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Fate of Zinc Oxide and Silver Nanoparticles in a Pilot Wastewater Treatment Plant and in Processed Biosolids

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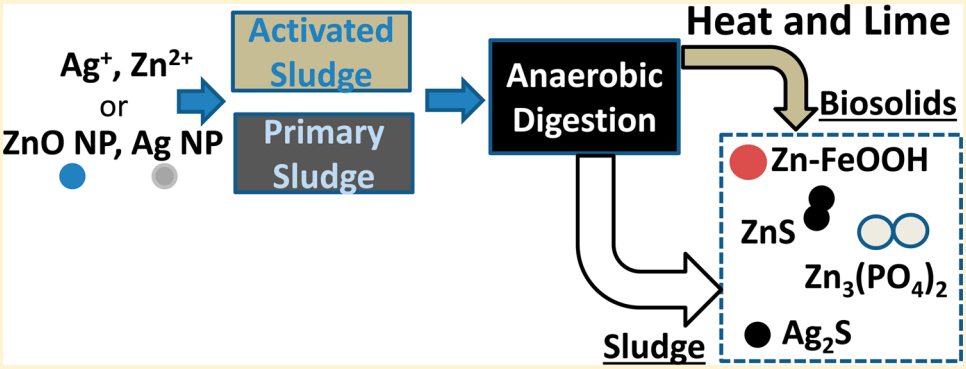
Supporting Information



ABSTRACT: Chemical transformations of silver nanoparticles (Ag NPs) and zinc oxide nanoparticles (ZnO NPs) during wastewater treatment and sludge treatment must be characterized to accurately assess the risks that these nanomaterials pose from land application of biosolids. Here, X-ray absorption spectroscopy (XAS) and supporting characterization methods are used to determine the chemical speciation of Ag and Zn in sludge from a pilot wastewater treatment plant (WWTP) that had received PVP coated 50 nm Ag NPs and 30 nm ZnO NPs, dissolved metal ions, or no added metal. The effects of composting and lime and heat treatment on metal speciation in the resulting biosolids were also examined. All added Ag was converted to Ag_2S, regardless of the form of Ag added (NP vs ionic). Zn was transformed to three Zn-containing species, ZnS, $Zn_3(PO_4)_2$, and Zn associated Fe oxy/hydroxides, also regardless of the form of Zn added. Zn speciation was the same in the unamended control sludge. Ag_2S persisted in all sludge treatments. $Zn_3(PO_4)_2$ persisted in sludge and biosolids, but the ratio of ZnS and Zn associated with Fe oxy/hydroxide depended on the redox state and water content of the biosolids. Limited differences in Zn and Ag speciation among NP-dosed, ion-dosed, and control biosolids indicate that these nanoparticles are transformed to similar chemical forms as bulk metals already entering the WWTP.

INTRODUCTION

Metallic silver nanoparticles (Ag NPs) and zinc oxide nanoparticles (ZnO NPs) are used in consumer products.[1] Due to the well-known antimicrobial properties of Ag^+, Ag NPs are used as antibacterial agents in textiles, cosmetics, personal care products, and water treatment products.[2] ZnO NPs are incorporated into products (e.g., sunscreen and cosmetics) and used for applications such as catalysts, semiconductors, solar cells, sensors, coatings, pigments, and optics.[3] According to the Project on Emerging Nanotechnologies (PEN), Ag and Zn rank first and fifth among the most prevalent nanomaterials in consumer products[4] and in the top five metals used for semiconductor NPs.[5]

The toxicity of Ag NPs and ZnO NPs has been extensively studied. Research investigating the toxicity of as-manufactured

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Ag NPs has demonstrated the potential for toxicity to a variety of different aquatic and terrestrial organisms including plants,[6] algae,[7] fungi,[8] vertebrates (zebra fish),[9] earthworms,[10] and bacteria. Similarly, as-manufactured ZnO NPs have been shown to be toxic to bacteria, various aquatic invertebrates, and earthworms.[11,12] However, as-manufactured Ag NPs and ZnO NPs are unlikely to persist in the environment due to their high tendency to undergo chemical transformations.[13,14] The partially or fully transformed products (e.g., Ag_2S, AgCl, ZnS, and $Zn_3(PO_4)_2$) will have different physical and chemical properties and toxicity potential.[3,7,15,16] To more accurately assess the environmental risks of Ag NPs and ZnO NPs, it is necessary to investigate the environmental transformations of Ag NPs and ZnO NPs in environments that are consistent with their anticipated release pathways and exposure routes. The major release pathway for metal based NPs in consumer products is through discharge to the sewer system. These materials are released to a wastewater treatment plant (WWTP) and accumulate in sludge produced during the wastewater treatment process.[17]

Several studies have demonstrated facile oxidation and sulfidation of Ag NPs to Ag_2S in laboratory reactors in the presence of dissolved oxygen and bisulfide.[7,18−20] Recently, Levard et al. reported that sulfidation of PVP coated Ag NPs was rapid (>50% sulfidation within 5 days) and was not inhibited by the PVP coating.[19] Lab studies also have demonstrated transformation of ZnO NPs to ZnS NPs in water at room temperature via dissolution and reprecipitation[3] or via outward growth of ZnS as a result of ZnO reacting with H_2S gas at the solid−gas interface,[21] as well as conversion of ZnO NPs to $Zn_3(PO_4)_2$ NPs in water in the presence of phosphate at room temperature and neutral pH.[15]

The fate and transformation end products of Ag NPs in WWTP sludge have also been investigated. In real WWTP sludge collected from a municipal plant in the U.S., Ag_2S NPs were found using transmission electron microscopy.[22] Kaegi et al. spiked Ag NPs directly into a large pilot scale plant or added them to samples collected from an activated sludge basin. One basin was aerated, and the other was not. No anaerobic digestion step was used in this study. They found that 85% of the Ag left the plant in the waste activated sludge and that Ag was present as Ag_2S and elemental Ag after five hours of reaction with the solids.[17] Kaegi et al. also reported high levels of sulfidation in Ag NPs in the sewer system prior to entering the WWTP.[23] Impellitteri et al.[24] introduced citrate stabilized Ag NPs into a pilot WWTP along with synthetic wastewater. They also added the same Ag NPs to waste activated sludge and to secondary clarifier solids from a real municipal WWTP. They found that all silver was associated with sulfide either as Ag_2S or as Ag sulfhydryl compounds within 24-h of exposure. No anaerobic digestion step was used in this study. Lombi et al. added Ag(0), AgCl, or Ag_2S NPs, to a mixture of primary and secondary clarifier solids, and digested them anaerobically for 30 days. They found that all Ag in the system was present as Ag_2S after 10 days, but found no evidence of Ag sulfhydryl compounds.[25] The absence of Ag-sulfhydryl compounds may have been a result of anaerobic digestion and a greater availability of sulfide compared to Impellitteri et al. Whitley et al. investigated the behavior of Ag in soils amended with Ag NP or $AgNO_3$ spiked WWTP sludge and found that, although both forms were extensively sulfidized, addition of NPs resulted in more Ag present as nanoscale colloids in soil pore water than addition of $AgNO_3$.[20]

There is only one report of the transformation behavior of ZnO NPs that was also added directly to anaerobic digester sludge recovered from a pilot WWTP.[26] Lombi et al. found that ZnO NPs had transformed completely to ZnS in the wet anaerobic digester sludge after 1 h. The ZnS in the wet sludge was completely transformed to a mixture of $Zn_3(PO_4)_2$ and Zn associated with Fe oxy/hydroxides upon drying (composting) the biosolids at 37 °C for 60 days. The present study adds Ag or ZnO NPs and the corresponding ions to the primary clarifier solids and to the activated sludge basin in proportions expected on the basis of these prior studies. The solids are then digested anaerobically for 15 days, and the speciation of metal in the resulting biosolids is determined. Thus, the present study is most similar to that of Lombi et al.,[25,26] but comparisons to the other prior studies without anaerobic digestion are also made.

In the United States, more than 50% of WWTP sludge is processed to biosolids that are land applied as fertilizer.[27] Usually, waste sludge from the WWTP anaerobic digester is dewatered and dried to 20−50 wt % solids content. The dewatered biosolids are then further dried, subjected to lime addition and heat treatment, and/or composted before being applied to agricultural fields. This sludge processing can potentially affect Ag and Zn speciation because the reported transformations (sulfidation, phosphatation, and dissolution) should be dependent on the redox conditions, the pH, the rates of transformations between species present, and the relative abundance of S to P in the sludge and/or biosolids. Data gaps still exist regarding the fate of Zn and Ag during sludge processing to produce Class A biosolids for fertilizer use following EPA guidelines.[28] Transformations in the presence of multiple competing ligands, after lime addition, and with alternating redox/moisture cycles have not been fully characterized.

In this study, the fate of ZnO and Ag NPs in a pilot WWTP is determined. The behaviors of the nanoparticulate phases are compared to an equivalent dose of metal in ionic form as soluble salts. NPs or salts are added to primary clarifier solids and the activated sludge basin; then, the primary clarifier solids and waste activated sludge are anaerobically digested to produce the final biosolids for analysis and for further treatment to produce Class A biosolids. The objectives of this study are to (1) determine the fate of ZnO and Ag NPs in a complete wastewater treatment process where NPs are added in as realistic a manner as possible and (2) compare chemical transformation products of the NP forms of the metals to their metal salts in wastewater treatment and sludge processing (heat/lime addition and composting at different moisture contents) to determine if there are "nano" specific effects to consider.

■ MATERIALS AND METHODS

Ag and ZnO NPs. Ag NPs were synthesized as described previously.[29] A TEM image of the Ag NPs from the batch used for this study is available in the Supporting Information (Figure S1). Briefly, the Ag NPs were 52 ± 29 nm in diameter based on 786 particles counted in TEM images. The Ag NPs are stabilized with a 55 kDa average molecular weight polyvinylpyrrolidone (PVP) and were synthesized and purified as described previously.[29] The intensity average hydrodynamic diameter of the PVP-stabilized Ag NPs was 80 nm (Supporting Information, Figure S2a). The zeta potential of the Ag NPs is −6.1 mV at pH 7 in 10 mM $NaNO_3$. The ZnO NPs (Nanosun P99/30) were donated by Micronisers (Dandenong, Australia).

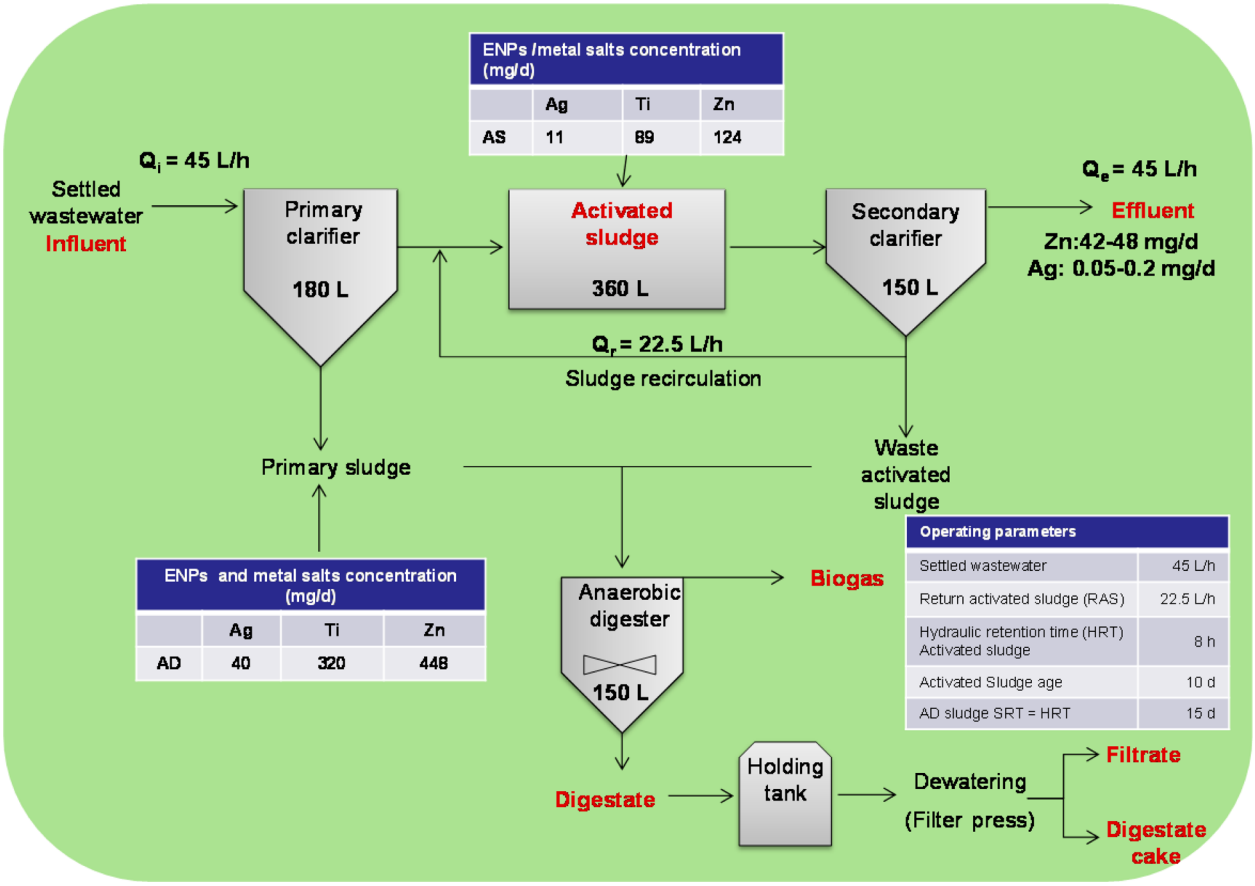

Figure 1. Schematic of the pilot WWTP for production of primary biosolids in the pilot WWTP in Cranfield University. Biosolids tested came from the anaerobic digester. Red fonts indicate sampling points. AS: Activated sludge. AD: Anaerobic digester.

Detailed characterization of these particles was previously reported.[3] The primary particle size of the ZnO NPs was 30 ± 10 nm based on TEM measurements of 100 particles. The intensity average hydrodynamic diameter of the ZnO NPs in DI water at pH = 7 was 253 nm (Supporting Information, Figure S2b). There is little to no coating on the ZnO NPs, which allows them to aggregate at circumneutral pH where little electrostatic stabilization is expected. The zeta potential of the ZnO NPs is +15 mV ± 1 mV at pH 7 in 10 mM $NaNO_3$. The Ag and ZnO NPs were selected because they are being used in a large-scale, multi-institution, international effort (Transatlantic Initiative for Nanotechnology and the Environment, TINE) to determine their fate during wastewater treatment and in soils amended with NP containing biosolids generated from the WWTP. The NPs were used as received.

Pilot Wastewater Treatment Plant (WWTP). The experiments were designed to produce biosolids from WWTP simulating conditions where ZnO and Ag NPs had been added to the plant influent. NPs or metal were dosed to the primary sludge and to the activated sludge basin, followed by anaerobic digestion of primary clarifier solids and waste activated sludge. In both cases, the behavior of the NPs was compared to that of a similar concentration of ionic species, added the same way. This best mimics the conditions expected in a WWTP plant and produces the most representative metal speciation possible. It also enables comparison with previous studies where NPs were added to digester sludge or biosolids to determine if differences exist between these two dosing methods.

Sludge was generated in a pilot plant located at Cranfield University, UK. A schematic layout of the WWTP is provided in Figure 1. The pilot WWTP consisted of three parallel lines each containing a primary clarifier (180 L), an aerated tank (360 L), a secondary clarifier (150 L), and an anaerobic digester (150 L). This enabled the same influent to be used for the ion, NP, and control sludge production. The hydraulic retention time was 8 h, and the activated sludge age was 10 days. The layout and residence times are similar to a full-scale wastewater treatment plant. Ag and Zn nanoparticles and metal salts were dosed into the respective activated sludge tanks continuously during the course of the experimental run (60 weeks between 2011 and 2012). Ag- and Zn-amended solids were fed to the anaerobic digesters in batch mode once per day during the same time period. The 40 kg of dry sludge (160 kg of wet sludge at 25 wt % solids) produced from each plant was collected for further study: first shipped to Rothamsted Research in the UK for drying and homogenizing and then dispatched to the US.

Influent Wastewater Properties and Dosing. The inflow rate was 45 L/h. The inflow pH was 6.4 (±0.24), and the background Ag and Zn concentrations in the influent were 0.3 ± 0.2 μg/L and 136 ± 35 μg/L, respectively. (Metal concentrations arriving at Cranfield's treatment works are higher than those in average raw municipal wastewaters due to industrial activity in the area it serves.) Stock suspensions of 0.01 mg/L Ag and 0.12 mg/L Zn in forms of either metal NP or metal salts were dosed to the activated sludge stage at a rate of 11 mg/d for Ag and 124 mg/d for Zn. Along with Ag and

Zn, 89 mg/d TiO_2 was also dosed but is not the focus of this study. Forty mg/d Ag and 448 mg/d of Zn were dosed to the primary sludge. The primary and the waste activated sludge were then mixed and digested anaerobically for 15 days. The digesters were kept at 35 °C using heated jackets from LMK Thermosafe, Haverhill, UK. The biosolids produced were passed through a filter press (a common process at full scale treatment plants), which brought the solids percentage up from ∼2% to ∼25%. The biosolids were dried in a greenhouse at ambient conditions. For metal concentrations in effluent of the pilot WWTP, two spot samples were taken 2 weeks apart, measured in duplicate using ICP-MS after acid digestion.

Sludge Characterization. The dried sludge (with less than 5% moisture content determined gravimetrically) from control runs (no added metal), runs with metal salts added, and runs with metal NPs added are named control, ion, and NP added sludge. These runs used the same wastewater and were run simultaneously. The dried sludges were characterized for pH, total metals (Fe, Al, Cu, Zn, Ag), total C and N content, NH_4^+, and selected anions (F^-, Cl^-, NO_3^-, NO_2^-, SO_4^{2-}, and PO_4^{3-}). The sludge pH was measured in 0.01 M $CaCl_2$ solution (1:5 solid/solution). Anions and NH_4^+ were measured in water extracts collected by placing 0.2 g of sludge and 2 mL of deionized water (DI) into a 15 mL centrifuge tube and mixing on a reciprocating shaker table on high for 1 h.[30] The resulting suspensions were allowed to settle for 30 min and were subsequently centrifuged at 3220*g* for 20 min. The supernatant was removed and diluted, after which an aliquot was removed for ammonium analysis via the phenol hypochlorite method.[31] For anion analysis via ion chromatography, extracts were cleaned up prior to analysis using Hypersep C18 columns (Thermo Fisher Scientific Corporation, Waltham, MA, USA) to remove hydrophobic organic matter. Anions were determined using isocratic elution with KOH on a Dionex AS11HC anion exchange column (Thermo Fisher Scientific). Analytical runs contained intercalibration verification samples (ICV), duplicate dilutions, and spike recovery samples.

The concentrations of selected metals were measured in 0.1 g of dried sludge samples digested in 9 mL of nitric acid and 3 mL of hydrochloric acid, both concentrated trace-metal grade, using a MARS microwave digestion system (CEM, Matthews, NC, USA) according to USEPA method 3052.[32] Digestion sets contained a sludge standard reference material (2781; National Institute of Standards and Technology, Gaithersburg, MD). The Zn, Ag, Cu, Fe, and Al concentrations in each digestate were measured using inductively coupled plasma mass spectrometry (ICP-MS) using an Agilent 7500cx ICP-MS (Agilent, Santa-Clara, CA, USA). Analytical runs contained ICV samples, duplicate dilutions, and spike recovery samples. Carbon and nitrogen content of the sludges were analyzed by flash combustion of approximately 20 mg of sample using a Flash EA 1112 series NC Soil Analyzer (Thermo Fisher Scientific Corporation). Analytical runs contained laboratory control samples (LCS) of known C and N content.

Sludge Aging to Produce Biosolids. The air-dried sludge (control, ion, and NP added) received directly from the pilot WWTP is referred to as "oxic". To assess the effects of redox environment on metal speciation in the sludge, these samples were wetted and allowed to age at 37 °C for one week prior to determining metal speciation. This resulted in anoxic conditions (ORP = −200 mV) and provided a sludge presumed to be similar to the wet sludge leaving the filter press. The anoxic aged set is referred to as "reduced". Using both the "oxic" and the "reduced" sets of NP amended biosolids, the effects of composting with different redox conditions and moisture content, as well as the effect of lime addition and heating, were investigated in laboratory reactors.

To simulate wetting and drying during composting, both the "oxic" and the "reduced" NP added biosolids were aged at 50 °C for 3 weeks in an oven exposed to air. To assess the effect of moisture content during composting, the biosolids were wetted with DI water to different amounts (30%, 50%, and 80% of water holding capacity summarized in Table S1, Supporting Information) during simulated composting. These moisture contents cover the typical range expected for composting of biosolids[33] and are consistent with similar experiments that have been conducted on biosolids.[26] The moisture content was adjusted back up to the desired value periodically during the 3-week composting cycle.

To simulate biosolid formation by lime addition and heat treatment of sludge, both the "oxic" and the "reduced" NP amended sludges were amended with lime using a lime/sludge w/w ratio (dry mass) of 1/10. This is approximately two times higher than lime doses commonly used for biosolids treatment in the U.S. Water was added to the dried sludge/lime mixtures to create slurries containing 20 wt % solids. The lime dosed sludges were heated to either 72 °C for 1 h or 24 °C for 72 h to follow common practices for producing class A biosolids.[34]

Determination of Ag and Zn Speciation in Biosolids. The speciation of Zn and Ag in sludges and biosolids was examined using X-ray absorption spectroscopy (XAS). Zn K-edge X-ray absorption spectroscopy spectra were collected at the Stanford Synchrotron Radiation Lightsource (SSRL) on beamline 4-3 in a liquid N_2 filled cryostat in fluorescence mode. Ag K edge XAS spectra were collected at SSRL beamline 11-2, also in a liquid N_2 filled cryostat in fluorescence mode. Samples were first homogenized by cutting and grinding and then pelleted. An N_2-cooled Si (220) double-crystal monochromator was detuned by 30% for harmonic rejection, and energy calibration was performed with a Zn metal or Ag metal foil placed after the I1 transmitted beam detector. XAS spectra of the following reference compounds were collected for Zn: bulk ZnS (sphalerite), nano ZnS, $Zn_3(PO_4)_2$, Zn-cysteine, ZnO, $Zn_5(CO_3)_2(OH)_6$, Zn adsorbed onto Fe oxy/hydroxides, Zn substituted Fe oxy/hydroxides (prepared as previously reported[35]), and $ZnCO_3$; for Ag, reference compounds include: Ag_2S, AgCl, Ag_3PO_4, AgBr, AgI, Ag-cysteine, Ag_2O, Ag NP, Ag adsorbed onto Fe oxy/hydroxides and Ag substituted Fe oxy/hydroxides, and Ag_2CO_3. Linear combination fitting (LCF) of the X-ray Absorption Near Edge Structure (XANES) region (−30 to +100 eV around the Zn absorption edge of 9659 eV) was performed for Zn using the SIXPack interface to the IFEFFIT XAFS analysis package.[36] LCF analysis of the EXAFS spectra (k = 2 to 9) was also performed for Ag using SIXPack. In linear combination (cycle) fitting, species are only included when adding that species to the fit decreased the R value (fit residual) by more than 20%.

RESULTS AND DISCUSSION

The fate of Zn and Ag in biosolids produced from the pilot WWTP amended with either NP or ionic forms of metal was determined. We focused on metal speciation in the biosolids because over 92% of the Zn and 99% of the Ag added was found with the solids leaving the plant. The concentration of Zn and Ag in the effluent leaving the secondary clarifier was approximately 40 mg/L for Zn and less than 1 mg/L for Ag

(Supporting Information, Table S3). The speciation on metal in the effluent was not determined.

Characterization of Sludges. WWTP sludge is a complex mixture of organic matter and inorganic salts. The pH and common anions in sludge (e.g., phosphate and sulfate) that can affect Zn and Ag speciation in the biosolids were characterized (Table 1). The control sludge has no added metal, and the "NP" and "Ion" columns are those dosed with Ag and ZnO NPs or ionic forms of Ag and Zn, respectively. The measured pH of the sludges ranged from 6.5 to 6.8, suggesting that the doses of metal used did not significantly upset the pH of the WWTP process. Control sludge contained 900 mg/kg of Zn metal (dry mass), consistent with Zn loadings observed in municipal WWTPs.[37] The Zn concentration increased to 3800 mg/kg and 4900 mg/kg with addition of ZnO NPs and Zn^{2+}, respectively. Similarly, Ag in control sludge was quite low (3.5 mg/kg) and increased to 272 mg/kg and 359 mg/kg upon addition of NP and Ag^{+}, respectively. Concentrations of Cu, Fe, and Al were similar across the three sludges (Table 1). Total C, S, and P were consistent with expectations for municipal biosolids (total C content is typically 40−44 wt %, total S content is 1−1.2 wt %, and total P is 1−1.4 wt %).[38] Concentration of phosphate ranged from 650 to 900 mg/kg in porewater separated from the biosolids. Sulfate ranged from 4100 to 6500 mg/kg in porewater separated from the biosolids. The presence of Fe, PO_4^{3-}, and S (especially if reduced) have a significant influence on the observed speciation of Zn and Ag in the sludge and resulting biosolids as discussed next.

Table 1. Characterization and Chemical Composition of Control (No Added Metal), NP Amended, and Ion Amended Sludge

biosolid	control	NP	ion
pH[a]	6.5	6.7	6.8
total C (%)	44.6 ± 0.6[b]	40.2 ± 0.2	43.8 ± 0.3
total N (%)	3.7 ± 0.1	4.1 ± 0.07	4.1 ± 0.04
total P (%)	1.2	1.4	1.0
total S (%)	1.1	1.2	1.0
Fe (mg/kg)	12135 ± 347	17710 ± 1143	10314 ± 402
Al (mg/kg)	4230 ± 392	5030 ± 190	4679 ± 336
Cu (mg/kg)	344 ± 15	434 ± 49	396 ± 18
Zn (mg/kg)	900 ± 83	3795 ± 214	4889 ± 402
Ag (mg/kg)	3.5 ± 1.2	272 ± 8	359 ± 21
F^- (mg/kg)	862 ± 14	654 ± 5	630 ± 23
Cl^-(mg/kg)	522 ± 16	546 ± 8	498 ± 40
SO_4^{2-} (mg/kg)	6492 ± 678	5723 ± 128	4072 ± 167
NO_3^- (mg/kg)	123 ± 64	95 ± 7	203 ± 232
PO_4^{3-} (mg/kg)	904 ± 36	649 ± 25	881 ± 25
NH_4^+ (mg/kg)	1098 ± 146	1234 ± 53	1065 ± 80

[a]The number of analyses (n) was 1 for pH, total P, and total S; $n = 3$ for all others. [b]Values are mean ± standard error of three measurements.

Zn Speciation in Oxic and Reduced Sludge. The Zn K edge XANES spectra of the air-dried oxic sludge, the reduced sludge that was wetted and allowed to "age" at 37 °C for one week, the sludge that was composted under aerobic conditions with multiple wetting/drying cycles, and the lime/heat treated biosolids are shown in Figures 2, S5, and S6, Supporting Information. XANES spectra are used in the Zn analyses rather than EXAFS spectra because the XANES spectra for the most relevant model compounds (i.e., Zn associated Fe oxy/hydroxides and $Zn_3(PO_4)_2$) were readily distinguishable (Figure 2), whereas the EXAFS spectra of these compounds are not as readily distinguishable. Thus, the near edge region provides more sensitivity to distinguish between Zn species. A collection of XANES spectra of all the model compounds used in this study is provided in the Supporting Information (Figure S4).

As shown in Figure 2 (left), the XANES spectra of the dried sludge from the WWTP were all similar, regardless of the amount or added form of Zn. According to the LCF results (Table 2), Zn is present as a combination of ZnS, Zn−Fe oxy/hydroxide, and $Zn_3(PO_4)_2$. In all three samples, ZnS ranged from 23% to 38%, Zn−Fe oxy/hydroxide ranged from 42% to 55%, and $Zn_3(PO_4)_2$ ranged from 21% to 22%. There are several important findings to note with this resulting speciation. First, there was no evidence of the presence of the initial ZnO NPs that had been added to the pilot WWTP in the resulting sludge. Second, the form of Zn (NP vs ion) that was added did not significantly affect the types of Zn species present in sludge relative to controls with no added Zn. This result is very similar to the findings of a recent report examining Zn speciation in biosolids resulting from addition of Zn ion or ZnO NPs to primary clarifier and waste activated sludge solids, followed by

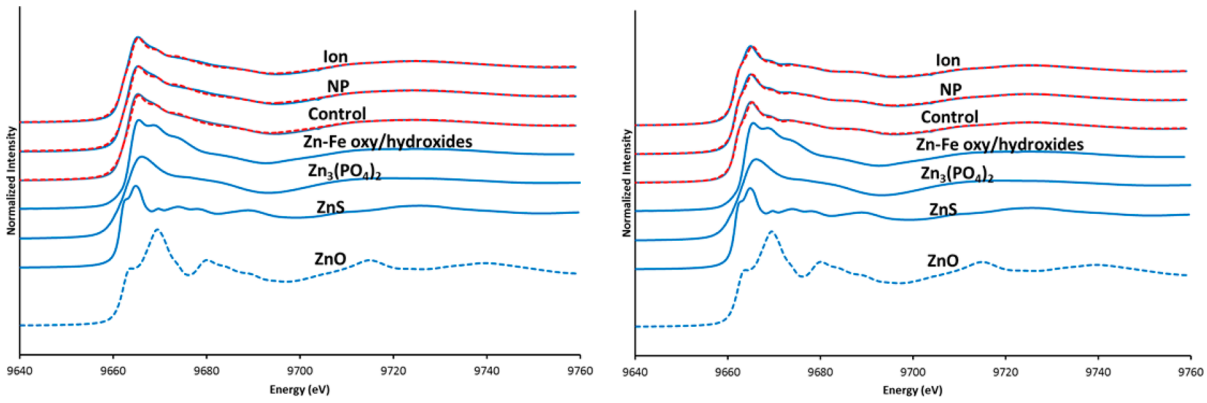

Figure 2. Normalized Zn K-edge XANES spectra of the (left) oxic air-dried biosolids from the pilot WWTP and (right) reduced biosolids after 1 week further anaerobic aging at 37 °C biosolids: control, NP, and ion-amended. Dotted line shows the best 3-component (Zn-Fe oxy/hydroxides, $Zn_3(PO_4)_2$, and ZnS) linear combination fit of reference spectra as shown below. Spectrum for the ZnO model compound is also included to demonstrate its absence.

Table 2. Best Fit Ag and Zn Speciation As Identified by Linear Combination Fitting (LCF) of K-Edge EXAFS (Ag) and XANES (Zn)[a]

metal dosed	treatment	Ag_2S (%)	R	ZnS (%)	Zn-Fh (%)	$Zn_3(PO_4)_2$ (%)	sum	R
		Oxic						
control (no metal)	none	ND		23	55	21	99	0.0003
Ag/ZnO NP	none	104	0.042	30	48	22	100	0.0002
Ag^+/Zn^{2+}	none	110	0.09	21	42	21	100	0.0002
		Reduced						
control (no metal)	7-d aged wet at 37 °C	ND		62	16	23	101	0.0001
Ag/ZnO NP	7-d aged wet at 37 °C	95	0.1	62	11	27	100	0.0001
Ag^+/ Zn^{2+}	7-d aged wet at 37 °C	102	0.1	65	4	31	100	0.0001
		Composting						
Ag/ZnO NP (reduced)	3 wk aerobic composting at 50 °C with 30% WHC	NA		53	21	26	100	0.0001
Ag/ZnO NP (reduced)	3 wk aerobic composting at 50 °C with 50% WHC	94	0.07	51	24	25	100	0.0001
Ag/ZnO NP (reduced)	3 wk aerobic composting at 50 °C with 80% WHC	NA		58	22	20	100	0.0001
Ag/ZnO NP (oxic)	3 wk aerobic composting at 50 °C with 30% WHC	NA		29	49	24	102	0.0003
Ag/ZnO NP (oxic)	3 wk aerobic composting at 50 °C with 50% WHC	95	0.07	24	51	25	100	0.0002
Ag/ZnO NP (oxic)	3 wk aerobic composting at 50 °C with 80% WHC	NA		26	55	19	100	0.0002
		Heat/Lime						
Ag/ZnO NP (oxic)	lime and heated to 24 °C for 3 d	NA		28	48	26	102	0.0002
Ag/ZnO NP (reduced)	lime and heated to 24 °C for 3 d	NA		51	25	23	99	0.0001
Ag/ZnO NP (oxic)	lime and heated to 72 °C for 1 h	110	0.06	34	41	25	101	0.0002
Ag/ZnO NP (reduced)	lime and heated to 72 °C for 1 h	110	0.09	53	22	24	99	0.0001

[a]ND: not detected due to low Ag concentration. NA: not available. Lower concentration Ag samples require more beam time than Zn and fewer could be analyzed. WHC: water holding capacity.

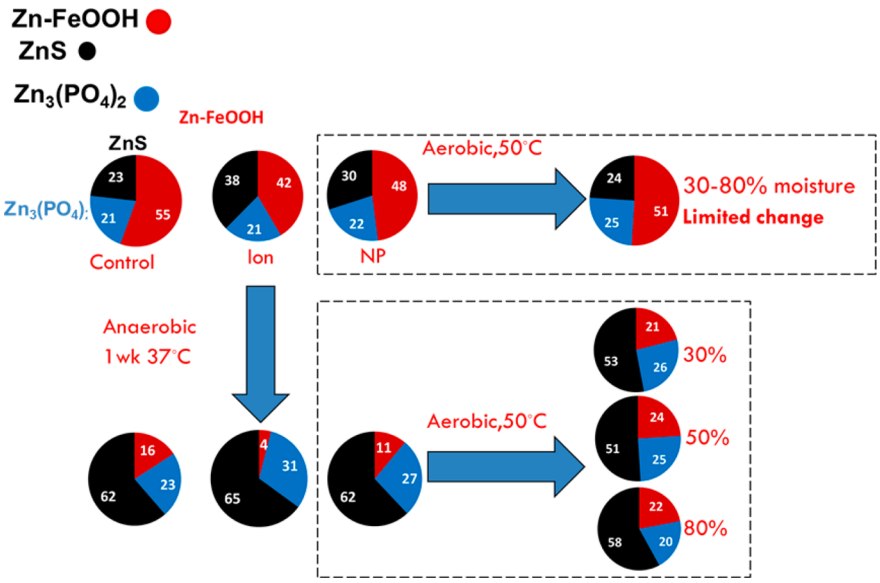

Figure 3. Pie charts showing the speciation of Zn in sludge as identified by LCF of Zn K-edge XANES spectra of oxic and reduced sludges and NP-amended sludges after they were composted at 50 °C for 3 weeks with different moisture contents (30%, 50%, and 80% of the water holding capacity).

anaerobic digestion.[26] However, there are some differences in the proporitons of species observed that may be attributed to the method used to dry the sludge. Lombi et al. took samples from the digester at different time points. They found that Zn occurred primarily as Zn−S in freeze-dried sludge, with ∼70% ZnS after 10 days and ∼90% ZnS after 30 days. This is significantly higher than the amount of ZnS found here. This may be because the biosolids in this study were air-dried prior to analysis rather than freeze-dried. This air drying process likely resulted in greater oxidation of the sulfur in the ZnS and its conversion to other Zn species than during lyophilization which is done under a vacuum. We also observed subtle differences in the proportions of Zn species present depending on the form of metal dosed. The Zn^{2+} dosed biosolids have the most ZnS and the least Zn−Fe oxy/hydroxide among the three treatments. This implies that Zn^{2+} is more available for sulfidation than ZnO NPs and other forms of Zn when added to WWTP influent.

During composting, sludge will undergo frequent wetting and drying cycles and will therefore be subject to different redox conditions than noncomposted biosolids. To assess whether or not redox cycling affects Zn speciation, we wet and

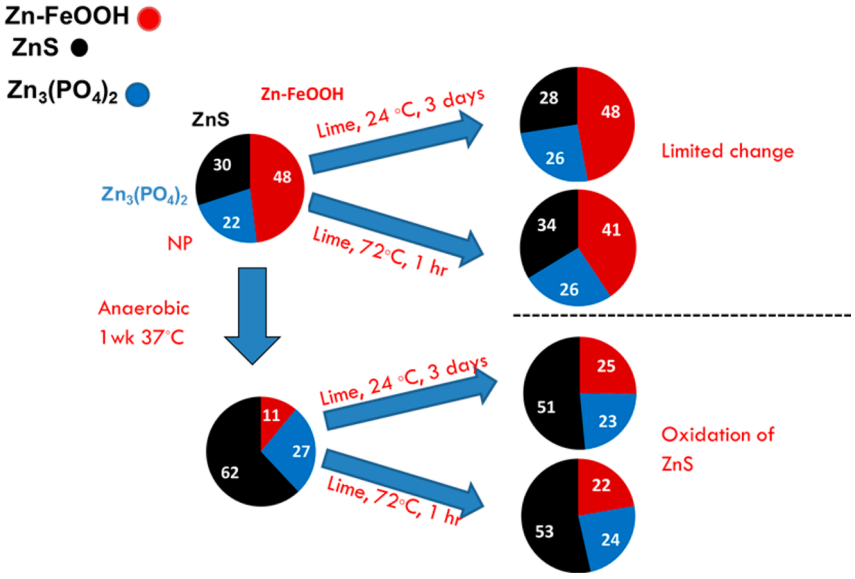

Figure 4. Speciation of Zn in biosolids formed from lime and heat treatment of oxic and reduced Zn and Ag NP amended sludges as identified by LCF of Zn K-edge XANES spectra.

then incubated the biosolids at 37 °C for 1 week. The XANES spectra and LCF analysis of this reduced sludge is reported in Figure 2 (right) and Table 2. In all three samples, this treatment increased the amount of ZnS in the biosolids (ranged from 62% to 65%) by about 30−40%. Conversely, the amount of Zn−Fe oxy/hydroxide in the samples decreased. The amount of $Zn_3(PO_4)_2$ in the biosolids remained relatively stable, ranging from 23% to 31%. Thus, the increase of ZnS is at the expense of Zn−Fe oxy/hydroxide, suggesting that the Zn in the biosolids is relatively labile depending on the redox conditions. It is unconfirmed that the ZnS is present as nanoscale particles, but our prior work (Ma et al.[3]) showed that the sulfidation of 30 nm ZnO NPs (the same particles as those added to the WWTP) resulted in 3 to 5 nm sized ZnS nanoparticles and the best LCF results were obtained using nano-sized ZnS model compounds. This suggests that the ZnS is nano-sized. The small size of the ZnS NPs may explain the relatively high lability of Zn in the biosolids.

A 3-week incubation at 50 °C simulating biosolids composting of either the oxic or reduced NP-amended biosolids altered the Zn speciation (Figure 3 and Table 2). In simulated composting, biosolids were repeatedly brought back up to 30%, 50%, or 80% of the water holding capacity to assess the effect of moisture content during wetting/drying on the resulting Zn speciation. We anticipated that increasing moisture content would reduce access to oxygen and lead to the formation of inorganic sulfide through biological sulfate reduction, affecting the redox state of the sludge and Zn speciation. For the reduced NP-amended sludge (i.e., those that had been wetted and incubated for a week and that contain a relatively larger amount of ZnS), simulated composting decreased the amount of ZnS by 4−11%. This decrease of ZnS was compensated by a less than 10% increase of Zn−Fe oxy/hydroxide and $Zn_3(PO_4)_2$, respectively. For the oxic NP-amended biosolids (i.e., those with less initial ZnS), the change in Zn speciation during composting is much less noticeable (Figure 3). Moisture content during simulated composting had a relatively small effect on Zn speciation, although higher moisture content did better preserve ZnS relative to the lower moisture content. This is likely due to lower redox at higher moisture content (−50 mV at the higher moisture compared to +305 mV for the lowest moisture content vs Ag/AgCl reference electrode) and continued sulfate reduction in the high moisture content sludge. Our results indicate persistence of some ZnS, regardless of drying or composting conditions after 3 weeks. This contradicts a prior report of Zn speciation in sludge showing complete disappearance of ZnS after 2 months of composting.[26] It is possible that longer composting times under oxic conditions used here may ultimately result in complete loss of ZnS as previously reported. However, persistence of ZnS in the air-dried (oxic) sludge, which had less than 5% moisture content, suggests that some ZnS may persist in these sludges.

The effect of lime addition and heating on Zn speciation was also studied, and the results are shown in Figure 4 and Table 2. For the biosolids formed from the oxic sludge, both heat and lime treatments (24 °C for 3 days and 72 °C for 1 h) did not significantly change Zn speciation. Addition of lime and heat to the reduced sludge decreased the proportion of ZnS and increased the proportion of Zn−Fe oxy/hydroxide, whereas the percentage of $Zn_3(PO_4)_2$ was unaffected (Figure 4), likely due to its fully oxidized state and low aqueous solubility. As with the oxic sludge, both of the lime/heat treatments used also had a similar effect on Zn speciation in the reduced sludge.

Ag Speciation in WWTP Sludge and Biosolids. Ag K edge EXAFS taken on the same sludges was used to identify relevant Ag species under the same conditions as described for Zn. Since the concentration of Ag in control sludge was only 3 mg/kg, XAS spectra could not be measured for those samples. The Ag concentration in NP and ion amended sludges were between 200 and 400 mg/kg (Table 1). Ag K edge EXAFS data for different biosolid samples are shown in Figure 5. In all cases, regardless of treatment and conditions, Ag is present largely as Ag_2S over the time scales studies here (Table 2). Cycle fitting of all model compounds tested (Ag_2S, Ag NP, AgCl, Ag_3PO_4, Ag-cysteine, AgBr, AgI, Ag_2SO_4, Ag_2CO_3, Ag-acetate, and Ag−Fe oxy/hydroxide) indicated that inclusion of other components did not significantly improve the fit.

The predominance of Ag_2S is consistent with previous reports of Ag speciation in WWTP sludge,[17,23,25,24] particularly the study that also digested the solids anaerobically.[25] This is

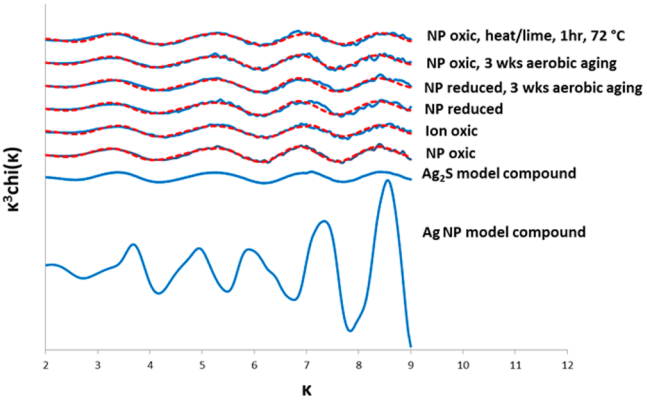

Figure 5. Ag K-edge EXAFS spectra of the Ag NP and Ag_2S model compound, pristine Ag NP and Ag ion amended oxic sludges and resulting biosolids, reduced sludges and resulting biosolids, and after composting. Dotted lines show the best 1-component (Ag_2S model compounds) linear combination fitting.

also consistent with expectations given the very high affinity of silver for sulfide ($Ag_2S_{(s)} \rightarrow 2Ag^+ + S^{2-}$, log $K = 10^{-51}$).[19] However, there remains potential for oxidative dissolution of Ag_2S nanoparticles in sludges over very long times.

Environmental Implications. This study indicates that both Ag and Zn, either natively present in municipal wastewater or added in NP or bulk metal form, transform significantly during the WWTP process. We observed no Ag(0) or ZnO NPs in sludges leaving the wastewater treatment or in the biosolids resulting from heat and lime treatment. Rather, effluent sludges consist of Ag in the form of Ag_2S and Zn in the form of Zn−Fe oxy/hydroxide, $Zn_3(PO_4)_2$, and ZnS. Zn is labile in biosolids processing, forming ZnS, Zn−Fe oxy/hydroxide, and $Zn_3(PO_4)_2$ in ratios that depend on redox conditions and moisture content during drying. Ag_2S persisted under various biosolids processing procedures. Thus, the transformation processes for Ag and ZnO NPs are rapid enough to completely transform during the WWTP process, and Ag_2S, Zn−Fe oxy/hydroxide, $Zn_3(PO_4)_2$, and ZnS appear to be the relevant species for toxicity and fate testing for exposures assumed to derive from application of wastewater treatment plant biosolids.

These observed transformations will likely affect the potential for toxicity from exposure to Ag(0) and ZnO NPs. Due to its extremely low solubility, Ag_2S is less toxic than Ag(0).[7,39] Compared to Ag, Zn will likely be more available in WWTP sludge and biosolids since Zn appears to be more labile than Ag, with an ability to change speciation with redox state. A very important discovery is that there is very little difference in the speciation of Ag and Zn in sludge and biosolids amended with ionic and nanoparticle forms of the metals. Further, for Zn, there is little difference between NP-amended sludge and control (unamended) sludge. Thus, there are no apparent nanospecific effects on the speciation of the metals as measured by XAS. This might lead to the conclusion that biosolids land application regulations should not be impacted by the use of nano versus non-nano Zn and Ag. However, a recent study[20] showed that there are differences in the partitioning and mobility of Ag between NP and $AgNO_3$ in biosolid amended soils even though the EXAFS spectra were similar among these treatments. This may be a result of structural differences in the species formed form NP addition vs ionic addition, even though the metal speciation is similar. Structural information (a microscopic investigation) for the metal nanoparticles formed in biosolids is still needed. Furthermore, Colman et al.[40] showed greater effects of Ag NPs on microbial community structure and function than for $AgNO_3$ in terrestrial mesocosms dosed with spiked WWTP sludge. Further studies of metal mobility and toxicity are required to confirm the tentative conclusion that existing regulations are protective of Ag and ZnO NPs. Finally, it appears that, for Zn and Ag, methods that add those materials directly to anaerobic digester sludge adequately capture the behavior of these metals in the WWTP sludge and resulting biosolids from sludge treatment. This makes testing new materials easier.

■ ASSOCIATED CONTENT

Supporting Information

Additional figures and tables on TEM, DLS of the pristine Ag and ZnO NPs, water holding capacity of the biosolids, Ag/Zn/Cu concentrations in the biosolids, setup picture of the pilot WWTP, Zn model compounds XANES spectra, Zn K edge XANES of the composting and heat/lime samples with fittings, and plant effluent metal concentrations. This material is available free of charge via the Internet at http://pubs.acs.org.

■ AUTHOR INFORMATION

Notes

Any opinions, findings, conclusions or recommendations expressed in this material are those of the author(s) and do not necessarily reflect the views of the NSF or the EPA. This work has not been subjected to EPA review and no official endorsement should be inferred.

The authors declare no competing financial interest.

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