Drastic Change in Zinc Speciation during Anaerobic Digestion and Composting: Instability of Nanosized Zinc Sulfide

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ABSTRACT. Zinc (Zn) is a potentially toxic trace element that is present in large amounts in organic wastes (OWs) spread on agricultural lands as fertilizer. Zn speciation in OW is a crucial parameter to understand its fate in soil after spreading and to assess the risk associated with agricultural recycling of OW. Here, we investigated changes in Zn speciation from raw OWs up to digestates and/or composts for a large series of organic wastes sampled in full-scale plants. Using extended X-ray absorption fine structure (EXAFS), we show that nano-sized Zn sulfide (nano-ZnS) is a major Zn species in raw liquid OWs and a minor species in raw solid OWs. Whatever the characteristics of the raw OW, anaerobic digestion always favors the formation of nano-ZnS (>70% of zinc in digestates). However, after 1 to 3 months of composting of OWs, nano-ZnS becomes a minor species (<10% of zinc). In composts, Zn is mostly present as amorphous Zn phosphate and Zn sorbed to ferrihydrite. These results highlight (i) the influence of OW treatment on Zn speciation and (ii) the chemical instability of nano-ZnS formed in OW in anaerobic conditions.

Introduction

Organic waste (OW) production is steadily increasing as a result of the growth of the world population[1] and there is thus an urgent need for sustainable OW management. OWs have different origins: agricultural (livestock slurries, harvest residues), urban (sewage sludge), industrial (food industry by-products) and municipal waste (OW collected from private households).[2] Among the

solutions for sustainable OW management, anaerobic digestion (AD) is gaining interest because biogas can be produced by biological decomposition of organic matter[2] and because of the value of the organic residue (called digestate) as fertilizer.[3] The short term advantages of applying digestate on cropped soils have already been evidenced,[4] but recently, questions have been raised about the long-term impact of the accumulation of trace elements (TEs) in soils after recycling of digestate.[5] With increasing interest in anaerobic digestion (AD) worldwide, it is crucial to determine the risk associated with agricultural recycling of digestate.

Among all the TEs present in OWs, zinc (Zn) is the most applied on agricultural lands.[6,7] In particular, sewage sludge and livestock manures used as amendments have relatively high concentrations of Zn (321-4753 mg/kg).[8-11] As Zn has been shown to be hazardous for plants,[12-15] regulations have been introduced to limit the environmental impact of OW recycling. However, the regulations are based on total Zn (and more generally on TE) concentrations in OW,[16,17] whereas Zn eco-toxicity not only depends on the total concentration but also on Zn speciation, which determines its bioavailability.[18] For instance, different toxicity mechanisms have been described when *Escherichia coli* were exposed to nanoparticulate ZnO or dissolved Zn.[19]

Recent studies using X-ray absorption spectroscopy (XAS) have shown that part of Zn is present as zinc sulfide (ZnS) in raw OWs[20,21] and digestates.[20,22-26] This technique is thus suitable to determine Zn speciation in a complex matrix such as OW.[27,28] Surprisingly, the proportion of ZnS present in OWs is wide, ranging from 0 to 100% in raw OWs[20,21] and 0 to 96% in digestates.[20,22-25] These differences can be explained by several factors including (i) the type of reactor sampled (lab-scale *vs.* full-scale reactors), (ii) the origin and the nature of the OW (agricultural *vs.* urban, solid *vs.* liquid) and/or (iii) the processing steps that may differ from one laboratory/site to another (e.g. hydraulic retention time, storage conditions). All these parameters can affect the physical-chemical conditions of the systems, which, in turn, control the speciation of Zn present in raw or digested OWs.

Some studies have reported that Zn K-edge extended X-ray absorption fine structure (EXAFS) spectra in OWs are (*i*) less structured than for a crystalline ZnS, and (*ii*) are similar to what is observed for 3-4 nm ZnS nanoparticles (referred to hereafter as nano-ZnS).[20,21,26] The presence of nano-sized and not well-organized ZnS in OW is thus suspected. This result is important when assessing the agro-environmental risks of agricultural recycling of OWs. Indeed, although nano-ZnS eco-toxicity has been poorly studied, nanoparticles are generally known for their increased reactivity and associated toxic effects in comparison with their bulk analogs.[29]

As the increased reactivity of nanoparticles favors their dissolution and transformation,[29] the chemical stability of nano-ZnS during treatment needs to be assessed. Recently, it was shown that ZnS present in sewage sludge digestates was partly or totally oxidized during composting[22-25] suggesting low stability of ZnS formed during AD. Moreover, no available information is available on Zn speciation in the intermediate products (solid/liquid fractions of digestates) even though these may also be spread on soils.

We conducted a systematic study to investigate the speciation of Zn in raw OWs and treated OWs in a wide range of full-scale OW treatment plants chosen to represent the most common origins of OW (agricultural, urban, industrial OW) and treatment processes (AD, composting). Special care was taken to sample OWs throughout the process to assess changes in Zn speciation during treatment. Nine full-scale treatment plants that process agricultural, urban and industrial OW were sampled and X-ray absorption spectroscopy was used to investigate the speciation of Zn in raw and treated OWs.

Experimental section

Preparation of organic waste samples

Organic wastes were sampled in nine different full-scale treatment plants distributed throughout France. The plants were chosen to represent the most common types of raw OW used as feedstock for anaerobic digestion (AD) and composting: agricultural waste (livestock effluents, crop residues, etc.),

84 urban wastes (sewage sludge, catering and major-food retailer wastes) and industrial wastes (from the
85 food industry). For the sake of clarity, the treatment plants are separated into three groups based on the
86 origin of the raw OW used as feedstock: Urban (sewage sludge), Agri (agricultural waste), Central (mix
87 of different types of OW digested in a centralized plant). Depending on the plant, OWs are treated by
88 AD and/or composting. Some of the AD plants apply a post-treatment to the digestate: solid/liquid
89 separation, pellet making and/or composting. For example, the Urban 2 plant includes a pelletizing unit
90 (drying, liming and pressing into pellets) to process the solid digestate. For AD, three types of reactors
91 are used depending on the dry matter (DM) content: wet reactors (DM < 16%), semi-dry reactors
92 (DM=[16-22%]) and dry reactors (DM=[22-40%]).[30] All digested OW were processed in wet reactors
93 except Agri 2 (digestate DM content 19%). Another specificity of this plant the temperature (45°C) used
94 is higher than the mesophilic temperature (32-42°C) used in all the other digesters. The characteristics of
95 the OW treatment plants are listed in Table 1.

		Anaerobic digestion			Composting	
Site	Composition of raw organic wastes	Reactor Size (m^3)	Duration (days)	Solid/liquid separation characteristics	Duration (weeks)	Co-substrate
Urban-1	Sewage sludge	6200	29	Filter press – 19m^3/h	4	Green waste
Urban-2	Sewage sludge	6000	34	Centrifugation – 20 m^3/h	12	Green waste
Urban-3	Activated and centrifuged sewage sludge	-	-	-	6	Green waste
Agri-1	Pig slurry + co-substrates [1]	950	38	Centrifugation – 8m^3/h Solid digestate stockpiled	12	None
Agri-2	Solid cow manure + co-substrates [2]	740	42	-	-	-
Agri-3	Solid cow manure	-	-	-	n/a	None
Central-1	Livestock effluents[3] + sewage sludge + food industry waste + harvest residues	1600	70	Screw press – n/a Solid digestate stockpiled	-	-
Central-2	Pig slurries + food industry waste + harvest residues + poultry manure	6000	30	Centrifugation – 8 m^3/h	-	-
Central-3	Catering and waste from major food retailers + cow slurries + food industry waste + wine making waste	8000	90	-	-	-

96 Table 1: Description of the treatment plants

97 [1]harvest residues, grass clippings

98 [2]harvest residues, fecal matter from cattle intestines, grass clippings, poultry litter

99 [3] solid cow, sheep and horse manure - cow and pig slurries

At each full-scale plant, around 3 kg of dry matter of OWs were sampled at different steps of the treatment. At the seven AD plants (Urban 1-2, Agri 1-2 and Central 1-2-3), OW was sampled just before AD (samples called "raw OW") and just after AD (samples called "digestate"). When a digestate post-treatment was included in the process, the intermediate products and the final products (ready to apply as fertilizer) were sampled (solid/liquid fractions of digestate called "solid digestate" and "liquid digestate" respectively, pellets and compost). At the two composting plants (Agri-3, Urban-3), raw OW was sampled before composting, and compost was sampled. All the samples were frozen at -20 °C, at the latest three days after on-site sampling.

Physical-chemical analyses

Dry matter (DM) content of the OW samples was determined at 60 °C by weight (+/- 0.2%). pH was measured directly in liquid OW samples. The pH of solid OW samples was measured after water extraction (ratio of OW to water volume 1:5) according to the ISO 10390 standard. Each pH value is given with an uncertainty of +/- 0.1. Oxidation-reduction potential (ORP) was measured in liquid samples only using a platinum wire electrode (Hamilton Polilyte Plus ORP Arc 120) with +/- 20 mV uncertainty. OW samples were freeze-dried, ground and homogenized for digestion using a mixture of HF, HNO_3 and $HClO_4$ (ISO 14869-1). Next, concentrations of trace elements were determined using an inductively coupled plasma mass spectrometer (ICP-MS iCAP^{TM} Q Thermo Scientific). Before each element was analyzed, the method of quantification was checked using certified reference material (sewage sludge (BSR 146R) and soil (LAOM CRM 7004)). Measurement uncertainty was less than 15%. Total carbon (ISO 10694) was determined by dry combustion (Dumas) with an elemental NC 2100 Soil Analyzer (Thermo Electron Corp.) The method of analysis was validated using certified reference material (Orchard Leaves Standard 502-055). Uncertainty was less than 5%. Inorganic carbon (ISO

10693) was determined (uncertainty +/-2%) with a Bernard Calcimeter by adding HCl and measuring released CO_2. Organic carbon (Corg) was deduced from the difference between total and inorganic carbon. Uncertainty of Corg concentration (+/- 7%) was determined by adding absolute uncertainties of total C and inorganic C. One replicate was performed for each OW and each parameter.

Preparation of samples and reference compounds for EXAFS analysis

When fresh and freeze-dried OW samples were compared using Zn K-edge EXAFS spectra, no influence of the freeze-drying process was observed (Figure S1). Therefore, all OW samples were freeze-dried before EXAFS analysis. Potential changes in Zn speciation during the aging of freeze-dried samples were also assessed. No change in Zn speciation was observed after three weeks of aging at room temperature (Figure S2). However, after six months and one year of aging of the freeze-dried sample, Zn speciation in OW was altered. To avoid these changes, all freeze-dried samples were stored at -20 °C until EXAFS analysis. Indeed, at -20 °C, there was no change in Zn speciation after 6 months of aging (Figure S3). Freeze-dried OW samples were cryo-ground before analysis (Retsch MM400).

Nano-ZnS reference compound was synthesized by mixing sodium sulfide (Na_2S) and zinc chloride ($ZnCl_2$) solutions at a molar ratio S/Zn of 1 to obtain a final ZnS concentration of 4 g/L. After eight days of agitation, the suspension was dialyzed (MWCO 1kDa) using ultrapure water for two days to remove excess Na^+ and Cl^- ions. The final nano-ZnS suspension was freeze-dried and the resulting solid was analyzed by X-ray diffraction (X'Pert Pro MPD, Panalytical). A crystallite size of 3 +/- 0.4 nm was determined using the Scherrer equation[31] (Figure S4). A commercial ZnS with a crystallite size of 20 nm (Figure S4) was purchased from Sigma Aldrich. Amorphous zinc phosphate (Am-Zn-phosphate) was synthesized following the protocol of Bach et al.[32] Briefly, $NaHPO_4^-$ (0.010 M, pH adjusted to 11.9) and $ZnCl_2$ (0.015 M) solutions were

mixed and immediately centrifuged at 12,544 g for 5 min and rinsed three times with acetone (> 99%, VWR). The resulting solid was dried under vacuum. The X-ray diffraction (Figure S5) pattern is in accordance with the result obtained by Bach et al. (2015).[32] Reference compounds were diluted in polyvinylpyrrolidone powder, ground and pressed into pellets for EXAFS analysis.

EXAFS spectra acquisition and analysis

Zn K-edge X-ray absorption spectra were recorded at the SSRL (Stanford, USA) on the 11-2 beamline and at the ESRF (Grenoble, France) on the BM30B (FAME) beamline. Each spectrum was measured at liquid helium temperature to prevent the beam damaging the samples. On both beamlines, the spectra of the OW samples were measured in fluorescence mode with a 30-element solid state Ge detector. The spectra of the reference samples were measured in transmission mode. One spectrum is the average of two to nine scans, depending on the concentration of Zn and the noise level. Each scan was measured on a different spot on the pellet to limit beam damage. Energy calibration was performed using metallic Zn reference foil (absorption edge defined at 9659 eV). Normalization and data reduction were performed according to standard methods[33] using Athena software.[34]

A library of Zn reference compound spectra was used to identify Zn species in the OW. Least square linear combination fitting (LCF) was performed for each OW spectrum over a k-range of 2.5 - 10.6 $Å^{-1}$ using Athena software.[34] The library of Zn reference compounds consisted in nano-ZnS, Am-Zn-phosphate, commercial ZnS (see previous section) and reference compounds described elsewhere[20,35-39] (Zn-cysteine, Zn-histidine, Zn-malate, Zn-sorbed to ferrihydrite (Zn-FeOx), Zn-methionine, Zn-cryptomelane Zn-phosphate, Zn-phytate, Zn-Goethite, Zn-oxalate-hydrate, Sphalerite, Smithsonite, Zincite, Zn hydroxide (Figure S6)). Residual factor of LCF was

calculated as follows: R = $\Sigma(k^3\chi(k)_{exp} - k^3\chi(k)_{fit})^2/\Sigma(k^3\chi(k)_{exp})^2$. At each step of the fitting, an additional reference spectrum was added if the two following conditions were true: the R factor decreased by 20% or more and the additional reference had a contribution equal to or higher than 10% among Zn-species. The uncertainty of this LCF method was estimated at +/- 15%.[33]

Results and Discussion

Changes in physical-chemical characteristics of OWs during treatment

The physical-chemical characteristics of OWs during treatment were identified as these parameters can control the speciation of Zn.

Table 2: Characteristics of raw and treated OWs (DM= dry matter, ORP=oxidation reduction potential, Corg=organic carbon). The concentrations of all the elements are expressed on a dry matter (DM) basis. Values are given +/- 15% for [Zn], [P] and [Fe], +/- 7% for [Corg], +/- 20 mV for ORP, +/- 0.1 for pH and +/- 0.2% for DM)

Treatment plant	Sample	DM %	pH	[Zn] mg/kg	[Fe] g/ kg	[P] g/ kg	[Corg] %	ORP (mV)
Urban-1	Raw OW	3.4	6.3	701	49.28	33.80	40	-213
	Digestate	3.1	7.3	822	56.73	39.21	34	-248
	Solid digestate	18.7	6.6	807	87.80	38.92	32	
	Solid digestate + green waste	41.4	6.9	275	25.56	11.00	43	
	Compost	58.2	7.0	423	37.77	17.54	30	
Urban-2	Raw OW	5.3	5.2	475	30.63	20.74	50	-138
	Digestate	2.7	7.6	694	48.61	43.01	37	-249
	Solid digestate	16.4	6.8	696	52.20	40.01	37	
	Pellets	91.7	12.2	543	41.48	33.43	29	
	Compost	96.6	7.75	711	62.04	31.58	27	
Urban-3	Raw OW	17.8	5.9	566	5.23	27.39	49	
	Compost	65.1	7.0	452	10.64	21.42	26	
Agri-1	Raw OW	5.1	6.4	398	2.11	13.72	48	-187
	Digestate	2.5	7.9	723	3.13	16.27	35	-233
	Liquid digestate	2.9	8.1	1 241	4.40	17.48	36	-262
	Solid digestate	36.8	6.8	547	5.34	34.51	34	
	Compost	59.7	6.8	697	8.96	42.27	22	
Agri-2	Raw OW	37.3	8.8	70	0.42	5.27	46	
	Digestate	19.0	9.1	244	3.74	12.32	38	
Agri-3	Raw OW	23.0	9.2	83	1.27	4.79	43	
	Compost	38.9	9.3	116	1.65	5.14	42	
Central-1	Raw OW	8.0	5.7	325	18.13	13.13	42	-177
	Digestate	11.0	7.9	701	40.61	21.22	32	-241
	Liquid digestate	5.8	7.8	663	38.02	19.71	31	-255

	Solid digestate	26.5	7.6	178	14.19	8.60	47	
Central-2	Raw OW	14.7	6.3	533	18.10	16.45	57	-209
	Digestate	7.4	8.5	841	27.89	32.07	40	-347
	Solid digestate	23.5	6.2	995	32.96	27.43	41	
Central-3	Raw OW	28.0	4.7	64	2.79	5.62	37	-112
	Digestate	7.4	7.9	201	9.05	14.36	34	-341

184 *Dry Matter, organic carbon and pH*

185 The range of dry matter (DM) content in the raw OWs used in this study was wide (ranging
186 from 3 to 37%, Table 2), reflecting the diversity of raw OWs used in the full-scale treatment
187 plants. Corg and DM content in the digestate were lower than in raw OW, due to the organic
188 matter released as biogas during AD: 9 to 30% of initial Corg in the raw OW is converted into
189 CH_4 and CO_2 (main gas phases of biogas),[2] which is in agreement with previous results.[20] Urban-
190 1 and Central-1 are exceptions to this trend, possibly be explained by the heterogeneity of OW
191 samples over time.[40] Similarly, Corg content decreased during composting: 3 to 47% of initial
192 Corg (in raw OW for Urban-3, Agri-3, solid digestate for Urban-1, Agri-1 or pellets for Urban-2)
193 was converted into CO_2 by carbon mineralization. Despite this C loss, DM content increased
194 after composting due to greater water loss during the aerobic process. pH values ranged from 4.7
195 to 9.2 in raw OWs and increased after AD due to the degradation of volatile fatty acids, as
196 observed in many studies.[8,9,41-43]

197 *Zn concentration*

198 The concentration of Zn (Table 2) in raw OWs ranged from 64 to 701 mg/kg. This wide range
199 can be explained by the different origins of the raw OW. Sewage sludges (raw OW in Urban-1, -
200 2, -3) tended to have high concentrations of Zn (475 – 701 mg/kg) as described earlier.[9-11] In

agricultural wastes (Agri-1, -2, -3), we observed high variability of Zn concentrations among treatment plants. The Zn concentration in raw OW sampled from Agri-1 was relatively high (398 mg/kg) whereas it was lower in samples from Agri-2 and Agri-3 (70 and 83 mg/kg, respectively). Raw OW from Agri-1 contained 80% of pig slurry whereas raw OWs from Agri-2 and Agri-3 contained solid cow manure. The Zn concentration in pig slurries is always significantly higher than in cattle slurries[8,44] because of the addition of Zn in pig feed and low intake by the animal.[45] In samples from the centralized AD plants (Central-1, -2, -3), we also observed a wide range of Zn concentrations among sites. Raw OWs with high Zn concentration were those that partly comprised pig slurries (Central-1: 325 mg/kg) and/or sewage sludge (Central-2: 533 mg/kg) in contrast to Central-3 (64 mg/kg) that contained neither. These results show that the concentration of Zn in raw OWs is driven by the origin of the OW.

Similar changes in Zn concentrations during OW treatments (AD or composting) were observed whatever the plant considered. Zn is conserved during AD while organic matter is lost. Therefore Zn concentration in digestates was higher than in raw OW, confirming previous observations.[9,20,42] Likewise, Zn concentrations increased following composting as reported by Knoop et al.[42] The only exception was Urban-3 compost where the decrease in Zn concentration could be due to dilution caused by the addition of green waste.

Change in Zn speciation during treatment

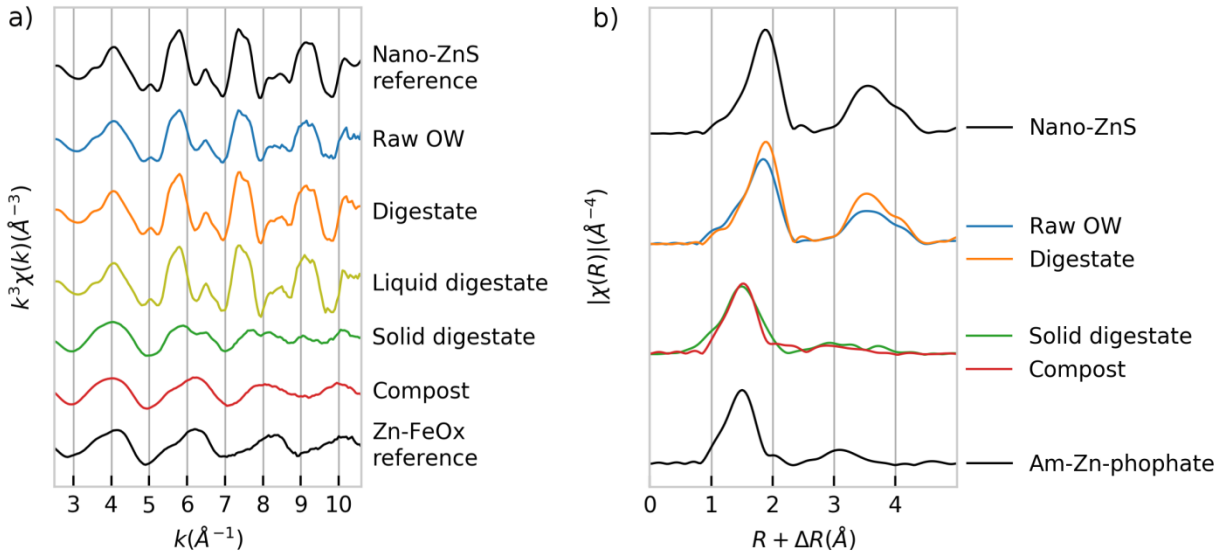
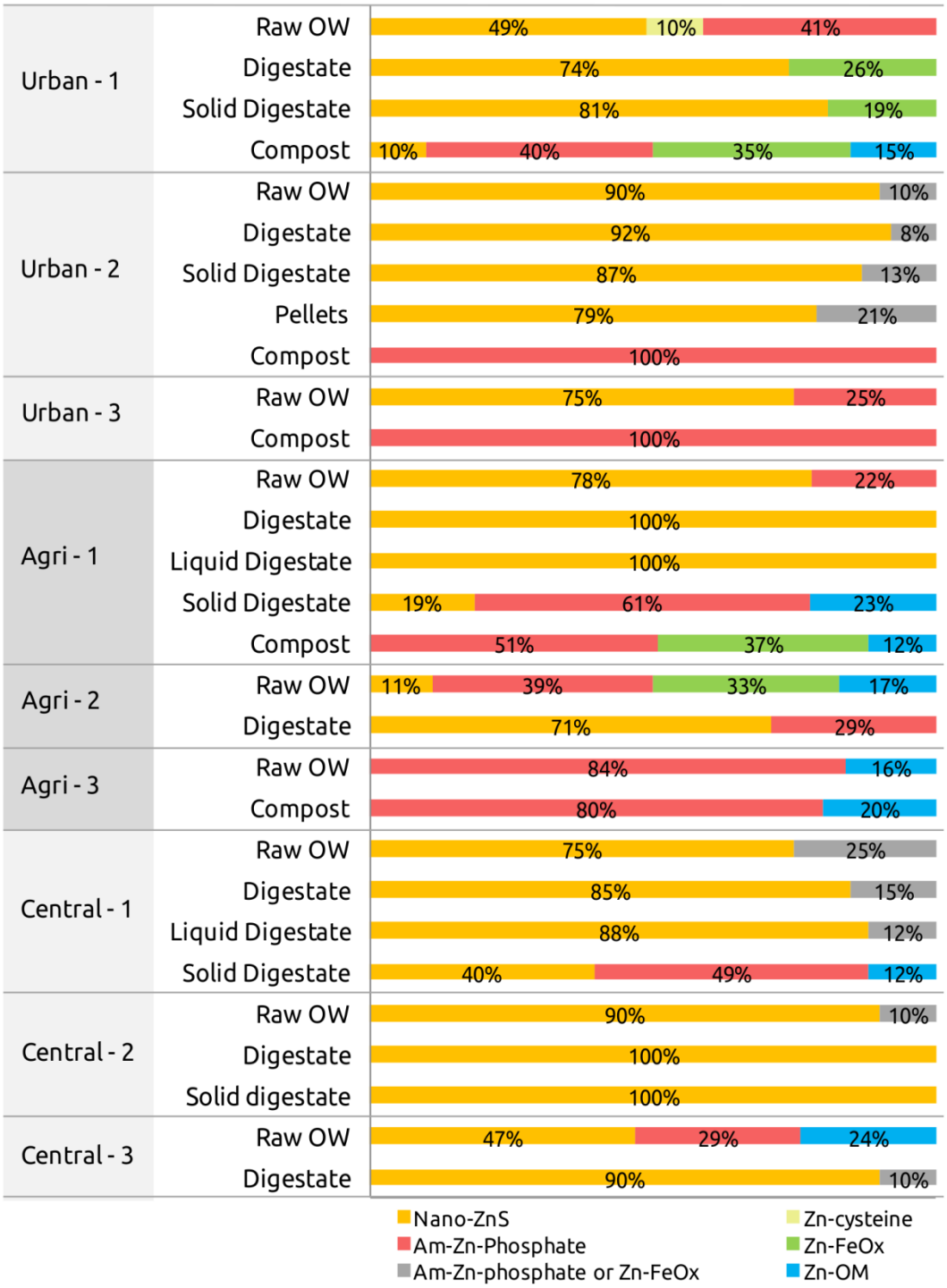


Figure 1: (a) Zn K-edge extended X-ray absorption fine-structure spectroscopy (EXAFS) spectra and (b) respective Fourier transforms (FT) of raw and treated OWs at different steps of the treatment at the Agri-1 site. Nano-ZnS and Zn-FeOx reference compounds have been added for EXAFS comparison.

First, a visual comparison of EXAFS spectra and the corresponding Fourier transforms (FT) of the samples at different steps of the treatment already provides qualitative insight into the change in Zn speciation during the process. Zn K-edge EXAFS spectra (Figure 1 (a)) are characteristic of Zn atomic environment (number of neighboring atoms, their distance from absorbing Zn atom, static disorder). Fourier transform of EXAFS spectra (Figure 1 (b)) gives an atomic radial distribution function for which the peak position is specific to an interatomic distance (from a Zn atom) and peak intensity depends on the number of neighboring atoms and static disorder. As an example, Figure 1 shows spectra and the corresponding FT obtained at the Agri-1 plant. Data for the eight other plants are presented in supplementary information SI-4.

EXAFS spectra of raw OW and digestate from Agri-1 strongly resembled the spectrum of the nano-ZnS reference compound (Figure 1 (a)). Centrifugation of Agri-1 digestate resulted in two fractions with contrasted Zn speciation. The EXAFS spectrum of the liquid digestate was very similar to the spectrum of the nano-ZnS reference compound whereas the solid fraction of the digestate tended to be closer to the spectrum of the Zn-FeOx reference compound. The FT can be used to distinguish the shift from a Zn—S contribution for raw OW, digestate and liquid digestate to a Zn—O contribution for solid digestate and compost. In addition to this visual inspection of the spectra, linear combination fitting (LCF) was performed to obtain quantitative results on Zn speciation in OWs in all the samples. The modeled spectra of all the samples were compared to experimental spectra (Figure S7). The quantitative LCF results (expressed as a percentage of each Zn species) obtained for samples from the nine treatment plants are shown in Figure 2. Experimental data were modeled with 1 to 4 references and total contributions ranging from 91 to 107%. The contributions of Zn species were normalized to 100% for easier comparison. R-factor of the LCFs ranged from 0.0057 to 0.0292. For the sake of clarity, reference compounds for Zn bound to organic matter (Zn-malate, Zn-histidine or Zn-methionine, see Table S1 and Figure S6) are represented as "Zn-OM". Also, in some cases, when the contributions of amorphous Zn phosphate (Am-Zn-Phosphate) and Zn bound to ferrihydrite (Zn-FeOx) were less than 25%, it was impossible to differentiate between them (Figure S8). In this particular case, they are represented as "Am-Zn-phosphate or Zn-FeOx".



252

253 Figure 2: Zn speciation determined from the linear combination fitting of the Zn K-edge

254 extended X-ray absorption fine-structure spectroscopy data for all raw and treated OWs.

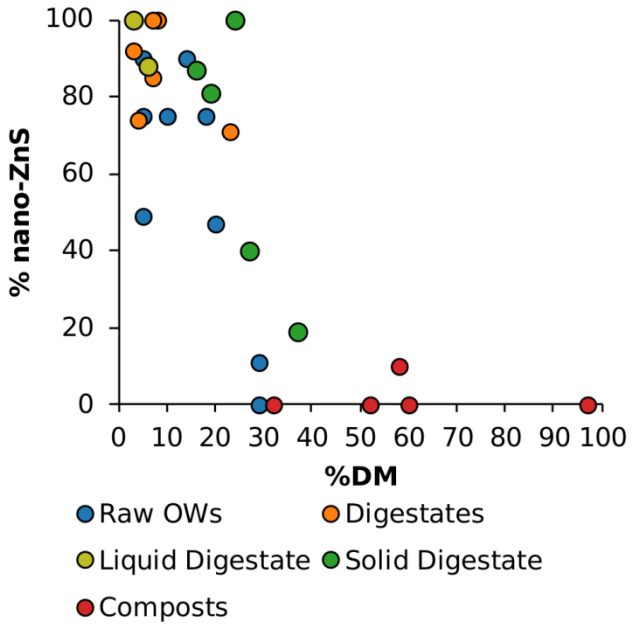


Figure 3: Percentage of nano-ZnS determined by linear combination fitting of the Zn K-edge extended X-ray absorption fine-structure spectroscopy data as a function of dry matter (DM) content for all raw and treated OWs (except for the pellets from Urban-2 as the treatment consisting of drying, liming and pressing is specific).

Zn speciation in raw OWs

Nano-ZnS was found to be the main Zn species in raw OWs (ranging from 47 to 90%) except in samples from Agri-2 (11%) and Agri-3 (no detection). The relatively wide range of nano-ZnS may be due to the nature of OW and more importantly to their different physical-chemical storage conditions (DM content and ORP). Indeed, Figure 3 reveals an opposite trend between the percentage of nano-ZnS in raw OW and their DM content. Interestingly, samples from Agri-2 and Agri-3, which did not contain significant amounts of nano-ZnS, had the highest DM content of all the raw OW samples (37 and 23% DM, respectively) (Figure 3). We were unable to measure an ORP value for these samples. However, it is probable that storage at such high DM

content favors aerobic conditions. Conversely, reducing conditions in raw liquid OWs from Urban-1,2,3, Agri-1, and Central-1,2 were favored, as shown by ORP values (-213 to-138 mV). This could enhance sulfur reduction and hence the formation of nano-ZnS with the presence of sulfate reducing bacteria (SRB).[46-48] The second main Zn species in raw OWs was amorphous Zinc phosphate (Am-Zn-phosphate) which accounted for 22 to 84% of Zn species. This is in agreement with the high affinity of Zn for phosphate compounds.[49] Hopeite (crystalline zinc-phosphate) has already been evidenced in previous studies as a major Zn phase in OW.[20,23,24]

Zn speciation in digestates

Whatever the raw OW, nano-ZnS was the dominant Zn species in the digestates. The contribution of nano-ZnS ranged from 71 to 100% in the digestates (Figure 2). What is more, the proportion of nano-ZnS always increased after AD (except in samples from Urban-2) (Figure 2). The more reducing conditions in the digestate compared with in raw OWs, as illustrated by ORP values (from -213 to -112mV in raw OWs and from -347 to-233 mV in digestates, see Table 2), may favor the formation of zinc sulfide. Indeed, AD conditions favor the presence of sulfate reducing bacteria (SRB), which convert sulfate to sulfide.[50]

Zn speciation in the liquid and solid fractions of the digestate

Zn speciation in the liquid and solid fractions of digestate was influenced by the storage conditions. The speciation of Zn in the solid fractions of digestate from Agri-1 and Central-1 had low percentages of nano-ZnS and differed from that of the digestates, whereas nano-ZnS were still present in samples from Urban-1, Urban-2 and Central-2. Such differences can be explained by the different sampling conditions. Agri-1 and Central-1 solid digestates were sampled in the storage pile whereas the other solid fractions of digestates were sampled directly at the end of the

separation process. When sampled in the storage pile, OWs had been exposed to the atmosphere for some days so nano-ZnS may have been oxidized. Nano-ZnS was detected in the liquid fraction after phase separation (Agri-1 and Central-1), which can be explained by the low DM content of the liquid digestates (3 and 6%, respectively). In addition, the storage of liquid digestate without mixing maintains anaerobic conditions, as shown by the ORP values (-262 and -255mV, respectively). Nano-ZnS speciation was preserved after Urban-2 solid digestate was pelleted, probably because the pellets were sampled immediately after processing and exposure to the atmosphere was thus limited. These results highlight the reactivity of ZnS formed in OW and the influence of OW storage and sampling conditions on Zn speciation.

Zn speciation in composts

In compost, Zn speciation differed dramatically from Zn speciation in digestates (Figure 2). Nano-ZnS was no longer observed in any of the composts, (except in Urban-1 compost in which 10% of Zn remained in the form of sulfide). This means that nano-ZnS initially present in raw OW and formed during AD disappeared after four to 12 weeks of composting (Table 1). Previous studies on sewage sludge composted at laboratory scale[24,25] and sewage sludge composted at full scale[23] still revealed ZnS or Zn-cysteine (amino acid containing thiol side chain) as a minor part of Zn speciation in compost (26 - 30% for ZnS and 6 -21%, for Zn-cysteine). The main species of Zn in all the composts studied was Am-Zn-phosphate (from 40 to 100%). The second main species was Zn-FeOx present in composts from Urban-1 and Agri-1. Zn associated with organic matter (OM) was present in three composts (Urban-, Agri-1 and Agri-3) as a minor species (12 to 20% of Zn). Other studies on Zn speciation in compost of sewage sludges have shown that the main Zn phase is Zn-FeOx (35-71%), followed by hopeite (crystalline Zn-phosphate, 14-31%).[23-25] There are therefore significant differences in results

obtained in previous work and the present study. These discrepancies can be explained by the introduction of a new reference compound spectrum in the EXAFS library and LCF, the amorphous Zn phosphate that matches the speciation of Zn in the composts we studied very well. To our knowledge, this is the first evidence for the presence of amorphous zinc phosphate in composted OWs. The presence of such amorphous compounds could be explained by the inhibition of phosphate crystallization due to the presence of large amounts of organic matter in the composts.

The nano-size of ZnS explains its ephemeral nature

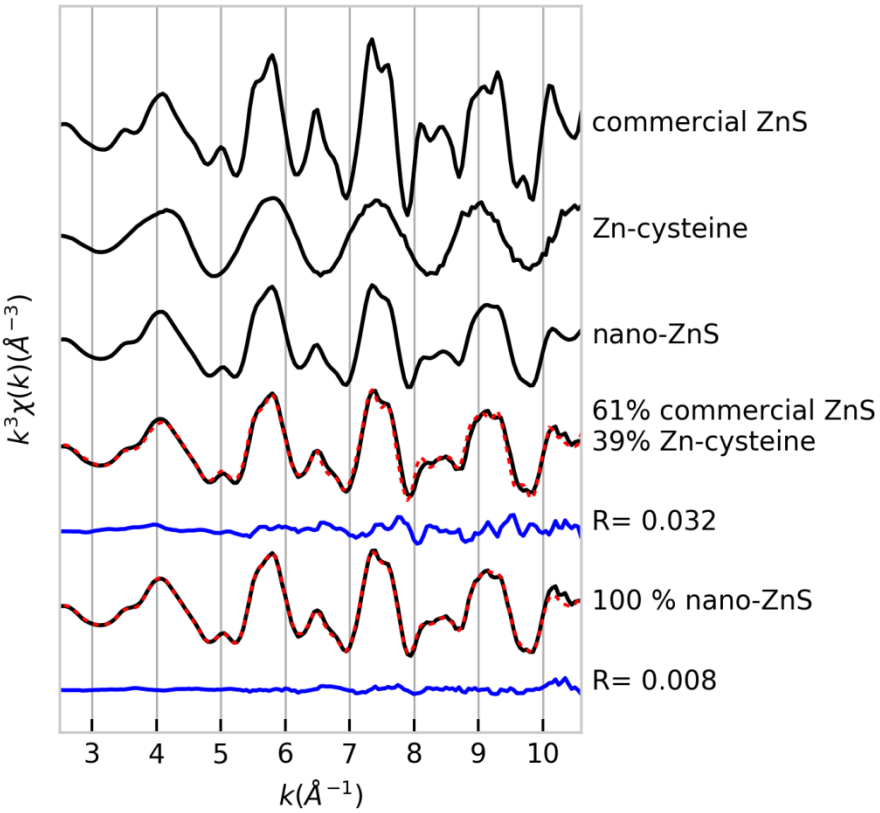


Figure 4: Comparison of Zn K-edge extended X-ray absorption fine-structure spectroscopy spectra of Agri-1 digestate, synthesized nano-ZnS and commercial ZnS.

EXAFS spectra of OW samples containing ZnS strongly resembled nano-ZnS reference spectra. Figure 4 shows that oscillations at 5, 6.5, 8.5 and after 10$Å^{-1}$ are much less intense for the Agri-1 digestate spectrum than for commercial ZnS. Visually, Agri-1 digestate spectrum is very similar to the nano-ZnS spectrum and this similarity was observed in all OWs containing ZnS (Figure S7). In addition, the nano-ZnS reference compound was always selected by LCF calculation to model the experimental signal unlike the commercial ZnS compound, which was never selected (Table S1 for detailed results of LCF). The EXAFS signal for nano-ZnS had smaller oscillations than the commercial ZnS due to a smaller number of Zn atoms in the second coordination shell. Indeed, the contribution of under-coordinated Zn atoms located at the surface to the EXAFS signal increases with a decrease in particle size. Thus when we detected ZnS in raw OWs and digestates, we suspected ZnS would be nano-sized.

As the EXAFS technique gives averaged information on Zn local environment, this nano-ZnS EXAFS signature probably reflects the average distribution of ZnS of different sizes and crystallinities that is equivalent, on average, to the 3 nm-nano-ZnS reference used in this study. Indeed, the less intense EXAFS oscillations for the Agri-1 digestate compared to commercial ZnS could be due to the presence of both crystalline ZnS (such as commercial ZnS) and Zn-cysteine, which is less structured. This combination was tested by LCF to fit Agri-1 and indicated a significantly higher residue (+300%) than the fit with nano-ZnS (Figure 4).

Legros et al.[20] also used a nano-ZnS reference spectrum to model OW spectra of lab-scale raw OWs and digestates composed of pig slurry, sewage sludge and municipal wastes. Our results extend the observation of a nano-ZnS signature in raw OWs and digestates from different origins and for samples taken from full-scale plants. The previous imaging of nano-ZnS (2.5 to 7.5 nm)

348 in digested sewage sludge[26] reinforces the hypothesis of the presence of nano-ZnS in OW
349 samples.

350 Anaerobic digestion and anaerobic storage of OW create environmental conditions that are
351 favorable for nano-ZnS precipitation. For example, sulfate-reducing bacteria (SRB) can be
352 present in AD digesters because of anaerobic conditions and high sulfate concentration.[50] Nano-
353 ZnS has been observed in in-situ biofilms containing a majority of SRB.[46,51] The presence of
354 SRB in liquid medium can also cause precipitation of nano-ZnS with crystalline defects.[47] The
355 mechanism of SRB influence on nano-ZnS precipitation is not known and might be specific to
356 SRB or an indirect mechanism. Indeed, organic compounds found in bacterial medium or
357 synthesized by bacteria also influence the size of ZnS-particles. For example, simple organic
358 compounds (cysteine and histidine) have been shown to limit ZnS particle growth.[52,53]Thus,
359 nano-ZnS precipitation in OW is consistent with these previous in-situ observations and bench-
360 scale experiments with SRB and organic compounds.

361 The drastic change in Zn speciation from a majority of nano-ZnS in raw OWs and digestates to
362 a majority of Am-Zn-phosphate in some solid digestates and in all the composts (Figure 2, Table
363 S1) reveals the low stability of nano-ZnS. Some authors already demonstrated that ZnS formed
364 in OW are much less stable after full-scale or bench-scale composting.[22-24] This behavior is
365 unexpected compared to what is observed with macroscopic ZnS (i.e. sphalerite). Indeed,
366 sphalerite has been shown to remain stable in the soil for years. Only 0.5% of spiked natural
367 sphalerite had dissolved after one year of incubation in the soil.[54] Voegelin et al.[55] observed 26 to
368 97% of dissolution of commercial 25-40 nm (crystallite size) ZnS spiked in four different soils
369 after four years.

370 The low stability of nano-ZnS particles in OWs is probably explained by their small size.
371 Indeed, nanoparticles have a higher proportion of atoms at the surface, making them more
372 reactive and prone to oxidative dissolution. Moreover, for a size of 3.4 nm, Gilbert et al. showed
373 that nano-ZnS present structural disorder[56] which can increase surface reactivity[57] and enhance
374 oxidative dissolution. Since ZnS were identified as particles with an average crystallite diameter
375 of about 3 nm in OWs in this study, we assume that the nano-sized ZnS formed in OW explain
376 their instability as soon as they are exposed to aerobic conditions.

377 **Environmental implications**

378 Since Zn speciation determines its availability in amended soil,[35] it is crucial to assess Zn
379 speciation in OWs before spreading in order to better predict the fate of Zn in soil. OWs are
380 spread on cultivated soil as fertilizer in raw, digested and/or composted form. Our study
381 evidences the impact of treatment on Zn speciation. Nano-ZnS is the main phase in OWs
382 characterized by reducing conditions (digestates, liquid OWs, liquid digestates, fresh solid
383 digestates). Very few studies have assessed the fate of this species in the soil after OW
384 spreading. Formentini et al.[21] showed that after spreading of pig slurry, in which nano-ZnS
385 accounted for 100% of the Zn speciation, on a clay soil, all the Zn accumulated in the top 30 cm
386 and was distributed between Zn-OM (41%), Zn-kaolinite (38%) and Zn-FeOx (23%). Zn applied
387 to the soil via pig slurry spreading was no longer present in the form of nano-ZnS. Isaure et al.[58]
388 found that Zn originating from the dissolution of ZnS formed in a sediment was mostly present
389 as Zn sorbed to ferrihydrite in a calcareous and silty soil 18 months after deposition of the
390 sediment on the soil. Thus, soil characteristics appear to influence the fate of Zn after dissolution
391 of nano-ZnS, as shown by Voegelin et al.[55]

Amorphous Zn-phosphate is the main phase in samples that have been exposed to oxygen due to their high DM content and storage conditions (solid OWs, composted digestates and OWs, stockpiled solid digestates) followed by Zn sorbed to ferrihydrite. To our knowledge, no results are available about the fate of amorphous zinc phosphate in soil since this phase has not previously been evidenced. Regarding Zn sorbed to ferrihydrite, Tella et al.[35] showed that Zn availability increases in OWs-amended clay soil. Zn speciation in these OWs was dominated by Zn sorbed to ferrihydrite and Zn desorption from Fe oxides has been suggested as the mechanism behind the release of Zn in soil solution. Unfortunately, such studies are still scarce and do not cover all the characteristics/properties of the soils that can interact with the fate of TEs. However, the present study provides general trends about the influence of treatments on Zn speciation that could be used for decision-making to limit the environmental impact of agricultural recycling.

Associated content

Supporting Information. Results on the influence of sample handling on Zn K-edge EXAFS spectrum; Characterization of reference compounds used for linear combination fitting of Zn K-edge EXAFS spectra; Detailed results of Zn K-edge extended X-ray absorption fine-structure spectroscopy linear combination fitting.

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