

# INVESTIGATION OF COPPER AND ZINC SPECIATION IN PIG SLURRY BY A MULTITECHNIQUE APPROACH

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## 1 INTRODUCTION

According to the Food and Agriculture Organization of the United Nations (FAO, 2007), world pig production has risen from around 830.6 million heads in 1997 to some 989.9 million heads in 2007, which represents a 19% increase, with a concomitant increase in the quantity of pig slurry produced. For instance, French pig breeding farms (14 million pigs produced) generate about 24 million t of pig slurry annually (Pauwels et al. 2005). Pig slurry spreading in the fields is the most common way of managing this waste. As elements essential for plant life (i.e. nitrogen (N), phosphorus (P) and potassium (K)) are found in pig slurry, it can thus be used as a fertilizer in crop fields. Unfortunately, pig slurry also contains heavy metals.

Among the heavy metals found in pig slurry, there is a high quantity of copper (Cu), i.e. 360–800 mg kg<sup>-1</sup> dry matter (dm) and zinc (Zn), i.e. 500 to 1900 mg kg<sup>-1</sup> dm (Jondreville et al. 2002; Nicholson, et al., 2003). Cu and Zn additions (under CuO and/or CuSO<sub>4</sub> and ZnO and/or ZnSO<sub>4</sub> forms) are currently authorized, even at very high concentration, in pig feeds (20 to 220 mg kg<sup>-1</sup> dm for Cu and 50 to 250 mg kg<sup>-1</sup> dm for Zn) as an essential micronutrient additive for pig feeds (Directive 70/524/EEC). Pigs assimilate very little Cu and Zn, so 80–90% Cu and Zn is excreted and found in pig slurry.

It has been demonstrated that intensive pig slurry spreading can have impacts on field ecosystems. Heavy metals can accumulate at the soil surface (Novak, et al., 2004), and this accumulation, through the enhancement of heavy metal mobility, can therefore ultimately lead to surface water and/or groundwater contamination (6). Hence, it is essential to assess heavy metal speciation in pig slurry, i.e. determine the bearing phases, atomic coordination, and oxidation state of heavy metals, to evaluate their mobility. This is an essential contribution in order to determine the overall environmental impact of pig slurry spreading in crop fields.

Pig slurry is a complex matrix composed of liquid (urine + water) and solid (feaces) phases in which organic (C, N) and inorganic (P, K) chemical elements are present. Studying the speciation of Cu and Zn in pig slurry is thus a scientific challenge that can be addressed through an *in situ* multi-technique approach. The unique feature of the present study is that it involved a combination of techniques to investigate Cu and Zn speciation in pig slurry. This study was conducted in two steps: characterization analyses first focused on raw pig slurry, and then, due to the great complexity of the sample, size fractionation was performed in order to possibly separate individual Cu and Zn species.

Size fractionation was first carried out to account for the complexity of pig slurry. We then used SEM-EDS to identify sites where Cu and Zn were colocated with other elements. Chemical microanalyses were performed at a laboratory-scale with micro X-ray fluorescence spectroscopy ( $\mu$ XRF) to locate Cu within pig slurry and identify spatial correlations between elements. The results were compared with those obtained through geochemical calculation of the pig slurry lagoon geochemical system. This calculation was performed to determine the Cu major species in the pig slurry. Finally a more detailed insight into Cu and Zn speciation was obtained using synchrotron-based X-ray absorption spectroscopy (XAS). XAS is one of the most known structural *in situ* techniques for direct determination of the speciation of chemical elements in complex matrix. This technique includes X-ray absorption near-edge structure spectroscopy (XANES), which provides global information on the oxidation state, three-dimensional geometry, and coordination environment of elements under investigation and

extended X-ray absorption fine structure spectroscopy (EXAFS), which provides information on the distances, coordination number, and species of the neighbors of the absorbing atom.

## 2 MATERIALS AND METHODS

### 2.1 Pig slurry sample

In pig breeding, the extent of Cu and Zn addition to pig feed varies with the pig maturity stage and Cu-Zn concentration in pig slurry is maximal during the fattening stage. Pig slurry produced during this specific stage was chosen for the present study. Apart from the high Cu and Zn concentration, the studied pig slurry parameter values (pH 7.7, 8.35% dry matter, etc.) were comparable to those reported in the literature.

### 2.2 Size fractionation

80 g of raw pig slurry (PS raw) were filtered through different sieves using pure water. The sieve sizes were 1000, 630, 355, 200, 50, and 20  $\mu\text{m}$ . Retentates were dried at 60°C for 24 h. Six size fractions were then obtained: PS sup 1000 (with particle sizes over 1000  $\mu\text{m}$ ), PS 630-1000 (with particle sizes ranging from 630  $\mu\text{m}$  to 1 000  $\mu\text{m}$ ), PS 355-630, PS 200-355, PS 50-200 and PS 20-50. Samples passed through the 20  $\mu\text{m}$  sieve could not be directly filtered at 0.45  $\mu\text{m}$  due to the problem of sieve fouling. Therefore, these samples were centrifuged for 27 h at 11 000 rpm. The centrifugation residue contained particles of over 0.45  $\mu\text{m}$  in size, providing the PS0.45-20 size fraction. The supernatant was filtered at 0.45  $\mu\text{m}$  (with less than 1% loss on the sieve) to give the PSinf0.45 fraction. These samples were also dried for 24 h at 60 °C. Size fractionation of the pig slurry was performed in triplicate, and each corresponding fraction was mixed.

### 2.3 Scanning electron microscopy with X-ray microanalysis (SEM-EDS)

Before analysis, the sample was metallised with a carbon metal-spray operator (Balzer MED 010). Micrographs and chemical microanalyses of the sample were obtained using a Philips XL30 SFEG scanning electron microscope coupled to an Oxford Instrument energy dispersive spectrometer. The SEM was operated at 15 kV with a counting time of 60 s per point. With SEM-EDS, chemical microanalysis results can be obtained with a spatial resolution around 2-5  $\mu\text{m}$  and a penetration depth of about the same range, but this technique is not always effective for locating trace elements because of its insufficient sensitivity (Zn detection limit of 10000 mg kg<sup>-1</sup>, depending of the type of matrix). SEM-EDS chemical microanalyses were then performed on selected size fractions (PS0.45-20) because these samples exhibited higher Zn content.

### 2.4 Laboratory-based $\mu$ XRF coupled with Chemometrics

The measurements were carried out on a HORIBA XGT-5000 microscope equipped with an X-ray tube producing a finely focused and high-intensity beam with a 10  $\mu\text{m}$  spot size. The X-ray beam was generated with an Rh X-ray tube at an accelerating voltage of 30 kV, with a current of 1 mA. X-ray emission from the irradiated sample was detected with an Energy-dispersive X-ray (EDX) spectrometer equipped with a liquid nitrogen cooled high purity Si detector. Elements detected by  $\mu$ XRF ranged from Mg (12) to U (92). The detector resolution was 145 eV at the Mn K $\alpha$  emission line.  $\mu$ XRF analysis detection limits can reach 100 mg kg<sup>-1</sup>, depending of the nature of the matrix and the atomic weight of the element analyzed. Sample to be analyzed was finely ground and then pressed as a 13 mm diameter pellet. 45  $\mu$ XRF spectra were recorded for each sample with the incident beam randomly pointed on 45 spots on the pellet surface (total counting time of 1000 s per spectrum).

In order to analyze these spectra properly, a self-modelling mixture analysis technique, called SIMPLISMA (SIMPLe-to-use Interactive Self-modelling Mixture Analysis) was used (Windig and Guilment, 1991).

The spectrum recorded by  $\mu$ XRF is actually a “mixture spectrum” as the incident X-ray beam penetrates thought the sample and the collected fluorescence signal is coming from all the phases contained in the probed volume. Thus it could be difficult to establish unambiguously spatial correlations between elements (trace and major elements). The SIMPLISMA algorithm decomposes the data matrix (i.e. the 45 pig slurry  $\mu$ XRF spectra) into pure contributions due to each of the components in the system, and it can elucidate and characterize components in a mixture. The algorithm assumes that there is a wavelength with significant intensity contributions from only one of the components (pure component) in the mixture. This wavelength is called the pure variable. If the pure variable can be found for every component in a mixture, then the intensity at these wavelengths can be used to resolve the corresponding spectra through a least squares fit.

## 2.5 X-ray absorption spectroscopy (XAS)

Cu K-edge XANES spectra were collected on the SAMBA beamline at the SOLEIL storage ring (France) run at 2.75 GeV with a current of 200 mA. XANES analysis of our specific samples was very time consuming and we were thus not able to analyze all the size fractions. Therefore, PSraw and four size fractions PS 0.45-20, PS 20-50, PS 50-200, and PS 355-630 were analyzed. These size fractions were chosen because they represent the majority of the copper mass in pig slurry. Spectra acquisition was performed at liquid nitrogen temperature to improve the signal/noise ratio by decreasing thermal motion of atoms, and to avoid sample oxidation. Measurements were carried out in the fluorescence mode with a seven-element solid-state germanium detector. Each spectrum is the sum of three recordings ranging from 150 eV below to 400 eV above the absorption edge of Cu at 8979 eV. XANES spectra were acquired from 8959 eV to 9029 eV with a 0.2 eV monochromator step (i.e. from 20 eV below to 50 eV above the absorption edge) and an integration time of 4.0 s per point. The photon energy of the experimental spectra was calibrated using a Cu foil (threshold energy was taken at the zero-crossing point of the second derivative of the spectrum). The data reduction was accomplished using Athena software. In order to quantitatively compare the intensity of absorption features in various compounds, all experimental Cu K-edge XANES spectra were reduced by background subtraction with a linear function in the pre-edge region and with a quadratic polynomial function in the post-edge region, and normalized using the point of inflection of the first EXAFS oscillations as a unit.

Quantitative application of XANES to geochemical systems requires determination of the proportions of multiple chemical species that contribute to the measured spectrum. Indeed, the XANES spectrum of the unknown sample is a weighted sum of all species spectra present; the atomic fraction of each metal species can be obtained by a linear combination fits (LCF) of this spectrum to reference spectra. XANES spectra analysis is based on comparisons with several reference compounds of well-known crystal structure. Reference compounds are chosen from a large panel of potential chemical species that could contribute to explaining the coordination, oxidation state and atomic environment of Cu within pig slurry. Cu K-edge XANES spectra for 13 reference compounds were recorded. In this database were inorganic and organic compounds with various Cu oxidation states (Cu(0), Cu(I) and Cu(II)). The purity of the mineral reference compounds was checked by XRD. These spectra were all subjected to accurate energy calibration and carefully normalized. In this study, we used a combination of principal component analysis (PCA), target transformation (TT), and linear combination fitting (LCF) to fit Cu K-edge XANES spectra of pig slurry samples. The goodness of fit was assessed with the normalized sum-square (NSS) equation.

Zn K-edge X-ray absorption spectra were recorded at room temperature on a FAME beamline at ESRF in fluorescence mode using a 30 element solid-state detector, and on an X32-A2 beamline at NSLS (in transmission mode), depending on the Zn concentration. The energy was calibrated on both beamlines using a Zn foil (threshold energy taken at the zero-crossing point of the second derivative spectrum). EXAFS  $k^3\chi_{Zn}(k)$  spectra were Fourier transformed from the k to R space using Kaiser-Bessel apodization windows. This procedure results in pseudo-radial distribution functions (RDF) uncorrected from phase shift functions. RDF peaks are therefore displaced by about 0.3-0.4 Å with respect to the crystallographic distances. The contributions of the first atomic shell were filtered by back Fourier transforms. In complex matrices like pig slurry, this approach may be unsuitable for fitting the second shell around Zn because of the numerous second neighbour nature, distance and number possibilities. Alternatively, since the EXAFS spectrum of the unknown sample is a weighted sum of all species spectra present, the atomic fraction of each metal species can be obtained by linear combination fits (LCF) of this spectrum to reference spectra within a 2.6-11.0 Å<sup>-1</sup> k-range. The minimum number of reference spectra needed to fit the unknown sample was determined by principal component analysis (PCA). We also verified that the progressive introduction of component during the spectral reconstruction decreases the NSS (see NSS definition in S3 supporting information) at least 20%. PCA was performed using SixPack software and EXAFS  $k^3\chi_{Zn}(k)$  spectra in the range 2.6 to 11.0 Å<sup>-1</sup>. Relevant reference compounds were identified via target transformation and the SPOIL function in a large collection of pure Zn minerals and species.

## 3 RESULTS AND DISCUSSION

### 3.1 Zn distribution in particle-size fractions

Cu and Zn analysis of particle-size fractions obtained from pig slurry led to recoveries of 108 and 98% respectively. First, only 0.3% of total Cu and 0.2% of total Zn was in PSinf0.45, which was potentially the most mobile size fraction. Second, most of the Cu and Zn were detected in the PS 0.45-20 fraction (78% of total Cu and 75% of total

Zn). The PS 0.45-20 fraction also contained the greatest share of Fe (51% of total Fe), S (48% of total S), Ca (33%) and P (28%). This indicated that there could be a correlation between these elements and Cu/Zn.

### 3.2 Chemical microanalysis

We used SEM-EDS to perform exploratory chemical microanalyses and identify Cu/Zn distributions in PS0.45-20, i.e. the size fraction with the highest Cu/Zn concentrations. Regions of interest were selected on the images and EDS spectra were then recorded to determine their elemental composition. Four phases were identified (in duplicate at least) according to the following elemental composition: P/Mg, Ca, Zn and O, and Cu/Zn/S.

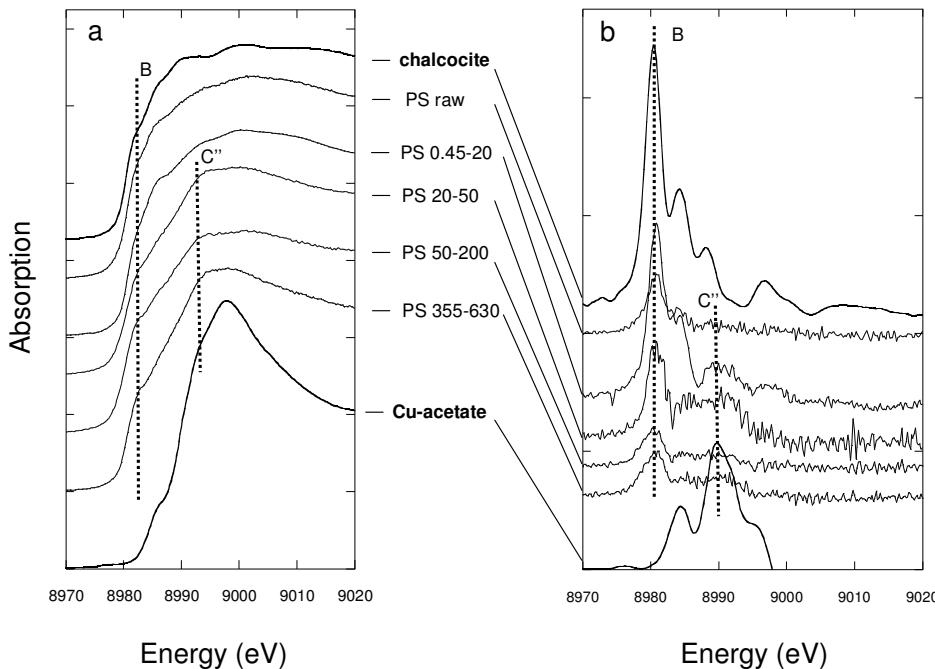
Zn co-located with oxygen could be explained by the presence of ZnO (e.g. zincite, a pig feed supplement) or Zn hydroxides. Zn could be co-located with phases containing sulfur, e.g. ZnSO<sub>4</sub> (a pig feed supplement), but also ZnS (sphalerite).

### 3.3 Localisation of Cu in Pig Slurry

Raw pig slurry was analyzed by  $\mu$ XRF in order to localise Cu and then determine the nature of the potential Cu-bearing phase(s). The initial dataset (45  $\mu$ XRF spectra recorded with a 10  $\mu\text{m}$  X-ray beam) was resolved by the SIMPLISMA procedure into pure components and their contributions in the original spectra. The most interesting finding showed co-localisation of Cu with S, suggesting the presence of an S-rich Cu-bearing phase

### 3.4 XANES Cu Speciation on the Molecular Scale

Figure 1 presents the normalized Cu K-edge XANES spectra of the pig slurry samples (PS raw and 4 size fractions) and two selected reference compounds. In all the samples the presence of a shoulder at 8982 eV (Feature B) and the absence of a pre-edge from 8977 to 8978 eV (feature A) indicated the presence of Cu(I) in the sample. PSraw and PS0.45-20 showed a Cu K-edge XANES pattern very close to the chalcocite spectrum. But, on the spectra of PS20-50, PS50-200 and PS355-630, a shoulder (C'') is observed. The peak C'' resulted from the 1s  $\rightarrow$  4p transitions in Cu(II) compounds in square planar symmetry like Cu(II)-acetate.



**FIGURE 1 (a) Normalized Cu K-edge XANES Spectra of Pig Slurry Samples (b) Corresponding First Derivatives of Cu-K edge XANES Spectra**

The LCF fits were satisfactory for all samples, with very low NSS values. All size fraction samples were fitted by a high proportion of Cu(I)<sub>2</sub>S (from 72 to 90 %) and a smaller proportion of Cu(II)-acetate (10–28%). The sum of the linear combination fit results for size fractions weighted by the mass of each size fraction reached 91% Cu(I)<sub>2</sub>S and 9% Cu(II)-acetate. Taking into account the LCF method uncertainty of 10 to 15%, this is consistent with Cu speciation in the raw pig slurry samples (Cu(I)<sub>2</sub>S = 96% and Cu(II)-acetate = 4%). Therefore, there is only a

minority of Cu(II) bound to organic matter in the pig slurry. The presence of Cu(I)<sub>2</sub>S in great majority in the pig slurry spectra LCF fit was attributed to chalcocite (Cu(I)<sub>2</sub>S) precipitation.

### 3.5 EXAFS Zn Speciation on the Molecular Scale

Complex matrices like pig slurry generally contain a disordered species distribution and a large panel of potential second neighbours. Therefore, we used a second "fingerprint" approach. The sample spectra were simulated by linear combinations of reference compound spectra. The PS raw EXAFS spectrum was reconstructed with 14% sphalerite 25% Zn-cysteine, 24% Zn-acetate acid and 37% amorphous Zn hydroxide. The large-size fractions (PS355-630; PS630-1000 and PSsup 1000) were reconstructed with 12-17% Zn-cysteine, 54-63% Zn-acetate, 22-24% amorphous Zn hydroxide and less than 4% of zincite or sphalerite. The intermediary-size fractions (PS20-50; PS50-200 and PS200-355) were reconstructed with 32-40% sphalerite, 38-40% Zn-acetate, 11-17% amorphous Zn hydroxide and 9-16% zincite. Finally, PS0.45-20 was reconstructed with 33% sphalerite, 27% Zn-acetate, 32% Zn-cysteine and 9% amorphous Zn-hydroxide.

## 4 CONCLUSIONS

In pig slurry, Cu is mainly in sulfide form (Cu<sub>2</sub>S) with a Cu(I) oxidation state. As copper sulfide precipitates in anoxic conditions (34), questions arise concerning the solubility of this mineral phase at the soil surface after pig slurry spreading. According to the litterature (Calmano et al., 1993; Al-Abed et al., 2006), we put forward the hypothesis that the solubility of copper sulfide from pig slurry would be very low in soil, even in aerobic conditions, when the soil pH is over 4.5. Cu accumulation at the soil surface resulting from intensive pig slurry spreading could thus be explained by the very low solubility of Cu<sub>2</sub>S.

Zn speciation within pig slurry revealed the presence of three main components: 49% Zn bound to organic matter, 37% amorphous Zn hydroxides and 14% sphalerite (Legros et al., 2010). The three forms of Zn bound to organic matter, Zn hydroxide and sphalerite were soluble under aerobic conditions and within the pH 5-8 range, which corresponds to the pH measured in many soils. It should be underlined that kinetic effects and potential interactions between soil compounds and pig slurry compounds were not taken into account in these latter observations. We therefore consider that, for the purposes of determining the long-term impact of pig slurry spreading, it would be essential to undertake long-term field experiments to assess the fate of Heavy Metals in soils following heavy organic waste applications.

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