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**BASIC AREA** - Article

# Effect of copper carbonate and zinc oxide applied to seeds on copper and zinc uptake by maize seedlings

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**ABSTRACT:** Seed treatment is an interesting alternative to deliver micronutrients to field crops. The aim of this study was to investigate the uptake of Cu and Zn by maize seedlings, with the application of the water-insoluble sources copper carbonate and zinc oxide as seed treatment. Treatments were composed of a control (untreated seeds), five doses of copper (0.14, 0.28, 0.56, 1.12 and 2.24 mg Cu·seed$^{-1}$) and zinc (0.55, 1.10, 2.20, 4.40 and 8.80 mg Zn·seed$^{-1}$) as well as five doses of copper and zinc combined (0.14, 0.28, 0.56, 1.12 and 2.24 mg Cu·seed$^{-1}$; 0.55, 1.10, 2.20, 4.40 and 8.80 mg Zn·seed$^{-1}$). Plants were cultivated in sand, under greenhouse conditions and, at the two-leaf stage (15 days), the root and shoot tissues dry mass and concentration of Cu and Zn were determined, which allowed to calculate accumulation and uptake efficiency of these micronutrients by maize plants. Seed treatment with copper carbonate and zinc oxide increased both root and shoot concentration and accumulation of Cu and Zn in maize seedlings, with two fully expanded leaves. Cu tended to accumulate in roots, while Zn was more evenly distributed among roots and shoots. Combined application of copper carbonate and zinc oxide resulted in lower uptake of both Cu and Zn by maize if compared to individual applications, with Cu uptake reduced in a higher extent.

**Key words:** *Zea mays* L., corn, seed treatment, micronutrients.

## INTRODUCTION

Application of fertilizers containing micronutrients via seed treatment can be considered an interesting option for field crops, in order to complement soil or foliar applications (Slaton et al. 2001; Farooq et al. 2012). The seed treatment operation allows a uniform distribution of fertilizers in a seed batch, which will correspond to an even distribution among plants in the field (Scott 1998). Other advantages are related to lower operational costs, earlier availability to plants and placement near the root system (Taylor et al. 1998; Scott 1998; Farooq et al. 2012).

Copper and zinc are commonly encountered at insufficient levels in many agricultural lands worldwide, with approximately half of the soils destined for cereal production in the world being zinc-deficient (Alloway 2008). This includes the Brazilian “Cerrado” biome, an important region for maize production (Abreu et al. 2007). Moreover, around 30 and 60% of the amount extracted of copper and zinc by maize, respectively, are exported with harvested grains, which demands replacement (Bender et al. 2013).

A variety of organic and inorganic sources of copper and zinc are available and used as fertilizers. These sources generally differ in terms of water solubility, which is an important parameter that determines their uptake by plants (Amrani et al. 1999; Shaver et al. 2007; Salanenka and Taylor 2011). Prado et al. (2007) compared two sources of zinc for maize seed treatment — zinc sulphate (water-soluble) and zinc oxide (water-insoluble) — and verified that the first is able to promote a higher uptake of zinc by plants. However, the same formulation reduced seedling emergence percentage, probably due to salinity excess, while zinc oxide did not present any harmful effect (Prado et al. 2007). Luchese et al. (2004) found similar results by testing a copper sulphate formulation (water-soluble) as maize seed treatment, with →

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a good uptake of copper by plants accompanied by toxic effects to seeds.

The application of different mineral nutrients together, in a single operation, is a common practice for field crops. Copper and zinc are both bivalent cationic metals that compete for the same site of entry during the uptake by plant roots, in a process named inhibitory competition (Malavolta 2006). Thus, understanding the uptake dynamics of these elements when applied together via seed treatment is crucial for the recommendation and development of fertilizers.

This study aimed to evaluate the uptake pattern of Cu and Zn in young maize plants, applied as seed dressing through water-insoluble sources. Parameters related to seed physiological quality and seedling growth were also analyzed.

## MATERIAL AND METHODS

The experiment was conducted with a seed lot of maize hybrid 2B688Hx (Dow AgroSciences, Jardinopólis, São Paulo, Brazil), with 98% of germination, 264.2 g of a thousand-seed mass and 8.2% of seed moisture content, evaluated according to the Rules for Seed Testing (Brasil 2009).

The sources of copper and zinc were composed of liquid suspensions of copper carbonate (0.5 g $Cu{\cdot}cm^{-3}$, density: 1.66 $g{\cdot}cm^{-3}$) and zinc oxide (1 g $Zn{\cdot}cm^{-3}$; density: 2 $g{\cdot}cm^{-3}$). Treatments corresponded to untreated seeds (control), five doses of copper (0.14, 0.28, 0.56, 1.12 and 2.24 mg $Cu{\cdot}seed^{-1}$), five doses of zinc (0.55, 1.10, 2.20, 4.40 and 8.80 mg $Zn{\cdot}seed^{-1}$) and five doses of copper and zinc combined (0.14, 0.28, 0.56, 1.12 and 2.24 mg $Cu{\cdot}seed^{-1}$ and 0.55, 1.10, 2.20, 4.40 and 8.80 mg $Zn{\cdot}seed^{-1}$). Seed treatment was performed with a "pan coater" equipment, containing a Leroy-Somer rotating motor (model LS71 0.75 Kw), which allowed a uniform coverage of maize pericarp.

After treatment, seeds were submitted to a seedling emergence test, conducted with four replicates of 50 seeds per treatment, sowed in polyethylene trays (0.47 × 0.30 × 0.11 m) filled with 8 $dm^3$ of fine sand, initially moistened at 60% of the water holding capacity. The trays were maintained in greenhouse, and final counting of emerged seedlings occurred at the tenth day after sowing.

Evaluations of root and shoot dry mass and uptake of copper and zinc by plants were conducted with four replicates of eight plants per treatment; plants were cultivated in plastic trays containing 32 cells of 200 $cm^3$, filled with fine washed sand. The trays were placed in greenhouse (natural light), and the substrate was irrigated with distilled water, in order to maintain 50 to 60% of the water holding capacity; the average maximum and minimum temperatures during the period of growth were 32 and 19 °C, respectively. After 15 days, with plants presenting two fully expanded leaves with no visible collar, they were carefully removed from the sand, rinsed in distilled water, separated into roots and shoots and oven-dried at 65 °C until constant mass. Dried samples were weighed in analytical scale (0.001 g) and ground in a Willey Mill (20-mesh sieve) in order to determine Cu and Zn concentrations by flame atomic absorption spectrometry, following procedures described by Malavolta et al. (1997).

The accumulation and uptake efficiencies were calculated using values of root and shoot tissues dry mass and copper and zinc concentration in these tissues; the first is calculated as the product of concentration and tissue dry mass, in micrograms per plant (Fageria 2009), while the second is calculated as the ratio of total element accumulated in the plant, in milligrams, and root dry mass, in grams.

All tests were conducted in a complete randomized design. The data was analyzed using the JMP® statistical software (SAS Institute, version 10). The results were firstly submitted to ANOVA and F-test and, in case of significance, submitted to comparison of means (Tukey's test, $p < 0.05$) and regression analysis.

## RESULTS AND DISCUSSION

Results of seedling emergence, presented in Figure 1, indicated no difference ($p < 0.05$) among treated and untreated seeds. Values of emerged seedlings ranged from 97 to 99%, considering all doses and combinations of copper carbonate and zinc oxide applied to seeds. Similarly, results of root and shoot dry mass (Figure 1) did not differ ($p < 0.05$) among treatments.

Despite not statistically significant, root dry mass of treatments containing only Cu, at doses equal or higher than 0.28 mg $Cu{\cdot}seed^{-1}$, was approximately 20% lower than the control and the treatment containing the lowest dose of Cu (0.14 mg $Cu{\cdot}seed^{-1}$), with values ranging from 57 to 72 mg per plant. It is interesting to note that the same amount of Cu co-applied with Zn did not reduce root dry mass in →

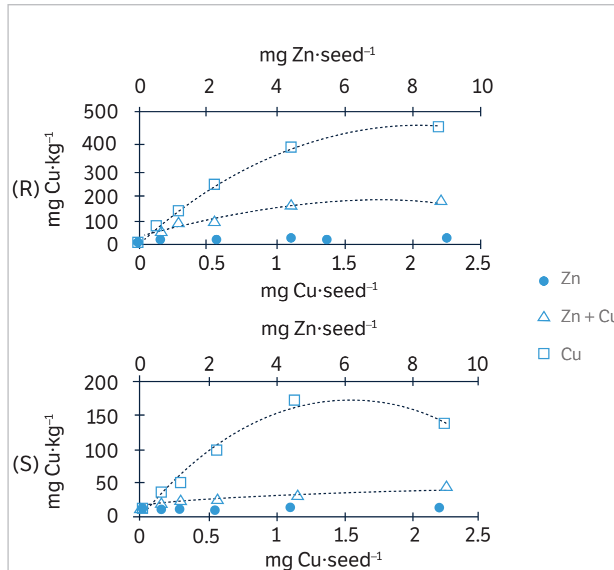
the same extent. Shoot dry mass showed to be unaffected by any of the treatments.

Cu concentration in root and shoot tissues increased as higher doses of copper carbonate were applied to seeds (Figure 2), when applied alone or mixed with zinc oxide. The Cu concentration in roots and shoots was approximately three- to four-fold higher when copper carbonate was applied alone, compared to the co-application with zinc oxide. Application of zinc oxide alone did not interfere on Cu concentration in maize tissues, both on roots and shoots.

Concentration of Zn in root and shoot tissues increased as higher doses of zinc oxide were applied to seeds (Figure 3), when applied alone or mixed with copper carbonate. In the cases where Zn and Cu were co-applied, the concentration of Zn in plant tissues were slightly lower, probably due to the inhibitory competition caused by the presence of Cu. Application of copper carbonate alone reduced zinc concentration in plant tissues.

In terms of Cu and Zn accumulation, similar patterns of concentration values were found, as presented in Figure 4. Again, individual applications resulted in higher accumulation of each element compared to the co-application, with a larger variation occurring for Cu. This is also confirmed by the results of uptake efficiency (Figure 5), with the efficiency of Cu uptake being considerably lower in the presence of Zn, while Zn uptake efficiency was reduced in a much lower extent due to the co-application with Cu.

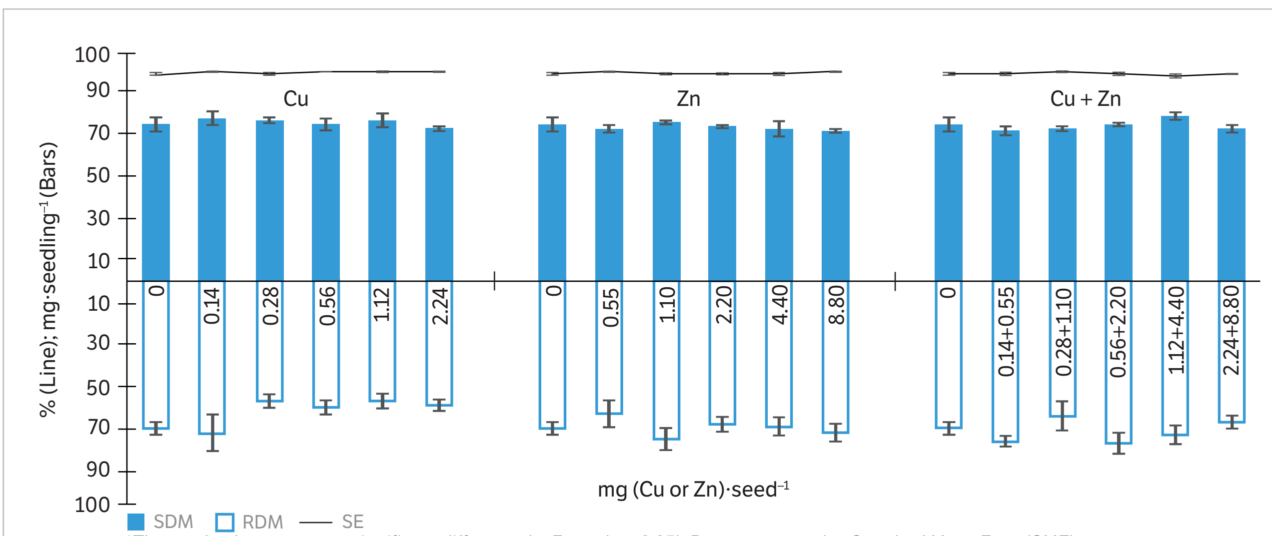
By the results obtained in this experiment, both copper carbonate and zinc oxide can be considered safe options




| | | | |
|---|---|---|---|
| R | Zn + Cu | $y = -12.86x^2 + 85.10x + 23.28$ | $R^2 = 0.93$* |
| | Cu | $y = -33.885x^2 + 245.014x + 6.746$ | $R^2 = 0.95$* |
| | Zn | $y = 12.99$ | |
| S | Zn + Cu | $y = -0.877x^2 + 10.025x + 16.424$ | $R^2 = 0,84$* |
| | Cu | $y = -22.894x^2 + 125.139x + 3.822$ | $R^2 = 0,95$* |
| | Zn | $y = 11.92$ | |

*Difference between means is significant by F-test ($p < 0.05$).

**Figure 2.** Copper concentration in root (R) and shoot (S) tissues of maize plants with two fully expanded leaves, according to each dose and combination of copper carbonate and zinc oxide applied to seeds.

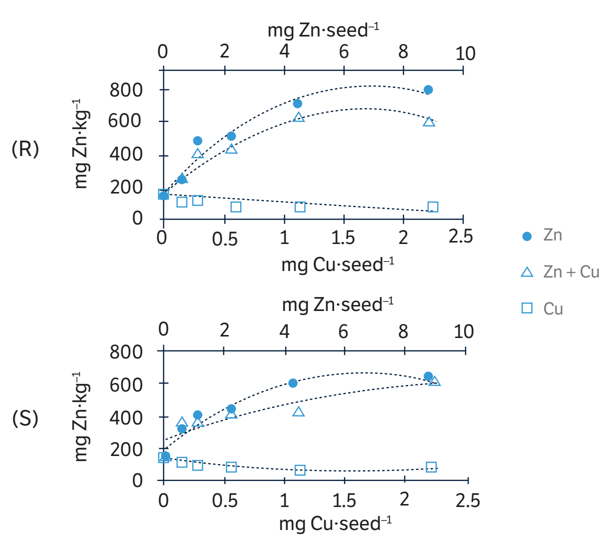



*The results do not present significant difference by F-test ($p < 0.05$). Bars correspond to Standard Mean Error (SME).

**Figure 1.** Shoot dry mass (SDM), root dry mass (RDM) and seedling emergence (SE) of maize plants with two fully expanded leaves, according to each dose and combination of copper carbonate and zinc oxide applied to seeds.

for maize seed treatment, with non-significant effect ($p < 0.05$) on seedling emergence and initial growth. However, comparing average results, plants tended to produce lower root biomass with the treatments containing only copper carbonate. These results differ from Luchese et al. (2004) and Prado et al. (2007), testing water-soluble sources based on copper and zinc sulphate salts, respectively. These authors verified that these sources reduced maize seed vigor, by negatively affecting seedling emergence and plants dry mass.

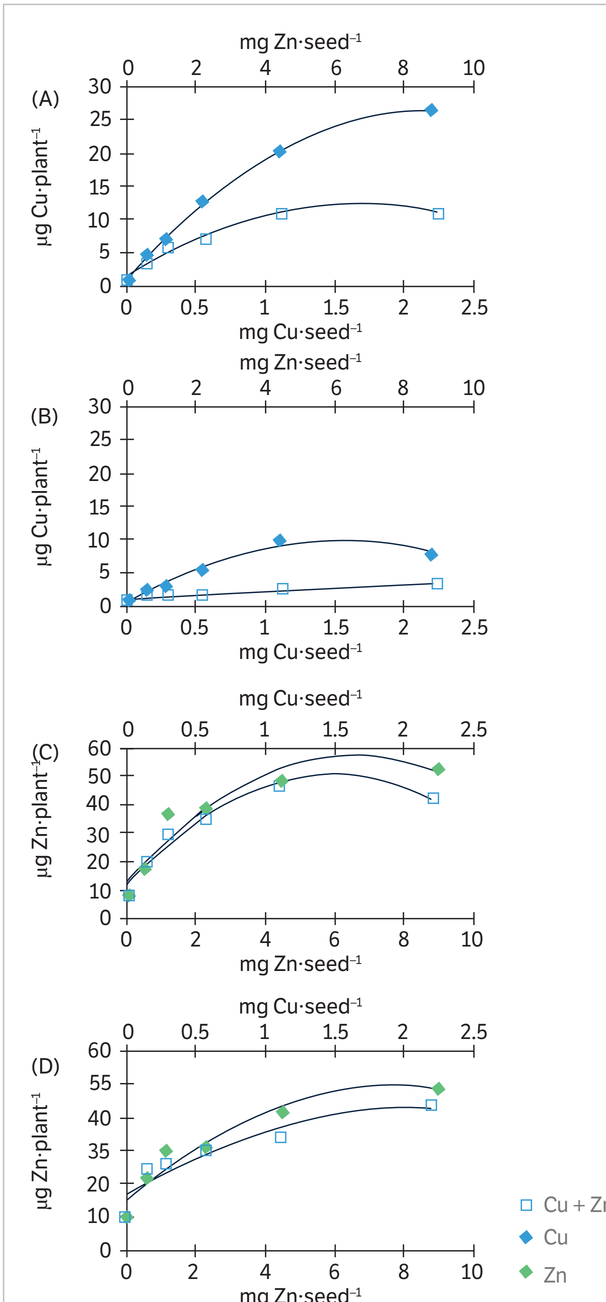
Both sources of Cu and Zn applied to seeds allowed a considerable uptake of these elements by maize. Compounds applied as seed treatment may be taken up by plants via three pathways: uptake through the seed structures, root absorption or uptake through the coleoptile (Quérou et al. 1997; Dias et al. 2014). Maize seed (botanically classified as caryopses fruit) has a selectively permeable characteristic, as verified by Dias et al. (2014), with non-ionic compounds being able to diffuse through the seed coat (pericarp), while ionic compounds are blocked.




| | | | |
|---|---|---|---|
| R | Zn + Cu | $y = -53.43x^2 + 317.28x + 192.66$ | $R^2 = 0.91$* |
| | Cu | $y = -19.82x + 140.32$ | $R^2 = 0.52$* |
| | Zn | $y = -61.04x^2 + 382.21x + 202.05$ | $R^2 = 0,90$* |
| S | Zn + Cu | $y = -16.601x^2 + 154.63x + 246.24$ | $R^2 = 0,79$* |
| | Cu | $y = 11.12x^2 - 58.26x + 133.41$ | $R^2 = 0,65$* |
| | Zn | $y = -52.11x^2 + 310.66x + 208.21$ | $R^2 = 0,91$* |

*Difference between means is significant by F-test ($p < 0.05$).

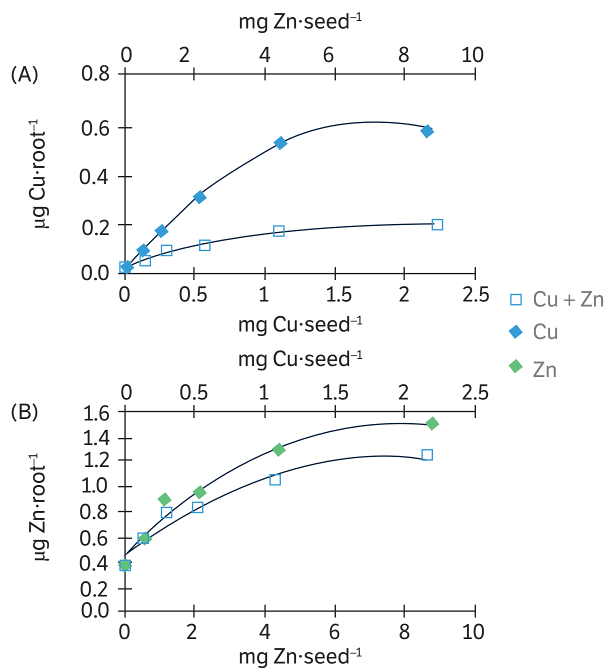
**Figure 3.** Zinc concentration in root (R) and shoot (S) tissues of maize plants with two fully expanded leaves, according to each dose and combination of copper carbonate and zinc oxide applied to seeds.




| | | | |
|---|---|---|---|
| A | Cu | $y = 0.0052x^2 + 0.023x + 0.001$ | $R^2 = 0.93$* |
| | Cu + Zn | $y = 0.0038x^2 + 0.0127x + 0.0014$ | $R^2 = 0.90$* |
| B | Cu | $y = 0.0038x^2 + 0.0119x + 0.0009$ | $R^2 = 0.93$* |
| | Cu + Zn | $y = 0.0008x + 0.0013$ | R2 = 0.82* |
| C | Zn | $y = -0.0011x^2 + 0.0139x + 0.0126$ | $R^2 = 0.96$* |
| | Cu + Zn | $y = 0.0011x^2 + 0.0129x + 0.0119$ | $R^2 = 0.90$* |
| D | Zn | $y = -0.0006x^2 + 0.009x + 0.016$ | $R^2 = 0.98$* |
| | Cu + Zn | $y = -0.004x^2 + 0.0062x + 0.0173$ | $R^2 = 0.95$* |

*Difference between means is significant by F-test ($p < 0.05$).

**Figure 4.** Copper and zinc accumulation in root (A and C) and shoot (B and D) of maize plants with two fully expanded leaves, according to each dose and combination of copper carbonate and zinc oxide applied to seeds.




| | | | |
|---|---|---|---|
| A | Cu | $y = 0.181x^2 + 0.661x + 0.0106$ | $R^2 = 0.97^*$ |
| | Cu + Zn | $y = -0.0438x^2 + 0.1699x + 0.0397$ | $R^2 = 0.93^*$ |
| B | Zn | $y = -0.0181x^2 + 0.275x + 0.462$ | $R^2 = 0.97^*$ |
| | Cu + Zn | $y = -0.0124x^2 + 0.197x + 0.465$ | $R^2 = 0.98^*$ |

*Difference between means is significant by F-test ($p < 0.05$).

**Figure 5.** Uptake efficiency of copper (A) and zinc (B) evaluated on maize plants with two fully expanded leaves, according to each dose and combination of copper carbonate and zinc oxide applied to seeds.

Modern maize cultivation practices, in general, are increasing average grain yields (Bender et al. 2013). Micronutrients such as Cu and Zn are exported from the soil in relatively great amounts by maize grains, which demands replacement. The application of these elements via seeds may be a feasible option to reduce operational costs, supply the initial phase of plants development and complement soil or foliar applications. This study demonstrates the possibility to significantly increase copper and zinc concentration in maize plants with the application of water-insoluble sources of these elements (copper carbonate and zinc oxide), without reducing seed quality.

In terms of elements partitioning, copper showed a proportionally higher accumulation in roots compared to zinc. This might consist in a plant mechanism to tolerate or avoid toxicity to excess copper. According to Broadley et al. (2012), roots are also a preferential site of copper accumulation in plants, with root growth generally being firstly inhibited than shoot growth under copper toxicity, as it was verified in this study by lower averages of root dry mass with copper-treated seeds. In the case of zinc, a similar distribution among roots and shoots was verified, even at higher doses applied to seeds.

Results of copper and zinc concentration and accumulation in plants show that zinc plays an important role in inhibiting copper uptake at excessive amounts by maize plants. Co-application of these elements seems to provide a more equilibrated uptake of both, mainly for copper. Moreover, individual applications of copper significantly reduced the uptake of zinc, which may result in zinc deficiency in certain situations.

## CONCLUSION

Seed treatment with copper carbonate and zinc oxide increased both root and shoot concentration and accumulation of Cu and Zn in maize seedlings, with two fully expanded leaves. Cu tended to accumulate in roots, while Zn was more evenly distributed among roots and shoots. The combined application of copper carbonate and zinc oxide resulted in lower uptake of both Cu and Zn by maize if compared to individual applications, with Cu uptake reduced in a higher extent.

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