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# Chitosan and Zinc Oxide Nanoparticle-Enhanced Tripolyphosphate Modulates Phosphorus Leaching in Soil

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**Abstract**

Phosphorus (P) loss from agro-ecosystems impinges upon P use efficiency by plants, and thereby constitutes both agronomic and environmental nuisances. Herein, we report on the potential for controlling P leaching loss and use in fertilizing crops, through repurposing and nano-functionalizing tripolyphosphate (TPP) as a sole P source. The developed TPP-Chitosan and TPP-Chitosan-ZnO nanofertilizers exhibited positive surface charges, 5.8 mV and 13.8 mV, and hydrodynamic sizes of 430 and 301 nm, respectively. In soil, nanoformulations of TPP-Chitosan and TPP-Chitosan-ZnO significantly reduced cumulative P leaching during 72 h, reaching 91 and 97%, reductions, respectively, compared to a conventional fertilizer, mono ammonium phosphate (MAP). Cumulative P leaching at 72 hours from these nanofertilizers were, respectively, 84 and 95% lower than from TPP alone. TPP-Chitosan-ZnO was, overall, 65% more effective in reducing P leaching, compared to TPP-Chitosan. Relative to MAP, wheat plant height was significantly increased by TPP-Chitosan-ZnO, 33.0%. Compared to MAP, TPP-Chitosan and TPP-Chitosan-ZnO slightly increased wheat grain yield by 21 and 30%, respectively. Notably, TPP-Chitosan-ZnO significantly decreased shoot P levels, 35.5, 47, and 45%, compared to MAP, TPP and TPP-Chitosan, respectively. Zn release over 72 h from TPP-Chitosan-ZnO was considerably lower, compared to a control, ZnO NPs, averaging respectively, 34.7 and 0.065 mg/L, which was 534 times higher for the former. Grain Zn was significantly higher in the TPP-Chitosan treatment, relative to MAP. TPP-Chitosan also significantly mobilized the resident K, S, Mg and Ca from soil into the plant, helping to improve the overall nutritional quality, and supporting a role for chitosan in nutrient mobilization. Taken together, our data highlights the potential for repurposing a non-fertilizer P material, TPP, for agricultural and environmental applications, and the effect of applying nanotechnology on such outcomes. Broadly speaking, the reduction in P loss is critical

for controlling the eutrophication of water bodies due to nutrient overload, and for sustaining the dwindling global P resources.

## 1. Introduction

Improving resource use efficiency is critical for sustaining the agricultural intensification needed to achieve food security for the ever-growing human population. Through its role in agricultural intensification, mineral fertilizers, particularly nitrogen and phosphorus (P), continue to play significant roles in meeting the increased food demand, while saving millions of hectares of marginal land, wildlife reserves, and forests from coming under cultivation. Specifically for P, despite its critical importance in plant development and productivity, its use by crops is characterized by very low efficiency. Over 70% of applied P is unavailable to plants due to fixation in the soil, runoff from soil surface, or leaching, into surface or underground waters [1,2]. Loss of P from the terrestrial environment could impair plant growth, affecting the biomass production that supports carbon sequestration in agricultural soils [2–4].

Phosphorus relations in agro-ecosystems is complicated. On the one hand, soils may contain so-called legacy P. However, only a small soluble fraction of the legacy P pool is available for plant uptake[2,5,6]. Complex interactions of P with soil components, such as iron and aluminum hydroxides in acidic soils and calcium in alkaline soils, account for this outcome. In addition, lack of moisture and the presence of clay particles also result in the immobilization of P in soil [7,8]. On the other hand, conventional P-based fertilizers (e.g., diammonium phosphate [DAP], monoammonium phosphate [MAP], and single or triple superphosphate [SSP/TSP]) are highly water soluble, resulting, as noted above, in P run-off and leaching [9,10] and creating a critical need for developing novel P fertilizers with reduced leaching tendency.

Tripolyphosphate (TPP; $Na_5P_3O_{10}$) is an inorganic P-containing compound currently with no significant crop production applications as a fertilizer. Globally, TPP is produced on a large scale, with approximately 2 million tons produced annually; the market share for the United States was estimated at 839,000 tons as of 2020 [11]. TPP is a component of numerous domestic and industrial products, including detergents (in countries where allowed). Based on its composition of sodium, P, and oxygen, TPP is inherently nontoxic to biological systems. In fact, it is classified as GRAS, ‘‘Generally Recognized As Safe’’, by the United States Food and Drug Administration and approved as a preservative in seafood, meats, poultry, and animal feeds. However, TPP is water soluble (solubility = 14.5 g/100 mL), hydrolyzing to P ions that contribute to the eutrophication of inland and coastal waters [12]. Notably, using its phosphate moieties, TPP can also bind strongly to metal cations, a property that can modulate its solubility. A TPP molecule contains approximately 25% P (as elemental P), compared to 20% P (as elemental P; 46% as $P_2O_5$) in DAP, and 27% P (as elemental P; 61% as $P_2O_5$) in MAP. Nevertheless, despite its comparable P content, TPP, unlike other similarly water-soluble P products, is not a conventional source of P fertilizer for crop production. A reason suggested for this is that TPP must first undergo hydrolysis to P ions for there to be plant available P; thus, its ability to meet early P needs of plants may be imperiled, compared to conventional WSP fertilizer forms [13].

Recent advances in nanotechnology suggest the possibility of manipulating the dissolution of P in fertilizers to decrease loss and increase use efficiency by plants [14,15]. Indeed, specific nanoscale or nano-enabled P fertilizers have been shown to be able to modulate P release dynamics, to potentially meet crop demand and reduce losses from imprecise supply [16–18]. With this view,

we hypothesize that TPP can be repurposed as a fertilizer when functionalization as a nanofertilizer, to modulate outcomes in soil-plant systems. Methods to incorporate nanotechnology into macronutrients like P include wet chemistry procedures such as ionic gelation, solution blending with nanopolymers, encapsulation with polymers, incorporation of nanoscale micronutrients followed by precipitation[16,19,20]**.** Among the polymers, use of chitosan is emerging as a prominent strategy for developing nano-enabled agrochemicals. Chitosan is a micro-scale polysaccharide obtained from the hard outer skeleton of aquatic organisms, including crab, lobster, and shrimp. Being a biopolymer, chitosan is of interest because it promotes the concept of sustainable green chemistry. It has high degradability, with no reported environmental hazard, unlike synthetic polymers that typically leave toxic residues following use. Chitosan has, thus, been used as a carrier for delivering active ingredients in biological systems due to this and several other beneficial properties: high permeability, excellent film-forming ability, and cost effectiveness [21,22]. Notably, the development of nano-scale chitosan can be achieved by a reaction with TPP in which the formation of nanostructures occurs via crosslinking of the negatively charged phosphate moiety of TPP and the positively charged amino moiety of chitosan. This complex formation results in the precipitation of the hitherto clear chitosan solution, indicative of the presence of nanostructures of TPP-Chitosan [23–25].

Zinc (Zn) is a critical metallic micronutrient in biological systems. Zn deficiency in humans is rife in many parts of the globe, which is attributed to the consumption of staple food crops grown in Zn-deficient soils. Not surprising, plant fertilization with Zn confers multiple benefits such as accelerating plant development and enhancing productivity under various growth conditions such as drought and disease, as well as enhancing grain Zn quality for potentially improved human

dietary Zn [26–35]. Notably, Zn has an antagonistic interaction with P [36] the ionic form ($PO_4^{3-}$) in soil, fertilizer-P precipitates with Zn ions, resulting in the formation of insoluble zinc phosphate [$Zn_3(PO_4)_2$] aggregates that affect the bioavailability and uptake of both P and Zn [37]. However, on the flip side, formation of insoluble Zn phosphate with P can help to regulate the high solubility of water-soluble P-fertilizers, to reduce losses. At the product development level, the negative interaction between P and metallic elements such as Zn has stifled the quest for developing P fertilizers with metallic micronutrients. However, TPP-Chitosan nanostructures have been used to deliver metallic nutrients to plants [24,38]. Thus, it can potentially be used to deliver Zn to plants, despite the negative Zn-P interaction. This is assuming that the presence of positively charged chitosan moieties will modulate the dynamics of the interaction between P and Zn in the plant-soil system. To this end, our main objectives for this study were to leverage the cross interactions among TPP, chitosan and Zn to formulate a composite fertilizer comprised of P and Zn, and to evaluate the product for effects on P leaching loss in soil and P and Zn accumulation by the plant.

## 2. Materials and Method

### 2.1 Synthesis and characterization of TPP-Chitosan-ZnO nanofertilizer

Commercial TPP as sodium tripolyphosphate ($Na_5P_3O_{10}$) was purchased from Alfa Aeser, Massachusetts, USA; chitosan was purchased from spectrum Chemicals, New Jersey, USA; and zinc oxide (ZnO) nanoparticles (18 nm) were purchased from US Research Nanomaterials Inc., Texas, USA. The ZnO-NPs were characterized using TEM in a previous study [31]. To prepare the TPP-Chitosan-ZnO nanofertilizer, TPP was dual nano-enabled in a two-step process. First, a TPP-Chitosan complex was prepared using an ionic gelation reaction as previously described [39]. Briefly, a 0.5% chitosan solution was prepared by dissolving 0.5 g chitosan powder in 100 mL of a 0.35% (w/v) acetic acid solution and allowing to stir overnight at room temperature at ~900 rpm.

The pH of the chitosan solution was adjusted to 3 by the addition of a 5 M KOH solution. Subsequently, TPP was dissolved in deionized water at a concentration of 0.08% (w/v) and equal volumes of the chitosan and TPP solutions were mixed under stirring (900 rpm) for 60 min at room temperature. During stirring, ZnO NPs were added to the TPP-Chitosan solution which generated a TPP-Chitosan-ZnO dual nano-enabled P-fertilizer. Our previous studies show Zn rates of 1-2% of ZnO powder to be optimal for improving plant productivity under soil exposure [30,31,40]. Accordingly, we selectively dosed an aliquot of the TPP-Chitosan nano-suspension with ZnO NPs at 2% Zn by weight of TPP. The TPP-Chitosan without and with ZnO nano-suspensions were centrifuged, and the supernatants collected using a filter paper. The semisolid portions were lyophilized to obtain a solid powder. Prior to drying, aliquots of the suspensions were characterized using standard analytical methods. Surface charge was determined using a zeta sizer (Zetasizer nano ZS90; Malvern Panalytical, UK); for hydrodynamic size by dynamic light scattering; and for shape and size imaging using TEM. The P and Zn concentrations of the suspensions were determined by Inductively Coupled Plasma- optical emission spectroscopy ICP-OES (Thermo Fisher iCAP 6500; Thermo Fisher Scientific, Waltham, MA). Fourier transform infrared (FTIR) spectra of prepared TPP-Chitosan nano-suspension samples were obtained using a FTIR spectrometer (INVENIO S, Bruker, Germany). The spectra were recorded in the wavelength region of 400 - 4000 $cm^{-1}$, with an average of 32 scans at a resolution of 4 $cm^{-1}$.

**2.2 Leaching of P in soil from TPP-Chitosan-ZnO Nanofertilizer**

The leaching of P in soil from the nanofertilizers was studied by incubating known amounts of the dry powders in soil known to be insufficient of readily bioavailable P and Zn (respectively, 347 and 21 mg/kg total P and Zn, based on nitric acid digestion; 18 and <2 mg/kg bioavailable P and Zn, based on water extraction; pH 6.07) and sampling periodically (up to 72 hours post incubation).

To this end, pots with 0.35 kg of dry soil were treated with 100 mg/kg P from the following five P sources: mono ammonium phosphate (MAP) as a conventional P-fertilizer, rock phosphate (RP), TPP, TPP-Chitosan, and TPP- ZnO-NP (2% Zn). The products were thoroughly and uniformly mixed into the potted soil and pots were then irrigated with 50 mL DI water to produce leachates for collection using plastic weigh boats (Fisher Scientific, Inc., Waltham, MA). Irrigation with this volume of water was conducted only during the leachate collection times of 1, 24, 48, and 72 hours after soil treatment with the P. The leachates were centrifuged at 5000 rpm for 15 min and filtered with a filter kit (Puradisc$^{tm}$ 25NYL, Whatman, Maidstone, United Kingdom), before being analyzed by ICP-OES for their P content.

The release of Zn from the TPP-Chitosan-ZnO nanofertilizer was studied in an aqueous medium over a 72-h period by adding 0.84 g of the product in 100 mL DI water, together with an equal amount of TPP-Chitosan, and a control treatment of the commercial ZnO NPs (25 mg ZnO/ L) used in the formulation. Samples were collected after 1 h and each subsequent 24 h, centrifuged (3900 RPM) for 20 min, and then analyzed for their Zn content using ICP-OES.

### 2.3 Evaluation of TPP-Chitosan-ZnO nanofertilizer on wheat

Evaluation of the nanofertilizer on plants was conducted using wheat, under greenhouse conditions. To this end, seeds of wheat (Hard Red Spring Wheat) were pre-germinated in a container filled with Pro-Mix BX soil (Premier Horticulture Inc., PA) for 2 weeks until the seedling stage. Uniformly germinated seedlings were selected and transplanted, one per pot, into 450 mL plastic pots containing 350 g of soil. The soil, pH 6.07, is considered insufficient of readily bioavailable P and Zn, respectively, 347 and 21 mg/kg total P and Zn, based on nitric acid digestion; and 18 and <2 mg/kg of bioavailable P and Zn, based on water extraction. Prior to loading in the pot, the

soil was treated with the different P-fertilizer products at 50 mg P/kg soil. The treatment consisted of manually homogenizing the P products in the soil. The P-fertilizer treatments included a conventional fertilizer control (MAP), TPP, TPP-Chitosan, and TPP-Chitosan-ZnO NP (2% Zn). Chitosan contains 20 g N/kg as determined in this study by a Nitrogen Analyzer (LECO FP628 Nitrogen Determinator). On that basis, N in all treatments was normalized to 100 mg/kg using urea to augment. Five replicates per treatment were established. The seedlings were allowed to grow under greenhouse conditions and were regularly watered with 50 mL DI water until the plants fully matured, 49 days post-transplanting. The plants were then harvested as in situ dry plant tissues, and grain biomass, shoot biomass, and plant end-of-life height were recorded for each plant. The grain and shoot dry biomass were measured with a portable balance (Mettler Toledo, Switzerland). The plant samples (separately, grain, shoot, and root) and soil samples were processed and analyzed for their P and Zn contents using ICP-OES, according to reported methods [41].

**2.4 Plant tissue elemental analysis using ICP-OES**

Samples of plant tissues were prepared for total P and Zn elemental analysis. To this end, the samples were dried in an oven at 70°C for 24 h and then ground to powder in a commercial coffee grinder. Subsequently, approximately 0.20 g of the ground samples were added into a digestion tube and digested in a digiPrep hot block (SCP Science, Quebec, Canada) at 115 °C for 45 min using a 5mL plasma pure $HNO_3$. Samples were then diluted to 50 mL with DI water after cooling to room temperature. Samples were analyzed for their P and Zn contents using ICP-OES. The standard reference material 1573a from the National Institute of Standards and Technology (NIST) was used to evaluate the accuracy of the digestion protocol for the plant samples.

**2.5 Statistical analysis**

All data were analyzed using SPSS 26 (SPSS 26, Chicago, IL). One-way ANOVA followed by Tukey test was used to compare the mean values from control (conventional fertilizer) and the

other treatments. The results were expressed as mean ± standard error (SE), and statistically significant differences were observed when $p \leqslant 0.05$.

## 3. Results

### 3.1 Characterization of TPP-Chitosan-ZnO nanofertilizer products

The hydrodynamic size and zeta-potential for TPP-Chitosan and TPP-Chitosan-ZnO were determined and are shown in Table 1. The TPP-Chitosan and TPP-Chitosan-ZnO exhibited a zeta-potential at +5.8 mV and +13.8 mV, and an average hydrodynamic size of 430 and 301 nm, respectively. These results indicate that the formulations possess low zeta-potential overall, and, thus, lower stability in suspension, but that TPP-Chitosan-ZnO NPs with the higher zeta-potential would be more stable than TPP-Chitosan.

Table 1. Hydrodynamic size and zeta-potential for applied materials.

| Material | Hydrodynamic Size (nm) | Zeta-Potential (mV) |
|---|---|---|
| TPP-Chitosan | 430 ± 24 | 5.8 ± 2.5 |
| TPP-Chitosan-ZnO | 301 ± 15 | 13.8 ± 1.3 |

The dry powders and TEM images of the TPP-Chitosan and TPP-Chitosan-ZnO NPs are shown in Figure 1. Both materials exhibited similar particle size and amorphous morphology, although the TPP-Chitosan-ZnO NPs were more clustered in morphology. The “branch structure” of both NPs had an approximate particle size of 20 nm, although large submicron structures are present, indicating material aggregation.

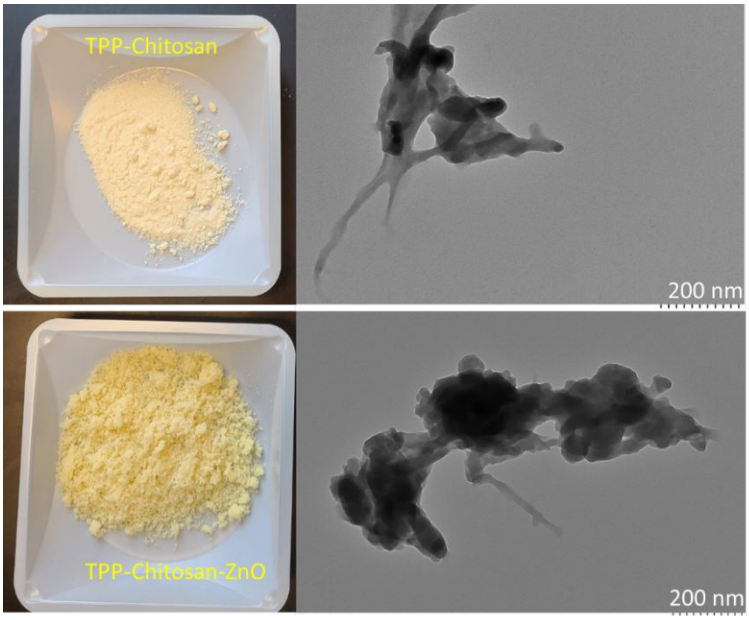


Figure 1. Photos of dry powders and TEM images of TPP-Chitosan and TPP- Chitosan-ZnO NPs.

The FTIR spectra of the TPP-Chitosan and TPP-Chitosan-ZnO NPs are shown in Figure 2. A typical band at 3,362 $cm^{-1}$ can be attributed to the -OH stretching vibration, which overlaps the stretching band of -NH, making it a broad band, as previously observed [42,43]. The absorption bands centered at around 1980, 1640, 1550 and 1375 $cm^{-1}$ and present for both TPP-Chitosan and TPP-Chitosan-ZnO could correspond to symmetric and asymmetric C=O stretching, respectively [42,44]. Besides, the peak at about 1050 $cm^{-1}$ indicates that the phosphate moiety of TPP is connected to the amine group of chitosan based on electrostatic interaction, which agrees with previous report [45]. After incorporation of ZnO NPs in TPP-Chitosan NPs, the peak at 1050 $cm^{-1}$ slightly shifted, suggestion a likely interaction of dissolved Zn ion with an -OH group in the TPP-Chitosan. Furthermore, two new absorption peaks at around 465 and 450 cm-1 were noticed, which correspond to the stretching mode of ZnO, and align with observations from previous studies [46,47]. However, the low content of Zn (2%) did not allow for better examination of these features.

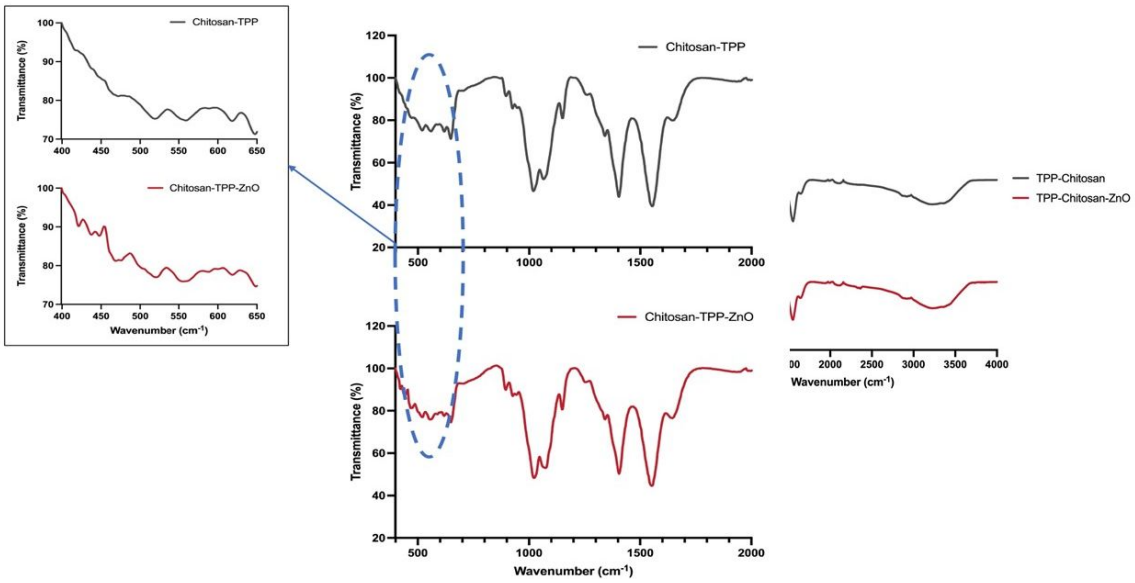


Figure 1. FTIR spectra of TPP-Chitosan and TPP- Chitosan-ZnO NPs (bottom panel). Arrowed left in the bottom panel is a projection of the spectra in the ZnO wavelength region.

### 3.2 Evaluation of TPP-Chitosan-ZnO nanofertilizer for P leaching loss in soil

The concentrations of P leached from the different treatments over 72 h is shown in Figure 3. Compared time wise (Figure 3a), leaching of P from RP was overall very low. The cumulative value increased significantly from about 0.007mg after 1 h to 0.04 mg after 72 h. Such overall low leaching from RP was not unexpected, given its widely reported insolubility [48]. For the conventional fertilizer (MAP), P leaching over time was also significant, increasing cumulatively from 0.6 mg after 1 h of incubation to 2.8 mg after 72 h. P leaching from the as-purchased TPP was high and significant as a function of time. The cumulative increase rose to 1.6 mg after 72 h, from a starting level of 1.2 mg. Leaching of P from TPP-Chitosan was significant over time, from a low of 0.16 mg after 1 h to a cumulative high of 0.26 after 72 h. For TPP-Chitosan-ZnO, overall P leaching was very low; level was from about 0.04 mg P after 1 h to about 0.09 mg P after 72 h. However, the increase was significant only after 48 hours.

When compared treatment or product wise, significant differences can be observed among the treatments at each time point (Figure 3b). As shown, after 1 h, P leaching levels from the as purchased TPP, conventional fertilizer (MAP), and TPP-Chitosan were, in that order, significantly different. Notably, leachates from these treatments were significantly higher than those from TPP-Chitosan-ZnO and RP, both of which were statistically similar. After 24 h, P leaching was significantly highest for MAP, followed by TPP, and by TPP-Chitosan, compared to TPP-Chitosan-ZnO and RP. Leaching after 48 hours was significantly highest for MAP, followed by TPP, while other treatments were similarly leached of P at a strongly lower rate. A similar trend was maintained after 72 h, with some notable differences that P leaching was more strongly suppressed with TPP than with MAP, and that leaching from TPP-Chitosan and TPP-Chitosan-ZnO increased at different rates, compared to RP. Taken together, these results indicate that the presence of chitosan, regardless of Zn status, very strongly suppressed P leaching, compared to both the conventional fertilizer (MAP), and TPP. Notably, TPP-Chitosan-ZnO suppressed P leaching to an overall greater extent compared to TPP-Chitosan.

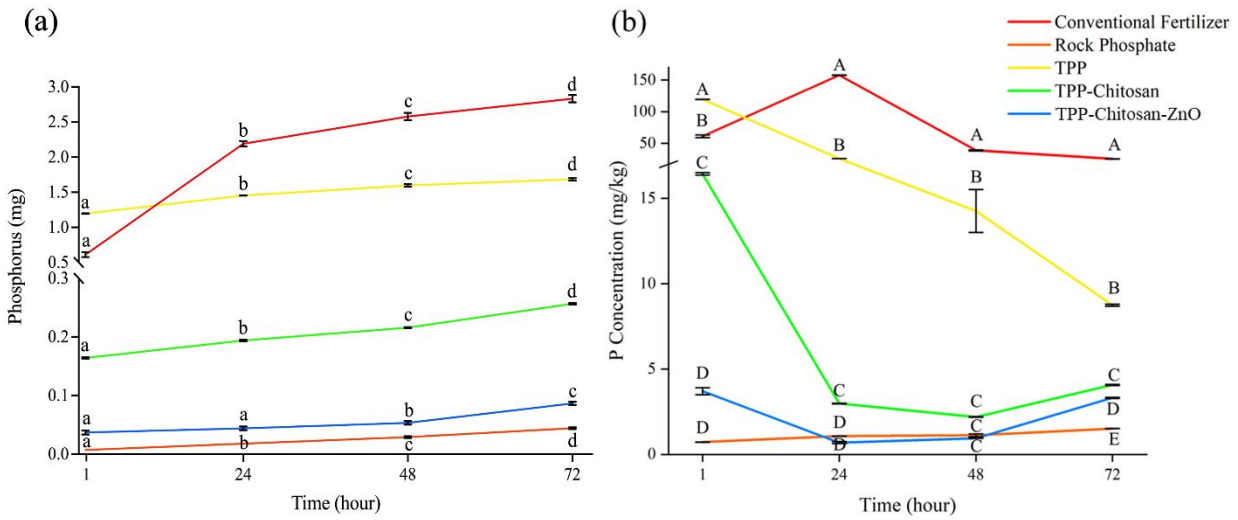


Figure 3. Phosphorus concentrations in leachates collected from treatments of conventional fertilizer (MAP), Rock phosphate, TPP, TPP-Chitosan, and TPP-Chitosan-ZnO for 72 hours. Values are means, and error bars correspond to the SEM (n=3). Values shown by different letters are significantly different at $p \leq 0.05$ (one-way ANOVA with a Tukey post hoc test). (a) Cumulative leaching measured for each individual product as a function of time; (b) Comparison among the different treatments at the different time points.

Investigation of Zn release from the TPP-Chitosan-ZnO over 72 h shows low a release level of 0.04 mg Zn/L after 1 h of incubation in water that increased by 60, 87, and 105% during 44, 48, and 72 h, respectively (Table 2). In comparison, the level of Zn released from TPP-Chitosan after 1 h was 0.002 mg/L, which was 181% lower than the value for TPP-Chitosan-ZnO within the same period. The soluble Zn levels then increased by 100% after 24 h, and by 150% apiece after 48 and 72 h. Presumably, the Zn in this product is residual and inherently from the chitosan, which is a biogenic material not unexpected to contain nutrients such as Zn. Contrary to the TPP-Chitosan-ZnO and TPP-Chitosan formulations, Zn release from commercial ZnO NPs that was added as a control was significantly high, starting at 11 mg/L after 1 h incubation, and increasing by 77, 266, and 510% during 24, 48 and 72 h, respectively.

Table 2: release of soluble Zn from TPP-Chitosan-ZnO, compared to TPP-Chitosan and ZnO NPs. Values are mean and SE (n=3). Values shown by different letters are significantly different among the materials at the same time point ($p < 0.01$, one-way ANOVA with a Tukey post hoc test).

| **Material** | **Zn Release dynamics (mg/l/hr)** | | | |
|---|---|---|---|---|
| | 1 | 24 | 48 | 72 |
| TPP-Chitosan-ZnO | 0.04 ± 0.006b | 0.064 ± 0.005b | 0.075 ± 0.006b | 0.082 ± 0.005b |
| TPP-Chitosan | 0.002 ± 0.0007b | 0.004 ± 0.002b | 0.005 ± 0.002b | 0.005 ± 0.002b |
| ZnO | 11.1 ± 0.96a | 19.6 ± 0.45a | 40.6 ± 8.85a | 67.7 ± 1.19a |

### 3.3 Agronomic performance of TPP-chitosan-ZnO nanofertilizer

The wheat plant height, shoot fresh weight, and grain yield were recorded as agronomic parameters. As shown in Figure 4, plant height was significantly increased by TPP-Chitosan-ZnO exposure by 33.0%, compared with the conventional fertilizer, MAP. The heights of plants treated with TPP, and TPP-Chitosan were also higher, albeit insignificantly, than the conventional control by 18.8% and 17.1%, respectively. The plant shoot weight was not affected by any of the treatments; however, TPP-Chitosan and TPP-Chitosan-ZnO increased grain weight by 21.1% and 30.1%, respectively, compared with the conventional fertilizer. These values were not statistically significant due to the high variability observed.

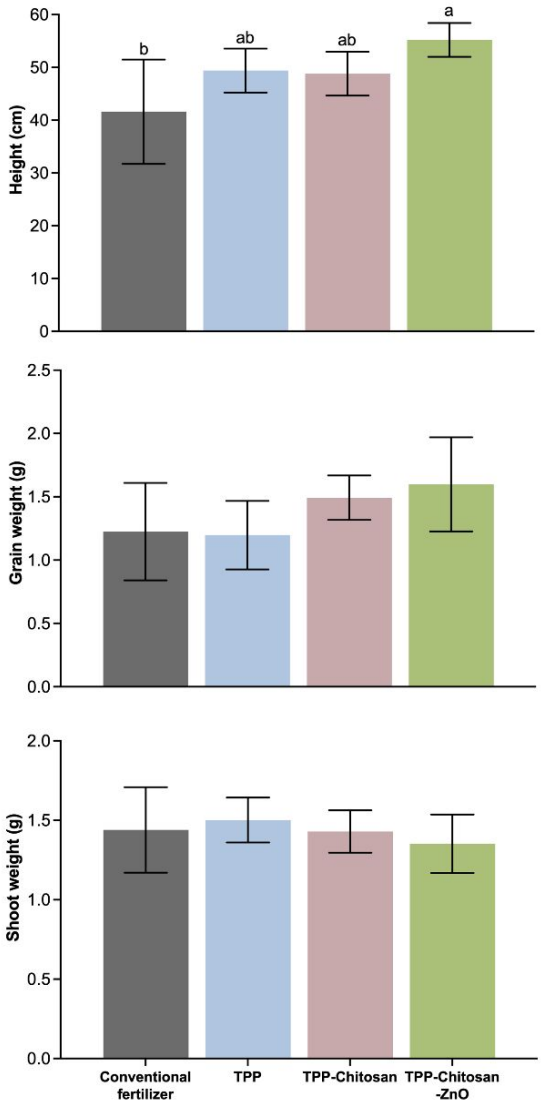


Figure 4. Vegetative and reproductive performance of wheat upon soil application of conventional fertilizer (MAP), TPP, TPP-Chitosan, and TPP-Chitosan-ZnO. Figure represents (a) plant height and (b) shoot weight, and (c) grain dry yield. Values are means, and error bars correspond to the SEM (n=5). Values shown by different letters are significantly different at $p \leq 0.05$ (one-way ANOVA with a Tukey post hoc test).

### 3.4 Uptake of P and Zn into wheat plant

As shown in Figure 5 (a), root P contents were higher in all the TPP-based treatments, albeit statistically insignificant, compared to the conventional fertilizer (MAP) treatment. Notably, TPP-Chitosan-ZnO significantly decreased shoot P levels, compared to other treatments; these levels

were decreased by 35.5%, 47, and 45%, compared to the conventional fertilizer, TPP and TPP-Chitosan, respectively. As with the root, grain P levels were insignificantly higher in all the TPP-based treatments, compared to the conventional fertilizer treatment, MAP. For Zn, no significant differences were observed for root and shoot tissue contents among the treatments, although levels tended to be higher with the TPP and TTP-Chitosan treatments. However, grain Zn was significantly higher in the TPP-Chitosan treatment, relative to MAP, with the TPP and TPP-Chitosan-ZnO treatments exhibiting median values.

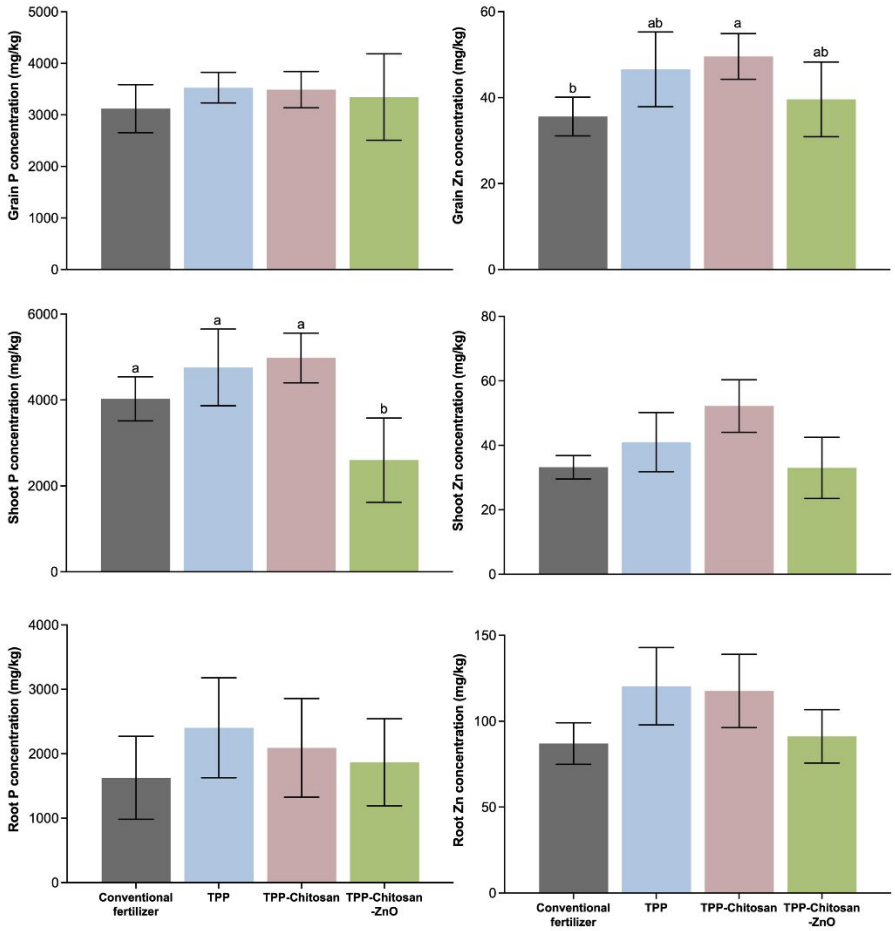


Figure 5. Phosphorus (left) and zinc (right) contents of wheat root, shoot, and grain following soil exposure to conventional fertilizer (MAP), TPP, TPP-Chitosan, and TPP-Chitosan-ZnO. Values are means, and error bars correspond to the SEM (n=5). Values shown by different letters are significantly different at $p \leq 0.05$ (one-way ANOVA with a Tukey post hoc test).

### 3.5 Effect of TPP-Chitosan-ZnO on native nutrients accumulation in plants

The levels of potassium (K), sulfur (S), magnesium (Mg) and calcium (Ca) in the above-ground plant tissues (shoot and grain) was used to assess the effect of the nanofertilizers on key native nutrients. Table 3 shows that wheat plants fertilized with TPP-Chitosan-ZnO contained greater K in the shoot, compared to TPP, and TPP-Chitosan by 20.7% and 25.4%, respectively. This coincided with a significant reduction in the postharvest soil K content by TPP-Chitosan-ZnO, 65.8%, compared with the conventional fertilizer, and by 54.1% and 57.3% compared with TPP and TPP-Chitosan, respectively. On the other hand, TPP-Chitosan significantly increased shoot S content by 40.1%, 33.4%, and 81.0%, compared with the conventional fertilizer, TPP, and TPP-Chitosan-ZnO, respectively. Similarly, plant exposed to TPP-Chitosan had significantly (67.9%) greater grain S, compared with the conventional fertilizer. Compared with TPP-Chitosan-ZnO, conventional fertilizer, and TPP, the TPP-Chitosan treatment also significantly increased shoot Mg accumulation by 39.3%, 36.2%, and 39.6%; and shoot Ca accumulation by 39.4%, 36.4%, and 38.0%, respectively.

Table 3. Contents of selected nutrient elements in wheat plant-soil system under TPP-Chitosan and TPP-Chitosan ZnO fertilization. Only changes with statistically significant changes are highlighted ($p \leq 0.05$).

| Plant tissue | Element | Treatment | Mean (mg/kg) | Standard Error | Significance |
|---|---|---|---|---|---|
| Shoot | K | TPP-Chitosan-ZnO | 30069.61 | 2439.3 | a |
| | | TPP | 37920.56 | 1416.5 | b |
| | | TPP-Chitosan | 40310.63 | 2381.4 | b |
| | S | TPP-Chitosan-ZnO | 2014.93 | 157.5 | a |
| | | Conventional fertilizer | 2604.27 | 131.6 | a |
| | | TPP | 2733.72 | 216.1 | a |
| | | TPP-Chitosan | 3647.38 | 299.8 | b |
| | Mg | TPP-Chitosan-ZnO | 1337.14 | 110.3 | a |
| | | Conventional fertilizer | 1363.61 | 91.8 | a |
| | | TPP | 1334.34 | 65.4 | a |

| | | | | | |
|---|---|---|---|---|---|
| | Ca | TPP-Chitosan | 1861.86 | 132.8 | b |
| | | TPP-Chitosan-ZnO | 3042.63 | 258 | a |
| | | Conventional fertilizer | 3110.82 | 129.3 | a |
| | | TPP | 3074.1 | 172.1 | a |
| | | TPP-Chitosan | 4241.77 | 359 | b |
| Grain | S | Conventional fertilizer | 1149.93 | 110.3 | a |
| | | TPP-Chitosan | 1931.63 | 267.9 | b |
| Soil | K | TPP-Chitosan-ZnO | 983.72 | 136.5 | a |
| | | Conventional fertilizer | 2876.33 | 255.7 | b |
| | | TPP | 2143.65 | 106.7 | b |
| | | TPP-Chitosan | 2303.75 | 190.2 | b |

## 4. Discussion

Phosphorus is subject to significant leaching loss, which is a topic of global concern considering the enormous ramifications for surface and underground water body pollution by. P loss due to surface run-off or leaching creates a quagmire for P utilization by plants, lowering its use efficiency. Notably, conventional P-fertilizers originate from natural reserves of RP that are not infinite [49,50]. However, as suggested in the leaching experiment reported in this study, use of RP for agricultural purposes would be limited by its poor insolubility. Hence, alternative P sources are direly needed for both supporting agriculture sustainably and reducing P leaching losses. We reasoned that such alternatives can be achieved from recycling processes and repurposing of other non-agricultural P-containing materials, for crop fertilization, to sustain the availability of natural P resources [2,5,13]. To this end, we herewith describe significant reductions in P leaching loss in soil based on P from a non-fertilizer P source, TPP, when formulated with chitosan with and without augmentation with Zn from ZnO nanoparticles.

A previous review discussed the potential rate limiting effect of tripolyphosphates (TPP) as a fertilizer, as it must first undergo hydrolysis to P ions for plant uptake [13]. This argument could be made in the backdrop that TPP and other linear polyphosphates can adsorb directly to metal

oxide surfaces without even hydrolyzing, which if the case, reduces TPP mobility, increases the formation of calcium phosphate [51], and ultimately affects TPP as a source of P in fostering early plant development. Nevertheless, Hamilton et al. [52] reported rapid dissolution of TPP within 48 h of incubation in a calcareous soil (pH 6.5), concomitant with rapid adsorption of the released P ions onto soil complexes [52]. The present study also showed rapid dissolution and decline in P levels in a chemically comparable soil as Hamilton et al. [52], in terms of the pH range, which would agree with a potentially similar fate for the P from TPP in our study. Notably, formulating TPP with chitosan not only initially lowered the initial dissolution drastically, but it also provided a more sustained release rate over the incubation period. The leaching reduction would have been achieved due to the strong intercalation of P with chitosan. This indicates that formulating TPP with chitosan could be a strategy to reduce early P loss and maintain P levels over a longer period, compared to water soluble P products, such as MAP and TPP.

Several studies have used chitosan for generating nanoscale material based on intercalation with TPP [20]. However, to our knowledge none of these studies investigated the utilization of the system for understanding P leaching from TPP-Chitosan formulations. One study involved additional P inclusion from NPKS fertilization and assessing P release from the TPP-Chitosan-NPKS in water (not soil) without a control of TPP-Chitosan alone [21]. They reported continuous release of P into the aqueous phase over a one-week period, which was not unexpected given the lack of complexity in the medium. Yet, the addition of extra P from NPKS would have complicated the evaluation of TPP as a sole P source when intercalated with chitosan. In that context, the present study provides novel insights on P leaching characteristics in soil from TPP as a function of chitosan intercalation.

Although several previous studies have examined the effects of TPP-Chitosan formulated with metal-based nanomaterials, such as copper (Cu), Zn and even silicon, in controlling the release profile of bioactive compounds [53–57], the release profile of P from such nano-enabled agrochemicals has not been investigated, to our knowledge. In this work, we observed further significant drop in P leaching when Zn was included (i.e., TPP-Chitosan ZnO NP treatment). Thus, the intercalation of TPP with chitosan appears to be further strengthened by Zn, to affect P release. This is an important finding showing that the slow-release properties of the synthesized nanofertilizer can have serious ramifications for lowering P environmental footprint. The nature of the TPP-Chitosan-ZnO NP was assessed by characterizing the product with a suite of analytical methods, alongside the TPP-Chitosan product. The high resolution of TEM makes it an excellent tool for identifying internal structures and particle size, as well as determining structure and encapsulation of metals in biopolymers such as chitosan [58]. The TEM results observed in this study indicated the "branch structure" of formed TPP-Chitosan and TPP-Chitosan-ZnO NPs, which are comparable in morphology to chitosan-TPP-Cu structures previously reported [22]. The aggregation, hydrodynamic sizes, and positive charges of the TPP-Chitosan vs TPP-Chitosan-ZnO indicate a greater structural stability (less potential to aggregate) induced by Zn and agrees with previous observations for similar chitosan-based nanofertilizers [59,60]. Addition of RP in the leaching study allowed to juxtapose the implication of the TPP-Chitosan-ZnO treatment. Based on the release kinetics results, as compared with the conventional fertilizer and TPP, TPP-Chitosan-ZnO exhibited strong P preserving propensity that more closely resembled that of RP. Earlier during incubation, the product leached relatively higher amount of P than RP, which could be due to the release of P from the surface of the formed NPs. However, P levels became indistinguishable

between these two treatments up to 48 hours of incubation. This strong inhibition of P release from TPP-Chitosan-ZnO could be explained by the fact that the P released after 24-hour was from the core of the nanofertilizer were the Zn also likely interacted. According to a previous study, the swelling properties of chitosan could lead to a time-dependent structural deformation of the nanostructure, resulting in the release of nutrients [58]. This could explain the uptick in P levels observed after 72 hours in the chitosan treatments, with or without Zn. Notably, metal oxides such as manganese, Cu and Zn have been used to curtail the mobility of P and vice versa, in soil or under laboratory conditions, which is a practical application stemming from the well-known phenomenon of P-metal complex formation that results in P immobilization [61,62], in this case zinc-phosphate. In this regard, the enhanced immobilization of P in the combined presence of chitosan and Zn could be a way of facilitating legacy P stocking in soil, although not without consequence for Zn, as would be discussed in a moment. The observed uptick after 72 hours suggests that a potential to tune P solubility from chitosan-Zn temporally. Ongoing studies involving different rates and ratios of TPP:chitosan:Zn in acid-neutral-alkaline soil systems would be required to unravel these aspects.

To assess the potential of TPP and its formulation with chitosan and Zn to support plant production as a P-source, agronomic parameters were recorded. In these studies, no Zn was provided in the treatments, except for TPP-Chitosan-ZnO, because we aimed to evaluate the products ‘‘as is’’, based on the outcome of the leaching studies. However, it is noteworthy that the indigenous soil amount of 2 mg/kg of bioavailable Zn proved to be an adequate source to supply the nutrient to the plant, as indicated in the control treatment. Nevertheless, the data also showed that plant growth in terms of height can be improved by TPP, significantly more so in the presence of chitosan and

Zn (i.e., TPP-Chitosan-ZnO), and that grain yield can also be potential improved. It seems plausible that this added Zn provided a plant growth stimulating effect, as also demonstrated in several previous studies involving low Zn amendments [e.g., 30-34, 40]. Prior studies have assessed TPP-chitosan formulations for effect on plant performance. For example, Kumaraswamy et al. [57] showed that soil application of TPP-Chitosan-Si NPs promoted maize vegetative indices (seedling growth, including shoot length, root length, and root number). A subsequent evaluation of the TPP-Chitosan-Si NPs under field condition demonstrated significant increase in yield. Compared to the current study, exposure to the products in the above study was by foliar, in a soil that received NPK fertilization. Therefore, the current observations where TPP is the sole P source show clearly that there is no penalty in plant performance for using TPP in place of MAP. Rather, there could be benefits for both vegetative and reproductive performances. We hypothesize that tuning the ratios of TPP:Chitosan:Zn will yield significant outcomes for these parameters; this is subject of future studies. In any case, a strategy to recycle TPP used for the earlier mentioned applications should be devised to repurpose the P for plant production.

The plant accumulation of P was not negatively impacted by using TPP; if anything, a slight positive impact was recorded, compared to MAP. This corroborates the agronomic findings and further supports the notion that TPP could serve as a sole P fertilizer source. However, shoot acquisition of P was affected in the presence of Zn. As earlier highlighted, the simultaneous presence of P and Zn is known to antagonize the plant accumulation of these nutrients by way of inhibiting their shoot uptake and/or grain translocation [37, 38,56]. Our findings agree with these studies in that P uptake into the shoot, and Zn translocation into the grain from the shoot, were to different extents hindered in the TPP-Chitosan-ZnO NP treatment, when compared to TPP-

Chitosan. One issue that arises from reduced P uptake in the presence of Zn is the question of how much P is required for maximizing plant metabolism, leading to the desired vegetative and reproductive performances. This argument was raised by Withers et al. [4] and reemphasized by Bindraban et al. [2, 5]. Considering these arguments and the level of P in the presence of Zn being still over 2500 mg P/kg dry plant mass for shoot, and above that for the grain, such a level of reduction should have no consequence on yield. This is corroborated by the plant vegetative and reproductive data. With respect to Zn, studies by Deshpande et al. [59] involving a similar formulation of Zn in a TPP-Chitosan complex reported increased uptake of Zn in wheat grain, compared to a TPP-Chitosan (no Zn) control treatment. However, several important differences can be identified, compared to the current study. First, in Deshpande's study, exposure of the wheat plant to the nanofertilizer was through foliar, implying that the distance to the reproductive sinks is shorter, compared to soil application. Secondly, foliar application bypasses the complexity of soil, thereby negating the effects of soil chemistry-induced formation of Zn-P complexes that affect nutrient uptake. Thirdly, the authors used a high concentration of Zn in the nanofertilizer, 20 mg/g (20, 000 mg/kg), compared to 2% Zn (= 20 mg Zn/kg) in the current study. And finally, the study was conducted in a sand matrix where P leaching would be high, compared to soil. Nevertheless, the grain Zn levels from the nanofertilizer treatments in both studies are similar, indicating that Zn-amended TPP-Chitosan nanofertilizers are capable of supplying Zn to wheat grain, an important criterion for food agronomic biofortification. We note the trend for reduced (albeit insignificant) Zn accumulation in the plant. Reduction in the Zn content of edible plant tissues, in this case the wheat grain, is an undesirable outcome for any Zn fertilizer regime. It is likely that the lower amount of Zn in the grain is resultant from the low amount of Zn used in the formulation, 2%. Nevertheless, further studies are required comparing Zn fertilization at different

rates and using conventional Zn fertilizers (Zn ions, bulk-scale ZnO) vs Zn-amended TPP-Chitosan to further our understanding of these interactions.

It has been speculated that chitosan upon being loaded into the plant tissue can be transported via the xylem, or phloem, if foliar applied, to distribute nutrients throughout the plant. Despite having a preponderance of positive charge, chitosan can mobilize cationic elements from soil to the plant.

Deshpande et al. [59] observed a strong shift in the TPP-Chitosan band to a higher wavenumber when Zn (from $ZnSO_4.7H_2O$) was added, suggesting that the mechanism of Zn interaction with TPP-Chitosan is based on complexation of ionic Zn with an –OH moiety. ZnO NPs is known to be soluble in soil suspensions [33], releasing Zn ions that could form similar interactions with –OH moieties during product preparation. Thus, these –OH moieties in TPP-Chitosan are potentially available for interaction with cations in the soil. Not surprisingly, the TPP-Chitosan facilitated the accumulation of several native nutrients in the tested soil, namely, K, S, Mg, and Ca. This clearly suggests that the TPP-Chitosan product was able to mobilize native soil nutrients into the plant. These findings concur with the reported role of chitosan for nutrient delivery or mobilization [24, 38]. Dhlamini et al. [23] demonstrated that soil exposure of maize to TPP-Chitosan NPKS could significantly improve the plant shoot and grain S content, as well as the shoot K content, compared to untreated crops. Notably the study was conducted with a TPP-Chitosan that contained added S and K. In contrast, the present study shows this potential for the first time in soil for non-added nutrients. Therefore, considering the poor nutrient use efficiency of most plants, the current finding should constitute an important aspect of soil fertility that should be further investigated for better management of soil nutrients. Indeed, as enunciated in Dimkpa

et al. [63], the use of biobased materials such as chitosan and others, including alginate, lignin and cellulose to enhance fertilizer use efficiency can contribute to simultaneously fulfilling the enhanced soil health and productivity objectives.

## 5. Conclusion

We have shown that TPP can serve as a source of P for plant growth and reproduction, as well as provide adequate P for plant use, compared to a conventional P fertilizer such as MAP. When TPP was formulated into a nano-enabled fertilizer, its attributes for supporting plant performance were not negatively affected in the presence of chitosan, a biopolymer of interest in nanotechnology applications. However, the TPP-Chitosan nanofertilizer considerably reduced P leaching loss in soil, compared to MAP and TPP, a desirable outcome that was further enhanced by inclusion of Zn in the formulation. Trend wise, leaching of P was lowered with time for MAP and TPP, whereas P leaching for TPP-Chitosan with and without Zn tended to be lowest between 24 and 48; significant upticks could be noticed with these two products. It would be indeed interesting to investigate leaching trends over a much longer period. As would be expected, Zn caused a lowering of P uptake into the plant shoot, although with no effect on grain P translocation. In contrast, Zn levels in the plant tissue was not significantly impacted by TPP. The nanofertilizer, particularly in the absence of Zn, mobilized native soil nutrients into the plant, helping to improve the overall nutritional quality. Figure 6 summarizes these key results, in terms of nutrient loss mitigation, agronomic performance, and differential effects on nutrient accumulation, as a function of TPP-Chitosan vs. TPP-Chitosan-ZnO, relative to MAP.

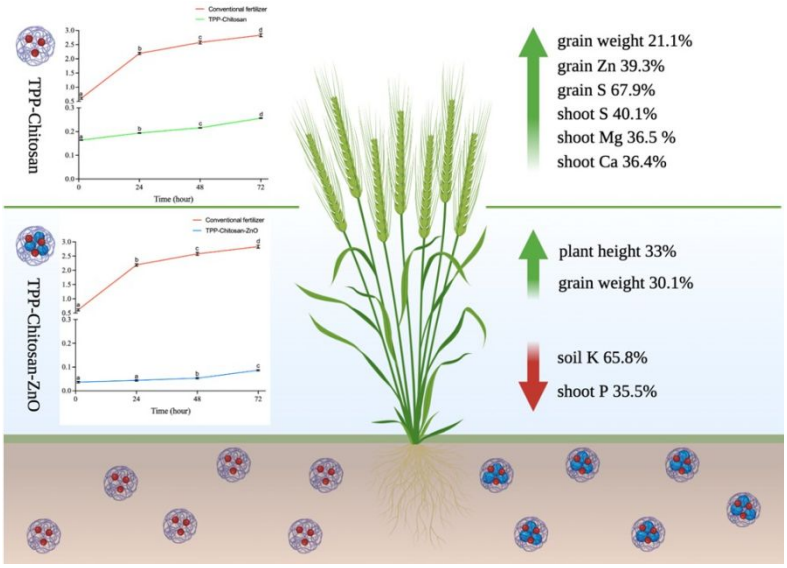


Figure 6. A generalized schematic representation of the reduction in P leaching loss by TPP-Chitosan and TPP-Chitosan-ZnO, relative to a conventional fertilizer, mono ammonium phosphate; and effects on plant performance and nutrient accumulation in wheat.

Taken together, our data clearly show the potential for repurposing a non-fertilizer P material for agricultural and environmental applications for reducing P loss. Reduction in P loss is critical for reducing eutrophication of water bodies due to nutrient overload, and for sustaining the dwindling global P resources. Notably the role of nanotechnology in enhancing such applications was also highlighted. Several studies to further elucidate these potentials are ongoing, including understanding run-off prevention potential of the described nanofertilizers using a rainfall simulator, as well as understanding leaching characteristics in an actual plant soil system.

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