ta(nv)	60	12	2	28	190	18	29	32	32	41	9	26	340	330
Ta(ag)	58	12	2	30	197	26	29	34	62	76	6	30	862	912
RP(nv)	22	22	2	56	156	306	12	4	22	32	1	42	621	633
RP(ag)	27	26	5	47	144	261	12	4	19	29	2	63	710	748
Pi(nv)	49	10	)	41	251	94	34	2	32	96	6	18	364	391
Pi(ag)	53	4		43	265	102	34	2	36	117	3	36	572	613

**Table 1.** Chemical, physical and mineralogical properties of the studied soils<sup>1</sup>.

<sup>1</sup> Standard deviation < 5%, n = 3.

 $^2$  The minerals were quantified in fraction <2  $\mu$ m; Kao=Kaolinite, Gib=Gibbsite, Geo=Goethite, Hem=Hematite

<sup>3</sup> The extraction followed EPA 3051a protocol, see more details in Chapter 7.

The homogeneity for these properties between the land uses secure that the chemical differences and soil behavior was created from soil management. The soils under native vegetation had low pH in water (4.5-5.8), low basic cations (45-52 mmol<sub>c</sub> kg<sup>-1</sup>) and P availability (6-18 mg dm<sup>-3</sup>), which are characteristic chemical properties of highly weathered soils. Conversely, agricultural soils samples present increases of these chemical characteristics. We highlighted the substantial increments in P availability (30-141 mg dm<sup>-3</sup>), which was reached over long-term fertilization practices.

The total Zn content were  $<63 \text{ mg dm}^{-3}$ , so above of environmental threshold limit (450 mg dm<sup>-3</sup>) established by Brazilian Environmental Agency (CONAMA, 2009) for remediation practices . However, there was an increment in metal content in agricultural soil in relation soil under native vegetation. Followed by increment in metal content, agriculture soils had considerable increment in Zn adsorption capacity (see details in the Chapter 7).

# 8.3.2. Zn chemical fractionation

The chemical procedure separated Zn-bearing fractions, which had affinity for different extractants solutions (Figure 1).



**Figure 1.** Relative distribution of total Zn in the fractions F1-F5 from soil. See details of fractionation procedures in table 2.

Overall, the mobile fraction (F1+F2) had an expressive contribution (22-61%) like a contaminated soil presented by (Voegelin et al., 2008), instead of geogenic Zn that is extracted in later steps. There was a weak tendency of a decrease in mobile fractions after long time incubation and concomitant increase in residual fraction. The easily soluble fractions are related to a potential metal mobility, which had relevance for leaching process and bioavailabity studies, whereas residual fraction is strongly retained to soil colloids.

Zhang et al. (2017) used soils with a wide range of different properties, and observed that after aqueous Zn was spiked to soils, it rapidly was partitioned between solution and the solid phase, which can be attributed to surface precipitation. Sayen and Guillon (2014) observed a decrease in Zn mobility in a calcareous soil over time by leaching experiments and a metal redistribution occurred from exchangeable sites to stronger binding sites, mainly from organic matter to primarily iron oxy(hydr)oxides. Using acid soils and ZnO and ZnS as contaminants, (Voegelin et al., 2011b) showed the decrease in Zn extractability in 0.01 M CaCl<sub>2</sub> concomitantly with the contaminants dissolution and formation of new Zn phases as layered double hydroxides (LDH), however, the main phases were Zn adsorbed on hydroxylinterlayered clay minerals and other species of Zn octahedrally coordinated.. The fractions F3 and F4 were not consistently affected by the incubation time.

Soil management practices had a strong influence in Zn distribution over the chemical fractionation. The new sites for Zn adsorption summed to precipitated forms due to higher pH values (e.g ZnOH;  $Zn_5(OH)_6(CO_3)_2$ ) could explain the increment in the metal retention in agricultural soil (Papadopoulos and Rowell, 1989) or , ,. However, as well as aging effect, agriculture practices decrease the mobile fraction (F1+F2), despite the highest initial Zn loading (Table 1), as suggested study by Xiang et al. (1995). Indeed, there was a increment of the proportion of residual fraction, notably in RP site. These results suggested a fast process that decreases Zn mobility and guides a new phase formation, which is strongly retained to the soil.

# 8.3.3. Zn speciation using XAS Spectroscopy 8.3.3.1. EXAFS refinement

The diverse chemical setting implies shifts in local coordination numbers, ligand types, and interatomic distances (Roberts et al., 2003). The EXAFS refinement disclosured some information about Zn surround environmental. The first-shell was dominated by the O as first neighbor (Figure 2a) responsible to strong oscillation. However, there was a considerable contribution of a second-shell, which performs a small oscillation around 2.66 Å

(Figure 2b) which could depict several options of neighbors as Zn, Al, Fe or Si (Scheinost et al., 2002) as well noise signal.



**Figure 2.** (a) The  $k^3$ -weighted  $\chi(k)$  of  $Zn^{2+}$  adsorbed in soil samples, (b) the corresponding Fourier transforms (not corrected for phase shift). The solid lines representing the experimental data and the dotted red lines the O-shell fit best fits. See adjusted fit parameters in table 3

The distance from Zn to O in first-shell is a good predictor of the Zn coordination (Waychunas et al., 2002). Zn–O bond lengths of  $\approx$ 1.96 Å typically represents 4-fold coordination, while Zn–O bond lengths of  $\approx$ 2.08 Å is typical of 6-fold coordination (Waychunas et al., 2002), which is also found for aqueous Zn<sup>2+</sup> spectra results (Scheinost et al., 2002). Under normal environmental conditions, Zn is tetrahedrally coordinated (e.g. hopeite (Zn-phosphate), Zn-sorbed ferrihydrite). For this coordination, EXAFS spectra are dominated by the first-shell Zn–O signal (figure 2) and could are reasonably similar (Jacquat et al., 2008), while adsorbed Zn species and aqueous are octahedrally coordinated. Adsorbed Zn could occur in tetrahedral or in octahedral coordination on organic and inorganic

substrates, depending on the type of sorbent and on surface coverage (Voegelin et al., 2005). See more detail in Roberts et al. (2003) and Waychunas et al. (2002).

Similar values were found for radial structure functions, about 2.03 Å, of Zn-O distances for all samples (Table 2). Nachtegaal and Sparks (2004) evaluated Zn adsorption on kaolinite, goethite and their mix as a function of pH, aging time, surface loading, and the extent of goethite coating. The authors concluded that the coordination number was approximately 6-fold coordination in all sorption samples, hence suggesting that Zn is octahedral geometry regardless the condition, similar to our results. Basically, Zn was retained in the soil in several phases, and then the atomic neighbor's signature should be overlapped, which damages the assessment of each phase.

**Table 2.** Zn *K*-edge EXAFS refinement results from first shell (Zn-O) fitting of Zn reference compounds and soil samples after Zn adsorption.

Sample	${{\bf S}_{0}}^{2}$	CN	R(Å)	$\sigma^2(\text{\AA}^2)$	Reduced $\chi^2$	$\Delta E_0$
Gu(nv)	0.82	5.8	2.05	0.010	1464	+1.88
Gu(ag)	0.88	6.3	2.04	0.013	1417	+0.85
Ta(nv)	0.89	5.5	2.03	0.010	21833	+1.62
Ta(ag)	0.91	5.9	2.01	0.013	23628	-0.02
RP(nv)	0.85	6.0	2.02	0.012	2186	+0.25
RP(ag)	0.89	5.4	2.03	0.012	10369	+1.84
Pi(nv)	0.85	6.2	2.03	0.011	1562	-0.43
Pi(ag)	0.86	6.1	2.01	0.012	1817	-0.41
Reference						
Zn-Goethite	0.95	6.72	2.06	0.012	36241	+0.64
Zn-Ferrihydrite	0.83	5.87	1.99	0.009	458	-0.61
Zn-Kaolinite	0.87	6.17	2.07	0.009	938	+1.46
Zn-Gibbsite	0.85	6.00	2.01	0.013	2386	+0.84
Zn-Humic Acid	0.88	6.22	2.06	0.011	5246	+0.77
Zn-Vermiculite	0.87	6.15	2.05	0.012	763	+0.09
Hopeite	0.84	4.99	1.97	0.010	10591	-0.18

 $S_0^2$  = amplitude reduction factor; CN = coordination number; radial structure functions or R = inter-atomic distance (±0.02Å for first shell; Scheidegger et al., 1997);  $\sigma^2$  (Å<sup>2</sup>) = Debye Waller factor;  $\Delta E_0$  = phase shift.

Other possible variable is the dried condition used for data acquisition. (Manceau and Gates, 1997) found a reduction from 6 to 4-fold coordination of surface Fe atoms as a result of dehydroxylation of ferrihydrite when there was a dried state. Molecules changes between wet and dry conditions are commonly reported in Infrared spectroscopy (Kang et al., 2008; Kang and Xing, 2007; Paul et al., 2005).

#### 8.3.3.2. PCA, TT, and LCF of EXAFS

The Zn K-edge EXAFS spectra of reference compounds used here (Figure 3) demonstrated spectral features among differences. Some feature could be used as a visual "fingerprint" of some reference. For example, Zn adsorbed on vermiculite the exhibited a splitting of the first EXAFS oscillation near 3-4 Å<sup>-1</sup> (Figure 3). The previous visual inspection should help to selected references that are good or wrong candidates to posterior fitting (Voegelin et al., 2005).



**Figure 3.** Zn K-edge EXAFS spectra of standards selected for LCF fitting. The standards represents  $Zn^{2+}$  adsorbed on soil minerals, humic acids, precipited zinc phosphate mineral and aqueous ions. See details in (Nachtegaal and Sparks, 2004) and (Nachtegaal et al., 2005).

PCA suggest that two principal components were enough to replicate the data set of the soil sample (Table 2SM), hence we used the two first components to evaluate standards fitting by TT approach. The TT indicated that the best candidates were Zn adsorbed on goethite; kaolinite pH 5, ferrihydrite, gibbsite pH 7, more fulvic and humic acids followed the

lower SPOIL values ( $\leq$  3). Secondary options are the references of Zn sorbed on vermiculate and hopeite (SPOIL values  $\leq$  4) (Supplementary material, Figure 3SM). The conclusion is that the appropriate reference spectra suggested by TT was higher than the minimum number of principal components (#2) from PCA (Table 2SM). The same event was verified by (Jacquat et al., 2008) and (Panfili et al., 2005).

Some references are too similar to be distinguishable from each other, and therefore the number of suitable standards (as determined by TT analyses) may exceed the number of standards identified by IND. Species that are present but always occur at roughly similar proportion in all samples (as the Zn-kaolinite, Figure 4) are not identified by the IND, but are still needed to get a good LC fit of the spectra. Indeed, Zn sorbed in fulvic and humic acids should have similarities for Zn adsorption binds, mainly on organic compounds. We chose humic reference rather be fulvic acid for posterior LCF due to better adjustment of individual "fits" for each soil sample (data not showed).



**Figure 4.** (a) Normalized Zn K-edge EXAFS spectra with LCF model fits over the energy range from 9640 to 9740 eV. Black line is the experimental data and red dotted line show the LCF model fits. (b) Zn speciation based on EXAFS LCF. All fits reached R-Factor < 0.14.

The LCF results are described in Figure 4. The standards proportion of LCF fit is presented in Figure 4b. The goodness-fit quality and details are presented in Table 3SM. The small R-factor number and the sum of all fractions close to 1 obtained in LCF results confirm that the LCF approach was suitable for data analysis. The presence and the proportion of standards reached by LCF results followed the soil mineralogical composition (Table 1). Hence, that ensures the robustness and efficiency of the methodology applied for spectrum analysis (PCA, TT, and LCF).

The sample from Gu site was the only one that had expressive fraction of "Zn sorbed Vermiculite" ( $\approx$ 27 %), similar to Jacquat et al. (2009) results, that founded a substantial fraction (29–84%) of Zn adsorbed on HIM in contaminated soil. The presence of this mineral was not confirmed. However, in XRD data of soil clay fraction ( $\leq$  2 µm) we can find minerals which have d-spacing  $\approx$ 14 Å (Figure 2SM), which are features of phyllosilicates 2:1 types (Pansu and Gautheyrou, 2006). The most probable was hydroxy-Al interlayered mineral (HIM) as vermiculite (Inda et al., 2010) or smectites (Caner et al., 2014), which are resistant to weathering process and persistent in soil systems even in advantages weathered soil process (Dixon et al., 1989).

The soil cultivation promoted the decrease in "Zn-Humic Acid" contribution in Gu (23 to 13%) Pi (41 to 33%) (Table 5) and which followed the considerable decrease ( $\approx$  39 and 50%, respectively) in the C content (Table 1). Hence, the organic compounds availability for Zn-adsorption decreased. Noteworthy, the Zn pointed as organic associated by wet-chemical fractionation was not sensitive for this shift. The association between low soil organic C content due to agriculture practices and the decrease Zn-organic fraction was not clear in Ta site, where even keeping a similar C content, the Zn-organic fraction went down in agriculture area. The specificity of organic compounds to Zn-association and their degradation by agriculture practices could be an explanation for that. However, furthers studies are desirable for this proposal.

The increment in "Zn-Kaolinite" (from 29 to 41%) fraction for Gu(ag) confirmed the preference to aluminum-silicates mineral association like kaolinite (Nachtegaal and Sparks, 2004), even in RP sites, where the kaolinite contribution for the clay fraction composition was lower (Table 1). Curiously, the "Zn-Ferrihydrite" had a strong contribution for Zn speciation. The considerable presence of ferrihydrite is unlikely in highly weathered soils as the studied here. The unique tetrahedrally coordinated standard used here was the Zn-Ferrihydrite (Table 3).Maybe other tetrahedrally coordinated species that were not added as standards could overestimate the Zn-Ferrihydrite proportion.

The crucial difference for Zn speciation introduced after the vegetal production was the presence the "Zn-P" species, average  $\approx 16\%$ , represented by the Hopeite mineral. The exception was Gu(ag) site, likely due to lower P availability (Table 1) than other agriculture soils here tested. Phosphate fertilizers are applied to decrease Zn mobility and others metals (Cao et al., 2003) in soil contaminated as remediation's practices, since that this promotes a metal-phosphate precipitation., . After treating a Zn/Pb contaminated soil, Baker et al. (2012) found a considerable proportion of "hopeite" both by  $\mu$ -EXAFS and  $\mu$ -XRD analysis. Here, we described the inverse process, because we applied Zn and ended up immobilized phosphate compounds.

Pérez-Novo et al. (2011) concluded that Zn adsorption in acid soils is higher in the presence of P, altering the distribution of Zn between site types. Ours results (Chapter 7) showed increment about 50% in Zn adsorption. Beyond that, the results of wet fractionation pointed out a lower mobile Zn fraction of agricultural soils than soils under native vegetation. Basically, adsorbed P increases the soil negative charge net (Li and Stanforth, 2000), which should increase the Zn adsorption capacity, by both inner-sphere (specific sites) and outersphere (electrostatic interaction) (Barrow, 1987)..Beyond the direct Zn-P mineral precipitation or adsorption on surface sites, there was a phosphate and Zn<sup>2+</sup> association commonly called as "ternary complex". This process is an important and efficient mechanism to increase the adsorption for both elements. Mechanistic approaches were applied by Liu et al. (2016) that evaluated the phosphate and Zn<sup>2+</sup> co-adsorption on ferrihydrite surface, and concluded that phosphate-bridged ternary complexes ( $\equiv$ Fe-P–Zn), incremented the total Zn adsorption five times at higher P loading. Similar results are described for  $\gamma$ -Al<sub>2</sub>O<sub>3</sub> system using and phosphate (Ren et al., 2015a) or even myo-inositol hexakisphosphate (Yan et al., 2018b).

#### 8.4. Environmental implication

Lands under vegetable crops in weathered tropical soil evaluated here already presented higher pH and low C content than native forest soils, beyond considerable P availability, since that elevated P fertilizers application are required to reach satisfactory P availability. Soil plays a substantial mission in the behaviors, fate, and toxicity of PTE in the environment contaminated sites as mining areas and or natural rich areas but also in soils destined to vegetable crops. When land use change from native vegetation to highly fertilized agricultural soil, aluminum-silicates minerals are notable Zn-sink regardless of soil origin and

land use. The tendency is Zn-organic matter fraction decreases and Zn associated with mineral phase and P increases.

Currently, many agriculture practices prescribe the maintenance and improvement of soil organic C. No-tillage, incorporation of organic residues (e.g. manure, sewage sludge etc.) and biochar are strategies for preserving soil functions, incrementing soil fertility and increasing C stocks. The consequences should be beneficial for agriculture production. Nevertheless, the Zn behavior and other elements such as Cu, Fe and Mn should be impacted by these management practices. The consequence could be higher metals availability for plants and higher metals mobility along the soil profile. Both consequences deserve further studies.

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# **Supplementary information**



**Figure 1.** Geographic location of the study areas in Brazil. The colors represent Guarapuáva-PR (black), Taquarituba-SP (red), Rio Paranaíba-MG (blue), and Piedade-SP (green). The geographic coordinates are available in Table 1SM.



**Figure 2SM.** Diffraction data from orientated soil clay-fraction (< 2 mm) of soils under native vegetation (dotted line) and under agriculture practices (solid line). The assigned peaks are referents to mineral phases: vermiculite (1.401 and 0.723 nm, likely hydroxyl-interlayered vermiculite), kaolinite (0.723, 0.356, and 0.238 nm), gibbsite (0.482 and 0.435 nm), goethite (0.411 and 0.269 nm), and anatase (\*= 0.350 and 0.238 nm).



**Figure 3SM.** Zn K-edge EXAFS spectra of selected Zn references (black lines) and target transforms (red line) calculated with the first two components obtained from PCA.

metal forms			
Fraction	Targeted forms	Extractant	Equilibration
F1	Soluble + exchangeable fraction	10 mL 0.1M MgCl <sub>2</sub>	shake for 1 h at room
			temperature
F2	Easily mobilizable fraction	10 mL 0.11M NaOAc	shake for 8 h at room
	(sorbed and bound at Fe, Al and		temperature
	Mn)		
F3 <sup>a</sup>	Low crystallized Al/Fe/Mn	20 ml of solution (0.175 M	shake for 2h in room
	oxides associated	$NH_4C_2O_4 + 0.1 H_2C_2O_4$ ), pH	temperature at dark
		3.25	
F4 <sup>a</sup>	Organic fraction (bound to soil	$2 \text{ mL } 0.02 \text{M } \text{HNO}_3 + 8 \text{ mL } \text{of}$	Pre-digestion for 4h, at
	organic matter)	30% H <sub>2</sub> O <sub>2</sub> (pH 2)	room temperature,
			eventually manual shake
			Heat to 80°C for 2h,
			eventually manual shake.
		After cooling, 10 mL 0.02M	shake for 30 min
		HNO <sub>3</sub>	
a Adapted from	$M_{a}$ Malformani at al (2000)		

Table 1SM. Scheme of sequential extraction procedures based on Tessier approach and targeted metal forms

<sup>a</sup> Adapted from Malferrari et al. (2009)

Component	Eigenvalue	Variance explained	Cumulative Variance	$IND^1$
#1	64.55	0.541	0.541	0.199
#2	21.65	0.181	0.722	0.159
#3	7.95	0.066	0.784	0.208
#4	6.73	0.056	0.845	0.297
#5	5.84	0.048	0.894	0.481
#6	5.54	0.046	0.941	0.891
#7	4.13	0.034	0.975	2.898
#8	2.89	0.024	1	-

**Table 2SM.** Results from principal component analysis of Zn K-edge EXAFS spectra for eight soil samples.

 $^{1}$ (IND) Indicator Error functions proposed by (Malinowski, 1991). Bold components were selected by IND criteria.

Table 3SM. LCF analysis of soil EXAFS spectra. See fitting in Figure 4.

			Reference co	mpounds				
Sample	Zn- Gibbsite	Zn- Kaolinite	Zn- Vermiculite	Zn- Humic Acid	Zn- Ferriydrite	Hopeite (Zn-P)	Sum (%)	R- factor
			%					
Gu(nv)	11	29	30	22	-	-	92	0.114
Gu(ag)	14	41	25	13	-	-	93	0.139
Ta(nv)	-	34	-	9	51	-	94	0.140
Ta(ag)	26	24	-	-	40	13	103	0.078
RP(nv)	26	41	-	-	41	-	108	0.120
RP(ag)	22	36	-	-	33	25	116	0.108
Pi(nv)	-	35	-	41	15	-	91	0.070
Pi(ag)	-	35	-	33	19	11	98	0.088