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RESEARCH PAPER

Production and evaluation of a co-granulated elemental sulfur-micronutrient fertilizer

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Abstract

The oxidation of elemental sulfur (ES) in soil and the resulting acidification can solubilize oxides containing micronutrients making them available to plants over time. In this study, we produced and evaluated a new fertilizer aiming to provide S, Zn, Mn and Cu in the same fertilizer granule (ES_micro). The effect of incorporating *Acidithiobacillusferrooxidans*in the granule (ES-micro_*Af*) was also evaluated. A granular ES_micro fertilizer with and without the S-oxidizing microorganism and a mixture of Mn, Zn and Cu as sulfates were applied to pots containing two soils with contrasting textures for a sequential crop cultivation.ES_micro fertilizer increased maize dry matter production as well as Zn and Mn uptake more than $ZnSO_4$ and $MnSO_4$fertilizers respectively, in clay soil.ES_micro had a residual effect to soybean cultivation for Zn in the sandy soil, and for Mn in the clay soil. The presence of *A. ferrooxidans*(ES-micro_*Af*) did not have any additional effect in terms of dry matter production and nutrients uptake, leading us to suggest thatnative soil microorganisms are effective to oxidize ES to S^{6+}. The ES-micro granular fertilizer can be a potential S, Zn, Cu, and Mn source to provide a strategic release of nutrients, keeping them available in the soil for more time.

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Introduction

The increasing use of concentrated NPK fertilizers with low (or none) S, combined with reduced atmospheric deposition, leaching losses and large-scale removal by high yielding crops have caused a global S deficiency (Stevenson and Cole, 1999; Eriksen and Askegaard, 2000; Scherer, 2001; Alvarez *et al.,* 2007; Horowitz and Meurer, 2007). Also, deficiencies of micronutrients such as Mn, Zn, and Cu, are considered a common problem throughout the world, especially in regard to crop productivity and human nutrition (Junior *et al*., 2000; Alloway, 2004; Cakmak, 2008). The use of soluble sources of these micronutrients, as chlorides or sulfates, has shown low agronomic effectiveness in some soil conditions. A fast release of these nutrients from soluble sources into the soil solution may favor their decreased plant availability within a short time when the soils display one of the following conditions: high pH, high organic matter content, high P content, chemisorption in clay minerals like kaolinite and iron and aluminum oxyhydroxides(Ritchey *et al.,* 1986; Abreu *et al.,* 1996; Atta *et al*., 1996; Junior *et al*., 2000; Bolan and Duraisamy, 2003).

Recently, elemental sulfur (ES) fertilizer has emerged as a S source for crops. This potential fertilizer is attractive due to its high S concentration (95-99% w w^{-1}) and residual effect over the growing season (Janzen and Bettany, 1987). Soluble S-sulfate fertilizers are effective in correcting S deficiencies; however, they are susceptive to leaching losses with a limited residual effect. Despite of the above-mentioned advantages of ES its agronomic effectiveness as a fertilizer is still questionable (Primo*et al*., 2012), mainly because it needs to be oxidized into (SO_4^{2-}) to become available to plants.

The oxidation kinetics depends on the microbial activity in the soil (Janzen and Bettany*,* 1987). The intensity of this reaction is related to soil properties, climatic conditions, and particle properties of the fertilizers (Janzen and Bettany, 1987; Lawrence and Germida, 1991; Donald and Chapman, 1998; Horowitz and Meurer, 2007).

Oxide micronutrient sources such as ZnO, MnO_2, and CuO, are a cheaper alternative because they require less processing compared to soluble sources. In addition, they are more environmentally friendly due to their low reactivity under normal soil conditions. However, they are not readily available to plants and need to be solubilized under acidic conditions. When applied in powder form mixed with other fertilizer particles, they are irregularly distributed in the field (Mortvedt, 1992). We are therefore hypothesizing that co-granulating ES with Zn, Mn, and Cu oxides is more or effective as using soluble sources, as sulfates.

The oxidation of ES in soil promotes acidification around the fertilizer granules and may increase the dissolution of micronutrient oxides (ZnO, MnO_2 and CuO). Thus, this process should gradually increase the release of these nutrients into the soil solution, achieving a better synchrony with plants´ demands. In addition, the slow release of Zn, Cu, and Mn in the soil solution may prevent their losses by chemisorption or precipitation. Therefore, the objective of this work was to investigate the performance of ES_micro fertilizers in terms of dry matter production and uptake of S, Mn, Zn, and Cu by a maize-soybean successive crop. We also evaluated the effects of adding oxidizing microorganisms (*A. ferrooxidans*) to the fertilizer.

Materials and methods

Fertilizer manufacturing

The elemental sulfur-micronutrient co-granulated fertilizer (ES_micro), composed of ES (74 % w/w), ZnO (4.5% w/w), MnO_2 (11.7% w/w), CuO (2.2% w/w), andsodium bentonite (Na-bentonite) (6% w/w), was produced through melting of ES on a heater plate at 140 °C, followed by the addition of micronutrient oxides. After cooling, the solid mixture was ground and sieved to obtain granules with a diameter ranging from 1.0 to 2.0 mm.ES_micro was also inoculated with *Aciditiobacillusferrooxidans* via a solution of 10^7 cells mL^{-1}, using 120 µL g^{-1} of ES-micro. Subsequently, the inoculated fertilizer (ES_micro_*Af*) was dried at 27 ± 3°C for 30 min.

Greenhouse trial

The performances of ES_microandES_micro_*Af* compared to soluble fertilizers (sulfates) were evaluated in a greenhouse experiment with a maize-soybean crop succession. We used two soils with contrasting clay contents.

The soils were collected from the top soil layer (0–20 cm), air-dried, and sieved to < 2 mm for physical and chemical analysis and < 4 mm for the experimental trial (Table 1).

A mixture of lime containing the proportion (w w^{-1}) 3/1 of $CaCO_3/MgCO_3$ was applied 30 d before planting in order to increase base saturation of each soil to 60%. After the incubation time, maize was cultivated in plastic pots containing 3 dm^3 of each soil. Prior to planting, we applied a basal fertilization containing 150 mg dm^{-3} of N as urea, 300 (clay soil) and 150 (sandy soil) mg dm^{-3} of P as triple-superphosphate, 180 mg dm^{-3} of K as KCl, 0.818 mg dm^{-3} of B as H_3BO_3, 0.150 mg dm^{-3} of Mo as $(NH_4)_6Mo_7O_{24}.4H_2O$.

Treatments were designed in a factorial scheme [(2 x 3) + 2)], evaluating two soil textures (sandy and clay soil), three fertilizers (ES_micro, ES_micro_*Af*,a mixture of Mn, Zn, and Cu fertilizer as sulfates), and two controls (absence of fertilization for both soils).

The experiment was randomized in random blocks with four replications; three plants composed the experimental unit. Fertilizers were applied in three points in the center of each pot, at 4 cm below the surface, in order to supply 120, 12, 6, and 3 mg dm^{-3} of S, Mn, Zn, and Cu, respectively. Treatments corresponding to the soluble sources were composed of a mixture of $CaSO_4.2H_2O$, $ZnSO_4.7H_2O$, $MnSO_4.H_2O$, and $CuSO_4$. $5H_2O$. Six maize (*Zea mays* L.) seeds were sown in each pot at a depth of 2 cm and the seedlings were thinned to three most uniform ones per pot after one week.

Pots were watered with distilled water to keep the water content at 80% of field capacity.

After 45 d of cultivation, plants were harvested by cutting the stems at the soil surface. The shoots were dried at 65 ± 5°C until weight stabilization (around 72 h) and weighted.

After maize cultivation, eight soybean (*Glicine max* L.) seeds were sown in undisturbed soil pots at a depth of 1 cm and. After 7 d each pot was thinned to three plants. We used the nitrogen-fixing bacteria *Bradyrhizobiumjaponicum*, with no N-application. Pots were watered with distilled water to keep the water content at 80% of field capacity. After 45 d, plants were harvested, dried at 65 ± 5°C until weight stabilization (around 72 h), and weighted.

The shoots from maize and soybean cultivation were grounded and representative samples were digested in a mixture of 3/1 $HNO_3/HClO_4$ (v v^{-1}). The extract was analyzed for S, Mn, Zn, and Cu in an ICP-OES (Perkim Elmer model 8300).

Data analysis

We used one-way analysis of variance (ANOVA). Differences among treatments (fertilizers) in each soil were evaluated using the Scott-Knott (1974) test adjustment at $p = 0.05$. All data were processed using SAS software, version 9.2 (SAS Institute Inc.)

Results

Dry matter production

Fertilizer applicationincreased dry matter production (DMP) of maize in both soils (Fig. 1). In the sandy soil, DMP increased by 1.14-fold in fertilized treatments, with no significant differences between treatments. In the clayey soil, application of ES_micro fertilizer increased DMP of maize by 1.12- and 1.15-fold compared to the control and sulfate source treatments, respectively (Fig.1).

There was no significant difference between ES_microandES_micro_*Af*fertilizers. Dry matter production of soybean (second cultivation) did not change among treatments, including the control (Fig. 1).

Sulfur uptake

Sulfur uptake by maize increased with the application of S fertilizers in both soils (Fig. 2). In clayey soil, the fertilizers increased S uptake by 1.19-fold compared to the control treatment, and there were no significant differences among fertilizers. In sandy soil, S uptake by maize was higher (1.24-fold) when fertilizer was applied as S-sulfate compared to ES_micro or ES_micro_*Af*(Fig. 2). Sulfur uptake by soybean did not change among fertilizers, including the control treatment (Fig. 2). The inoculation of ES_microfertilizers with *A. ferrooxidans* had no influence in terms of S absorption on both cultivations.

Table 1. Soils characteristics after lime incubation.

Soil (localization)	Sandy soil	Clay soil
Sand (g kg^{-1})	837	220
Silt (g kg^{-1})	21	60
Clay (g kg^{-1})	142	720
pH $^{(1)}$	6.7	5.7
pH $^{(2*)}$	5.9	5.9
Al^{3+} (cmol$_c$ dm^{-3})$^{(2)}$	0.0	0.0
(H + Al) (cmol$_c$dm^{-3})$^{(3)}$	1.6	2.1
Ca^{2+} (cmol$_c$ dm^{-3})$^{(2)}$	1.5	1.8
Mg^{2+} (cmol$_c$ dm^{-3})$^{(2)}$	0.4	0.5
P (mg dm^{-3})$^{(4)}$	1.8	0.8
K (mg dm^{-3})$^{(4)}$	15.0	11.0
Organic matter (dag kg^{-1}) $^{(5)}$	1.3	1.5
P-rem (mg L^{-1}) $^{(6)}$	30.4	11.2
S (mg dm^{-3})$^{(4)}$	9.2	6.2
Zn (mg dm^{-3})$^{(4)}$	0.7	0.6
Cu (mg dm^{-3})$^{(4)}$	0.1	3.5
Mn (mg dm^{-3})$^{(4)}$	5.9	44.8
Fe (mg dm^{-3})$^{(4)}$	86.1	26.8
B (mg dm^{-3})$^{(7)}$	0.2	0.1

(1), (2*) represent pH measured in water and KCl solution, using a soil/solution ratio of 1:2.5 (v v^{-1}). (2) Extracted with a KCl 1 mol L^{-1} solution. (3) Extracted with a calcium acetate solution 0.5 mol L^{-1} at pH 7.0. (4) Extracted with Mehlich-1 solution. (5) Organic matter= C * 1.724 Walkley Black (6) Remaining Phosphorus – used to evaluate soil P affinity (Alvarez V. *et al.*, 2000). (7) Hot water. For both soils, mineralogical analysis identified the presence of quartz in the sandy fraction; quartz, kaolinite, gibbsite, and goethite in the silt fraction and kaolinite, gibbsite, and goethite in the clay fraction. We used the DiffractometerPHNalytical, model X' PertPRO, Co Kα radiation (1.7889 Å).

Zinc, manganese, and copper uptake

We observed differences between fertilizers in terms of Zn, Mn, and Cu absorption by maize (first crop) and soybean (second crop) cultivations (Figs. 3, 4, and 5).

In clay soil, the application of ES_micro fertilizer increased Zn and Mn uptake of maize by 1.8 and 1.2-fold compared to the application of these nutrients as $ZnSO_4$ or $MnSO_4$, respectively (Figs. 3 and 4). Copper uptake by maize increased with the application of Cu fertilizers, but there were no significant differences among the sources. Although the fertilizers increased Zn and Cu uptake by maize, these effects were not observed for soybean (second crop) grown in clay soil (Figs 3 and 5).

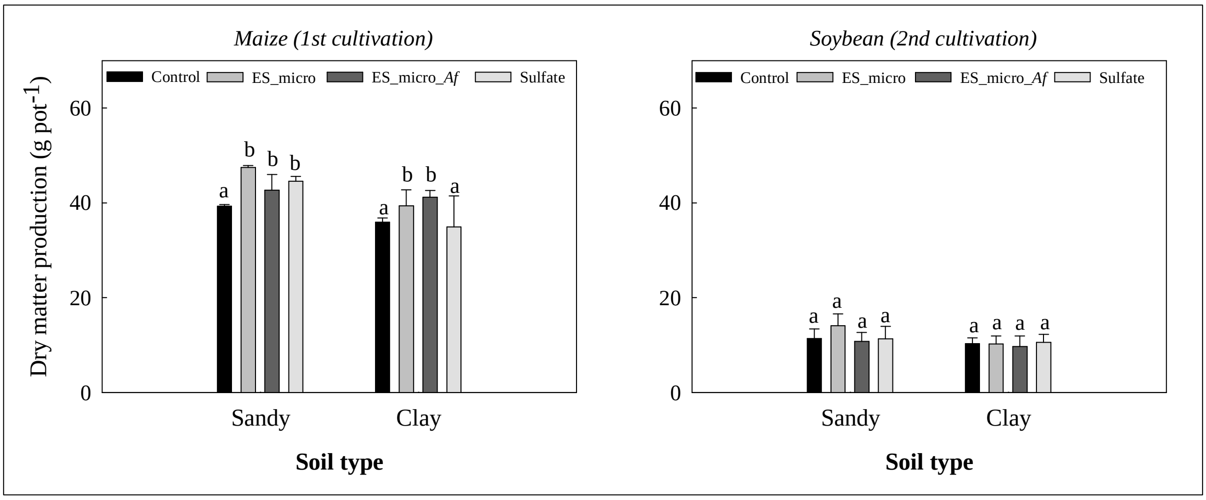

Fig. 1. Dry matter production of a sequential crop cultivation (maize-soybean) following S, Zn, Mn, and Cu soil fertilization. Elemental sulfur-micronutrient (ZnO, MnO_2, and CuO) co-granulated fertilizers with (ES_micro_*Af*) and without (ES_micro) inoculation with *Acidithiobacillusferrooxidans*, and soluble sources of S, Zn, Mn, and Cu (as sulfates) were applied in clay soil (72% clay) and sandy soil (14.2% clay). Same letter in column groups means non-statistical difference by Scott knott test at 5%. Cross-bars (I) represent the standard error with four replications.

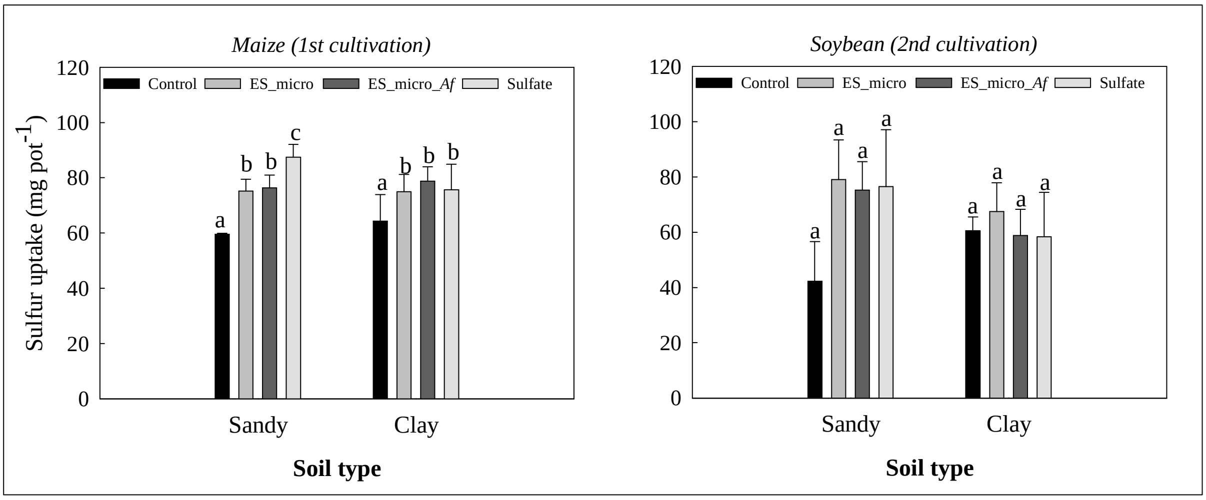

Fig. 2. Sulfur uptake of a sequential crop cultivation (maize-soybean) following S, Zn, Mn, and Cu soil fertilization. Elemental sulfur-micronutrient (ZnO, MnO_2, and CuO) co-granulated fertilizers with (ES_micro_*Af*) and without (ES_micro) inoculation with *Acidithiobacillusferrooxidans* and soluble sources of S, Zn, Mn, and Cu (as sulfates) were applied in clay soil (72% w w^{-1} clay) and sandy soil (14.2% w w^{-1} clay). Same letter in column groups means non-statistical difference by Scott knott test at 5%. Cross-bars (I) represent the standard error with four replications.

In contrast, ES_micro increased Mn uptake in soybean of up to 2.8-fold compared to $MnSO_4$ fertilizer (Fig. 4).

In sandy soil, Zn fertilizers increased Zn uptake of maize, but there were no significant differences among the sources (Fig. 3).

However, in the second cultivation, soybean plants fertilized with ES_micro absorbed 1.52 times more Zn than when this nutrient was supplied as $ZnSO_4$ fertilizer. Manganese uptake was higher for both crops when this nutrient was provided as $MnSO_4$ fertilizer.

The absorption of Mn by maize and soybean when this element was supplied as $MnSO_4$, was 5.30 and 2.1 times higher for maize and soybean cultivation, respectively (Fig. 4), in relation when Mn was applied as ES_micro and ES_micro_*Af* fertilizers.

We found no significant differences among fertilizers in terms of Cu absorption for both maize and soybean crops (Fig. 5).

Discussion

Agronomic performances of the fertilizers

Our results show that the use of ES co-granulated with Zn, Mn, and Cu oxides is a good alternative as it is easier to apply fertilizers in granule form. Additionally, these oxides are more cost-effective compared to soluble sources, such as sulfates, and their residual effects over time are desired.

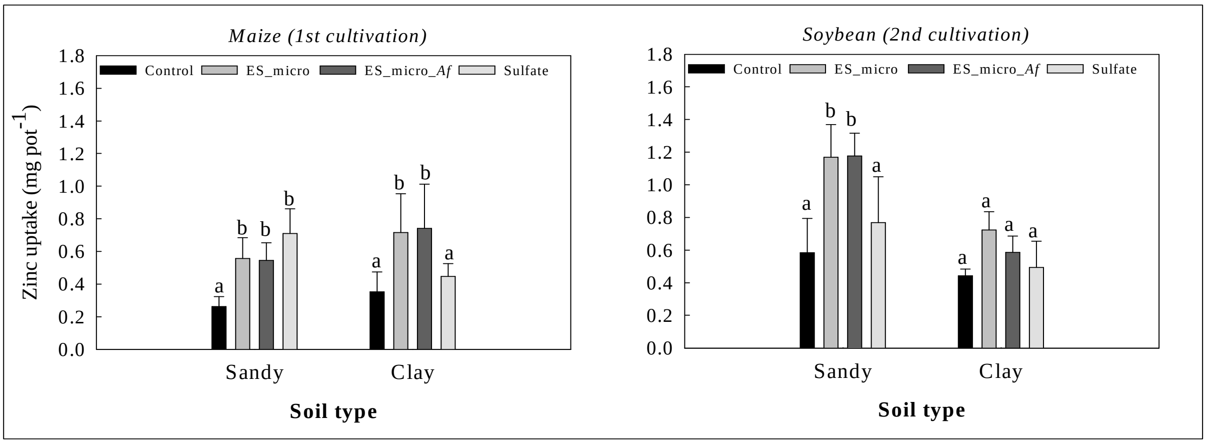

Fig. 3. Zinc uptake of a sequential crop cultivation (maize-soybean) following S, Zn, Mn, and Cu soil fertilization. Elemental sulfur-micronutrient (ZnO, MnO_2 and CuO) co-granulated fertilizers with (ES_micro_*Af*) and without (ES_micro) inoculation with *Acidithiobacillusferrooxidans* and soluble sources of S, Zn, Mn, and Cu (as sulfates) were applied in clay soil (72% w w^{-1} clay) and sandy soil (14.2% w w^{-1} clay). Same letter in column groups means non-statistical difference by Scott knott test at 5%. Cross-bars (I) represent the standard error with four replications.

However, the effectiveness of these sources in increasing soil nutrient concentrations and plant uptake needs to be better understood. Our study shows the effectiveness of ES_micro fertilizer for maize and soybean cultivations, highlighting its effects in clay soil. The best performance of ES_micro fertilizer compared to sulfates in clay soil was mostly related to higher Zn and Mn absorption promoted by this fertilizer. Thus, we suggest that the association between ES and ZnO or MnO_2 in clay soil represents an efficient method to supply Zn and Mn to maize cultivation.

In highly weathered clay soils, due to their high Zn and Mn chemisorption capacities, soluble sources of these elements, such as $ZnSO_4$ and $MnSO_4$, generally present low agronomic effectiveness. According to Borges and Coutinho (2004) and (Sims, 1986), the main Mn fractions in clay and sandy soil were attributed to Fe and Al oxides and exchangeable Mn, respectively.

Fertilizers as sources of S, Zn, Cu and Mn

As described above, the different fertilizer sources showed similar performances in terms of S uptake in maize cultivation. This indicates that elemental sulfur was oxidized in the soil.

The superiority of sulfate fertilizers in sandy soil in terms of S uptake can be associated to lower microbial activities in sandy soil in terms of the oxidation of elemental sulfur.

In addition, the use of sulfate fertilizers in agriculture has promoted expressive losses of S by leaching in rainy regions. Eriksen and Askegaard (2000) detected sulfate leaching of up to 45 kg ha^{-1}, which was closely related to the annual drainage volume.

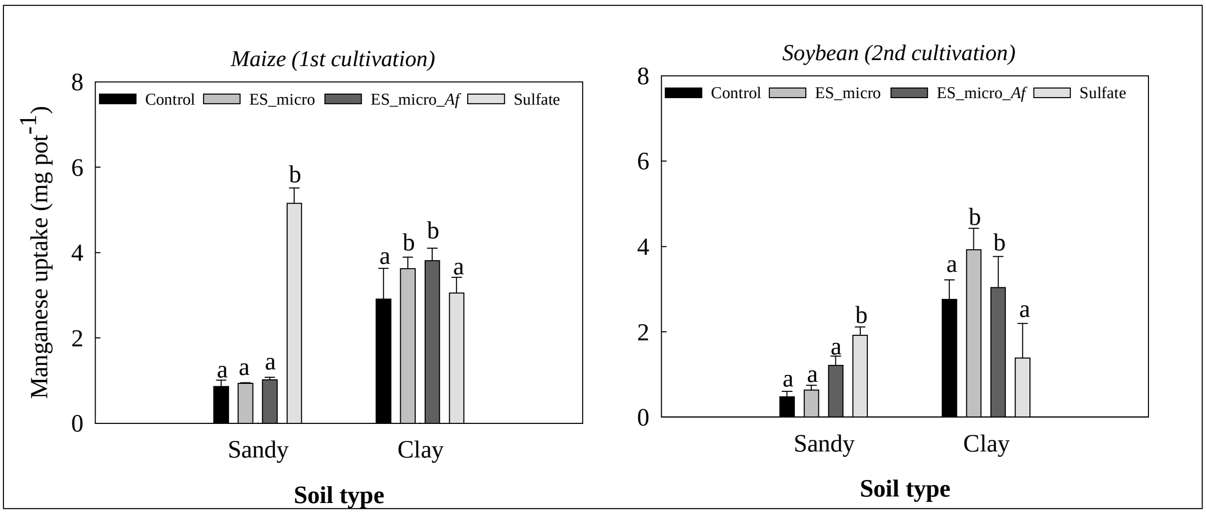

Fig. 4. Manganese uptake of a sequential crops cultivation (maize-soybean) following S, Zn, Mn, and Cu soil fertilization. Elemental sulfur-micronutrient (ZnO, MnO_2 and CuO) co-granulated fertilizers with (ES_micro_*Af*) and without (ES_micro) inoculation with *Acidithiobacillusferrooxidans* and soluble sources of S, Zn, Mn, and Cu (as sulfates) were applied in clay soil (72% clay) and sandy soil (14.2% clay). Same letter in column groups means non-statistical difference by Scott knott test at 5%. Cross-bars (I) represent the standard error with four replications.

We presume that gradual oxidation from S^o to S^{6+} (SO_4^{2-}) prevents sulfate leaching in soil conditions, improving its efficiency as fertilizer compared to soluble S sources, such as sulfates. According to our results, including the effect of ES_micro fertilizers in DMP, S, Zn, Mn, and Cu uptake (Fig.1, 2, 3, 4 and 5), these fertilizers were partially solubilized in the soil, as schematized by Eq. 1 and Eq. 2 for ZnO:

$$\boxed{S^o + 1.5\ O_2 + H2O\ (\textit{Microorganism}) \rightarrow SO_4^{2-} + 2H^+} \quad (1)$$

$$\boxed{ZnO + 2H^+ \rightarrow Zn^{2+} + H2O} \quad (2)$$

The dissolution of ZnO, MnO_2, and CuOmay be related to soil pH and oxidative dissolution of S^o, which produces acidity, as described in Eq. 1. However, we did not have treatments with the application of oxides in absence of S^o, to confirm that the solubilization of these oxides was really promoted by acidic conditions produced through oxidation of S^o.

For all treatments, *A. ferrooxidans* had no effect, suggesting that oxidation of S^o was promoted by microbes native to the soil.

Some studies identified that many microorganisms naturally occurring in the soil are capable of oxidizing S^o, including chemolithotrophic bacteria, such as *Acidithiobacillus*, *Thiomicrospira*, and *Thiosphaera* and heterotrophic bacteria, such as *Alcaligens*, *Paracoccus, Xanthobacter, Epicoccumnigrum, Alternariatenius, Aureobasidiumpullulans, Penicillium species, Aspergillus, Scolecobasidiumconstrictum*,and*Myrotheciumcinctum* (Wainwright *et al.*, 1986; Kuenen and Beudeker, 1982; Germida and Janzen, 1993; Shinde*et al.*, 1996; Friedrich *et al.*, 2001).Burgstaller*et al.* (1992) reported that H^+ from citric acid dissociation produced by *Aspergillussimplicissimum* is capable to solubilize ZnO. According to Sayer*et al.* (1995), strains of *Aspergillusniger* and *Penicilliumsimplicissimum*are capable to solubilize ZnO and $Zn_3(PO_4)_2$, due to their organic acid-producing capability.

In similar studies, manganese from MnO_2 or $MnSO_4$was applied to soybean and tomato cultivations, with $MnSO_4$ showing the best performance, which was attributed to its higher solubility (Abreu *et al.*, 1996; Fiskel and Mourkides

1955). However, in contrast to our study, these studies did not use acidifying sources to MnO_2;

thus we presume that the presence of S^o in the same fertilizer granules mixed with ZnO, MnO_2, and CuO is a key to solubilize these oxides. Soil pH measured at the end of maize cultivation (data not shown) did not change as a function of fertilizer type, suggesting that most of the H^+ produced by the oxidation of the S^o (Eq. 1) was consumed tosurrounding soil.

The residual effect provided by ES_fertilizer in soybean cultivation, as shown for Zn (sandy soil) and for Mn (clay soil), represents an important characteristic of this source in terms of agronomic efficiency over time in successive crops.

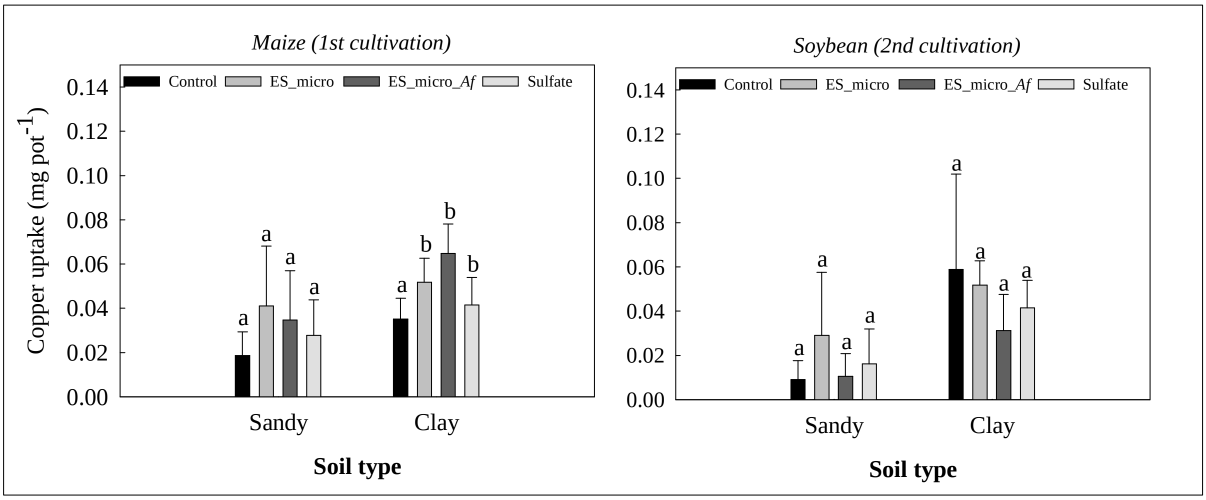

Fig. 5. Copper uptake of a sequential crop cultivation (maize-soybean) following S, Zn, Mn, and Cu soil fertilization. Elemental sulfur-micronutrients (ZnO, MnO_2, and CuO) co-granulated fertilizers with (ES_micro_*Af*) and without (ES_micro) inoculation with *Acidithiobacillusferrooxidans* and soluble sources of S, Zn, Mn, and Cu (as sulfates) were applied in clay soil (72% clay) and sandy soil (14.2% clay). Same letter in column groups means non-statistical difference by Scott knott test at 5%. Cross-bars (I) represent the standard error with four replications.

Conclusion

This research indicates that ES_micro fertilizer is an effective Zn, Cu and Mn source for maize and soybean successive crop. The residual effect of the ES_micro fertilizer represents an advantage, allowing higher nutrient availability for a longer period of time.

Thus, this fertilizer may be an effective product with similar or higher performance than soluble sources of S, Zn, and Mn. Trials should be developed to evaluate the performance of ES_micro under field conditions. The experimental use of Zn, Mn, and Cu oxides, if applied separately from S^o in future works, will allow discovering if their apparent solubilization is due to S^o oxidation, as presumed here. We did not find any supporting evidence that adding *A. ferrooxidans*in ES_microfertilizerincreases the oxidation of ES or the dissolution of Zn, Mn and Cu oxides.

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