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Sulfur and Zinc Availability from Co-granulated Zn-Enriched Elemental Sulfur Fertilizers

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ABSTRACT: Acidification by oxidation of elemental sulfur (ES) can solubilize ZnO, providing slow release of both sulfur (S) and zinc (Zn) in soil. For this study, a new granular fertilizer with ES and ZnO was produced and evaluated. The effect of incorporating microorganisms or a carbon source in the granule was also evaluated. Four granulated ES−Zn fertilizers with and without S-oxidizing microorganisms, a commercial ES pastille, $ZnSO_4$, and ZnO were applied to the center of Petri dishes containing two contrasting pH soils. Soil pH, $CaCl_2$-extractable S and Zn, and remaining ES were evaluated at 30 and 60 days in two soil sections (0−5 and 5−9 mm from the fertilizer application site). A visualization test was performed to evaluate Zn diffusion over time. A significant pH decrease was observed in the acidic soil for all ES−Zn fertilizer treatments and in the alkaline soil for the *Acidithiobacillus thiooxidans*-inoculated treatment only. In agreement with Zn visualization tests, extractable-Zn concentrations were higher from the point of application in the acidic (62.9 mg dm^{-3}) compared to the alkaline soil (5.5 mg dm^{-3}). Elemental S oxidation was greater in the acidic soil (20.9%) than slightly alkaline soil (12%). The ES−Zn granular fertilizers increased S and Zn concentrations in soil and can provide a strategically slow release of nutrients to the soil.

KEYWORDS: *Aspergillus niger, Acidithiobacillus thiooxidans, sulfur oxidation, ZnO, micronutrients, Zn diffusion*

INTRODUCTION

Sulfur (S) and zinc (Zn) deficiencies in soils are common nutrient problems throughout the world, with respect to both crop productivity and human nutrition.[1−3] Zinc deficiency is widespread and most common in calcareous and sandy soils.[4−6] Sulfur deficiency has become more prevalent in the past decades due to a decrease in incidental inputs through atmospheric deposition and fertilizers and an increase in crop S removals. Therefore, S- and Zn-containing fertilizers are applied to large agricultural areas to increase crop yields and food quality. For S fertilization, gypsum, ammonium sulfate, and elemental S (ES) are the most common sources, whereas Zn is provided mainly as zinc sulfate or zinc oxide. Although ES is unavailable for plant uptake, it is oxidized in the soil to its plant-available form, SO_4^{2-}-S.

The oxidation of ES in soils occurs quickly if finely divided ES is mixed through soil.[7,8] However, finely divided ES tends to have a high production cost and can pose a fire or explosion hazard during handling.[9] Furthermore, applying fine ES particles to soil is impractical using current farmer fertilizer application practices, and mixing it with soil is not compatible with no-tillage practices. As S^0 oxidation is a biological process, soil properties that affect microbial abundance and activity also affect the oxidation rate. For instance, oxidation of ES was found to be positively correlated with pH, organic matter (OM) content, microbial populations, and activity of soil.[10] Higher OM content,[11,12] soil pH between 5.4 and 8.0,[11] and soil moisture close to field capacity are expected to maximize S oxidation rates,[13,14] as well as temperatures between 30 and 40 °C.[15]

The oxidation of ES occurs under aerobic conditions and is an acid-producing process. Many microorganisms can oxidize ES (S^0) to SO_4^{2-}, but the most efficient are the chemoautotrophic bacteria *Acidithiobacillus thiooxidans* and *Acidithiobacillus ferrooxidans*.[16−18] However, these organisms are almost absent in most agricultural soils[7,19,20] due to soil chemical properties and the absence of an energy source for these microorganisms in aerated soil. Heterotrophic microorganisms, both bacteria and fungi, can also be important ES oxidizers in agricultural soils.[20−22]

Elemental S is produced predominantly by recovery from the oil and gas industry,[23] and the low price of this concentrated S form has recently stimulated its wider use. Traditionally, ES is a primary source for sulfuric acid production that is then used to manufacture phosphates as well as other soluble fertilizers. When ES is used directly as a S source in fertilizer, it is often co-granulated with rock phosphate or granular fertilizers including urea, single and triple superphosphate, mono- and diammonium phosphate, and potassium chloride.[24] In addition, pastilles containing ES and bentonite (90% ES and 10% bentonite) have been made after a S melting process.[25]

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Zinc oxide is a cheap source of zinc, but it is not readily available to plants when banded or added in granular form.[26,27] Furthermore, the distribution of pure micronutrient granular fertilizers even dry blended with macronutrient fertilizers results in poor Zn distribution, which limits plant uptake. We hypothesized that co-granulating Zn with ES fertilizers may be beneficial to achieve adequate Zn distribution and, importantly, the acidity generated during the ES oxidation could enhance ZnO dissolution according to the following reaction, that is, the oxidation of 1 mol of ES supplies enough protons to dissolve 1 mol of ZnO:

$$S^0 + ZnO + {}^3/_2O_2 \rightarrow ZnSO_4$$

A fertilizer made of ES and ZnO would have the advantage of higher nutrient concentrations and lower cost compared to traditional soluble sources of S or Zn.

The objectives of the present work were to produce co-granulated ES−Zn fertilizers and evaluate ES oxidation and S and Zn availability in soils with contrasting pH. We also assessed the oxidation of ES in fertilizers enriched with sugar and known S-oxidizing microorganisms (*A. thiooxidans* or *Aspergillus niger*).

MATERIALS AND METHODS

Fertilizer Manufacturing. Elemental sulfur−zinc granular fertilizer (ES−$Zn_{control}$) with the composition ES (80%), ZnO (5%), sodium bentonite (Na-bentonite; 7.5%), sucrose (5%), and inulin (2.5%) was produced using a laboratory granulator with an atomized water spray addition. To assess if sugar had an effect on ES oxidation, an additional control fertilizer with no sugar (ES−$Zn_{nosugar}$) was produced with the following composition: ES (86.5%); ZnO (5.5%); Na-bentonite (8.0%). For the bacteria-inoculated fertilizer, sucrose was omitted and replaced by inulin to avoid inhibition of bacterial growth.[28] Inulin was also used to increase granule hardness.

Microorganisms. *A. thiooxidans* (ATCC19377; NCIMB 8343) and *A. niger* (ATCC 8740) cultures were grown on 9K and potato dextrose agar (PDA) media, respectively, both for 10 days prior to culture preparation. Then, the fertilizer was inoculated with microorganisms via a solution with 8.2×10^8 cells mL^{-1} of bacteria or 1.9×10^7 conidia mL^{-1} of fungus sprayed with dispersive Triton solution (0.1%) during the granulation process. Thus, ES−Zn fertilizers inoculated with *A. thiooxidans* (ES−$Zn_{thiooxidans}$) and *A. niger* (ES−Zn_{niger}) were produced. Prior to application, the number of bacterial cells and conidia in suspension was counted using a hemocytometer (depth, 0.1 mm; area, 0.0025 mm^2). The bacterial cells were stained using Crystal Violet staining reagent.[29]

The particle size for all dry compounds ranged between 20 and 75 μm. The granules were sized between 2.5 and 3.0 mm. The fertilizers were dried at 35 °C for 48 h and stored at room temperature (around 22 °C). Whole granule and hand-cut cross sections were imaged using a Leica Wild Optical Microscope M420 fitted with a Lumenera Infinity 4, 11 megapixel digital camera and Lumenera "Infinity Analyze" software. An FEI Quanta 450 FEG environmental scanning electron microscope (ESEM) was used to image cross sections and produce elemental maps of S and Zn to investigate nutrient distribution.

Petri Dish Experiments. Two agricultural soils with contrasting pH values were used because soil pH is an important factor affecting ES oxidation and Zn reactions in soil (Table 1). The soil orders are Alfisol (Monarto, South Australia) and Tenosol (Eneabba, Western Australia), as classified according to the Australian Soil Classification.[30] The soils were collected from the top layer (0−10 cm), air-dried, and sieved to <2 mm prior to use. A Petri dish experiment was carried out to evaluate the release of S and Zn from the fertilizers through chemical analyses and visualization of Zn diffusion (see below). The equivalent of 20 g of air-dried soil was placed in a Petri dish (55 mm diameter × 10 mm height), ensuring a flat soil surface.

Table 1. Selected Soil Characteristics

soil characteristic	Eneabba	Monarto
pH ($CaCl_2$)	5.5	7.0
pH (H_2O)	6.5	7.8
organic carbon (g kg^{-1})	15	10
total S[a] (mg kg^{-1})	84.5	99
total Zn[a] (mg kg^{-1})	3.5	29.5
total P[a] (mg kg^{-1})	49.5	127
clay (g kg^{-1})	27	80
silt (g kg^{-1})	12	70
fine sand (g kg^{-1})	180	680
coarse sand (g kg^{-1})	770	160

[a]Total S, Zn, and P measured after aqua regia digestion.

The four granulated ES−Zn fertilizers, ES−$Zn_{control}$, ES−$Zn_{nosugar}$, ES−Zn_{niger}, and ES−$Zn_{thiooxidans}$, and a commercial ES pastille (90% S and 10% bentonite) were evaluated. The treatments with pastille fertilizer was excluded from Zn diffusion visualization tests because they contained no Zn. A single granule or pastille of ca. 17 mg (about 14 mg of S and 0.7 mg of Zn) was applied in the center of the Petri dish by gently pushing it just below the soil surface. A $ZnSO_4$ solution or ZnO suspension, providing the same amount of Zn (0.7 mg Zn per dish), was used as reference treatment for Zn diffusion. Both were applied by pipetting 20 μL of a solution or suspension in a small hole made in the center of the Petri dish. Then, the soil was wetted to 80% field capacity by spraying. The Petri dishes were sealed with Parafilm to minimize water loss while maintaining aeration and incubated at 25 °C in the dark. After 1, 12, and 29 days of incubation, Zn diffusion was assessed following the method described by Degryse et al.[31] Briefly, a $CaCO_3$-impregnated filter paper (Whatman Nno. 1, 55 mm diameter), which acts as a sink for Zn, was placed on the soil surface. After 1−3 h of exposure (1 h for 1 day of incubation and 3 h for 12 and 29 days of incubation), the Zn captured on the paper was colored with dithizone reagent. The filter papers were left to air-dry and then scanned. Images were analyzed using GIMP (v. 2.6.11) software for image processing to quantify the pink area corresponding to the high Zn zone. The diffusion radius (DR) was calculated using the equation

$$DR = \sqrt{A/\pi}$$

where A is the area of the high Zn zone.

The remaining samples were incubated for 30 or 60 days prior to chemical analysis. Concentric soil samples at 0−5 mm (inner section) and 5−9 mm (outer section) from the fertilizer application point were collected using a cork borer, then air-dried and homogenized before chemical analysis.

Chemical Analyses. The pH and extractable S and Zn concentrations were determined in 10 mmol L^{-1} $CaCl_2$ at a liquid/solid ratio (L/S) of 5 L kg^{-1}. About 0.7 g (inner section soil, including the granule) or 1.25 g (outer section) of air-dried soil was shaken with $CaCl_2$ solution for 30 min.[32] Samples were centrifuged for 10 min at 4000*g*, and a subsample of the supernatant was filtered through a 0.45 μm filter. Sulfur and Zn concentrations in the filtered extracts were determined using inductively coupled plasma atomic emission spectroscopy (ICP-AES; PerkinElmer 7000 DV, US). The pH was determined on the remainder of the suspension after shaking.

Residual ES in the soil was extracted with chloroform.[33] About 0.7 g of air-dried soil from the inner section, including the granule, was shaken with 4 mL of water, to promote soil dispersion, and 8 mL of chloroform for 2 h. The suspension was centrifuged for 20 min at 4600*g*, and an aliquot of 40 μL of chloroform extract (bottom) was pipetted, diluted with methanol, and stored at −20 °C. The determination of ES was performed by high-performance liquid chromatography (HPLC, Agilent, USA). A standard stock solution of ES (500 mg L^{-1} S) was made by dissolving powdered ES in a mixture of chloroform and methanol at a ratio of 1:1. A standard curve from 0 to 30 mg L^{-1} S was obtained by diluting the stock solution with methanol.

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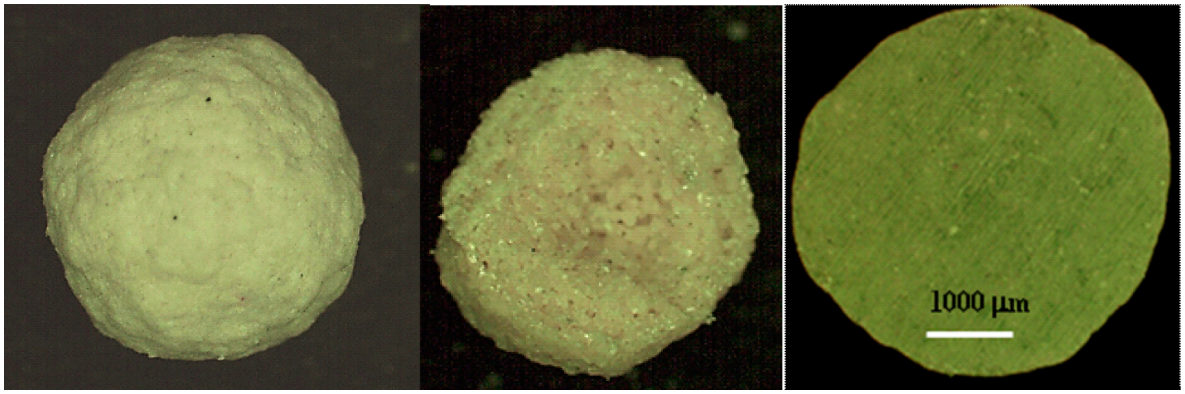

Figure 1. Optical microscope image of a whole (left) or sectioned (middle) ES−Zn granular fertilizer and a sulfur pastille (right).

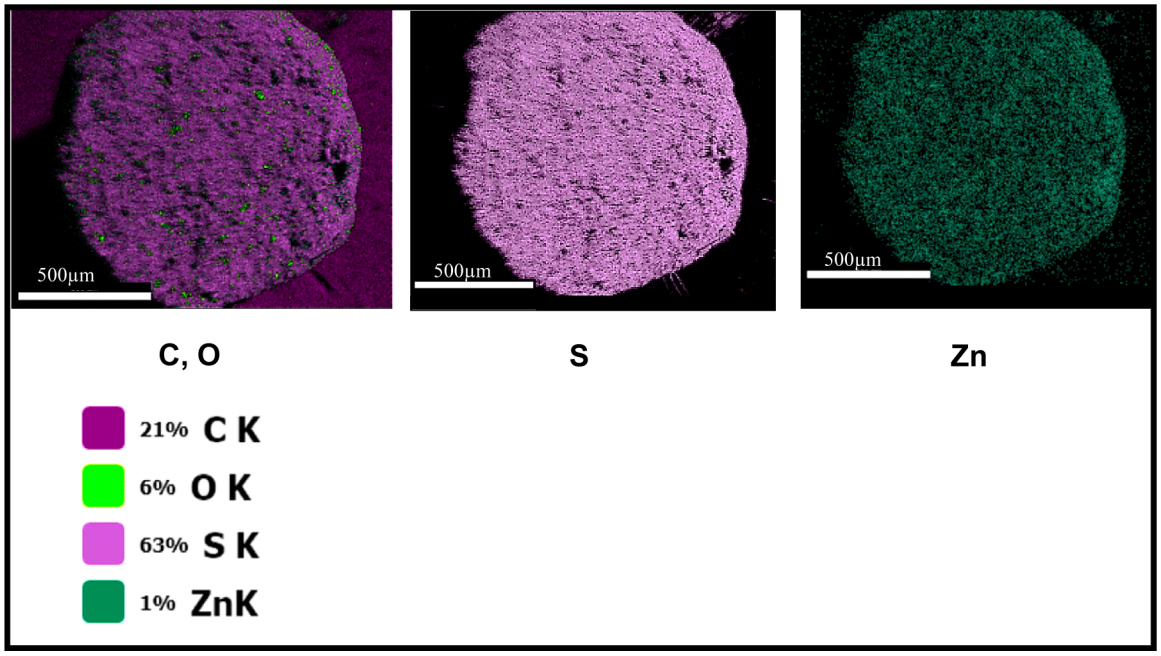

Figure 2. Elemental map images of granules obtained using scanning electron microscopy−energy dispersive X-ray analysis for the ES−Zn control sample in cross section showing the distribution of all elements (left), elemental S (middle), and Zn (right).

Sulfur oxidation was calculated using the amount of ES added and the amount recovered for each treatment by the following equation:

$$\text{S oxidation \%} = 100 - [(\text{ES recovery from treatment}/\text{ES recovery from fertilizer}) \times 100]$$

For ES−Zn fertilizers, ES recovery by the chloroform method ranged from 88.2 to 95.4% for measurements after 60 and 30 days of incubation, respectively. For the pastille fertilizer, ES recovery by the chloroform method ranged from 86.3 to 91.2% for measurements after 60 and 30 days of incubation, respectively.

Microorganism Survival. The survival of inoculated microorganisms in the granules was evaluated in a culture medium from 1 to 97 days after their inoculation. For the fungus-inoculated treatment, the granules were placed in Petri dishes filled with 1.2% PDA, and the characteristic *A.nbs niger* growth produced a positive visual result. To test the bacterial survival, we used the 9K medium, pH 2.8, with Thymol Blue indicator (0.05%). Changes in color from yellow to red indicated bacterial growth when compared to the no-bacteria granules as control.

Data Analysis. The experiments were carried out using randomized block designs with three replications. Statistical analysis was carried out by one-way ANOVA followed by general linear model using the Statistical Analysis System.[34] The differences among treatment means (fertilizers and soil) were analyzed using the LSMEANS procedure with the Tukey adjustment at $p \leq 0.05$. Pearson correlation analysis for S oxidation, pH, and $CaCl_2$-extractable S and Zn for the inner section was carried out by the correlation procedure using SAS.

RESULTS

Nutrient Distribution in the Granules. Elemental S−Zn fertilizer granules with a spherical shape were produced (Figures 1 and 2). The optical microscope image of the control sample shows that ES−Zn granules had higher porosity than the ES pastille, which is important for water penetration and microbial growth and activity when applied to soil. Scanning electron microscope images showed that both S and Zn were very well distributed throughout the granules and that the potential issue of segregation and agglomeration during production, especially for Zn, did not occur prior to, or during, granulation (Figure 2).

Zinc Diffusion. Zinc diffusion assessed by the visualization test showed large differences among treatments (Figures 3 and 4). The diffusion of Zn after 1 day was highest when Zn was

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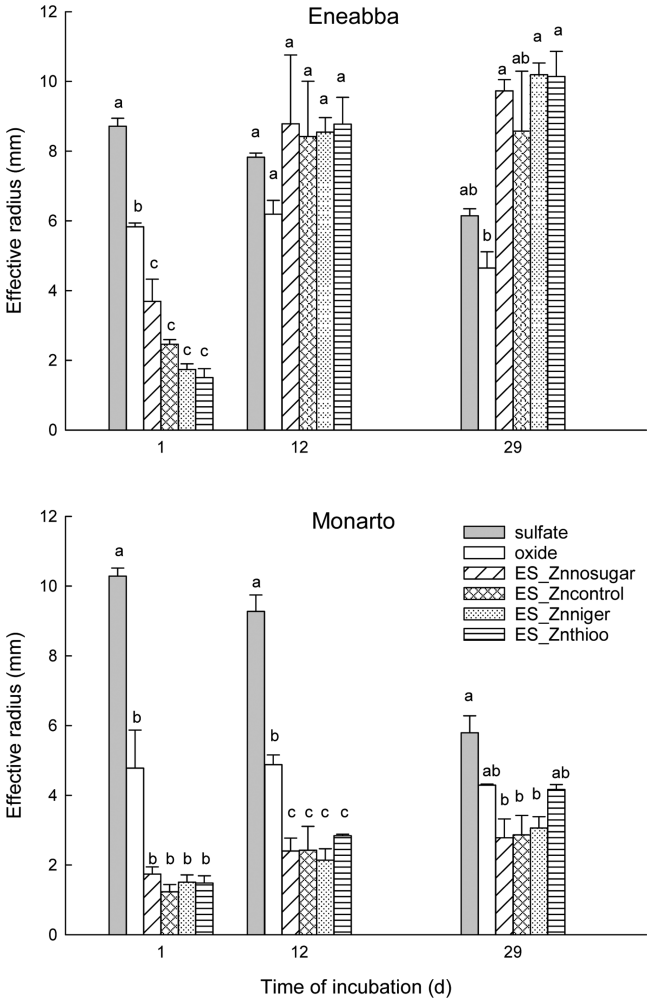

Figure 3. Radius of the high-Zn zone after 1, 12, or 29 days of incubation with Zn fertilizers placed in the center of the Petri dish in soil with low pH (Eneabba) and high pH (Monarto). Zinc was added to the soil at a rate of 0.7 mg as a single granule of elemental sulfur (ES)−Zn fertilizer or a concentrated solution and suspension of $ZnSO_4$ and ZnO, respectively. Different letters for each time of incubation indicate significant differences between treatments (Tukey test, $p \leq 0.05$). The least significant difference (LSD) of effective radius to compare ES fertilizers over time of incubation is 1.45 and 0.60 for Eneabba and Monarto, respectively. Vertical bars over each mean are standard errors of three replicates. See Materials and Methods for full description of the fertilizers.

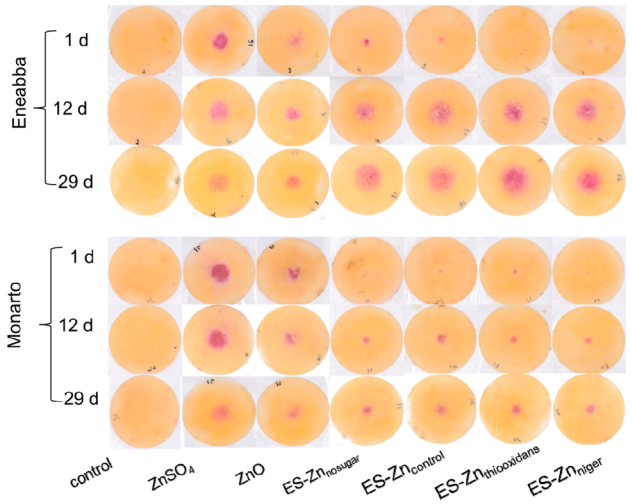

Figure 4. Visualization of Zn diffusion at 1, 12, and 29 days after addition of sulfur−zinc fertilizers in a sandy soil with a low pH (Eneabba) or a soil with high pH (Monarto). Zinc was added to the soil at a rate of 0.7 mg of Zn as a single granule of elemental sulfur (ES)−Zn fertilizer or a concentrated solution or suspension of $ZnSO_4$ or ZnO. See Materials and Methods for full descriptions of the fertilizers.

added as $ZnSO_4$ in both soils. However, the high Zn zones were similar after 12 days for all fertilized treatments in the low-pH soil (Eneabba). The ES−Zn fertilizers and ZnO had much higher diffusion after 12 days in the low-pH soil than in the high-pH soil (Monarto). In the high-pH soil, there was an increase in the high-Zn zone for ES−Zn fertilizers compared with a decrease for $ZnSO_4$ between 1 and 29 days (Figures 3 and 4).

Soil pH. Acidification was observed after 30 days of incubation in both soil sections when ES−Zn fertilizers were applied to Eneabba soil (low-pH soil), and the pH further decreased between 30 and 60 days (Table 2). In the Monarto soil, there was little change in pH. When ES−Zn fertilizer was inoculated with *A. thiooxidans*, there was a small but significant decrease in pH compared to the ZnO control in both soil sections at 60 days of incubation (Table 2). $ZnSO_4$ addition decreased the pH slightly compared to the control (at 60 days for Eneabba and both sampling times for Monarto), and ZnO increased the pH in the Eneabba soil (Table 2).

Extractable S and Zn. Application of ES−Zn fertilizers increased S and Zn extractable in the $CaCl_2$ extract in the inner section (0−5 mm) as well in the midsection (5−9 mm far from application point) in both soils (Tables 3 and 4). Although ES−Zn fertilizers did not induce significant changes in the pH of the Monarto soil (except ES−$Zn_{thiooxidans}$ at 60 days), Zn and S concentrations were increased in the inner soil section with their application.

Extractable S remained high in the midsection far from the application point of S-containing fertilizers in the Eneabba soil (Table 3). As expected, Zn concentrations were higher in the Eneabba soil (with low pH) than in the Monarto soil, which has a slightly alkaline pH (Table 4). Moreover, as shown in the Zn visualization test (Figures 3 and 4), this higher Zn concentration resulted in a wider Zn diffusion zone in the Eneabba soil compared with the Monarto soil.

Zinc diffusion was greater in the Monarto soil for the $ZnSO_4$ treatment than for the other treatments, as evident from the higher Zn concentrations in the midsection (Table 4) and as was also indicated by the Zn visualization results (Figures 3 and 4). There was little difference between the ES−Zn fertilizers in the Monarto soil, except for the ES−Zn inoculated with *A. thiooxidans*, which showed higher Zn and S concentrations than the other treatments (Tables 3 and 4).

In the Eneabba soil, S concentrations were generally lower for the ES pastille than for the ES−Zn fertilizers. There was little difference in S or Zn concentrations between the ES−Zn fertilizers. The presence of sugar induced high Zn concentration in the inner soil section at 30 days of incubation, but this effect was not consistent over time (Table 4).

Residual ES and Oxidation Rate. Elemental S oxidation was higher in the Eneabba soil than in the Monarto soil ($p \leq 0.05$), and it was significantly increased in the presence of *A. thiooxidans* at 30 days of incubation in both soils (Table 5). At this time, 20.9 and 12.0% of applied ES were oxidized, which

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Table 2. Soil pH in a 10 mmol $CaCl_2$ L^{-1} Extract for Soil in <5 mm (Inner Section) or 5−9 mm (Outer Section) Concentric Sections from the Fertilizer Application Point at 30 and 60 Days of Incubation[a]

	pH			
	30 days of incubation		60 days od incubation	
fertilizer	inner section	outer section	inner section	outer section
Eneabba Soil				
control	5.30cb	5.30abc	5.55b	5.53a
$ZnSO_4$	5.37b	5.41ab	5.31c	5.18b
ZnO	6.33a	5.46a	6.39a	5.50a
ES−$Zn_{nosugar}$	5.05d	5.14bcd	4.89d	4.97c
ES−$Zn_{control}$	5.08 cd	5.02d	4.83de	4.94c
ES−Zn_{niger}	4.95d	5.03 cd	4.80de	4.91c
ES−$Zn_{thiooxidans}$	4.95d	4.97d	4.69e	4.92c
ES pastille	5.12 cd	5.08 cd	4.80de	4.92c
av fertilizers[b]	5.26*	5.16#	5.10	5.05
LSD[c]	0.25	0.27	0.13	0.11
CV[d] (%)	1.65	1.52	0.92	0.72
Monarto Soil				
control	7.09a	7.08b	7.05a	7.15a
$ZnSO_4$	6.85b	7.04b	6.86b	7.10a
ZnO	7.11a	7.10ab	7.11a	7.10a
ES−-$Zn_{nosugar}$	7.14a	7.10ab	7.13a	7.12a
ES−$Zn_{control}$	7.12a	7.11ab	7.09a	7.12a
ES−Zn_{niger}	7.14a	7.09ab	7.14a	7.14a
ES−$Zn_{thiooxidans}$	7.08a	7.12a	6.79b	7.00b
ES pastille	7.14a	7.13a	7.13a	7.13a
av fertilizers[b]	7.10*	7.10	7.04	7.10
LSD	0.10	0.09	0.16	0.07
CV (%)	0.29	0.18	0.83	0.36

[a]Different letters within a column indicate significant ($p \leq 0.05$) differences by Tukey test for each soil. [b]* and # indicate significant ($p \leq 0.05$ and $p \leq 0.10$, respectively) differences by F test between time of incubation for inner-section and outer-section for each soil. [c]Least significant difference ($p < 0.05$). [d]Coefficient of variation for ANOVA.

Table 3. Sulfur Extractable in a 10 mmol L^{-1} $CaCl_2$ Extract for Soil Taken from <5 mm (Inner Section) or 5−9 mm (Outer Section) Concentric Sections from the Fertilizer Application Point at 30 and 60 Days of Incubation[a]

	$CaCl_2$-extractable S (mg kg^{-1})			
	30 days of incubation		60 days of incubation	
fertilizer	inner section	outer section	inner section	outer section
Eneabba Soil				
control	10.7b	7.8c	9.9c	6.9b
$ZnSO_4$	24.0ab	23.1ab	27.4a	27.2a
ZnO	11.4b	7.1c	11.9bc	9.9ab
ES−$Zn_{nosugar}$	30.6a	29.0a	30.9a	29.3a
ES−$Zn_{control}$	36.2a	24.7ab	28.3a	26.7ab
ES−Zn_{niger}	31.8a	28.1a	27.6a	27.2a
ES−$Zn_{thiooxidans}$	34.6a	27.2a	35.0a	29.3a
ES pastille	29.4a	13.8bc	16.0b	15.1ab
av fertilizers[b]	28.3ns	21.9ns	25.3	23.5
LSD[c]	16.6	12.7	19.3	20
CV[d] (%)	22	18	29	31
Monarto Soil				
control	6.6c	4.8b	5.0b	4.8c
$ZnSO_4$	23.7a	26.9a	26.1a	25.3a
ZnO	5.8c	4.3b	4.3b	4.5c
ES−$Zn_{nosugar}$	10.3bc	5.1b	6.6b	5.0c
ES−$Zn_{control}$	14.6abc	6.7b	13.7ab	7.5c
ES−Zn_{niger}	13.5abc	6.3b	9.7b	7.7c
ES−$Zn_{thiooxidans}$	25.4a	12.1ab	19.7ab	14.6b
ES pastille	21.0ab	9.5b	15.1ab	6.5c
av fertilizers[b]	16.3ns	10.1ns	13.6	10.1
LSD[c]	12.7	17.3	15.5	3.2
CV (%)	27	54	43	10

[a]Different letters within a column indicate significant ($p \leq 0.05$) differences by Tukey test. [b]ns, nonsignificant ($p > 0.05$) differences by F test between time of incubation for inner section and outer section for each soil. [c]Least significant difference ($p < 0.05$). [d]Coefficient of variation for ANOVA.

corresponded to S oxidation rates of 95.6 and 54.8 μg day^{-1} S in the Eneabba and Monarto soils, respectively (Table 5). Elemental S oxidation increased over time in both soils; however, at 60 days of incubation it was lowest for the ES pastille fertilizer, with 10.1 and 6.9% oxidized in the Eneabba and Monarto soil, respectively. At this time in Monarto soil, the ES−$Zn_{thiooxidans}$ treatment had a higher ES oxidation (17.6% of applied ES) and oxidation rate (39.9 μg day^{-1} S) than the pastille, but there was no significant difference from other treatments. Adding sugar or *A. niger* in these ES−Zn fertilizers had no influence on S oxidation.

Given the relatively low percentage of ES oxidized, ES oxidation was calculated from the difference between two similar values (ES added and ES remaining), which inherently carries a large uncertainty. If there is no immobilization of sulfate-S, ES oxidation can also be calculated from the extractable SO_4-S concentrations. The added S in the $ZnSO_4$ treatment was fully recovered as $CaCl_2$-extractable S, indicating that there was little immobilization of S in these soils over this time frame. Assuming that there was no extra immobilization in the other treatments and that the soil >9 mm of the application point had the same $CaCl_2$-extractable S concentration as the remainder of the soil (because sulfate diffuses quickly in soil), it was estimated that up to 4% of added ES was oxidized. These values are more realistic for whole ES pastilles enclosed by soil (which cannot disintegrate into small ES particles), which have been found to oxidize at rates around 0.05% per day, corresponding to around 10 μg S day^{-1}.[35,36]

The oxidation of ES showed a significant negative correlation with soil pH and a positive correlation with extractable S of inner section soil (Table 6). Moreover, there was a negative and significant correlation between soil pH and extractable S or Zn. Extractable S and Zn had a positive and significant correlation (Table 6). There was no significant correlation between ES oxidation and extractable Zn using all data, but it was positive and significant in Monarto soil (Table 6).

DISCUSSION

Sulfur oxidation and consequent pH reduction around the granules can be an important strategy to increase Zn efficiency for soil application. Our results showed a decrease in soil pH around the ES granule in the Eneabba soil with lower pH (5.5). In the Monarto soil, which has a higher pH buffering capacity, only the treatment with *A. thiooxidans* showed a significant change in pH as a result of ES addition. The increase in diffusion of Zn with ES−Zn application after 12 days in the low-pH soil and at 29 days in the high-pH soil (see Figures 3 and 4), together with the increase in S and Zn extractable in 10

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Table 4. Zinc Extractable in 10 mmol L^{-1} $CaCl_2$ Extract for Soil Taken from <5 mm (Inner Section) or 5−9 mm (Outer Section) Concentric Sections from the Fertilizer Application Point at 30 and 60 Days of Incubation[a]

	$CaCl_2$ extractable Zn (mg kg^{-1})			
	30 days of incubation		60 days of incubation	
fertilizer	inner section	outer section	inner section	outer section
Eneabba Soil				
control	0.8c	0.5c	0.3b	0.25b
$ZnSO_4$	53.2b	34.7ab	30.3a	24.5a
ZnO	65.1ab	22.4b	47.5a	22.2a
ES−$Zn_{nosugar}$	59.1b	41.6a	36.2a	23.8a
ES−$Zn_{control}$	99.5a	41.9a	25.2a	21.9a
ES−Zn_{niger}	69.1b	53.0a	31.4a	25.7a
ES−$Zn_{thiooxidans}$	93.8ab	51.0a	25.3a	21.7a
ES pastille	0.7c	0.4c	1.2b	0.5b
av fertilizers[b]	62.9*	35.0*	24.7	18.0
LS[c]	30	18.7	23.5	19.9
CV[d] (%)	32	17	32	18
Monarto Soil				
control	0.1c	0.1b	0.1b	0.1b
$ZnSO_4$	9.0a	1.7a	6.8ab	1.75a
ZnO	5.2b	0.1b	4.4ab	0.65b
ES-$Zn_{nosugar}$	4.0b	0.1b	3.9ab	0.1b
ES−$Zn_{control}$	4.9b	0.1b	6.1ab	0.1b
ES−Zn_{niger}	4.7b	0.1b	4.8ab	0.1b
ES−$Zn_{thiooxidans}$	10.6a	0.2b	20.2a	1.85a
ES pastille	0.1c	0.1b	0.3b	0.1b
av fertilizers	5.5	0.3	5.8	0.55
LSD	3.3	0.7	18	1.0
CV (%)	28	67	60	61

[a]Different letters within a column indicate significant ($p \leq 0.05$) differences by Tukey test for each soil. [b]* indicates significant ($p \leq 0.05$) differences by F test between time of incubation for inner section and outer section for each soil. [c]Least significant difference ($p < 0.05$). [d]Coefficient of variation for ANOVA.

Table 5. Sulfur Oxidized (S_{Oxi}) and Oxidation Rate (OR) for Inner Section Soil after Incubation Time of 30 and 60 Days with Elemental Sulfur Fertilizers[a]

	30 days of incubation		60 days of incubation	
fertilizer	S_{Oxi} (%)	OR (μg S day^{-1})	S_{Oxi} (%)	OR (μg S day^{-1})
Eneabba Soil				
ES−$Zn_{nosugar}$	8.5b	41.0b	19.8ab	45.3ab
ES−$Zn_{control}$	7.5b	31.7b	18.2ab	41.8ab
ES−Zn_{niger}	9.6b	45.0ab	18.6ab	44.5ab
ES−$Zn_{thiooxidans}$	20.9a	95.6a	22.6a	52.3a
ES pastille	4.6b	23.0b	10.1b	22.0b
av[b]	10.2*	47.3*	17*	41.2*
LSD[c]	10.6	52	10.6	25
CV[d] (%)	34	36	21	22
Monarto Soil				
ES−$Zn_{nosugar}$	6.8ab	37.2ab	9.2ab	21.2ab
ES−$Zn_{control}$	3.8b	16.4b	10.0ab	23.9ab
ES−Zn_{niger}	9.1ab	42.2ab	8.4ab	19.1ab
ES−$Zn_{thiooxidans}$	12.0a	54.8ab	17.6a	39.9a
ES pastille	3.7b	17.5b	6.9b	15.9b
av[b]	8.3	33.6#	10.4	24
LSD	8.0	36	10.3	23.7
CV (%)	40	39	40	42

[a]Different letters within a column indicate significant ($p \leq 0.05$) differences by Tukey test. [b]* and # indicate significant ($p \leq 0.05$ and $p \leq 0.10$, respectively) differences by F test between time of incubation for inner-section and outer-section soil. [c]Least significant difference ($p = 0.05$). [d]Coefficient of variation for ANOVA.

Table 6. Pearson Correlation Coefficients for S Oxidation, pH, and $CaCl_2$-Extractable S and Zn for Inner Sections of Two Australian Soils with ES−Zn Fertilizers Applied[a]

variable	S oxidation	pH	extractable S	extractable Zn
General				
S oxidation		−0.489**	0.351**	0.197
pH			−0.690**	−0.569**
extractable S				0.605**
Eneabba Soil				
S oxidation		−0.521**	−0.097	−0.191
pH			0.229	0.349*
extractable S				0.410**
Monarto Soil				
S oxidation		−0.673**	0.354*	0.652**
pH			−0.328#	−0.864**
extractable S				0.305#

[a]**, *, and # indicate significant differences at $p \leq 0.01$, 0.05, and 0.1, respectively.

mmol L^{-1} $CaCl_2$, suggests increases in Zn availability in both soils.

Although ES is used as a S source in fertilizers, few fertilizer products have combined ES with cationic micronutrients. The oxidation of ES induces acidification, which may increase the availability of cationic micronutrients in soil. The $CaCl_2$-extractable Zn from co-granulated ES with ZnO was similar to or higher than that for the common Zn sources in fertilizers, $ZnSO_4$, or ZnO. Additionally, these fertilizers provide a progressive release of plant-available S. Fertilizers formulated using ES and ZnO have a high nutrient concentration and therefore should be cheaper to transport than other S or Zn fertilizers. Moreover, using raw materials such as ES and micronutrients in oxide forms will have less of an environmental impact for fertilizer production due to significantly less processing being involved in their manufacture. Thus, ES−Zn fertilizers may be a competitive source of both S and Zn for fertilizer application.

Considering the impractical application of ES to soil as a fine powder, granules or pastille forms are a more appropriate way for soil fertilization. However, incorporation of ES in granules or pastilles reduces the exposed surface area of ES and reduces S oxidation.[36] The effectiveness of ES as a granular or pastille fertilizer depends on oxidation rate, and residual effects should be understood for sequential annual crops or perennial species management.[37,38]

Interestingly, S oxidation was not correlated with extractable Zn in the acid soil, whereas it was in the high-pH soil. Soil acidity is a very important factor related to ZnO dissolution and consequent increase in extractable Zn concentration in soil.[39,40] Thus, the acidity that is generated by ES oxidation is only a small contributor to Zn dissolution from ES−Zn fertilizer in soils that are already acidic. On the other hand, a significant correlation between ES oxidation and extractable Zn in the high-pH soil (Monarto) shows how important ES oxidation is for ZnO dissolution and diffusion from the granule in these soil conditions. This means that ES oxidation is more useful in

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increasing Zn availability in high-pH soils, which is an important observation for soil-specific Zn nutritional management. Additionally, comparison of the distribution of Zn across the soil sections showed little difference between the ZnO and $ZnSO_4$ fertilizers (see Figures 3 and 4 and Table 4). This means that the low water solubility of Zn fertilizer in unbuffered laboratory tests underestimates potential Zn availability in acidic soils. On the other hand, soluble Zn fertilizer ($ZnSO_4$) provided higher Zn diffusion in the high-pH soil than the ZnO-based fertilizers (Monarto soil, see Figures 3 and 4). This was also clear from the $CaCl_2$-extractable Zn, which was significantly higher in the 5−9 mm section for the $ZnSO_4$ treatment than for the other treatments (see Table 4).

Soil pH is an important soil property in relation to Zn availability, and it is generally recognized as the main factor influencing Zn solubility and mobility across a wide range of soils.[39,41,42] However, other factors also play important roles, such as organic matter content,[43,44] type and content of clay minerals,[45] and soil moisture.[46] Zinc concentration and diffusion were much higher in the acidic Eneabba soil than in the slightly alkaline Monarto soil. When the soil pH was low and S oxidation contributed to a further pH reduction, Zn solubilization from oxide happened quickly, and it was free to diffuse through the soil, because adsorption is weak in acidic sandy soils. On the other hand, in the Monarto soil Zn solubilization from the oxide was limited by soil pH, S oxidation was lower, and it was not enough to reduce soil pH, except when ES−$Zn_{thiooxidans}$ was applied. In the ES−$Zn_{thiooxidans}$ treatment, the Zn concentration in the outer section was similar to that in the $ZnSO_4$ treatment after 60 days, suggesting that ZnO was nearly fully solubilized after 60 days. This can be explained by the fact that only 0.35 mg of S (or 2.5% of the S in the granule) needs to be oxidized to provide the acidity to oxidize the Zn present in the granule (0.7 mg of Zn as ZnO).

Although the autotrophic bacteria *A. thiooxidans* is the most important group of ES-oxidizing organisms, it is almost absent in most cropping soils.[47] These observations suggest there could be benefits from applying ES-containing fertilizers inoculated with these microorganisms.

Our study shows a potential of *A. thiooxidans* inoculation of ES−Zn for ES oxidation and consequently pH reduction even in a high-pH soil. However, additional research is needed to examine its real efficacy. On the other hand, we did not find enough evidence in this work to support the inclusion of *A. niger* in ES−Zn granules to increase ES oxidation in soil, although *A. niger* has been reported as an ES-oxidizing microrganism.[21,48,49]

The inoculation of ES-oxidizing microorganisms in fertilizers should explore the survival capacity over time because it is a key requirement for inoculated fertilizer production and efficacy when applied. Whrease *A. niger* survived in fertilizer granules for at least 4 months (data not shown), we could not evaluate with accuracy how long the *A. thiooxidans* survived in ES−Zn fertilizer granules at a range of temperatures.

Results for the addition of 5% sugar to increase ES oxidation in ES−Zn fertilizers were inconclusive. Adding a labile carbon may promote the activity of heterotrophic S-oxidizing microorganisms in soil.[22,50] Growth of S-oxidizing microorganisms around or inside the granules can increase the contact area to ES particles and accelerate its oxidation in soil. It is possible that general heterotrophic microorganisms in these low organic carbon soils may have outcompeted the S-oxidizers, and thus no measurable benefit in S-oxidation was observed by adding glucose. Future tests using different soils and higher sugar formulations should be undertaken to examine the effects of sugar in ES−Zn fertilizers. In addition, a plant growth test is needed to evaluate S and Zn uptake over time and measure the efficiency of co-granulated Zn−ES fertilizers.

This work demonstrates that ES−Zn granular fertilizers can be effective for soil application, given that both S and Zn concentrations increased in soil over time. Compared to ZnO only, elemental S−Zn application in a sandy acidic soil increased both S and Zn concentrations and diffusion of Zn away from the point of application, similar to soluble Zn−sulfate fertilizers. The ES−Zn application in a slightly alkaline soil increased S and Zn concentration more slowly than in the acidic soil. We did not find enough evidence to support the addition of sugar or inoculation with *A. niger* to increase ES oxidation. Although early ES oxidation was increased by adding the ES-oxidizing bacterium *A. thiooxidans*, the results were not conclusive in terms of its efficacy in ES fertilizers.

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