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# Nanoparticle and Ionic Zn Promote Nutrient Loading of Sorghum Grain under Low NPK Fertilization

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**ABSTRACT:** This study evaluated the effects of ZnO nanoparticles (NP) or Zn salt amendment on sorghum yield, macronutrient use efficiency, and grain Zn-enrichment. Amendments were through soil and foliar pathways, under "low" and "high" levels of nitrogen, phosphorus, and potassium (NPK). In soil and foliar amendments, grain yield was significantly ($p \leq 0.05$) increased by both Zn types, albeit insignificantly with soil-applied Zn at low NPK. Across NPK levels and Zn exposure pathways, both Zn types increased N and K accumulation relative to control plants. Compared to N and K, both Zn types had a mixed effect on P accumulation, depending on NPK level and Zn exposure pathway, and permitted greater soil P retention. Both Zn types significantly ($p \leq 0.05$) increased grain Zn content, irrespective of exposure pathway. These findings suggest a nanoenabled strategy for enhancing crop productivity, grain nutritional quality, and N use efficiency based on Zn micronutrient amendments, with potential implications for improved human and environmental health.

**KEYWORDS:** *ZnO nanoparticles, ionic Zn, macronutrient use efficiency, grain Zn nutritional quality improvement, sorghum*

## ■ INTRODUCTION

The macronutrients, nitrogen (N), phosphorus (P), and potassium (K), are among the most important plant nutrients. Nitrogen, in particular, is understood to have contributed to feeding more than half of the global human population.[1] However, these nutrients are characterized by very low plant uptake efficiencies[2,3] and significant environmental losses, which, together with N and P overuse in agriculture, leads to undesirable environmental effects such as greenhouse gas production, pollution of surface waters, and eutrophication.[4] There is clear evidence that inefficient uptake of N into plants correlates with increased N application rates.[5] Consequently, increased application results in more losses[6] and, potentially, enhanced negative environmental consequences. Accordingly, strategies to enhance NPK nutrient efficiencies in fertilizers by improving plant uptake are sorely needed. Micronutrients use could be one strategy for achieving increased macronutrient uptake by plants.[7] A recent study[8] demonstrated that the amendment of NPK-fertilized soil with composite micronutrient formulations of zinc (Zn), boron (B) and copper (Cu) increased soybean grain yield and resulted in significant increases in shoot and grain accumulation of N and K. In contrast, the micronutrients significantly decreased shoot P uptake, with little effect on P translocation to the grain. Although the later study was conducted under limited water availability where nutrient uptake is generally inhibited,[9] these findings suggest contrasting scenarios in the dynamics of N/K and P accumulation in the presence of micronutrients.

Micronutrients are conventionally packaged as salts, chelates, or as bulk oxide powders. More recently, however, packaging micronutrients as nanopowder (NP) has received increased attention. Nanoscale materials (1−100 nm size in any dimension) possess heightened reactivity due to their small size and enhanced surface area.[10] Thus, when packaged as NP, micronutrients could yield significant positive plant responses in a number of physiological pathways. Strategic use of nanoscale elements as fertilizers will require sufficient understanding of judicious doses based on crop and soil needs, use of proper and sustainable nano formulations, understanding of soil effects on nanoscale nutrient amendments, and the ability to remain effective over the life cycle of the crop of interest.[11,12] Along those lines, several recent review articles have articulated the benefits of nanoscale micronutrients to crop yield enhancement and resiliency to environmental stresses, as well as the fortification of crops with nutrients to enhance edible tissue nutritional quality.[7,12−15] For example, Raliya and Tarafdar[16] and Raliya et al.[17,18] reported increases in plant growth, yield, and nutrient content upon treatment with NP ZnO. Similarly, Subbaiah et al.[19] demonstrated that NP ZnO increased growth, yield, and Zn content of maize. Still, the agronomic effectiveness of NP compared to non-nano forms of micronutrients has yet to be fully resolved. For example, application of NP vs salts of Zn, B, and Cu in a composite formulation caused largely similar responses in the vegetative and reproductive performance of soybean, as well as in the plant accumulation of N, P, K, B, and Cu. Notably, Zn accumulation did differ, with the salts yielding significantly higher shoot and grain Zn than the NP amendment.[8]

The current study evaluates the influence of soil and foliar amendments of Zn in salt and NP forms on sorghum productivity and nutrient accumulation under low and high NPK treatment regimes. Sorghum was selected because it is an important yet relatively understudied cereal crop that is grown

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widely in different parts of the world, including the United States. We hypothesized that Zn fertilization will influence sorghum NPK utilization and serve as a useful nutrient management tool for sorghum production.

## ■ MATERIALS AND METHODS

**Soil Preparation.** A sandy loam soil, pH 6.87, was used in a pot experiment conducted under greenhouse conditions at the International Fertilizer Development Center (IFDC) in Muscle Shoals, Alabama. The nutrient content analysis of the soil was previously described, with Zn being critically deficient, at 0.1 mg/kg.[8] Pots were amended with 8 kg of soil, and basal NPK at high and low rates (Table 1) were mixed into the soil. The sources of NPK were ammonium nitrate, monocalcium phosphate, and sulfate of potash, respectively.

**Table 1. Experimental treatments and description**

| treatment | description |
|---|---|
| NPK-L | NPK at low rate (100:50:75 mg/kg soil) |
| NPK-L + Zn salt | NPK at low rate + Zn salt ($ZnSO_4{\cdot}7H_2O$) at 6 mg Zn/kg soil |
| NPK-L + ZnO NP | NPK at low rate + Zn nanoparticles (ZnO) at 6 mg Zn/kg soil |
| NPK-H | NPK at high rate (200:100:150 mg/kg soil) |
| NPK-H + Zn salt | NPK at high rate + Zn salt ($ZnSO_4{\cdot}7H_2O$) at 6 mg Zn/kg soil |
| NPK-H + ZnO NP | NPK at high rate + Zn nanoparticles (ZnO) at 6 mg Zn/kg soil |

**Experimental Setup.** The treatments in this study involved exposure of sorghum (*Sorghum bicolor*; var. 251) to Zn fertilization in NP or ionic (salt) form, and delivery was by soil or foliar application at two NPK regimes. NP powder of zinc oxide (ZnO; particle size 18 nm) was purchased from US Research Nanomaterials, Inc. (Houston, TX). The Zn salt ($ZnSO_4{\cdot}7H_2O$) was purchased from J.T. Baker (New Jersey). Six treatments were established as described in Table 1; the two NPK rates without Zn application represented the control treatments. All treatments were duplicated in soil and foliar Zn applications. Treatments were randomly assigned using a block design consisting of four replicated plants for soil or foliar application.

**Plant Material and Growth Condition.** Sorghum was used in the study; planting was done using three seeds per pot. However, the number of plants per pot was thinned down to one after seedling emergence. Treatments were applied at the V-6 leaf stage, 25 days after planting (DAP). Immediately prior to application, ZnO NP was weighed out based on Zn content and then suspended in water to generate a single stock solution at a final application rate of 6 mg Zn/kg soil (Table 1). During use, the suspension was kept under slow agitation to prevent particle aggregation. The salt equivalent, $ZnSO_4{\cdot}7H_2O$, was dissolved in water and added at the same Zn rate/kg soil as the NP ZnO. For soil application, a 100 mL of ZnO NP suspension or Zn salt solution was applied directly to the soil in which the plant was growing. For the foliar application, a drop of commercial detergent (Ecolab, Great Plains, TX) was added to the nano suspension or salt solution as surfactant to reduce surface tension of the products and aid in their sticking to the leaves. The four replicates of each foliar treatment were grouped to create enough canopy for foliar application. The groups were separated from the controls and soil-applied treatments and then sprayed with a total of 400 mL (100 mL per plant). After spraying, each group was allowed to air-dry for 10 min before being returned to the experimental block. The control treatment received 100 mL of water without the addition of Zn. Subsequent soil watering and other management practices for all treatments followed standard greenhouse plant growth procedures.

**Data Collection and Analysis.** Chlorophyll levels were measured using a SPAD (Soil-Plant Analyses Development) meter (Konica Minolta) approximately midway into the growth cycle. Subsequently, at anthesis, vegetative parameters including leaf number, plant height, tiller number, and panicle number were recorded. Upon physiological maturity, plants were harvested, separated into root, straw (stem and leaves), and panicle head; all tissues were dried at ∼60 °C to determine dry-matter yield. Subsequently, grains were separated from the panicle head, weighed, and the straw and grains were ground separately and analyzed for shoot nutrient uptake or grain nutrient translocation, respectively. For nutrient analysis, samples were ground, acid-digested, and subjected to standard plant tissue analytical methods: ICP-OES for K, Zn, Mg, Fe, B, Cu, and Mn; and Skalar segmented flow analyzer for N and P. In addition, the soils were sampled, cleaned of root debris, and used for the analysis of residual N and P.

A two-way analysis of variance (ANOVA; OriginPro 2017) was used to determine significant differences in crop responses to the treatments and in block effects, combined for both Zn types (NP or salt) and NPK levels (low and high). Dependent on the parameter, ANOVA was either combined or done separately with respect to Zn delivery methods (soil or foliar). A Fisher LSD means comparison was performed to further explore the differences with significant ($p \leq 0.05$) ANOVA.

## ■ RESULTS AND DISCUSSION

In this study, we used a low dose of Zn, 6 mg/kg, to evaluate the effects of micronutrient addition to NPK fertilization on sorghum. Plants were exposed to Zn as NP or ions (salt) in soil or foliar applications. From an agro-societal perspective, Zn has gained increased attention recently due to its deficiency in many soils around the world, its proven ability to improve crop productivity in soils that are unresponsive to NPK applications, and its role in human nutrition and health, which is critical for sections of the global human population.[12,20,21] We demonstrate here that Zn application increases grain yield, modulates NPK accumulation, and increases grain Zn content in sorghum, a relatively understudied crop among the cereals. These findings are discussed in detail in the following sections. To the best of our knowledge, this is the first study describing sorghum response to NP ZnO exposure, as well as the comparative effects of Zn types on the grain yield of this important cereal crop.

**Zinc Application Stimulates Chlorophyll Production in Sorghum.** The different NPK rates (low and high) did not affect sorghum leaf chlorophyll levels, as indicated by SPAD measurement (Figure 1). Upon Zn application, there was a significant increase in SPAD counts, indicating a positive effect of amendment on chlorophyll production as previously demonstrated for this crop.[22] In the present study, the effect of Zn on chlorophyll production did not differ between NP and ionic Zn treatments, between soil and foliar Zn treatments, nor

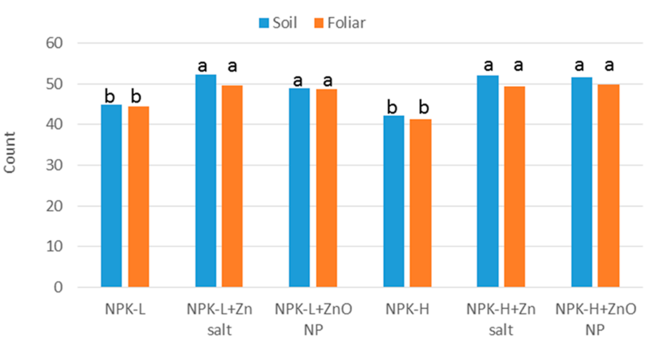


**Figure 1.** Effect of Zn fertilization on chlorophyll content of sorghum under low (L) and high (H) NPK applications. Combined for soil and foliar treatments, bars followed by different letters are significantly different at $P < 0.05$ ($n = 4$).



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under low and high NPK levels (Figure 1). Previously, an increase in chlorophyll production was shown with a Zn-containing micronutrient treatment in soybean;[8] both NP and ionic treatments in foliar application generated similar effects. In contrast, Raliya et al.[18] reported NP ZnO to be more effective in promoting chlorophyll formation than bulk (size ≥1000 nm) particles in mung bean. In the current study, the similarity in chlorophyll production data between the two forms of Zn is not surprising given likely dissolution of NP Zn in soil to the ionic form.[23−26] Mechanistically, the finding of Zn improvement of chlorophyll levels in sorghum is supported by studies in the literature linking its involvement with enzymes and proteins of the chloroplast and photosynthetic systems in other crops, including maize, rice, and mung bean.[18,27,28]

**Zn Type Differently Influences Sorghum Biomass.** There was no difference in biomass (straw, panicle, and total shoot dry weight) between the low and high NPK control treatments. However, Zn treatment differently influenced sorghum biomass; the straw, and total shoot dry biomass were increased by Zn salt, but only at the high NPK rate. In contrast, there were no significant effects of NP ZnO on biomass production (Figure 2). In the foliar treatment, the

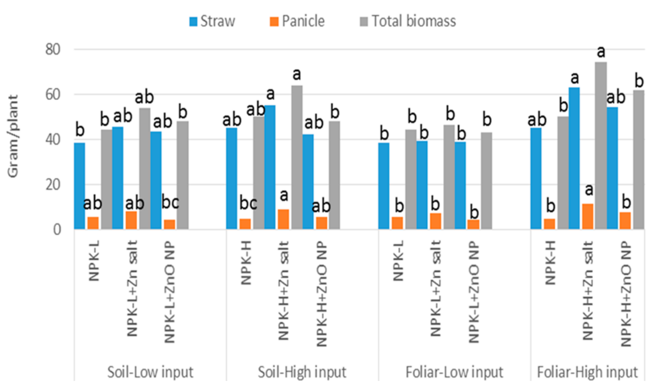


**Figure 2.** Effect of Zn fertilization on sorghum biomass production under different NPK regimes. Separately for soil and foliar treatments, bars followed by different letters are significantly different at $P < 0.05$ ($n = 4$).

findings were similar, with the Zn salt showed a stronger effect on total biomass (Figure 2). A prior study[22] reported that Zn salt application enhanced sorghum biomass with increasing K concentration; 60 kg K/ha (~30 mg K/kg soil) + 10 kg Zn/ha (~5 mg Zn/kg soil) resulted in 36−38% increase in dry matter yield. In the current study, regardless of the Zn type, 6 mg/kg Zn with 75 (low) or 150 (high) mg K/kg resulted in 9−21% and 24−49% increases in biomass, respectively. In rice, ionic Zn (~0.7−2.6 mg/kg) was more effective than particulate (bulk size) Zn for straw production.[29] In maize, Subbaiah et al.[19] showed that Zn salt was more effective in stimulating biomass production as compared to NP ZnO upon foliar exposure. However, for the comparison, this study tested excessively high amounts of Zn (2000 mg/kg soil), which is both unrealistic and unsustainable in crop production systems. In contrast, no difference was observed between Zn salt (25 mg Zn/kg soil) and NP Zn on tomato biomass.[30]

**Zinc Increases Sorghum Grain Yield.** For grain yield, there were no significant differences between the control (i.e., NPK-L vs NPK-H) treatments in soil and foliar applications (Figure 3). However, upon Zn fertilization, we observed significant increases in grain yield. Under low NPK, NP ZnO

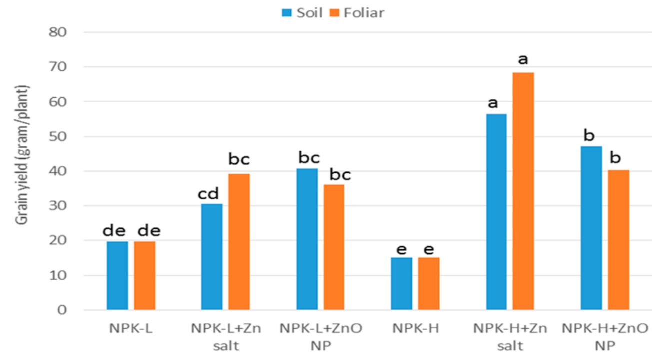


**Figure 3.** Effect of Zn fertilization on sorghum yield under low (L) and high (H) NPK applications. Combined for soil and foliar treatments, bars followed by different letters are significantly different at $P < 0.05$ ($n = 4$).

was more efficient than Zn salt at increasing sorghum grain yield (33% more grain) in the soil treatment; a similar effect was noted in the foliar application. When compared to the low NPK with Zn, grain increases were generally greater at the high NPK. Here, Zn salt increased yield more than NP Zn; 20 and 70% more grain in soil and foliar treatments, respectively. Although statistically insignificant, each Zn type seemed to have a different effect, depending on the application route: the foliar route was 21% more effective for the salt, while the soil route was 17% more effective for NP ZnO (Figure 3). Notably, few studies describing the effect of Zn on sorghum grain yield were available in the literature. In one study,[31] treatment of NPK-fertilized sorghum with a Zn salt (3 kg Zn/ha-containing formulation of secondary and micronutrients [Ca:Mg:S:Zn:B] lowered sorghum yield in two African field sites but enhanced maize yield in several other field sites; some of the increases were quite dramatic when compared to NPK alone. However, unlike the present study, no direct role for Zn can be isolated from that study due to the multinutrient nature of the treatments. In other crops, comparing crop seed/grain yield effects between NP and ionic Zn reveals that overall effects can be quite inconsistent. Prasad et al.[32] reported greater yield with NP than salt Zn in peanut (*Arachis hypogaea* var. K-134); Subbaiah et al.[19] showed Zn salt to be more effective than NP Zn for maize; and Elmer and White[33] found Zn salt to increase tomato yield components to a greater extent than NP Zn. Our recent study with a NP formulation composed in large part of Zn showed that NP and ionic Zn were similarly effective in stimulating soybean grain yield.[8] The current findings with sorghum suggest that the influence of Zn type on productivity may be dependent on NPK levels and Zn application route: yield was higher with NP ZnO when soil-applied under low NPK, while yield was higher with Zn salt under high NPK, regardless of Zn application route.

**Zinc Fertilization Promotes Nitrogen Accumulation in Sorghum.** Not surprisingly, nitrogen accumulation in sorghum was influenced by initial N dose; higher dose resulted in higher shoot N uptake, grain translocation, and ultimately, higher total N content (Figure 4). Zn treatment influenced N accumulation dynamics in different ways, and varied with application route (soil or foliar). Shoot N uptake was unaffected by soil-applied Zn at low N, but was reduced by Zn at high N, particularly for Zn salt. With foliar-applied Zn, shoot N uptake under the low N regime was reduced, albeit insignificantly, by both Zn types, but was unaffected under high N treatment. In contrast, grain translocation of N from the shoot was significantly enhanced by


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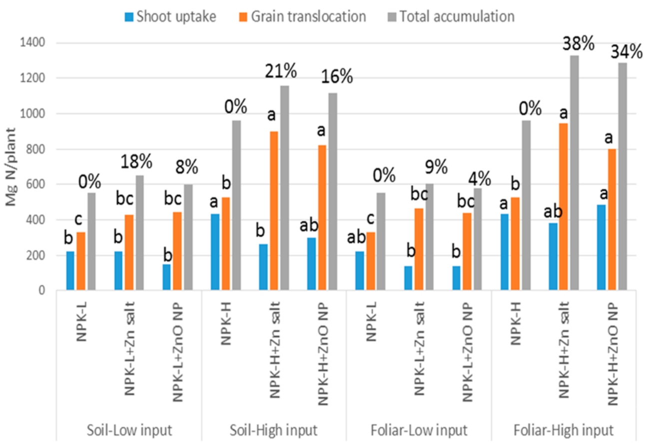


**Figure 4.** Effect of Zn fertilization on shoot uptake and grain translocation of N in sorghum under different NPK regimes. Separately for soil and foliar treatments and for shoot and grain measurements, bars followed by different letters are significantly different at $P < 0.05$ ($n = 3$). Total accumulation represents the sum of uptake and translocation under each condition, with % increase compared between NPK alone (0%) and NPK under each Zn treatment to show % increase or decrease in N levels.

Zn at both N application rates. This effect was greater at the high N rate under both soil and foliar Zn applications, but did not vary significantly according to Zn type. Ultimately, Zn treatment promoted N accumulation, resulting in between 4 and 48% more N accumulated in the plant (Figure 4). In all cases, there was a tendency for Zn salt to promote more N accumulation than nano ZnO. Notably, foliar Zn application promoted more N accumulation at the high N rate, while soil Zn application enhanced N accumulation at the low N rate. Taken together, Zn positively influencing overall N accumulation is in agreement with previous studies. For example, Sahrawat et al.[34] reported that soil-applied Zn salt (10 kg Zn/ha), in combination with boron and sulfur, increased sorghum shoot and grain N accumulation at 60 kg N/ha application. Similarly, Zn salt application at 10 kg Zn/ha enhanced N uptake by 38% when sorghum was fertilized with N at 120 kg/ha, along with various levels of K.[21] In another study, treatment of soybean with a formulation of Zn, B, and Cu, showed that Zn played a dominant role in enhancing overall accumulation of N.[8] However, the influence of treatment on shoot-grain N dynamics differs between soybean and sorghum (current study); unlike sorghum, increased shoot uptake correlated with increased grain translocation in soybean. Conversely, Puga et al.[35] found no influence of Zn application on N grain content of sorghum; specifically, a foliar application of 0.2 mg Zn/kg soil or soil applications of 1−12 mg Zn/kg soil with N at 30 kg/ha (60 mg/kg) did not increase N levels in the grain. However, it should be noted that the Zn content of the soil was 0.5 mg/kg, with a pH of 5.5. This soil Zn level, though low, may not have been suitable to induce multiple Zn responses. Moreover, the acidic pH of the studied soil could stimulate Zn bioavailability, moderating the effect of externally applied Zn. Also, in Puga et al.[35] study shoot N uptake data were not provided; these data may have offered insight into explain how Zn might have influenced N dynamics prior to grain translocation. Nevertheless, induction of N uptake by Zn may be related to a direct action of the micronutrient on N uptake, as shown in wheat,[36] with occurs with increased biomass. In the present study, overall crop productivity (biomass + grain) increase was found with Zn application, although to different degrees; this implies more need for N to meet the crop's physiological requirements. Alternatively, prior studies[37,38] have shown how Zn can be used to enhance N use efficiency by acting as nitrification inhibitors to lower rates of N transformation to nitrous oxide or nitrate, and thus, lower loss of N to the atmosphere or surface water. Intuitively, when N loss is reduced, its uptake by plant is enhanced.[4] From a broader perspective, the use of Zn to enhance N uptake demonstrates a clear role for this element in an integrated N management strategy.[7]

**Effect of Zinc on Phosphorus Accumulation Is Dependent on Zinc Delivery Route.** As with N, the uptake and translocation of P was NPK dose-dependent. With Zn fertilization, shoot uptake of P was significantly decreased by Zn salt under high and low P by both soil and foliar applications. Similarly, NP Zn strongly inhibited P uptake under all conditions except in the foliar application at high NPK, where the reduction was insignificant (Figure 5). Notably, presenting Zn by a NP foliar delivery mitigated the reduction of P uptake by Zn, similar to previous study.[8] Contrary to shoot uptake, grain P translocation was positively affected by Zn treatment. This effect was significant with NP ZnO under low NPK fertilization in the soil exposure, as well as in both soil and foliar exposures at the high P treatment. Zn salt stimulated grain P translocation to a greater degree than NP ZnO at the high NPK rate in both soil and foliar exposures. The effect of the two Zn types on P translocation was similar with the foliar treatment under low NPK (Figure 5). With regard to total P accumulation, the data show that when applied through the soil, Zn largely negatively affected P accumulation, reaching a maximum decrease of −11% with the NP ZnO. Only at the low NPK application with NP ZnO was total P accumulation not inhibited. Conversely, plant response to foliar Zn application was more complex; accumulation was negatively affected at low NPK (−4 to −8% decrease) but was increased (8−9%) at high NPK. Several prior studies have reported variable responses with respect to P accumulation in the presence of Zn. As with the current work, our previous study showed that Zn salt and

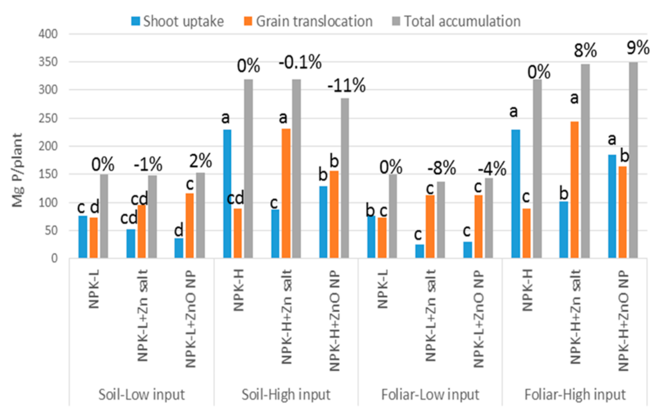


**Figure 5.** Effect of Zn fertilization on shoot uptake and grain translocation of P in sorghum under different NPK regimes. Separately for soil and foliar treatments and for shoot and grain measurements, bars followed by different letters are significantly different at $P < 0.05$ ($n = 3$). Total accumulation represents the sum of uptake and translocation under each condition, with % increase compared between NPK alone (0%) and NPK under each Zn treatment to show % increase or decrease in P levels.



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NP ZnO decreased shoot P uptake but tended to promote grain P translocation.[8] Similarly, Watts-Williams et al.[30] reported NP Zn (25 mg Zn/kg soil) lowered shoot P content in tomato when compared to Zn salt. In contrast, Zn salt (0.2–12 mg/kg soil) did not affect grain P content in maize and sorghum.[34,35] Furthermore, Moinuddin and Imas[22] reported overall stimulation of P uptake in sorghum by Zn application; however, shoot P concentration was lowered due to growth dilution from Zn-induced biomass increase. In our case, Zn application did not overall strongly enhance sorghum biomass production (Figure 2). As such, only negligible growth dilution would have occurred, especially at the lower NPK levels. Lastly, our finding with NP ZnO does not align with that of Raliya et al.[18] who found that NP ZnO stimulated P uptake. However, it is important to note that their study dealt with native P mobilization induced by Zn, rather than with amended P, where formation of zinc phosphate would likely be promoted.

After plant harvest, more residual P was present in the soil treated with Zn than without; 37.0 ± 6.9 mg/kg for salt, 36.4 ± 5.8 mg/kg for NP, and 26.6 ± 1.1 mg/kg for control, representing 37–39% more residual P in the Zn-treated soil. The pots used in the growth studies have holes drilled in their bottom to collect irrigation water leaching from them. As such, the occurrence of P "run-off" is likely. However, mechanistically, Zn will precipitate with P ions to form zinc phosphate,[39] which is highly immobile, and presumably resulted in less leaching of the macronutrient. We note that such potential mitigation of soil runoff of P by Zn was not observed for N: no difference in residual N levels were found based on Zn treatment (data not shown). At the same time, the formation of zinc phosphate in soil could limit root uptake of P, as our data and those of Watts-Williams et al.[30] indicate. The subsequent mechanism by which grain translocation of P is enhanced in sorghum upon Zn amendment is yet to be resolved. While this may well be due simply to the enhanced grain mass caused by Zn that would then serve as more sink for P, it also seems plausible that Zn may be stimulating metabolic pathways involved in grain translocation of both N and P. This topic is of great interest for further research that we are currently investigating. Understanding the mechanistic basis for Zn-induced promotion of macronutrient loading in the grain could have significant ramifications for sorghum-based human/animal Zn nutrition.

**Zinc Fertilization Promotes Potassium Accumulation in Sorghum.** Overall K accumulation in the plant increased with increasing concentration of applied NPK. The shoot uptake of K was unaffected by Zn treatment at low NPK application, regardless of the Zn exposure route. In contrast, shoot K uptake was stimulated by Zn at high NPK application. Zn salt was generally more effective than NP ZnO in promoting K uptake, regardless of exposure route (Figure 6). Grain translocation of K was positively influenced by Zn treatment under all conditions. The effect varied with Zn type, with NP Zn being more effective when soil-applied at low NPK, and Zn salt being more effective at high NPK when soil or foliar applied. Ultimately, this increase in grain K was 11–44% when Zn was soil-applied, and was 6–38% when Zn was foliar-applied. Increased K accumulation upon Zn treatment agrees with some previous reports on sorghum,[22] where Zn influenced K uptake in a K dose-dependent manner, but disagrees with other reports,[35] where Zn fertilization had no effect on K grain content in sorghum. Furthermore, we previously demonstrated that Zn-containing micronutrient formulations enhanced K

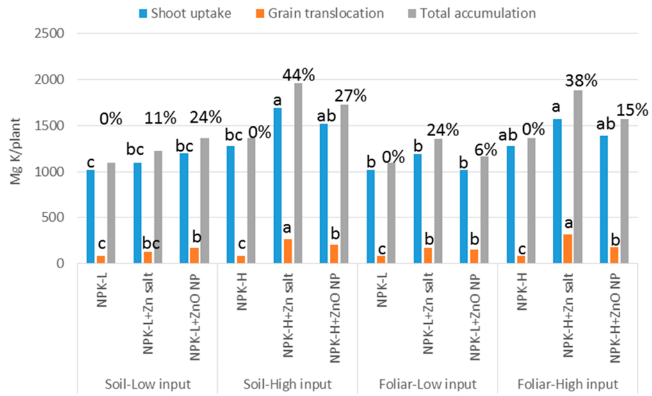


**Figure 6.** Effect of Zn fertilization on shoot uptake and grain translocation of K in sorghum under different NPK regimes. Separately for soil and foliar treatments and for shoot and grain measurements, bars followed by different letters are significantly different at $P < 0.05$ ($n = 3$). Total accumulation represents the sum of uptake and translocation under each condition, with % increase compared between NPK alone (0%) and NPK under each Zn treatment to show % increase or decrease in K levels.

uptake in soybean; specifically, soil application of the micronutrient salt was better at mobilizing K than foliar application, but the salts were not more effective than NP micronutrients in this regard.[8] In that study, grain K translocation was not significantly influenced by the Zn-containing micronutrient formulations, which is contrary to Zn treatment on sorghum in the present study.

**Zinc Fertilization Improves Zn Content of Sorghum.** There was no difference in Zn accumulation between the high and low NPK control treatments. Not surprisingly, enhanced zinc uptake was evident in plants upon Zn treatment. Zn salt showed a slightly high uptake from soil compared to NP ZnO, under both NPK levels (Figure 7). Similarly, upon foliar

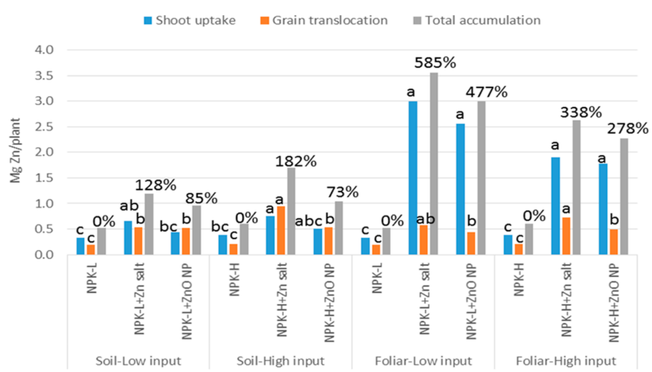


**Figure 7.** Zn shoot uptake and grain translocation in sorghum under low and high NPK regimes. Separately for soil and foliar Zn treatments and for shoot and grain measurements, bars followed by different letters are significantly different at $P < 0.05$ ($n = 3$). Total accumulation represents the sum of uptake and translocation under each condition, with % increase compared between no Zn (0%) and Zn treatments to show % increase in Zn levels.

application several-fold higher Zn was detected in the shoot of the Zn-treated plants when compared to the controls. Although shoot Zn levels between Zn salt and NP ZnO treatments were statistically equivalent under both low and high NPK, the Zn salt tended to permit more Zn in the leaves than the NP form (Figure 7). Zn translocation to grain followed shoot Zn uptake


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patterns; greater Zn was translocated into the grain when the Zn was supplied in the salt form. This trend occurred regardless of NPK rate or Zn delivery route. In fact, the difference between Zn salt and NP ZnO was significant at the high NPK rate under both soil and foliar amendments (Figure 7). Ultimately, Zn treatment resulted in 73−585% more total Zn accumulated by the plant when compared to the control treatments. Total accumulation of Zn from foliar treatment was noticeably greater than accumulation from the soil. However, the higher shoot Zn content of the foliar-treated plants did not result in greater grain translocation than plants supplied with Zn from the soil (Figure 7). Given this, some fraction of the measured Zn in the shoots of foliar-treated plants may be surface-adsorbed Zn, rather than Zn internalized into the shoot tissue. Our finding of improved Zn content upon exposure of sorghum to Zn agrees with previous studies involving the crop and Zn salt.[22,34,35] Nevertheless, the average accumulation of Zn from exposure in the present study was 18 times (shoot only) higher than previously reported in the shoot[22] and 23 times higher in the shoot + grain in another report;[34] this is in spite of comparable levels of Zn treatment, 5 mg Zn/kg soil (∼10 kg Zn/ha) vs 6 mg Zn/kg soil, that were used.

The experimental soil contained 0.1 mg/kg Zn, as measured by DTPA extraction. From this soil, the control-treated plants extracted about 0.06 mg Zn/kg soil (0.5 mg Zn/8 kg soil; Figure 7), representing 60% of the extractible Zn. In the case of soil applied Zn, of the 6 mg Zn applied to a one-plant pot, only about 1.22 mg (when averaged from all Zn application treatments, minus all control treatments) could be accounted for inside the plant. Thus, only about 20% of the soil-applied Zn was recovered in the plant. Data from Watts-Williams et al.[30] indicate that Zn in soil was more efficiently taken up (into shoot) from Zn salt than NP ZnO in one variety of tomato, but this effect was not evident for a different cultivar. Similar to that study, our report found that packaging Zn as salt was more effective in delivering Zn to sorghum than packaging as a NP. Nevertheless, viewed broadly, our data with sorghum clearly indicate an overall low use efficiency for Zn in both salt and NP forms. However, the sorghum Zn use efficiency data are greater than use efficiency for tomato, where <2% of Zn (<0.5 mg Zn per plant from a 25 mg Zn exposure to each plant) was recovered from Zn salt, and even less from the NP form.[30] These findings confirm that there is need to design better Zn delivery strategies, such as physically coating and/or combining the Zn and N fertilizers.[12,29]

In contrast to soil application, our findings for foliar application differ from those of Subbaiah et al.[19] for maize, where more Zn uptake and translocation (to the cob, but not to the grain) from NP Zn than salt Zn were observed. Importantly, the authors used a much higher Zn level (2000 mg/kg) in their study. As already noted, only a small fraction of the Zn translocated to the grain from the leaves, suggesting that a large proportion of the measured Zn of the foliar-treated plants is leaf surface-adsorbed. This indicates that leaf surface may serve as a significant sink for Zn, essentially paralleling the above-discussed soil tendency to retain Zn. However, in terms of implications for human Zn nutrition, leaf surface adsorption of Zn may not have a negative consequence for leafy vegetables that are directly consumed, although this would depend on nutrient losses during surface washing.

Our study suggests that the effectiveness of delivering Zn to grain through soil vs foliar application is unresolved, although Joy et al.[20] attributed greater efficacy to foliar Zn treatment in several grain crops. Here, we report similar grain Zn levels by soil and foliar applications of salt and NP Zn. These results are in agreement with prior findings for soybean and sorghum,[8,35] although, the study of Puga et al.[35] described using 5−60 times more Zn in soil, compared to foliar, application. However, Wang et al.[40] reported increased Zn translocation to corn and wheat grains from foliar application than from the soil Zn. Collectively, these results suggest that foliar application may not always provide an additional advantage in terms of improving grain Zn content when compared to soil application; the exception may be if it is timed strategically to coincide with early seed development.[41] However, late application of Zn may negate the benefit for grain yield enhancement.

Notably, we did not observe inhibition of Zn accumulation from Zn salt with increasing P treatment, as previously reported for sorghum.[42] However, lowered Zn was found at the higher P level with the ZnO NPs. Viewed from the perspective of soil Zn treatment (which ignores the issue of surface adsorption by the foliar pathway), it can be seen that Zn accumulation increased with P application. However, the increase was not necessarily linear; with increased P dose, Zn level in the salt increased linearly at 0 to 128−182%, but in the NP form increased nonlinearly from 0 to 85−73%. In contrast to Oseni's[42] work with Zn salt, Subbaiah et al.[19] showed in maize that Zn uptake was promoted by both Zn salt and ZnO NP in the presence of P. It is likely that differences in observed Zn−P interactions are dependent on soil chemistry variabilities that determine the promotion or otherwise of Zn phosphate formation. Previously, Lv et al.[39] showed that phosphate inhibits the dissolution of NP ZnO, while promoting transformation into insoluble zinc phosphate. Notably, significant formation of zinc phosphate occurs in the root tissues of grain cereals,[25] potentially limiting uptake of dissolved Zn ions. Thus, we suggest that while ions from Zn salt were more readily available for plant accumulation from soil, the presence of added P more negatively affected Zn dissolution from NP ZnO, which could have increased with higher P treatment, leading to reduced Zn accumulation when compared to the Zn salt.

**Zn Fertilization Differently Regulates Soil Nutrient Acquisition.** The effect of Zn application on the acquisition of unadded nutrients, namely Mg, Fe, B, Cu, and Mn, by sorghum was assessed in the above-ground tissues. The data are presented as % change from the control (no Zn) at each NPK level and for each nutrient (Table 2). The data show that, dependent on Zn type and/or Zn application route, nutrient acquisition from soil by the plant was mixed for Mg and Mn, inhibitory for Fe, and largely stimulatory for B and Cu. Notably, Zn salt was significantly more inhibitory to Fe accumulation than NP ZnO. Upon averaging each nutrient from all Zn treatments (across Zn types, application routes and NPK rates), a net positive effect on nutrient acquisition was found for Mg, Mn, Cu, and B, while a net negative accumulation was observed for Fe. In prior studies, Fe and Mn uptake by bean decreased with increasing Zn or Cu uptake (each root applied),[43,44] supporting the data in the present study for Fe in sorghum. Antagonistic reactions in plant roots between certain elements is driven by imbalances in nutrient ratios and strong competition for uptake through root-localized, shared metal ion transporters.[43,44] Interestingly, the current data indicate that antagonism toward root uptake of nutrients can happen even if the competitive nutrient (in this case Zn) is applied directly to the shoots.


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**Table 2. Effects of Zn Application on Soil Nutrient Acquisition by Sorghum under High and Low NPK Regimes**

| | accumulation of nutrient in shoot + grain (% change from NPK)[a] | | | | |
|---|---|---|---|---|---|
| treatment | Mg | Fe | Cu | Mn | Mg |
| Soil, low NPK | | | | | |
| NPK | – | – | – | | – |
| NPK + Zn salt | −2 | −23 | +22 | −2 | −4 |
| NPK + Zn nano | −4 | −1 | +19 | +17 | +5 |
| Soil, high NPK | | | | | |
| NPK | – | – | – | | – |
| NPK + Zn salt | −6 | −7 | +23 | +26 | +4 |
| NPK + Zn nano | +13 | −2 | 0 | +11 | +4 |
| Foliar, low NPK | | | | | |
| NPK | – | – | | | - |
| NPK + Zn salt | −6 | −64 | +16 | +15 | −41 |
| NPK + Zn nano | −1 | −25 | +9 | +28 | −15 |
| Foliar, high NPK | | | | | |
| NPK | – | – | – | | – |
| NPK + Zn salt | +27 | −37 | +25 | +67 | −4 |
| NPK + Zn nano | +20 | −9 | +20 | +26 | +9 |
| net accumulation[b] | +4.0 | −2.1 | +0.1 | +0.1 | +0.52 |

[a]Values for each nutrient are % change from the control of averaged total accumulation (shoot uptake and grain translocation). [b]$[\sum(\text{NPK} + \text{Zn})/x - \sum \text{NPK}/x]$, where $x$ = treatment mean.

Viewed more broadly, enhanced nutrient acquisition may immediately benefit the crop in terms of available nutrient supply for plant growth. However, this phenomena also has implications for soil nutrient stocks that may constrain future crop production, especially where fertilizer application is not a routine agronomic practice, as in many parts of Sub-Saharan Africa. Thus, irrespective of Zn type and Zn delivery route, the findings are quite striking for Mg and Fe; however, the effects were less definitive for Mn, Cu, B, where soil levels of these elements are not likely to be significantly affected Zn fertilization. For Mg, the enhanced uptake indicates its potentially significant removal from soil. In the short term, the Mg nutritional quality of the crop may be enhanced by Zn fertilization but over time, with continuous enhanced removal lowering Mg soil content, subsequent Mg fertilization may be needed. For Fe, under a continuous Zn fertilization regime, the antagonistic effect of Zn on Fe accumulation could lead to low crop Fe content. In this instance, foliar Fe application could be a strategy to remedy Fe deficiency in the plant. The effect of Zn application on Fe and Mg could be quite significant for their soil and plant nutrient inventories, and as such, additional investigation into the mechanistic bases of these molecular interactions is needed prior to implementation of strategies in the field.

A few remarks on the different Zn types for agronomic evaluations is worth making. NP ZnO was effective at increasing sorghum yield in the current study; however, farmers will clearly prefer the most affordable Zn type to achieve yield increases. The most common and affordable fertilizer Zn type is bulk ZnO powder. Moreover, owing to the fact that the main fate of particulate Zn (nano and bulk ZnO alike) is dissolution into ions, using bulk ZnO powder may be more appropriate than Zn salt for evaluating the relative effectiveness of NP ZnO. In this case, Zn salt, already in the dissolved state, will more readily become bioactive, unlike particulate Zn that would have to dissolve first, prior to any interactions with the plant. Thus, it is not surprising that Zn salt was more effective overall in this study as compared to NP Zn. In any case, including both bulk particulate and ionic Zn comparisons in future studies would allow for determining both particle-size-specific and dissolution-dependent impacts of Zn.

Taken together, this study demonstrated that Zn in both NP and salt form positively influenced sorghum productivity and grain nutritional quality under low and high NPK inputs. Our finding for the low NPK system is particularly interesting, considering that in many parts of the globe, NPK fertilizers are underused for reasons related to resource and fertilizer availability. In such cases, the addition of Zn together with low NPK rates can be a strategy to significantly increase yield, contingent upon soil testing to determine Zn needs. In high N use production systems, the enhancement of N accumulation induced by Zn has implications for N loss mitigation and downstream effects on greenhouse gas ($N_2O$) production and climate change. Under the conditions described in this work, Zn salt appears to be a more effective option than nano Zn for enhancing yield, N use, and grain Zn quality in sorghum; however, for this crop, applying the Zn to the shoot appears equivalent to soil application.

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### Notes

The authors declare no competing financial interest.

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