



HAL
open science

Anaerobic Digestion Alters Copper and Zinc Speciation

Samuel Legros, Clément Levard, Claire-Emmanuelle Marcato-Romain,
Maritxu Guiresse, Emmanuel Doelsch

► **To cite this version:**

Samuel Legros, Clément Levard, Claire-Emmanuelle Marcato-Romain, Maritxu Guiresse, Emmanuel Doelsch. Anaerobic Digestion Alters Copper and Zinc Speciation. *Environmental Science and Technology*, 2017, 51 (18), pp.10326-10334. 10.1021/acs.est.7b01662 . hal-01643555

HAL Id: hal-01643555

<https://hal.science/hal-01643555v1>

Submitted on 21 Nov 2017

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.



Open Archive TOULOUSE Archive Ouverte (OATAO)

OATAO is an open access repository that collects the work of Toulouse researchers and makes it freely available over the web where possible.

This is an author-deposited version published in : <http://oatao.univ-toulouse.fr/>
Eprints ID : 18528

To link to this article : DOI :10.1021/acs.est.7b01662
URL : <http://dx.doi.org/10.1021/acs.est.7b01662>

To cite this version : Legros, Samuel and Levard, Clément and Marcato-Romain, Claire-Emmanuelle and Guiesse, Agnès Maritchù and Doelsch, Emmanuel *Anaerobic Digestion Alters Copper and Zinc Speciation*. (2017) *Environmental Science & Technology*, vol. 51 (n° 18). pp. 10326-10334. ISSN 0013-936X

Any correspondance concerning this service should be sent to the repository administrator: staff-oatao@listes-diff.inp-toulouse.fr

Anaerobic Digestion Alters Copper and Zinc Speciation

Samuel Legros,^{*,†,‡,§} Clément Levard,[§] Claire-Emmanuelle Marcato-Romain,^{||} Maritxu Guiresse,[⊥] and Emmanuel Doelsch^{‡,§}

[†]CIRAD, UPR Recyclage et risque, 18524 Dakar, Senegal

[‡]CIRAD, UPR Recyclage et risque, F-34398 Montpellier, France

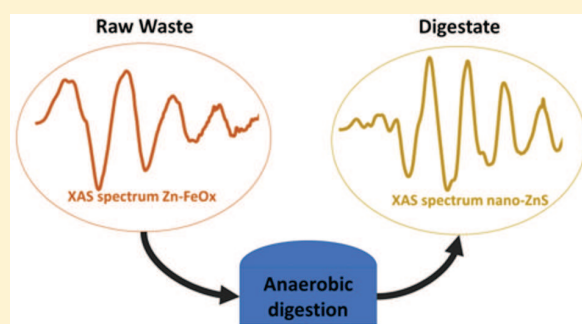
[§]Aix Marseille Université, CNRS, IRD, Collège De France, CEREGE, Aix en Provence, France

^{||}Université de Toulouse, UPS, LBAE (EA 4565), IUT 'A', 24 rue d'Embaquès, F-32000 Auch, France

[⊥]EcoLab, INPT, UPS, Université de Toulouse, UMR CNRS 5245, F-31326 Castanet Tolosan, France

Supporting Information

ABSTRACT: Anaerobic digestion is a widely used organic waste treatment process. However, little is known on how it could alter the speciation of contaminants in organic waste. This study was focused on determining the influence of anaerobic digestion on the speciation of copper and zinc, two metals that generally occur at high concentration in organic waste. Copper and zinc speciation was investigated by X-ray absorption spectroscopy in four different raw organic wastes (predigestion) and their digested counterparts (postdigestion, i.e., digestates). The results highlighted an increase in the digestates of the proportion of amorphous or nanostructured copper sulfides as well as amorphous or nanostructured zinc sulfides and zinc phosphate as compared to raw waste. We therefore suggest that the environmental fate of these elements would be different when spreading either digestates or raw waste on cropland.



1. INTRODUCTION

Waste production has risen 10-fold over the past century, and it is predicted that by 2025 it will double again to reach 6 million t/day.¹ Solutions must therefore be found to deal with this worsening situation. Organic waste (e.g., pig slurry, sewage sludge, etc.) accounts for a significant share of global waste production, and can be recycled via two main routes.

The first main route is agricultural recycling. Organic waste (OW) has fertilizing properties (e.g., high N, P and K concentration) and can be used as an alternative to chemical fertilizers.² However, high pollutant concentrations may also be found in OW, including trace elements (e.g., copper (Cu) and zinc (Zn)) that are particularly worrisome.³ OW amended soils can have trace elements accumulated in their surface layers.^{4,5} The fate of trace elements in cropfield soils after OW amendment is a key issue since their accumulation could induce phytotoxicity and groundwater quality degradation. Prediction of this fate could be enhanced by determining trace element speciation in OW.^{6–9}

The second main route is energy recovery. OW can undergo anaerobic digestion (AD) to produce biogas (mainly composed of methane) that can be used to generate electricity and heat. AD has recently been receiving attention in a sustainable development framework since the energy it produces is renewable. Moreover, AD preserves N, P, and K, so digested waste (i.e., digestates) can also be spread on cropland, like the

raw counterpart.¹⁰ However, during AD, organic substance decomposition results in a decrease in OW total mass and a relative increase in the trace element concentration.¹⁰ It is thus essential to monitor the total trace element concentration in digestates while, more importantly, identifying the chemical forms in which trace elements occur, since these forms determine their mobility, bioavailability, and ecotoxicity.^{11–13}

Whether AD could induce some change in trace element speciation is still a matter of debate in the literature. Only a few studies have investigated changes in trace element speciation following AD. Regarding Cu, two recent chemical extraction studies demonstrated that AD has no effect on Cu speciation in sewage sludge.^{14,15} For Zn, Dong et al.¹⁵ showed that AD has no effect on Zn speciation in sewage sludge, while Dąbrowska et al.¹⁴ showed an increase in the proportion of the organic matter/sulfide fraction. Marcato et al.¹⁶ combined sequential extraction and biochemical fractionation and highlighted Cu and Zn conversion into potentially less mobile fractions in digested pig slurry compared to raw pig slurry. However, the same authors also used a biological approach (Cu and Zn

Table 1. Element Concentrations in Raw Waste and Digestates

		Corg	Ntot	C/N	P	S	Fe	Cu	Zn
		% ^a	%		g/kg	g/kg	g/kg	mg/kg	mg/kg
pig slurry	PS	39.9	4.71	8.47	43.9	5.72	2.84	664	553
	PS_D	34.4	4.86	7.08	47.3	7.26	3.18	788	842
municipal waste fine fraction	MWFF	35	1.53	22.9	3.84	3.83	8.77	84.8	369
	MWFF_D	30	1.81	16.6	5.92	4.28	12.1	136	634
sewage sludge	SS	41.8	7.91	5.28	26.1	0.61	6.07	350	668
	SS_D	34.7	5.23	6.63	37.3	3.60	9.84	487	988
sewage sludge mix green waste	SSGW	42.8	7.62	5.62	22.8	1.03	5.05	294	553
	SSGW_D	34.7	5.5	6.31	40.1	3.56	10.4	501	842

^aThe element concentrations are all expressed on a dry matter basis.

uptake by *Zea mays* and *Vicia faba*) and found no differences between digested and raw pig slurry.¹⁶

These inconsistencies in the findings of the different studies could be attributed to chemical extraction shortcomings, including incomplete dissolution of the target phase, and/or dissolution of the nontarget species. In these studies, the obtained fractions were dependent on the chosen extraction procedure.¹⁷ Substantial uncertainties on the impact of AD on trace element speciation in OW hence remain. In this study, we assessed the impact of AD on Cu and Zn speciation in four different OWs. X-ray absorption spectroscopy (XAS) was used to compare Cu and Zn speciation in raw OW (predigestion) and the digestate (post-AD). XAS is one of the most widely known structural techniques for direct determination of speciation of trace elements present in complex matrix, even at low concentration. The accuracy of this approach has been previously demonstrated for OWs.^{8,9,18}

2. EXPERIMENTAL SECTION

2.1. Sample Collection and Preparation. Four wastes were analyzed in the study, before and after anaerobic digestion: pig slurry (PS), sewage sludge (SS), sewage sludge mixed with green waste (SSGW) and municipal waste fine fraction (MWFF).

PS has been described in Marcato et al.¹⁰ SS was collected in an experimental municipal plant in the APESA technical platform (www.apesa.fr). MWFF was obtained by sieving (<10 mm) the biodegradable fraction of household waste collected in southwestern France. Green waste, mainly composed of leaves and grass clippings, was ground and sieved through a 10 mm mesh sieve, then mixed with sewage sludge to produce SSGW.

AD of PS has been described in Marcato et al.¹⁰ Briefly, the anaerobic reactor was run at mesophilic temperature (37 °C), with a retention time of about 15 days. For the three other wastes (SS, MWFF, and SSGW), three mesophilic (39 °C) stirred tank reactors in the APESA technical platform were used. In each digester, the organic loading rate was maintained at 1 g organic matter (OM) L⁻¹·d⁻¹, with a hydraulic retention time of about 35 days. Eh was maintained lower than -300 mV, and the pH was always within the 7.0–8.2 range.

Note that, throughout the manuscript, the abbreviations (PS, SS, MWFF, and SSGW) refer to raw OW, whereas all information related to the digestate are annotated with “D” (e.g., PS_D for digested pig slurry).

2.2. Chemical Analysis. Total carbon (ISO 10694) and total nitrogen (ISO 13878) were determined by dry

combustion with an elemental NC 2100 Soil Analyzer (Thermo Electron Corp.) Inorganic carbon was measured in a Shimadzu carbon analyzer and organic carbon was deduced from the difference between total and inorganic carbon.

Ground and homogenized samples were digested using a mixture of HF, HNO₃ and HClO₄ (ISO 14869-1:2001; Soil quality—dissolution for determination of the total element content). Major element and trace element concentrations were determined using an inductively coupled plasma optical emission spectrometer (ICP-OES Vista-PRO, Varian Inc.) with an axially viewed plasma system and a charge coupled device detector. Two kinds of checks were performed for each element analysis using certified reference material (Reference material No. 146R: Sewage sludge of industrial origin produced by the Community Bureau of Reference, Commission of the European Communities) with five repeat measurements of each sample signal. The variation coefficient for both of these checks had to be less than 5%. Internal control samples and CRM samples (Certified Reference Materials from Bureau of Analyzed Samples Ltd.) were used to improve the quality of the results according to the ISO 9001 certification of the laboratory (AFAQ, number QUAL/2000/13516b). The measurement uncertainty was less than 10%.

2.3. X-ray Absorption Spectroscopy. Zinc K-edge (9659 eV) and Cu K-edge (8979 eV) X-ray absorption spectra were recorded at the Stanford Synchrotron Radiation Lightsource (SSRL) on beamline 11–2. X-ray absorption spectroscopy (XAS) measurements were performed on the eight OWs (raw wastes and digestates). Spectra acquisition was performed on freeze-dried samples pressed into thin pellets at liquid nitrogen temperature to prevent X-ray sample damage. Measurements were carried out in fluorescence mode with a 100-element solid-state Ge detector. Each scan was focused on a different specimen position to reduce the risk of beam damage and obtain representative spectra. The energy was calibrated using Zn and Cu metallic foils (threshold energy taken at the zero-crossing point of the second derivative spectrum). Each spectrum was at least the average of three scans. The normalization and data reduction were performed according to standard methods¹⁹ using Athena software.²⁰

There are two regions in a spectrum obtained with XAS: X-ray absorption near edge structure spectroscopy (XANES) and extended X-ray absorption fine structure spectroscopy (EXAFS).

XANES spectra provide overall information on the oxidation state, three-dimensional geometry, and coordination environ-

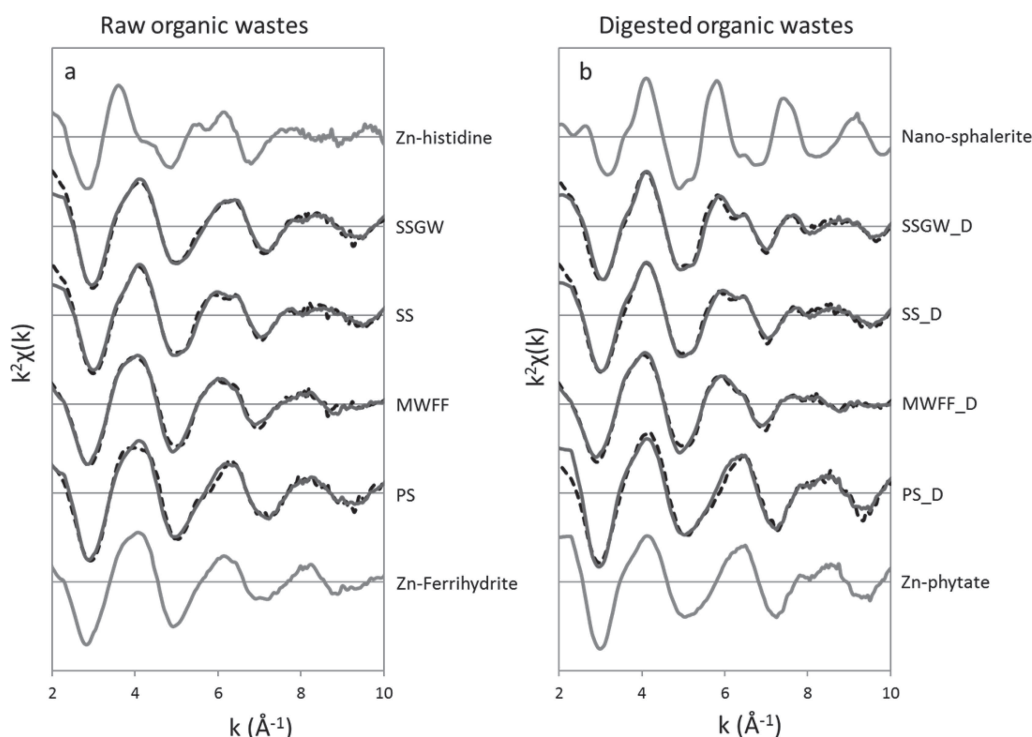


Figure 1. Zinc K-edge extended X-ray absorption fine structure spectra (solid line) and their respective LCF fits (dashed lines) for (a) the raw wastes and (b) digestates. The spectra of the references are shown for comparison.

ment of elements under investigation. Here, XANES spectra were used only for Cu. Indeed, Cu presents two oxidation states (+I and +II) that could be identified with XANES spectroscopy. The XANES spectra of Zn were not discriminant in this study, presumably because Zn only has one oxidation state. Therefore, the XANES results for Zn were not presented.

EXAFS provides information on the distance between the target element and atomic neighbors, the number of atomic neighbors, and the nature of atomic neighbors. This information enables identification of the target element bearing phases. EXAFS was thus used for Cu and Zn. The procedure used in this study to fit the Zn and Cu EXAFS spectra of each of the raw wastes and digestate samples has been described previously.⁶ Briefly, this procedure is based on a combination of principal component analysis (PCA), target transformation (TT), and least-squares linear combination fitting (LCF). Details on PCA, TT, and LCF results are given in the [Supporting Information \(SI\) \(Part SI-2\)](#), in addition to the complete Cu and Zn model compound library (Part SI-3). Note that the LCF fitting uncertainty was estimated at 10%.²¹

3. RESULTS AND DISCUSSION

3.1. Anaerobic Digestion Influence on Element Concentrations. The concentrations of selected major and trace elements in raw waste and digestates are shown in [Table 1](#).

Concerning organic carbon, all of the studied paired samples (raw waste + digestate) showed the same pattern. The organic carbon concentration was 14–20% lower after AD. This indicated the conversion of organic carbon into biogas mostly consisting of CO₂ and CH₄ and the reduction of the OM content in the waste during digestion.

Concerning the total nitrogen concentration (N_{tot}), two patterns were noted. First, in PS and MWFF, the N_{tot} concentration increased by 3% and 18%, respectively, after AD, inducing a decrease in the C/N ratio. Conversely, in SS and SSGW, the N_{tot} concentration decreased by 34% and 28%, respectively, after AD, increasing in the C/N ratio. SS and SSGW had a high quickly mineralizable nitrogen content.

Concerning the major elements P, S, and Fe, all of the studied wastes showed the same pattern. The concentration was higher after digestion (NB: analysis findings for the other major elements are presented in [SI Table SI-1](#)). The increase ranged from 8% (for P in PS) to 490% (for S in SS). Note that there were relative increases in these element concentrations. Indeed, their total mass was preserved during the AD process, while the total waste mass decreased. AD therefore preserved or even improved the potential fertilizing value of the waste.

Concerning the trace elements Cu and Zn, all of the studied wastes showed the same pattern. The Cu and Zn concentrations were higher after AD (NB: analysis findings for the other trace elements are presented in [SI Table SI-2](#)). The increase ranged from 19% (for Cu in PS) to 72% (for Zn in MWFF). Similarly to the pattern noted for major elements, the trace elements concentrations underwent relative increases, thus highlighting the potential risk concerning trace elements in digestates. Note, however, that Cu and Zn concentrations were still below the threshold limits set in European environmental protection legislation, particularly in soil when sewage sludge is used in agriculture (86/278/EEC). Indeed, this limit is 1750 mg.kg⁻¹ for Cu and 4000 mg.kg⁻¹ for Zn. Besides, it is now recognized that the total trace element concentration in waste is not sufficient to properly assess the risk when used in agriculture. Further information on their speciation is required.

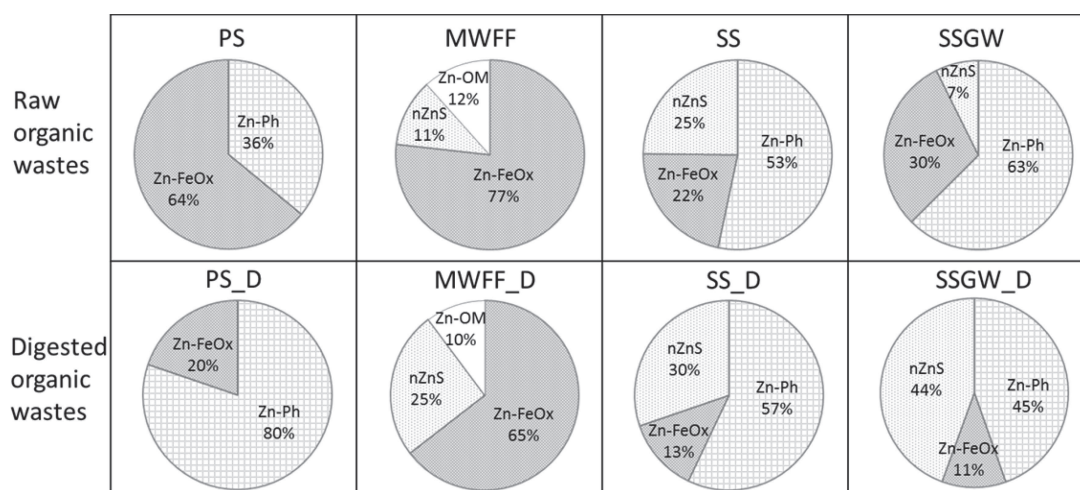


Figure 2. Pie charts showing Zn speciation in raw organic waste (first line) and digestates (second line). Zn–FeOx = Zn sorbed on Fe oxyhydroxide, Zn–Ph = Zn sorbed on phosphate, Zn–OM = Zn bound to organic matter, nZnS = nanoparticulate Zn sulfide.

3.2. Zinc Speciation in Raw Waste and Digestates. The Zn K-edge EXAFS spectra of the raw wastes and their digested counterparts are presented in Figure 1. The LCF results were satisfactory for all samples, with low normalized sum-square (NSS) values ranging from 1.53 to 3.38, and high recovery ranging from 99% to 112% (details on the LCF results are presented in the SI, Table SI-7).

The EXAFS spectra of four references were necessary to fit the Zn K-edge EXAFS spectra of all the samples (Figure 1), that is, Zn adsorbed on ferrihydrite, Zn phytate, nanosphalerite, and Zn complexed to histidine (see SI Table SI-9 for further details on the EXAFS structure of all of the Zn references used in this study). The EXAFS spectrum corresponds to a local chemical structure. Therefore, the interpretation of this spectrum in an actual Zn bearing phase must be done with caution, while taking into account the consistency of this species with regard to the physicochemical properties of the sample.

First, the reference Zn adsorbed on ferrihydrite was interpreted as a more generic bearing phase, that is, Zn sorbed on iron oxyhydroxide (Zn–FeOx). Indeed, the local structure of Zn sorbed on ferrihydrite with ≈ 4 atoms of O at ≈ 1.97 Å and $\approx 1-4$ atoms of Zn/Fe at ≈ 3.4 Å^{22,23} was similar to that of Zn sorbed on several iron oxyhydroxides. Besides, it has been shown that Zn has a high sorption affinity for several iron oxyhydroxides.^{22,23} Finally, the physicochemical conditions in a pig slurry lagoon or a sewage treatment plant are favorable for the precipitation of several iron oxyhydroxides.²⁴ The use of Zn sorbed on ferrihydrite as a model compound is therefore environmentally relevant and representative of Zn sorbed on an oxyhydroxide surface (Zn–FeOx).

The EXAFS spectrum of Zn phytate presented a local structure with four oxygen atoms at 1.96 Å and one phosphorus atom at 3.08 Å.²⁵ This structure was very similar to that observed when Zn was sorbed on phosphate minerals.²⁶ The physicochemical conditions in a pig slurry lagoon or a sewage treatment plant are also favorable for the precipitation of phosphate minerals,⁶ which have been identified as a Zn bearing phase in OW^{27,28}. The selection of this reference in LCF was therefore interpreted as involving a Zn sorbed on phosphate minerals (Zn-Ph).

The EXAFS spectrum of nanosphalerite (i.e. sphalerite of around 3 nm in size) presented a local structure with four S atoms at 2.34 Å and \approx six Zn atoms at 3.8 Å.²⁹ This local structure differed from that of the four bulk sphalerite S atoms at 2.34 Å and 12 Zn atoms at 3.83 Å.⁶ Sphalerite nanoparticles thus exhibited a high fraction of under-coordinated surface atoms. The average neighbouring nanosphalerite Zn atoms was lower than for bulk sphalerite, leading to a less structured EXAFS spectrum (see SI Figure SI-6). The bulk sphalerite spectrum was also used as a reference in the LCF fitting, but the results obtained with nanosphalerite were consistently better. Zn sulfides have already been observed in OW^{6,18} and were found to be nanostructured only in sewage sludge²⁹ and pig slurry.⁹ Batch experiments in which the physicochemical conditions were similar to those of OW suggested the occurrence of a Zn sulfide nanoparticulate state. Indeed, Lau et al.³⁰ suggested that the high OM content of OW hinders Zn sulfide crystal growth, which would lead to the formation of Zn sulfide nanoparticles. The selection of this reference in LCF was therefore interpreted as being due to the presence of a nanoparticulate Zn sulfide bearing phase (nZnS). Besides, our results showed that this Zn sulfide nanoparticulate state might be common in OW.

The EXAFS spectrum of the reference Zn complexed to histidine presents a square planar local structure with four N atoms at 2.05–2.19 Å.³¹ This structure corresponds to the interaction of Zn with natural OM functional groups.³² This structure was thus interpreted as being Zn bound to OM (Zn–OM).

Figure 2 presents the Zn distribution among the bearing phases, adjusted to 100% for easier comparison. The Zn speciation of PS included a majority of Zn–FeOx (64%) and Zn–Ph (36%). The Zn speciation of MWFF included a majority of Zn–FeOx (77%) and two minor species, that is, nZnS (11%) and Zn–OM (12%). The Zn speciation of SS and SSGW includes a majority of Zn–Ph (53–63%), a significant proportion of Zn–FeOx (22–30%) and nZnS (7–25%).

Zn speciation was dominated by inorganic species in all samples. The Zn–FeOx species was present in all wastes. Zn–FeOx represented a high proportion in SS and SSGW (22–30%) and was the major species in PS and MWFF (64–77%), confirming the results of previous studies where Zn was always

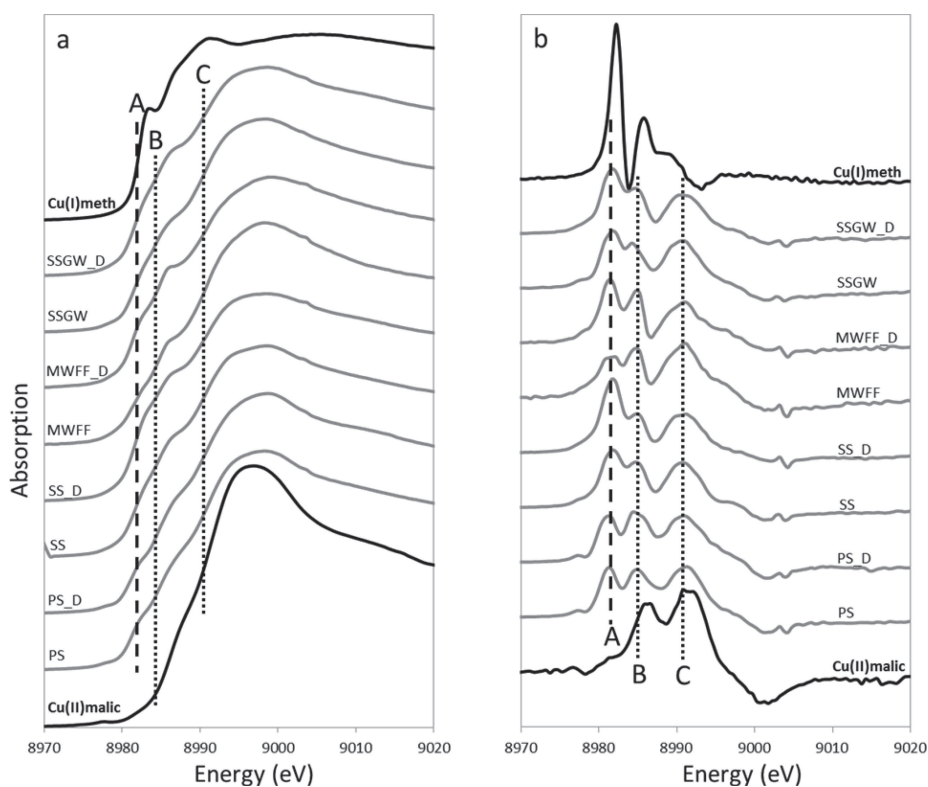


Figure 3. (a) Copper K-edge X-ray near edge structure spectra, and (b) their respective first derivative functions for all raw waste and digestates. The reference spectra are shown for comparison. Cu(II)malic = Cu(II)malic, Cu(I)meth = Cu(I)methionine.

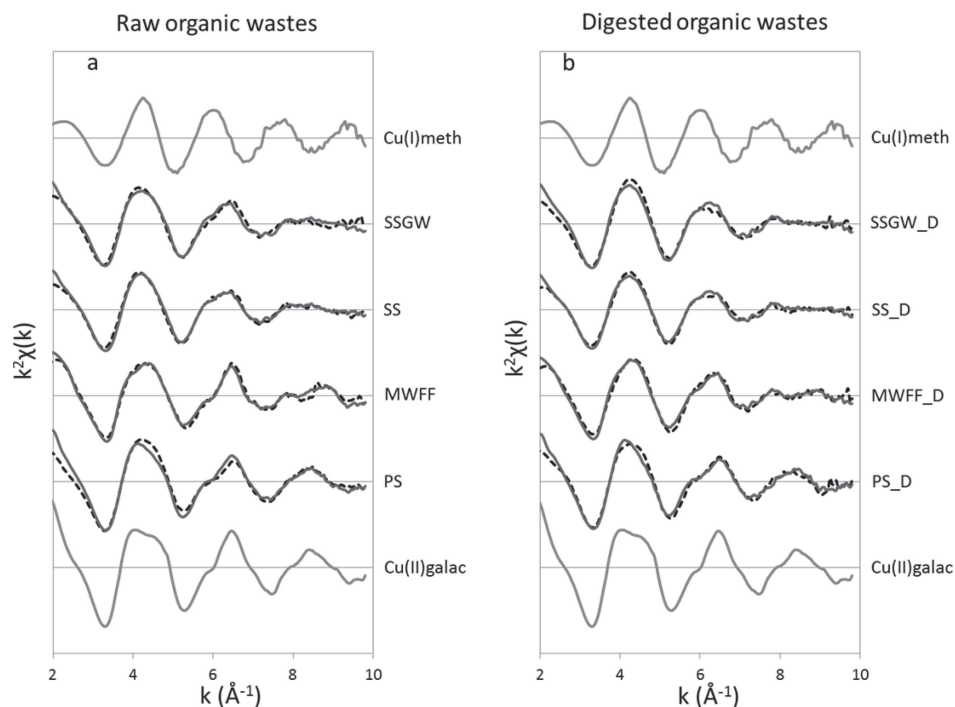


Figure 4. Copper K-edge extended X-ray absorption fine structure spectra (solid line) and their respective LCF fit (spheres) for (a) the raw wastes and (b) digestates. The reference spectra are shown for comparison; Cu(II)galac = Cu(II)galacturonic acid and Cu(I)meth = Cu(I)methionine.

partially associated with iron oxy-hydroxides in wastes.^{18,28,33} The Zn-Ph species was also present in all raw wastes except in MWFF and it was the major species in SS and SSGW (53–

64%). The absence of Zn-Ph in MWFF was consistent with the relatively low amount of total P measured in the sample compared to the three others (Table 1). The nZnS species was

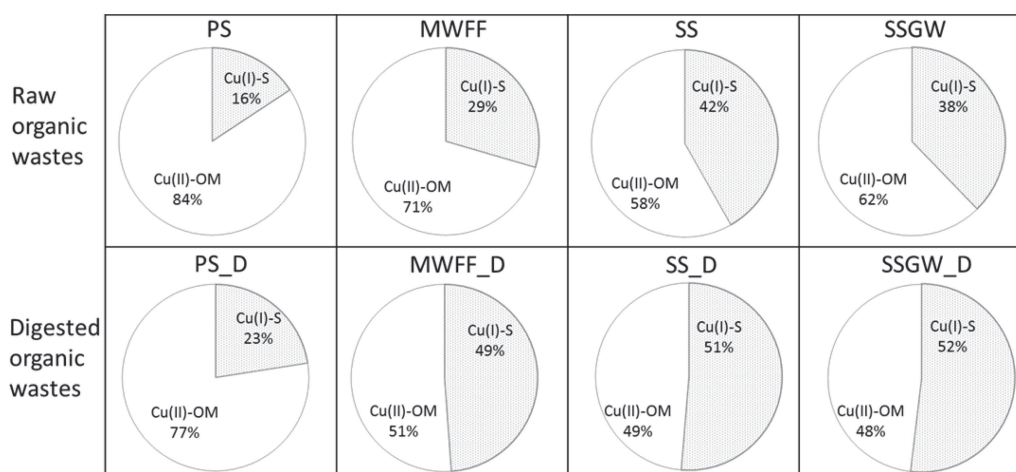


Figure 5. Pie charts showing the speciation of Cu of raw organic waste (first line) and digestates (second line). Cu(II)-OM = Cu(II) bound to organic matter and Cu(I)-S = Cu(I) bound to sulfur.

present in all raw wastes except in PS, but the proportion of this species was relatively low (7–25%). It seemed that the role of sulfide phases might have been markedly underestimated in Zn speciation in OW. Indeed, Zn sulfide minerals have only been observed in OW in the few studies using XAS.^{6,29} Standard sequential chemical extraction methods underestimate the sulfide fraction, since it is extracted along with OM fractions.³⁴ Zn species bound to organic species were only present in a minor proportion (12%) in MWFF, thus confirming the minor role played by OM in Zn speciation in OW.

AD changed Zn speciation in all the wastes studied except for SS (Figure 2). In PS_D, the proportion of Zn-Ph increased sharply from 36% to 80%. The proportion of Zn sulfide had increased in the other wastes. The proportion of Zn sulfide in digestates was 1.2- to 6.3-fold higher than in raw waste. Indeed, the anoxic conditions in the AD were favorable for Zn sulfide precipitation. The amount of Zn sulfide in SS also increased after AD. However, this increase was not significant regarding the uncertainty of the LCF fitting ($\pm 10\%$).

Previous findings concerning the effect of AD on Zn speciation were not consistent. On one hand, AD was shown to have no effect on Zn speciation in sewage sludge,¹⁵ while on the other chemical extractions revealed that AD induced an increase of the proportion of the OM/sulfide fraction.^{14,16,35} Our results were in agreement with the latter studies, whereas the analysis of the XAS results overcame the confusion between organic matter species and sulfide species.

3.3. Copper Speciation in Raw Waste and Digestates.

The normalized Cu K-edge XANES spectra of all raw waste and digestates are presented in Figure 3, with two reference compounds for comparison: Cu(I) complexed to methionine and Cu(II) complexed to malic acid.

Differences were noted among the near-edge spectra and inflections in the absorption edge (A, B, and C) corresponding to peaks in the first derivatives (Figure 3b). These inflections provided information on the Cu oxidation state. Indeed, Feature A, at 8981 eV, corresponds to the $1s \rightarrow 4p$ transitions for Cu(I) compounds. No Cu(II) reference had this inflection point (or first maximum of the first derivative) between 8980 and 8985 eV.²⁴

Consequently, the presence of a shoulder between 8980 and 8985 eV in the Cu absorption edge spectrum indicated the

presence of Cu(I) in the sample. Features B (8984 eV) and C (8990 eV) correspond respectively to the $1s \rightarrow 4p$ and $1s \rightarrow$ continuum transitions for Cu(II) compounds.^{24,36} Features A, B and C were observed on the spectra of all waste samples studied, indicating that two Cu (Cu(I) and Cu(II)) oxidation states were present in all the samples.

The Cu K-edge EXAFS spectra of the raw wastes and their digested counterparts are presented in Figure 4. The LCF results were satisfactory for all samples, with low NSS values ranging from 1.10 to 3.96 and high recovery ranging from 87% to 106% (details on the LCF results are presented in the SI, Table SI-8).

For copper, the LCF results revealed the contribution of four reference spectra: Cu(II)phtalocyanine, Cu(II)histidine, Cu(II)galacturonic acid and Cu(I)methionine (Figure 4). In the Cu(II)phtalocyanine and Cu(II)histidine references, Cu presented an oxidation state of 2, surrounded by four oxygen and/or nitrogen atoms in square planar symmetry. In the Cu(II)galacturonic acid reference, Cu presented an oxidation state of 2 surrounded by six oxygen atoms in distorted octahedral symmetry (see SI Table SI-10 for more details). These two types of structure corresponds to the interaction of Cu(II) with natural OM functional groups.³⁷ Therefore, those three references were pooled in “Cu(II)-OM”, representing a pool of Cu with an oxidation state of 2 complexed to OM.

The last Cu(I)methionine reference corresponded to a pool of Cu with an oxidation state of 1 bound to sulfur: “Cu(I)-S”. This Cu(I)-S pool could correspond to two types of species. First, Cu(I)-S would correspond to Cu complexation with thiol (-SH) functional groups in the OM. Indeed, OW has a high OM content by definition and the thiol functional group has high affinity for Cu.³⁸ Second, Cu(I)-S would correspond to Cu sulfide precipitation.

Cu speciation has been extensively studied in soil. In oxidized conditions, Cu(II)-OM is considered to be the dominant form of Cu, while five- to six-membered oxygen ring chelates formed by closely spaced carboxyl and hydroxyl groups were shown to be the dominant form of Cu(II)-OM complexes. At low Cu-to-C ratios (<0.005), nitrogen-containing functional groups are likely also involved in Cu(II) complexation.³⁹ In reducing conditions, Cu(I)-S is the dominant form of Cu. In sulfide poor environments, the OM thiol functional group is the dominant

form of Cu(I)-S, whereas in sulfide rich environments, Cu(I)-thiol complexation may be less relevant as inorganic sulfide is assumed to compete with organic ligands.³⁹

In this study, we found that the distribution among the different Cu species observed in soil could be to some extent applied to OW.

The pH and anoxic conditions and high sulfur concentration of a pig slurry lagoon or a sewage treatment plant are favorable for the precipitation of chalcocite (Cu₂S).²⁴ However, the EXAFS spectrum of chalcocite has also been used as a reference in LCF fitting and was not selected. The EXAFS spectrum of Cu(I)methionine is quite similar to that of chalcocite (see SI Figure SI-1) and Cu sulfide has already been detected in pig slurry²⁴ and sewage sludge.²⁷ Cu(I)-S could thus represent an amorphous Cu sulfide phase with a disordered local structure similar to that of Cu(I)methionine. This hypothesis has been well documented in the literature, for instance Villa-Gomez et al.⁴⁰ suggested that the instantaneous reaction kinetics and low Cu sulfide solubility over the entire pH range makes Cu sulfide precipitation prevail over the formation of another Cu species, while also leading to the formation of amorphous Cu sulfide precipitates. The studies of Shea and Helz⁴¹ and Patrick et al.⁴² also demonstrated the precipitation of amorphous Cu sulfide in batch experiments at room temperature and in anaerobic conditions. Besides, similar to nanoparticles of Zn sulfides, the high OM content of OW could hinder chalcocite mineral crystal growth and lead to the formation of nano or amorphous Cu sulfide.

Figure 5 presents the Cu distribution among the bearing phases, adjusted to 100% for easier comparison. Cu speciation was similar in all of the raw waste samples studied. Cu(II)-OM was the major species in all of the raw waste samples studied (58–83%). The speciation of Cu in raw waste was thus dominated by Cu(II) complexed to organic species. This was consistent with previously published findings where OM was considered to be the major (and often only) Cu bearing phase in waste.¹⁶ Indeed, OW is mainly composed of OM and Cu is known to have strong affinities for OM.¹⁶

However, Cu(I)-S was also detected in all of the raw wastes studied at high proportions (17–42%). Like Zn, the role of sulfide phases might have been markedly underestimated in the Cu speciation in OW. Indeed, Cu sulfide has only been observed in OW in the few studies that have been carried out using XAS.²⁴

AD altered Cu speciation in all of the wastes studied, with a similar pattern. The proportion of Cu(I)-S increased to even become the major species in SS_D and SSGW_D. The proportion of Cu(I)-S in digestates was 1.2- to 1.7-fold higher than in raw waste. This was consistent with the sharp decrease in OM content during AD. Besides, the anoxic conditions in AD were also favorable for the reduction of Cu and for Cu(I) sulfide precipitation.^{24,43}

Contradictory findings have been reported in the literature concerning the influence of AD on Cu speciation due to differences in the extraction methods used. Dabrowska and Ronsiska¹⁴ and Dong et al.¹⁵ observed no influence, while Marcato et al.¹⁶ observed a modification in Cu speciation. The results presented here are in agreement with the data reported by Marcato et al.,¹⁶ but contradict those of Dabrowska and Ronsiska¹⁴ and Dong et al.¹⁵ Indeed, when using sequential chemical extraction, the sulfide fraction is extracted together with the organic matter fraction,⁴⁴ so redistribution between those two species is impossible to observe.

3.4. Environmental Implications. Cu and Zn speciation in raw waste differs from that in the digestate. In raw waste, speciation is largely dominated by oxidized species (e.g., Cu(II)-OM or Zn-FeOx) while in digestates other species (e.g., Cu(I)-S, Zn-Ph or nZnS) also have a high proportion and can even be the major species. Raw waste and digestates are often spread on crop fields. Therefore, questions arise concerning the behavior of their Cu and Zn bearing phases at the soil surface after spreading.

Concerning Zn-FeOx, the sorption/desorption mechanisms of Zn from waste-derived Fe oxyhydroxide in soil conditions drive the Zn mobility and bioavailability.⁸ Desorption is favored below pH 6⁴⁵ and the presence of OM increases the desorption, likely because of chelating effects.¹¹

Concerning nZnS, the oxidation and solubilizing mechanisms of Zn sulfide species likely have a marked impact on Zn mobility and bioavailability. Zn sulfide precipitates in anoxic conditions, and its solubility is low, with solubility constants of 2×10^{-25} .⁴³ However, it has been shown that Zn sulfide can be soluble under aerobic conditions within the pH 5–8 range, which corresponds to the pH measured in many soils.^{46–48} Besides, Voegelin et al.⁴⁹ showed that ZnS dissolved more slowly in the acidic soils than in the near neutral and the calcareous soil.

Concerning Cu(II)-OM, waste OM mineralization by soil microorganisms has a substantial influence on Cu mobility and bioavailability.⁸ The highest OM mineralization rate in soil is obtained in wet aerobic conditions from 25 to 35 °C⁵⁰ over the entire pH range, but the rate gradually decreases below pH 6.⁵¹

Concerning Cu(I)-S, Cu mobility and bioavailability are principally determined by the oxidation and solubilizing mechanisms of Cu sulfide species. Chalcocite (Cu sulfide) precipitates in anoxic conditions and its solubility constant (2.5×10^{-48}) is very low.⁴³ However, the reactivity and fate of Cu sulfide in soil remains to be determined, but the pH and redox conditions likely have an important role.^{52,53}

Cu and Zn species in digestates seem to reduce the mobility and bioavailability of Cu and Zn compared to Cu and Zn species in raw waste. This suggests that AD could reduce the risk associated with Cu and Zn. It should, however, be underlined that kinetic effects and potential interactions between soil compounds and OW were not taken into account in the previously cited assessments of Cu and Zn species solubility. Besides, Zn sulfides and Cu sulfides observed in this study presented a disordered structure, indicating that they are amorphous or they are nanostructured, which would have a marked influence on their solubility. A more detailed study on the solubility kinetics of amorphous and/or nanoparticulate Cu and Zn sulfides in soil upon which OW has been spread is needed in order to determine whether AD actually reduces the trace element associated risk in OW spreading.

■ ASSOCIATED CONTENT

📄 Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: [10.1021/acs.est.7b01662](https://doi.org/10.1021/acs.est.7b01662).

Complementary analysis on raw waste and digestates; details concerning Zn and Cu K-edge EXAFS spectra data treatment, PCA, TT and LCF; Reference compound database for XAS; Comparison of EXAFS spectra of mineral and organic sulfides; Complementary XAS information for samples (PDF)

AUTHOR INFORMATION

Corresponding Author

*Phone: +221 33 849 33 17; fax: +221 33 849 16 75; e-mail: samuel.legros@cirad.fr.

ORCID

Samuel Legros: 0000-0001-7081-6679

Emmanuel Doelsch: 0000-0002-7478-4296

Notes

The authors declare no competing financial interest.

ACKNOWLEDGMENTS

We are grateful to APESA (Philippe Pouech, Camille Lagnet) for providing the samples. This study was part of the DIGESTATE project, funded by ANR (Agence Nationale de la Recherche, France) under Grant ANR-15-CE34-0003-01. We acknowledge the Stanford Synchrotron Radiation Light-source (SSRL) for provision of synchrotron radiation facilities. Use of the SSRL, SLAC National Accelerator Laboratory, is supported by the U.S. Department of Energy, Office of Science, Office of Basic Energy Sciences under Contract No. DE-AC02-76SF00515.

REFERENCES

- (1) Hoornweg, D.; Bhada-Tata, P.; Kennedy, C. Waste production must peak this century. *Nature* **2013**, *502*, 615–617.
- (2) Goss, M. J.; Tubeileh, A.; Goorahoo, D. A Review of the Use of Organic Amendments and the Risk to Human Health. *Adv. Agron.* **2013**, *120*, 275–379.
- (3) Belon, E.; Boisson, M.; Deportes, I. Z.; Eglin, T. K.; Feix, I.; Bispo, A. O.; Galsomies, L.; Leblond, S.; Guellier, C. R. An inventory of trace elements inputs to French agricultural soils. *Sci. Total Environ.* **2012**, *439*, 87–95.
- (4) Legros, S.; Doelsch, E.; Feder, F.; Moussard, G.; Sansoulet, J.; Gaudet, J. P.; Rigaud, S.; Doelsch, I. B.; Macary, H. S.; Bottero, J. Y. Fate and behaviour of Cu and Zn from pig slurry spreading in a tropical water-soil-plant system. *Agric., Ecosyst. Environ.* **2013**, *164*, 70–79.
- (5) Formentini, T. A.; Mallmann, F. J. K.; Pinheiro, A.; Fernandes, C. V. S.; Bender, M. A.; da Veiga, M.; dos Santos, D. R.; Doelsch, E. Copper and zinc accumulation and fractionation in a clayey Hapludox soil subject to long-term pig slurry application. *Sci. Total Environ.* **2015**, *536*, 831–839.
- (6) Legros, S.; Doelsch, E.; Masion, A.; Rose, J.; Borschneck, D.; Proux, O.; Hazemann, J. L.; Saint-Macary, H.; Bottero, J. Y. Combining size fractionation, scanning electron microscopy, and X-ray absorption spectroscopy to probe zinc speciation in pig slurry. *J. Environ. Qual.* **2010**, *39* (2), 531–540.
- (7) Doelsch, E.; Deroche, B.; Van de Kerchove, V. Impact of sewage sludge spreading on heavy metal speciation in tropical soils (Réunion, Indian Ocean). *Chemosphere* **2006**, *65* (2), 286–293.
- (8) Tella, M.; Bravin, M. N.; Thuriès, L.; Cazevielle, P.; Chevassus-Rosset, C.; Collin, B.; Chaurand, P.; Legros, S.; Doelsch, E. Increased zinc and copper availability in organic waste amended soil potentially involving distinct release mechanisms. *Environ. Pollut.* **2016**, *212*, 299–306.
- (9) Formentini, T. A.; Legros, S.; Fernandes, C. V. S.; Pinheiro, A.; Le Bars, M.; Levard, C.; Mallmann, F. J. K.; da Veiga, M.; Doelsch, E. Radical change of Zn speciation in pig slurry amended soil: Key role of nano-sized sulfide particles. *Environ. Pollut.* **2017**, *222*, 495–503.
- (10) Marcato, C. E.; Pinelli, E.; Pouech, P.; Winterton, P.; Guisresse, M. Particle size and metal distributions in anaerobically digested pig slurry. *Bioresour. Technol.* **2008**, *99* (7), 2340–8.
- (11) He, Z. L.; Yang, X. E.; Stoffella, P. J. Trace elements in agroecosystems and impacts on the environment. *J. Trace Elem. Med. Biol.* **2005**, *19* (2–3), 125–140.
- (12) van Leeuwen, H. P.; Town, R. M.; Buffle, J.; Cleven, R. F. M. J.; Davison, W.; Puy, J.; van Riemsdijk, W. H.; Sigg, L. Dynamic Speciation Analysis and Bioavailability of Metals in Aquatic Systems. *Environ. Sci. Technol.* **2005**, *39* (22), 8545–8556.
- (13) Smith, S. R. A critical review of the bioavailability and impacts of heavy metals in municipal solid waste composts compared to sewage sludge. *Environ. Int.* **2009**, *35* (1), 142–156.
- (14) Dąbrowska, L.; Rosińska, A. Change of PCBs and forms of heavy metals in sewage sludge during thermophilic anaerobic digestion. *Chemosphere* **2012**, *88* (2), 168–173.
- (15) Dong, B.; Liu, X.; Dai, L.; Dai, X. Changes of heavy metal speciation during high-solid anaerobic digestion of sewage sludge. *Bioresour. Technol.* **2013**, *131* (0), 152–158.
- (16) Marcato, C.-E.; Pinelli, E.; Cecchi, M.; Winterton, P.; Guisresse, M. Bioavailability of Cu and Zn in raw and anaerobically digested pig slurry. *Ecotoxicol. Environ. Saf.* **2009**, *72* (5), 1538–1544.
- (17) Doelsch, E.; Moussard, G.; Macary, H. S. Fractionation of tropical soilborne heavy metals-Comparison of two sequential extraction procedures. *Geoderma* **2008**, *143* (1–2), 168–179.
- (18) Donner, E.; Howard, D. L.; de Jonge, M. D.; Paterson, D.; Cheah, M. H.; Naidu, R.; Lombi, E. X-ray Absorption and Micro X-ray Fluorescence Spectroscopy Investigation of Copper and Zinc Speciation in Biosolids. *Environ. Sci. Technol.* **2011**, *45* (17), 7249–7257.
- (19) Doelsch, E.; Basile-Doelsch, I.; Rose, J.; Masion, A.; Borschneck, D.; Hazemann, J.-L.; Saint Macary, H.; Bottero, J.-Y. New Combination of EXAFS Spectroscopy and Density Fractionation for the Speciation of Chromium within an Andosol. *Environ. Sci. Technol.* **2006**, *40* (24), 7602–7608.
- (20) Ravel, B.; Newville, M. ATHENA, ARTEMIS, HEPHAESTUS: data analysis for X-ray absorption spectroscopy using iFFFIT. *J. Synchrotron Radiat.* **2005**, *12*, 537–541.
- (21) Doelsch, E.; Basile-Doelsch, I.; Rose, J.; Masion, A.; Borschneck, D.; Hazemann, J. L.; Macary, H. S.; Bottero, J. Y. New combination of EXAFS spectroscopy and density fractionation for the speciation of chromium within an andosol. *2006*, *40*, (24), 7602–7608.
- (22) Waychunas, G. A.; Fuller, C. C.; Davis, J. A. Surface complexation and precipitate geometry for aqueous Zn(II) sorption on ferrihydrite I: X-ray absorption extended fine structure spectroscopy analysis. *Geochim. Cosmochim. Acta* **2002**, *66* (7), 1119–1137.
- (23) Cismasu, A. C.; Levard, C.; Michel, F. M.; Brown, G. E., Jr. Properties of impurity-bearing ferrihydrite II: Insights into the surface structure and composition of pure, Al- and Si-bearing ferrihydrite from Zn(II) sorption experiments and Zn K-edge X-ray absorption spectroscopy. *Geochim. Cosmochim. Acta* **2013**, *119* (0), 46–60.
- (24) Legros, S.; Chaurand, P.; Rose, J.; Masion, A.; Briois, V.; Ferrasse, J. H.; Macary, H. S.; Bottero, J. Y.; Doelsch, E. Investigation of copper speciation in pig slurry by a multitechnique approach. *Environ. Sci. Technol.* **2010**, *44* (18), 6926–6932.
- (25) Sarret, G.; Saumitou-Laprade, P.; Bert, V.; Proux, O.; Hazemann, J. L.; Traverse, A.; Marcus, M. A.; Manceau, A. Forms of zinc accumulated in the hyperaccumulator *Arabidopsis halleri*. *Plant Physiol.* **2002**, *130* (4), 1815–1826.
- (26) Lv, J.; Zhang, S.; Wang, S.; Luo, L.; Huang, H.; Zhang, J. Chemical transformation of zinc oxide nanoparticles as a result of interaction with hydroxyapatite. *Colloids Surf., A* **2014**, *461*, 126–132.
- (27) Donner, E.; Brunetti, G.; Zarcinas, B.; Harris, P.; Tavakkoli, E.; Naidu, R.; Lombi, E. Effects of Chemical Amendments on the Lability and Speciation of Metals in Anaerobically Digested Biosolids. *Environ. Sci. Technol.* **2013**, *47* (19), 11157–11165.
- (28) Ma, R.; Levard, C.; Judy, J. D.; Urline, J. M.; Durenkamp, M.; Martin, B.; Jefferson, B.; Lowry, G. V. Fate of Zinc Oxide and Silver Nanoparticles in a Pilot Wastewater Treatment Plant and in Processed Biosolids. *Environ. Sci. Technol.* **2014**, *48* (1), 104–112.
- (29) Kim, B.; Levard, C.; Murayama, M.; Brown, G. E.; Hochella, M. F. Integrated Approaches of X-Ray Absorption Spectroscopic and Electron Microscopic Techniques on Zinc Speciation and Characterization in a Final Sewage Sludge Product. *J. Environ. Qual.* **2014**, *43* (3), 908–916.

- (30) Lau, B. L. T.; Hsu-Kim, H. Precipitation and Growth of Zinc Sulfide Nanoparticles in the Presence of Thiol-Containing Natural Organic Ligands. *Environ. Sci. Technol.* **2008**, *42* (19), 7236–7241.
- (31) Ferrer, P.; Jiménez-Villacorta, F.; Rubio-Zuazo, J.; da Silva, L.; Castro, G. R. Environmental Influence on Zn–Histidine Complexes under No-Packing Conditions. *J. Phys. Chem. B* **2014**, *118* (11), 2842–2850.
- (32) Sarret, G.; Manceau, A.; Spadini, L.; Roux, J.-C.; Hazemann, J.-L.; Soldo, Y.; Eybert-Bérard, L.; Menthonnex, J.-J. Structural Determination of Zn and Pb Binding Sites in *Penicillium chrysogenum* Cell Walls by EXAFS Spectroscopy. *Environ. Sci. Technol.* **1998**, *32* (11), 1648–1655.
- (33) Xiaoli, C.; Shimaoka, T.; Xianyan, C.; Qiang, G.; Youcai, Z. Characteristics and mobility of heavy metals in an MSW landfill: Implications in risk assessment and reclamation. *J. Hazard. Mater.* **2007**, *144* (1–2), 485–491.
- (34) Peltier, E.; Dahl, A. L.; Gaillard, J. F. Metal speciation in anoxic sediments: When sulfides can be construed as oxides. *Environ. Sci. Technol.* **2005**, *39* (1), 311–316.
- (35) van Hullebusch, E. D.; Utomo, S.; Zandvoort, M. H.; Lens, P. N. Comparison of three sequential extraction procedures to describe metal fractionation in anaerobic granular sludges. *Talanta* **2005**, *65* (2), 549–558.
- (36) Kau, L. S.; Spira-Solomon, D. J.; Penner-Hahn, J. E.; Hodgson, K. O.; Solomon, E. I. X-ray absorption edge determination of the oxidation state and coordination number of copper. Application to the type 3 site in *Rhus vernicifera* laccase and its reaction with oxygen. *J. Am. Chem. Soc.* **1987**, *109* (21), 6433–6442.
- (37) Manceau, A.; Matynia, A. The nature of Cu bonding to natural organic matter. *Geochim. Cosmochim. Acta* **2010**, *74* (9), 2556–2580.
- (38) Laglera, L. M.; van den Berg, C. M. G. Copper complexation by thiol compounds in estuarine waters. *Mar. Chem.* **2003**, *82* (1–2), 71–89.
- (39) Fulda, B.; Voegelin, A.; Maurer, F.; Christl, I.; Kretzschmar, R. Copper Redox Transformation and Complexation by Reduced and Oxidized Soil Humic Acid. 1. X-ray Absorption Spectroscopy Study. *Environ. Sci. Technol.* **2013**, *47* (19), 10903–10911.
- (40) Villa-Gomez, D. K.; van Hullebusch, E. D.; Maestro, R.; Farges, F.; Nikitenko, S.; Kramer, H.; Gonzalez-Gil, G.; Lens, P. N. L. Morphology, Mineralogy, and Solid–Liquid Phase Separation Characteristics of Cu and Zn Precipitates Produced with Biogenic Sulfide. *Environ. Sci. Technol.* **2014**, *48* (1), 664–673.
- (41) Shea, D.; Helz, G. R. Solubility product constants of covellite and a poorly crystalline copper sulfide precipitate at 298 K. *Geochim. Cosmochim. Acta* **1989**, *53* (2), 229–236.
- (42) Patrick, R. A. D.; Mosselmans, J. F. W.; Charnock, J. M.; England, K. E. R.; Helz, G. R.; Garner, C. D.; Vaughan, D. J. The structure of amorphous copper sulfide precipitates: An X-ray absorption study. *Geochim. Cosmochim. Acta* **1997**, *61* (10), 2023–2036.
- (43) Michard, G.; Allègre, C. J. Comportement géochimique des éléments métalliques en milieu réducteur et diagrammes (LogS, pH). *Miner. Deposita* **1969**, *4*, 1–17.
- (44) L’Herroux, L.; Le Roux, S.; Appriou, P.; Martinez, J. Behavior of metals following intensive pig slurry applications to a natural field treatment process in Brittany (France). *Environ. Pollut.* **1997**, *97* (1–2), 119–130.
- (45) Farley, K. J.; Dzombak, D. A.; Morel, F. M. M. A surface precipitation model for the sorption of cations on metal oxides. *J. Colloid Interface Sci.* **1985**, *106* (1), 226–242.
- (46) Carbonell-Barrachina, A. A.; Rocamora, A.; García-Gomis, C.; Martínez-Sánchez, F.; Burló, F. Arsenic and zinc biogeochemistry in pyrite mine waste from the Aznalcóllar environmental disaster. *Geoderma* **2004**, *122* (2–4), 195–203.
- (47) Isaure, M.-P.; Manceau, A.; Geoffroy, N.; Laboudigue, A.; Tamura, N.; Marcus, M. A. Zinc mobility and speciation in soil covered by contaminated dredged sediment using micrometer-scale and bulk-averaging X-ray fluorescence, absorption and diffraction techniques. *Geochim. Cosmochim. Acta* **2005**, *69* (5), 1173–1198.
- (48) Stanton, M. R.; Gemery-Hill, P. A.; Shanks Iii, W. C.; Taylor, C. D. Rates of zinc and trace metal release from dissolving sphalerite at pH 2.0–4.0. *Appl. Geochem.* **2008**, *23* (2), 136–147.
- (49) Voegelin, A.; Jacquat, O.; Pfister, S.; Barmettler, K.; Scheinost, A. C.; Kretzschmar, R. Time-dependent changes of zinc speciation in four soils contaminated with zincite or sphalerite. *Environ. Sci. Technol.* **2011**, *45* (1), 255–261.
- (50) Stemmler, S. J.; Loyaux-Lawnczak, S.; Berthelin, J. Effet de la teneur en eau d’un sol sur la réduction bactérienne d’oxydes de fer. *C. R. Geosci.* **2004**, *336* (13), 1171–1179.
- (51) Adams, F.; Martin, J. B. *Liming Effect on Nitrogen Use and Efficiency*; American society of agronomy: Madison, United States of America, 1994; p 426.
- (52) Calmano, W.; Hong, J.; Förstner, U. Binding and mobilization of Heavy metals in contaminated sediments affected by pH and redox potential. *Water Sci. Technol.* **1993**, *28* (8–9), 233–235.
- (53) Al-Abed, S. R.; Hageman, P. L.; Jegadeesan, G.; Madhavan, N.; Allen, D. Comparative evaluation of short-term leach tests for heavy metal release from mineral processing waste. *Sci. Total Environ.* **2006**, *364* (1–3), 14–23.