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Anaerobic Digestion Alters Copper and Zinc Speciation

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* 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
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.\(^ {6,9,18}\)

### 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, HNO\(_3\) and HClO\(_4\) (ISO 14869-1:2001; Soil quality—digestion 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\(^ {\text{®}}\), 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 diode detector. Each spectra was at least the average of three scans. The normalization and data reduction were performed according to standard methods\(^ {11}\) 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-

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**Table 1. Element Concentrations in Raw Waste and Digestates**

<table>
<thead>
<tr>
<th>Waste Type</th>
<th>Corg (% dry weight)</th>
<th>Ntot (% dry weight)</th>
<th>C/N</th>
<th>P (g/kg)</th>
<th>S (g/kg)</th>
<th>Fe (g/kg)</th>
<th>Cu (mg/kg)</th>
<th>Zn (mg/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pig slurry</td>
<td>39.9</td>
<td>4.71</td>
<td>8.47</td>
<td>43.9</td>
<td>5.72</td>
<td>2.84</td>
<td>664</td>
<td>553</td>
</tr>
<tr>
<td><strong>PS_D</strong></td>
<td>34.4</td>
<td>4.86</td>
<td>7.08</td>
<td>47.3</td>
<td>7.26</td>
<td>3.18</td>
<td>788</td>
<td>842</td>
</tr>
<tr>
<td>Municipal waste fine fraction</td>
<td>MWFF</td>
<td>35</td>
<td>1.53</td>
<td>22.9</td>
<td>3.84</td>
<td>3.83</td>
<td>8.77</td>
<td>369</td>
</tr>
<tr>
<td><strong>MWFF_D</strong></td>
<td>30</td>
<td>1.81</td>
<td>16.6</td>
<td>5.92</td>
<td>4.28</td>
<td>12.1</td>
<td>136</td>
<td>634</td>
</tr>
<tr>
<td>Sewage sludge</td>
<td>SS</td>
<td>41.8</td>
<td>7.91</td>
<td>5.28</td>
<td>26.1</td>
<td>0.61</td>
<td>6.07</td>
<td>350</td>
</tr>
<tr>
<td><strong>SS_D</strong></td>
<td>34.7</td>
<td>5.23</td>
<td>6.63</td>
<td>37.3</td>
<td>3.60</td>
<td>9.84</td>
<td>487</td>
<td>988</td>
</tr>
<tr>
<td>Sewage sludge mix green waste</td>
<td>SSGW</td>
<td>42.8</td>
<td>7.62</td>
<td>5.62</td>
<td>22.8</td>
<td>1.03</td>
<td>5.05</td>
<td>294</td>
</tr>
<tr>
<td><strong>SSGW_D</strong></td>
<td>34.7</td>
<td>5.5</td>
<td>6.31</td>
<td>40.1</td>
<td>3.56</td>
<td>10.4</td>
<td>501</td>
<td>842</td>
</tr>
</tbody>
</table>

---

\(^{6}\)The element concentrations are all expressed on a dry matter basis.
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. 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%.

3. RESULTS AND DISCUSSION

3.1. Anaerobic Digestion Influence on Element Concentrations. The concentrations of selected major and trace elements in raw waste and digestate 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 (Ntot), two patterns were noted. First, in PS and MWFF, the Ntot concentration increased by 3% and 18%, respectively, after AD, inducing a decrease in the C/N ratio. Conversely, in SS and SSGW, the Ntot 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.
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 ≈1−4 atoms of Zn/Fe at ≈3.4 Å 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. Finally, the physicochemical conditions in a pig slurry lagoon or a sewage treatment plant are favorable for the precipitation of several iron oxyhydroxides. 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. This structure was very similar to that observed when Zn was sorbed on phosphate minerals. The physicochemical conditions in a pig slurry lagoon or a sewage treatment plant are also favorable for the precipitation of phosphate minerals, which have been identified as a Zn bearing phase in OW. 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 ≈ six Zn atoms at 3.8 Å. 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. 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 and were found to be nanostructured only in sewage sludge and pig slurry. 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. 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. This structure corresponds to the interaction of Zn with natural OM functional groups. 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...
partially associated with iron oxy-hydroxides in wastes.\textsuperscript{18,26,33} 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.
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.  

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 (CuS). 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 Figure 1) and Cu sulfide has already been detected in pig slurry and sewage sludge. 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. 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 Helz and Pattrick et al. 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. Indeed, OW is mainly composed of OM and Cu is known to have strong affinities for OM. 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.  

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. 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 Ronsisaka and Dong et al. observed no influence, while Marcato et al. observed a modification in Cu speciation. The results presented here are in agreement with the data reported by Marcato et al., but contradict those of Dabrowska and Ronsisaka and Dong et al. Indeed, when using sequential chemical extraction, the sulfide fraction is extracted together with the organic matter fraction, 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. Desorption is favored below pH 6 and the presence of OM increases the desorption, likely because of chelating effects. 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 $2 \times 10^{-25}$. 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. Besides, Voegelin et al. 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. The highest OM mineralization rate in soil is obtained in wet aerobic conditions from 25 to 35 °C over the entire pH range, but the rate gradually decreases below pH 6. 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^-14) is very low. 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.

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.

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)
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 Lightsource (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.

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