Radical change of Zn speciation in pig slurry amended soil: key role of nano-sized sulfide particles

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ABSTRACT

Spreading livestock manure as fertilizer on farmlands is a widespread practice. It represents the major source of heavy metal(loid)s (HM) input in agricultural soils. Since zinc (Zn) is present at high concentrations in manure, it poses special environmental concerns related to phytotoxicity, groundwater contamination, and introduction in the food chain. Therefore, investigations on the fate and behavior of manure-borne Zn, when it enters the soil environment, are necessary to predict the environmental effects. Nevertheless, long-term field studies assessing Zn speciation in the organic waste matrix, as well as within the soil after manure application, are lacking. This study was designed to fill this gap. Using SEM-EDS and XAS analysis, we reported the following new results: (i) ZnS made up 100% of the Zn speciation in the pig slurry (the highest proportion of ZnS ever observed in organic waste); and (ii) ZnS aggregates were about 1- μ m diameter (the smallest particle size ever reported in pig slurry). Moreover, the pig slurry containing ZnS was spread on the soil over an 11-year period, totaling 22 applications, and the resulting Zn speciation within the amended soil was analyzed. Surprisingly, ZnS, i.e. the only species responsible for a nearly 2-fold increase in the Zn concentration within the amended soil, was not detected in this soil. Based on SEM-EDS and XAS observations, we put forward the hypothesis that Zn in the pig slurry consisted of nano-sized ZnS crystallites that further aggregated. The low stability of ZnS nanoparticles within oxic and complex environments such as the studied soil was the key explanation for the radical change in pig slurry-borne Zn speciation after long-term amendments.

2-LINE SUMMARY

Nano-sized ZnS particles accounted for 100% of the Zn speciation in the pig slurry (PS). PS-borne Zn underwent rapid conversion after spreading on the soil and was subsequently bound to soil minerals and OM.

Keywords: Trace elements; agricultural recycling; environmental impact; spectroscopy; microscopy.

1. INTRODUCTION

There were nearly 1 billion pigs in the world in 2013. In the same year, China was the largest producer, with 475.9 million pigs, followed by the United States (64.8 million) and Brazil (36.7 million) (FAO, 2013). Pig manure is thus produced in massive quantities. In 2008, swine activity generated more than 900 million m³ of pig slurry (PS) in the European Union alone (EC, 2014). Spreading manure as fertilizer on agricultural lands is the most common destination. Nevertheless, soil pollution by nitrate, phosphorus and heavy metal(loid)s (HM) has raised concern (Legros et al., 2013; Mallmann et al., 2012; Hernández et al., 2006). Whereas mobility and transfer mechanisms of the former have been substantially studied and are fairly well understood, the fate of exogenous HM in soils is still under ongoing investigation.

Zinc (Zn) is added to livestock feed owing to its growth and antibiotic attributes (Jondreville et al., 2003). However, most of this Zn is excreted in the manure and subsequently spread on the soil (Revy et al., 2003). Livestock manure currently represent 78% and 51% of total Zn inputs in agricultural soils in France and China, respectively. In China, Zn concentrations in pig manure have nevertheless increased about sixfold from the early 1990s to 2003 (Romeo et al., 2014 and references therein). Increased Zn concentrations may lead to phytotoxicity, introduction in the food chain via accumulation in plant tissues and grains, or groundwater contamination through leaching (Lipoth and Schoenau, 2007; Gräber et al., 2005; L'Herroux et al., 1997).

HM speciation in wastes and waste-amended soils is a key factor in determining their mobility, bioavailability, and potential toxicity in the soil-water-plant system. Speciation takes into account chemical and physical properties such as the element isotopic composition, oxidation state, coordination and molecular structure (Reeder et al., 2006). Therefore,

speciation-based investigations of the fate of organic waste-borne Zn is necessary to better predict the environmental effects of this widespread recycling practice (Tella et al., 2016).

Sequential extraction methods are most used to assess Zn speciation. This approach has been applied to examine organic waste (L'herroux et al., 1997) and organically-amended soils, mostly using controlled bench scale and short-term conditions (Guan et al., 2011; Plekhanova, 2012; Qiao et al., 2003; Hseu, 2006). Only a few studies have involved long-term field experiments (L'Herroux et al., 1997; Asada et al., 2012; Girotto et al., 2010). Although sequential extractions are straightforward and easy-to-use, they have major shortcomings, such as incomplete dissolution of target phases and/or dissolution of non-target species (Doelsch et al., 2008). Therefore, the obtained fractions, and consequently the research findings and interpretation, depend on the chosen extraction procedure.

On the contrary, XAS is a powerful structural technique for direct determination of trace element speciation in complex samples. XAS has been used in a few studies to assess HM speciation within PS (Legros et al., 2010a; Legros et al., 2010b; Lin et al., 2013) and soils amended with both sewage (Kirpichtchikova et al., 2006) and biosolids (Mamindy-Pajany et al., 2014). Nevertheless, XAS studies to assess Zn speciation within soils after PS amendment via long-term field experiments are lacking.

The present study takes into account a unique combination of three approaches. We assessed Zn speciation: (i) in the PS matrix and within the soil that had received PS amendments; (ii) by using a combination of state-of-the-art physical techniques: X-ray powder diffraction (XRD), scanning electron microscopy with X-ray microanalysis (SEM-EDS), and XAS; (iii) in a long-term field experiment (11 years). We considered qualitative and quantitative characterization of each Zn species detected in the PS and within the soil to obtain a comprehensive description on Zn behavior throughout the waste recycling process, from slurry storage until its fate within the soil after 11 years of land application.

2. MATERIALS AND METHODS

2.1 Field experiment and soil characterization

The experiment was conducted in the demonstration field of the *Cooperativa Regional Agropecuária de Campos Novos* (Copercampos), in Campos Novos, Santa Catarina State,

southern Brazil. The basaltic soil is classified as Rhodic Hapludox (USDA, 2003), composed of 67.8% clay, 30.8% silt and 1.4% sand (Mallmann, 2013).

Pig slurry had been spread in field plots at different application rates since the year 2000. A control soil was also monitored, with soil characteristics and crop management identical to those of the PS-amended soil, except for the absence of PS application. For the present study, the control soil and the soil that had received the highest PS application rate – $200 \text{ m}^3 \text{ ha}^{-1} \text{ year}^{-1}$ – were selected for analysis, in order to provide the most marked contrast in Zn concentration between treatments. Moreover, we recently demonstrated that most of the Zn added to the soil via PS amendment had accumulated in the soil surface horizon (Formentini et al., 2015). Samples from the 0–5 cm soil layer were therefore used in the present study. Sampling was performed in June of 2011, after 11 years of soil amendment and 22 PS applications (two per year). Details on the soil characterization, crop rotation, seeding, sampling and Zn concentration through the soil profile may be found elsewhere (Formentini et al., 2015; Mallmann, 2013; Veiga et al., 2012).

2.2 XRD analysis

The mineral composition of the soil was identified with an X-ray diffractometer (X'Pert Pro, Panalytical) running at 40 kV and 40 mA, using Co K α radiation ($\lambda=1.79 \text{ \AA}$) with a linear detector (X'Celerator) and a secondary flat monochromator. Samples were ground in an agate mortar and placed on zero background silicon sample holders with a few drops of ethanol. A counting time of 5.5 s per 0.033° step was used for 2θ in the $4\text{--}80^\circ$ range. Phase identification was performed using XRD data analysis software (X'pert PRO Highscore plus) and its powder diffraction database (ICDD-PDF2).

2.3 Pig slurry characterization

Pig slurry was obtained from an anaerobic lagoon reservoir located on a pig finishing farm in Campos Novos, Brazil. The average physico-chemical characteristics of the PS added to the soil during the experiment were: 7.3 pH, 2.25% dry mass content, and 43.0 g m^{-3} of Zn (Formentini et al., 2015). For the present study, a fresh PS sample was collected from the same anaerobic lagoon reservoir that had provided the samples used in the soil amendments. One

portion of this sample was subjected to a size fractionation procedure, and fractions were dried to assess the dry matter content and Zn concentration in each fraction (Section 2.4). Another portion was subjected to the same particle size fractionation procedure, except that the obtained fractions were not dried and were used in liquid form for SEM–EDS analysis (Section 2.5). Finally, another portion of raw PS was freeze-dried (-53°C), ground with a mortar and pestle, and pressed into pellets for powder EXAFS analysis, along with the soil samples (Section 2.6). The anaerobic conditions in the sample were preserved by collecting it near the bottom of the lagoon, with storage in a sealed plastic container without air bubbles, in the dark at 4°C prior to analysis.

2.4 Particle size fractionation of pig slurry

Filtration thresholds of $20\text{ }\mu\text{m}$, $0.2\text{ }\mu\text{m}$ and 10 kDa were used, leading to four particle size fractions: $>20\text{ }\mu\text{m}$, $20\text{--}0.2\text{ }\mu\text{m}$, $0.2\text{ }\mu\text{m--}10\text{ kDa}$, and $<10\text{ kDa}$. Forty grams of liquid raw PS were passed through a $20\text{ }\mu\text{m}$ nylon net filter (Whatman). The retained solid fraction was dried (60°C , 48 h) and weighed, and it represented the $>20\text{ }\mu\text{m}$ fraction. The filtrate was subjected to tangential flow filtration. The filtration system (KrosFlo, Spectrum Laboratories Inc.) was mounted with a $0.2\text{-}\mu\text{m}$ pore size modified polyethersulfone (mPES) hollow filter and fed at 10 ml min^{-1} . The system was adjusted for a filtration flow of 1 ml min^{-1} (10% of the feeding flow) to allow sample recirculation. The permeate end was then closed and the system was fed with 10 ml of demineralized water for recirculation in order to recover the retentate containing the $20\text{--}0.2\text{ }\mu\text{m}$ -size fraction. This retentate was dried and weighed. The permeate was then passed through a 10-kDa pore size mPES hollow filter, which was mounted on the same filtration system, as a replacement for the previous filter. The same filtration procedure was conducted and the retentate containing particles in the $0.2\text{ }\mu\text{m--}10\text{ kDa}$ range was collected, dried and weighed. Lastly, the permeate containing the $<10\text{ kDa}$ fraction (virtually lacking solid particles) was collected, acidified and stored prior to analysis.

The $>20\text{ }\mu\text{m}$ fraction was not further analyzed due to its low (and insufficient) mass recovery, i.e. below 2% of the PS dry matter. Dried samples of the raw PS (10 g of liquid raw sample were dried at 60°C for 48 h) and the $20\text{--}0.2\text{ }\mu\text{m}$ and $0.2\text{ }\mu\text{m--}10\text{ kDa}$ fractions were subjected to acid digestion according to the EPA 3051A protocol (USEPA, 2007). The digested samples and the $<10\text{ kDa}$ fraction were analyzed for their Zn concentrations using ICP-MS (NexION 300X, PerkinElmer).

2.5 SEM-EDS analysis of the pig slurry

Liquid samples of the 20–0.2 μm particle-size fraction (filtered as described in Section 2.4) were analyzed using SEM–EDS. This fraction was selected because it contained most of the dry matter and Zn content (see Section 3.2). A small portion of sample was sonicated gently and a droplet was deposited onto a holey-carbon-coated 200 mesh Cu-TEM grid. The sample was allowed to dry on the grid for a few hours prior to analysis. The scanning electron microscope (Philips XL30 SFEG, North Billerica, MA) was operated at 15 kV accelerating voltage with a counting time of 60 s per point. Elemental analysis was performed with an energy dispersive spectrometer (Oxford Instruments, Oxfordshire, UK).

2.6 EXAFS

Zinc K-edge X-ray absorption spectra of the PS and soil samples were recorded on the FAME beamline at the ESRF, Grenoble, France. Finely ground samples were pressed into pellets and analyzed at liquid He temperature to prevent sample damage by photoreduction. The signal was acquired in fluorescence mode using a 30 element solid-state Ge detector (CANBERRA Industries Inc.). The spectra were the sum of 5 to 16 scans, depending on the Zn concentration and the noise level. E_0 was set at 9664.6 eV for normalization and extraction of EXAFS spectra. Normalization and data reduction were performed according to standard methods (Doelsch et al., 2006) using Athena software (Ravel and Newville, 2005).

Previously background subtracted and normalized EXAFS spectra were subject to a least-square linear combination fitting (LCF) procedure over the k -range 2–9.9 $\text{\AA}^{-1}$. A library of Zn model compounds was used to identify and quantify the Zn-bearing components in the samples. The residual parameter [$Res = \Sigma (k^2 \chi_{exp} - k^2 \chi_{model})^2 / \Sigma (k^2 \chi_{exp})^2$] and visual analysis of the fits were used as the fit criterion. LCF scenarios using only one and up to five fitting components were successively tested and evaluated based on the *Res* value and by visual observation of the quality and improvement of LCF when adding an extra component to previous best fits. LCFs with $n + 1$ components were retained if the *Res* parameter was decreased by more than 20% as compared to the fit with n components.

The sum of component weights in the best LCFs ranged from 90% to 112%. Deviations from 100% may arise from variations in amplitudes due to overabsorption during spectra recording or from slight differences between the structure of Zn species existing in the samples

and in the reference materials (Jacquat et al., 2009a; Manceau et al., 2002). Linear combination results were normalized to 100% for the comparison of the relative speciation between samples.

The library of Zn model compounds included: (i) primary and secondary Zn minerals and precipitates, i.e. synthetic ZnS nanoparticles (see Section 2.7), bulk ZnS (Aldrich), willemite (Zn_2SiO_4), parahopeite [$\text{Zn}_3(\text{PO}_4)_2 \cdot 4(\text{H}_2\text{O})$], zincite (ZnO), franklinite (ZnFe_2O_4), zinc hydroxide [$\text{Zn}(\text{OH})_2$], smithsonite (ZnCO_3), zinc sulphate (ZnSO_4), zinc phosphate [$\text{Zn}_3(\text{PO}_4)_2$], zinc nitrate [$\text{Zn}(\text{NO}_3)_2$] and zinc aluminate (ZnAl_2O_4); (ii) mineral surface sorption complexes at different loadings, i.e. Zn-HIM (hydroxy-interlayered mineral), Zn sorbed kaolinite, Zn sorbed ferrihydrite, Zn sorbed goethite, Zn sorbed birnessite, Zn sorbed kerolite, Zn-LDH (layered double hydroxide) and Zn sorbed hydrotalcite; and (iii) Zn-organic complexes: Zn-malate, Zn-cysteine, Zn-acetate, Zn-citrate, Zn-phytate, Zn-oxalate, Zn-malate, Zn-methionine, Zn-histidine, Zn-phthalocyanine, Zn-glycine, Zn-galacturonic acid and Zn-pectin. For convenience, only the spectra of model compounds used in the best LCF scenarios to simulate the PS and soil sample spectra were presented and discussed (Sections 3.2.2 and 3.3.2).

2.7 Synthesis and characterization of ZnS nanoparticles

ZnS nanoparticles were synthesized by mixing high purity ZnCl_2 and Na_2S in N_2 -purged deionized water. The Zn^{2+} concentration was set at 0.04 M, and different S^{2-} concentrations were used in order to obtain ZnS particles of different sizes. Three S/Zn molar ratios were used: 0.9, 1.1 and 3.0. After precipitation, the ZnS suspensions were aged for 48 h in the dark, at room temperature, with constant stirring. The final suspension pH levels were 3.6, 5.6 and 12.8, respectively. To remove excess salts, the suspensions were centrifuged at 3488 g for 30 min. The supernatant was then removed and the ZnS nanoparticles were dispersed in 20 mL of deionized water using an ultrasound bath and vortex. To exclude oxygen, the whole procedure was performed in a glovebox containing 100% N_2 .

One portion of each suspension was diluted and 7- μL drops were laid on a 400-mesh carbon film. Particle sizes were obtained after transmission electronic microscopy (TEM) characterization using a JEOL JEM-2010 microscope with a 200 keV field emission source. The ZnS particle sizes were 2.49 ± 0.29 nm, 4.73 ± 1.11 nm, and 5.92 ± 0.64 nm, respectively for the initial S/Zn molar ratios of 0.9, 1.1 and 3.0 (Supporting Information). Hence, the synthesized ZnS nanoparticles are hereafter denoted $\text{ZnS}_{[2.5\text{nm}]}$, $\text{ZnS}_{[4.7\text{nm}]}$, and $\text{ZnS}_{[5.9\text{nm}]}$.

Another portion of each suspension was freeze-dried and the resulting powder was used for XRD analysis and to prepare thin PVP-diluted pellets for XAS analysis. XRD characterization was performed on the beamline 11-ID-B of the Advanced Photon Source (APS) at the Argonne National Laboratory (Lemont, USA). XAS spectra were recorded on the FAME beamline at ESRF, Grenoble, France. The spectra were obtained by merging two scans performed in transmission mode at liquid He temperature.

3. RESULTS AND DISCUSSION

3.1 Zn enrichment within the soil due to PS application

Figure 1 presents the Zn concentration within the 0–5 cm layer of the control and the amended soils, as well as the amounts of Zn added to the soil via PS application. The Zn concentration in the control soil was 100.4 mg kg^{-1} , corresponding to 51.7 kg ha^{-1} (soil bulk density_(0–5 cm) = 1.03 g cm^{-3} ; Mallmann, 2013). The amount of Zn added to the soil over 11 years of PS application, at $200 \text{ m}^3 \text{ ha}^{-1} \text{ year}^{-1}$, was 94.6 kg ha^{-1} . If the whole amount of pedogenic and exogenous Zn were retained within the 0–5 cm layer of the amended soil, the expected Zn concentration would be 146.3 kg ha^{-1} . However, the observed concentration corresponded to 96.9 kg ha^{-1} . This means that the PS additions caused a nearly 2-fold increase in the Zn concentration within the 0–5 cm layer of the amended soil. Excess Zn accumulated in deeper layers, i.e. up to 30 cm (Mallmann, 2013; Formentini et al., 2015).

Figure 1 – Zn concentration within the 0–5 cm layer of the control and amended soils, as influenced by the amount of Zn added to the soil via PS (adapted from Mallmann, 2013 and Formentini et al., 2015).

3.2 Pig slurry

3.2.1 Co-location of Zn and S in sub-micron particle size

The PS dry matter was distributed as follows: 1.9% in the >20 μm fraction, 74.6% in the 20–0.2 μm fraction, and 17.9% in the 0.2 μm –10 kDa fraction. The dry matter content in the <10 kDa fraction was negligible. The mass recovery of the size fractionation procedure was 94.4%. Assessment of the Zn concentration in the >20 μm fraction was not possible, due to its low dry matter content. In the <10 kDa fraction, only the dissolved Zn concentration was measured (9.6 $\mu\text{g L}^{-1}$ of the fraction). The Zn concentration in the 20–0.2 μm fraction was 708.6 mg kg^{-1} of dry matter, accounting for 91.8% of the Zn in the PS. The Zn concentration in the 0.2 μm –10 kDa fraction was 1.7 mg kg^{-1} of dry matter, representing only 0.2% of the total Zn.

The preponderance of Zn in the 20–0.2 μm fraction was in line with previous results. Legros et al. (2010a) reported 75% of Zn in the PS matrix in the 20–0.45 μm size-fraction. Masse et al. (2005) found 80% of PS-Zn in the 10–0.45 μm particle-size range.

Hence, the 20–0.2 μm fraction was selected for SEM–EDS analysis. After extensive examination, the single region on the analyzed sample surface that contained Zn in the elemental composition was selected as presented in Figure 2. The diameter of the two detected particles was about 1 μm (Figure 2b). It should be noted that the particles appeared to have a disordered long-range structure, especially particle #2. One possible explanation is that they consisted of micro-sized aggregates of smaller-(nano-)sized sub-units. Both contained mostly Zn and S in their elemental composition, but also low intensity peaks indicating the presence of Ca and K cations (Figure 2c). The co-location of Zn and S may be attributed to the presence of ZnS, which was previously observed in only one study (Legros et al, 2010a). We conducted EXAFS analysis to test this hypothesis.

Figure 2 – (a, b) Scanning electron microscopy micrograph of the 20–0.2 μm PS fraction; arrows indicate regions selected for energy dispersive X-ray spectroscopy (c).

3.2.2 Nano-sized ZnS particles in PS

The EXAFS spectra of the PS sample presented oscillation patterns in close agreement with the synthesized ZnS references. Figure 3a presents the EXAFS and Fourier transform (FT) spectra of the PS superposed by the spectra of the bulk (Aldrich) and the synthesized nano-ZnS with different particle sizes: $\text{ZnS}_{[2.5\text{nm}]}$, $\text{ZnS}_{[4.7\text{nm}]}$, and $\text{ZnS}_{[5.9\text{nm}]}$ (Section 2.7). The bulk ZnS spectrum was characterized by sharp and high amplitude EXAFS oscillations as compared to the PS sample and the nano-sized ZnS references. This was reflected in the FT spectra, in which the bulk ZnS displayed the highest intensity peaks. In bulk ZnS, the FT peak at 1.9 $\text{\AA}$ (not phase-shifted) indicated that the first Zn coordination shell was consisted of four S atoms located 2.34 $\text{\AA}$ from the central atom (Legros et al., 2010a). The FT peak centered at 3.7 $\text{\AA}$ originated from the Zn-Zn interaction in the second coordination shell, with a bonding distance of 3.87 $\text{\AA}$ and a coordination number of 12 (Roberts et al., 2002). Moreover, the second peak at 3.7 $\text{\AA}$ may have received a contribution from the Zn-S interaction in the third coordination shell.

The lower amplitude, sharpness (EXAFS) and intensity (FT) in spectra of the synthesized nano-ZnS references and the PS sample (compared to the bulk ZnS) may have been due to the greater proportion of under-coordinated Zn atoms located at the particle surfaces, causing a decrease in the average number of Zn neighbors. This is confirmed in the

second FT peak (centered at 3.7 Å) in Figure 3a, in which the peak intensity declines with the ZnS particle-size.

The spectral features of the PS sample and the ZnS_[4.7nm] reference presented the most pronounced similarity, both in the EXAFS and FT. This was confirmed by the LCF procedure. The best LCF for the PS sample was nearly perfectly adjusted to the sample spectra and was achieved by using only one component: ZnS_[4.7nm] (Figure 3b).

Figure 3 – (a) k^2 -weighted Zn K-edge EXAFS and FT spectra of the PS sample compared to bulk and nano-sized ZnS; (b) 1-component LCF for the PS sample.

Although EXAFS analysis may not be conclusive on particle-size determination, it may be assumed that the ZnS phase detected here in the PS had a relatively disordered structure compared to the bulk ZnS. We put forward the hypothesis that these ZnS particles were nano-sized. This is in agreement with our SEM-EDS analysis that showed the co-location of Zn and S in 1-μm units that should have been formed by aggregation of nano-sized particles (Section 3.1.1; Figure 2). A very similar trend was recently observed in a sewage sludge product (Kim et al., 2014). The authors used TEM-based techniques to report ZnS nanocrystals with a 2.5–7.5 nm diameter range, forming aggregates of a few hundred nm. Moreover, in the latter study, the LCF of the sewage sludge spectra was consistent with the Zn local structure of a 3-nm ZnS reference material.

A few studies have been conducted on Zn speciation in PS, notably via sequential extractions. L’Herroux et al. (1997) reported a mixture of Zn bound to carbonate and Zn bound to oxides, whereas Miaomiao et al. (2009) found a mixture of Zn bound to oxides, Zn bound to organic matter (Zn-OM), and Zn in the residual fraction. Legros et al. (2010a), in a study

involving combined SEM-EDS and XAS analysis, reported for the first time the presence of ZnS particles in the PS matrix, along with Zn-OM and amorphous Zn hydroxides. The ZnS contribution to Zn speciation was 14%, and particles were about 5- μ m diameter.

To our knowledge, the present study is the first: (i) in which ZnS accounted for 100% of the Zn speciation in the PS matrix, and (ii) to provide evidence that ZnS in the PS was nano-sized. The anoxic environment within PS lagoons is consistent with the formation of Zn sulfides by two possible mechanisms: chemical precipitation or bacteria-assisted sulfate reduction (Legros et al., 2010a). We attribute our novel findings to the special care we took to preserve the sample anaerobic conditions during the sampling and storage procedures (Section 2.3). This was not the case for other studies in which samples were vigorously shaken (L'Herroux et al., 1997), collected from a forced-aeration treatment process (Miaomiao et al., 2009), or in which no specific care was taken concerning sample oxidation (Legros et al., 2010a). Note also that precipitation of nano-sized ZnS particles might be favored in PS because of the high amount of OM that likely prevents the growth of ZnS particles by sorbing on their surfaces.

Furthermore, the PS containing 100% ZnS was spread on the soil over an 11-year period, totaling 22 applications. The resulting Zn speciation within the amended soil, as compared to the control soil and the PS, was then explored via EXAFS and XRD analysis.

3.3 Soil

3.3.1 Clays dominate the soil mineralogy

The X-ray diffractogram of the bulk soil is shown in Figure 4. The soil mineralogy was dominated by the phyllosilicates vermiculite and kaolinite, the aluminum hydroxide gibbsite, the Fe oxide hematite, and quartz. Therefore, vermiculite, kaolinite and hematite species were logical candidates with respect to potentially bearing Zn and were considered in the EXAFS analysis of the soil.

Figure 4 - X-ray diffractogram of the studied soil ($\lambda=1.79\text{\AA}$).

3.3.2 ZnS was not detected in the amended soil

The k^2 -weighted Zn K-edge EXAFS and FT spectra of the control soil and the amended soil, as compared to Zn sorbed on the selected references, are shown in Figure 5. The references presented in Figure 5 were selected from the large database of Zn reference materials presented in Section 2.6 based on the most representative Zn species used in the LCF procedure (to be presented in this section), while taking the soil composition into account (Section 3.3.1).

Both the control and amended soils displayed marked splitting in the first EXAFS oscillation, at $3.75\text{ \AA}^{-1}$ (dashed line “a”), coincident with the Zn-kaolinite reference. In this reference, splitting was due to the Zn-Al interaction in the second coordination shell, when Zn occupied dioctahedral vacancies in gibbsitic hydroxy-Al polymers. The Zn-Al interaction was reflected by the FT peaks at $2.6\text{ \AA}$ and $5.5\text{ \AA}$ (not phase-shifted, dashed lines “b” and “c”, respectively) (Jacquat et al., 2009a; Jacquat et al., 2009b; Schlegel & Manceau, 2007). Spectral signatures “a”, “b” and “c” were coincident in the soil samples and in the Zn-kaolinite reference, indicating that Zn-kaolinite may have dominated Zn speciation in the soil samples.

The spectra of Zn-ferrihydrite presented distinct oscillation patterns, reflecting the different Zn local environment in this Fe oxyhydroxide. The FT peak near $1.5\text{ \AA}$ (not phase-shifted, dashed line “d”) was slightly shifted to a shorter distance as compared to the Zn-kaolinite reference (dashed line “e”). Complexes on the ferrihydrite surface had Zn-O distances of around $1.97\text{ \AA}$ and coordination numbers consistent with tetrahedral oxygen, as opposed to Zn-O distances of around $2.11\text{ \AA}$ in octahedral coordination. Moreover, the FT peak at $3.2\text{ \AA}$ (not phase-shifted, dashed line “f”) arose from the Zn-Fe interaction in the second coordination shell, at $3.44\text{ \AA}$ distance (Waychunas et al., 2002). Therefore, Zn-ferrihydrite was

used as a proxy to represent tetrahedrally coordinated adsorbed/complexed Zn on Fe (oxyhydr)oxides (Voegelin et al., 2011), denoted “Zn-Fe (oxyhydr)oxide” hereafter.

In the Zn-malate complex, the first Zn-O coordination shell was octahedral, and the second shell was composed of C and O atoms. Since Zn-malate is a common organic acid present in soils, it was used as LCF proxy to account for Zn bound to organic matter (Sarret et al., 2002; Jones et al., 1996), denoted “Zn-OM” hereafter.

Figure 5 – k^2 -weighted Zn K-edge EXAFS and FT spectra of the control and amended soils as compared to the selected references.

The spectra of the control soil and the amended soil, superposed by their best LCF using the Zn reference database (Section 2.6), are shown in Figure 6. The best LCF for the control soil using two components used Zn-kaolinite and Zn-Fe (oxyhydr)oxide. Adding a third component (Zn-OM in the best fit) did not lead to a better reproduction of the sample spectra, and the decrease in the *Res* parameter was below 20%. Hence, the two-component LCF was the best solution. We attributed the contribution of the Zn-Fe (oxyhydr)oxide proxy reference to Zn-hematite, supported by the XRD observation of hematite in the soil composition (Section 3.1).

The LCF for the amended soil using two components was also achieved by combining the same references as in the control soil: Zn-kaolinite and Zn-Fe (oxyhydr)oxide. Nevertheless, adding a third component to the fit (Zn-OM) improved the reproduction of the sample EXAFS spectrum, notably in the first half of the main oscillation (pointing arrow “a”). Moreover, the

resulting FT spectrum in the three-component scenario showed an increased amplitude in the first peak, followed by a decrease and a shift to a higher distance in the second peak as compared to the two-component scenario (pointing arrows “b” and “c”). The visual improvement in the quality of the LCF when adding the third component was confirmed by the more than 20% decrease in the *Res* parameter as compared to the two-component LCF. Hence, the three-component LCF was the optimal solution.

Figure 6 – k^2 -weighted Zn K-edge EXAFS and FT spectra of the control and amended soils, and the optimal LCF for each sample.

The resulting Zn speciations in the control soil, amended soil and PS are presented in Figure 7. The concentration of each Zn species within the soil samples was obtained by multiplying the normalized LCF proportions with the total Zn content in each soil (Section 3.1). The control soil ($Zn = 100.4 \text{ mg kg}^{-1}$) contained 70% Zn-kaolinite (70.3 mg kg^{-1}) and 30% Zn-Fe (oxyhydr)oxide (30.1 mg kg^{-1}). The amended soil ($Zn = 188.1 \text{ mg kg}^{-1}$) contained 54% Zn-kaolinite (101.6 mg kg^{-1}), 27% Zn-Fe (oxyhydr)oxide (50.8 mg kg^{-1}) and 19% Zn-OM (35.7 mg kg^{-1}).

Hence, Zn species originally present in the control soil [Zn-kaolinite and Zn-Fe (oxyhydr)oxide], plus Zn-OM (not detected in the control soil) were responsible for the assimilation of exogenous Zn, in the following proportions: 36% Zn-kaolinite, 23% Zn-Fe (oxyhydr)oxide and 41% Zn-OM. It should be stressed that the ZnS contained in the PS was the only exogenous source of Zn within the amended soil. It caused a Zn concentration increase from 100.4 mg kg^{-1} in the control soil to 188.1 mg kg^{-1} in the amended soil (0–5 cm layer; Figure 1). However, this species (ZnS) was not detected in the amended soil. Besides the LCF

procedure, visual comparison between the spectral features of the ZnS-containing PS sample (Figure 3) and the amended soil (Figure 5) clearly confirmed the radical change in PS-borne Zn speciation after spreading on the soil. None of the EXAFS or FT spectral features coincided when comparing these two samples (PS vs amended soil). This rules out any possible underestimation or misinterpretation of the potential contribution of the PS-borne ZnS to Zn speciation in the amended soil.

Figure 7 – (a) Zn speciation within the control and amended soils and the PS; and (b) the resulting exogenous Zn distribution.

3.4 Environmental implication

The solubility constant of sphalerite (i.e. ZnS) is 2.93×10^{-36} (Robert et al., 1988). It would therefore be expected to be highly insoluble, although the solubility may be influenced by the media composition, pH and redox conditions (Moreau et al., 2004; Martínez & Motto, 2000). Indeed, particulate transfer of micrometer-sized ZnS was the predominant mechanism of Zn propagation within the soil 18 months after spreading a ZnS-rich dredged sediment (Isaure et al., 2005). Nevertheless, the authors observed Zn leaching peaks when the oxidative dissolution of ZnS was favored by non-flooding conditions. Voegelin et al. (2011) reported 26% to 75% dissolution of 25–40-nm crystallite ZnS after 4 years of soil incubation. ZnS dissolution

was slower in acidic soils, as compared to either a near neutral or a calcareous soil. Except for the calcareous soil, ZnS also dissolved more slowly compared to ZnO.

Moreover, reactivity of metal sulfides depends on the particle size, morphology, and crystal structure (Hochella et al., 2008; Liu et al., 2009), which may in turn be related to the formation process. The resistance of ZnS to re-oxidation was in the following order: sphalerite mineral > ZnS formed by SO_4 -reducing-bacteria > chemically precipitated ZnS (Peltier et al., 2011). The anoxic environment within PS lagoons is consistent with the formation of Zn sulfides by either bacteria-assisted sulfate reduction or chemical precipitation (Legros et al., 2010a). Indeed, the findings of full scale, pilot and benchtop studies revealed that Zn sulfidation was dominant during anaerobic digestion of wastewater and in sewage sludge (Kim et al., 2014; Lombi et al., 2012; Ma et al., 2014).

Reducing the size of ZnS nanoparticles from 100 to 1 nm radius theoretically increased their solubility in water by five orders of magnitude (Moreau et al., 2004). Strain-driven distortion has been observed for small disordered ZnS nanoparticles (~3-nm), which could potentially reduce their stability (Gilbert et al. 2004), as for instance observed in submicrometer ZnS phases present in river suspended particulate matter when exposed to oxic environments (Priadi et al. 2012).

Therefore, the radical change in PS-borne Zn speciation following land application observed in this study was consistent with the presence of nano-sized ZnS particles in the PS matrix. This was supported by: (i) our SEM-EDS (Section 3.2.1) and XAS (Sections 3.2.2 and 3.3.2) observations; and (ii) by previous literature data (presented in this section), highlighting the influence of the particle size and structure, as well as the type of environment (e.g. oxic or anoxic), on the stability of such particles.

4. CONCLUSION

In the present study, we: (i) identified, via SEM-EDS, aggregates of about 1- μm diameter in the PS matrix, composed mostly of Zn and S; (ii) reported, using EXAFS, that ZnS accounted for 100% of the Zn speciation, most likely at the nano-scale, thus supporting our SEM-EDS observations; and (iii) verified that the Zn applied to the soil via PS spreading was not present in the form of ZnS, after 22 applications over 11 years, although this was the only source of Zn during the period.

Considering the nature of Zn added to the pig feed (sulfate), the nature of the lagoon used in the pig waste treatment (anoxic), and the results reported here, the most likely explanation for our findings is that: (i) dissolved Zn species reacted *in situ* with reduced S to form ZnS nanocrystals in the anaerobic lagoon; (ii) the formation process (chemical precipitation or bacteria-assisted sulfate reduction) and the nano-scale of the PS-borne ZnS favored the oxidation of this species within the soil, releasing Zn that was subsequently retained by phyllosilicates, Fe (oxyhydr)oxides and OM in the soil.

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SUPPORTING INFORMATION AVAILABLE

TEM images and XRD characterization of synthesized ZnS nanoparticles used as reference materials for the EXAFS analysis of PS.

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Control Soil

(Zn=100 mg kg⁻¹)

Pig Slurry

(Zn=100% nano-ZnS)
22 applications
over 11 years

SEM-EDS

Amended Soil

(Zn=188 mg kg⁻¹)