# NPK fertilisers as agents for the biofortification of trace elements in wheat

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**Abstract**
Millions of people across the globe suffer from malnourishment as a result of deficiencies to trace elements. This study addressed whether different combinations and concentrations of NPK fertilisers can assist in the biofortification of Boron (B), Copper (Cu), Iron (Fe), Manganese (Mn) and Zinc (Zn) in wheat (*Triticum aestivum* cv Mace). In field and glasshouse trials, the uptake and accumulation of B, Cu, Fe, Mn and Zn differed when NPK fertilisers were applied in different combinations and rates. When N was applied in conjunction with P and K, grain B and Mn concentrations increased by 0.4 mg $kg^{-1}$ (≈40%) and 5-22 mg $kg^{-1}$ (up to 56%), respectively. Conversely, when N was applied in conjunction with P and K grain Cu and Zn concentrations decreased by 2-4 mg $kg^{-1}$ (20-40%) and 10 mg $kg^{-1}$ (16-28%) respectively relative to when N was supplied on its own. These observations highlight that management of NPK fertilisers and residual P and K concentrations in soil can assist in improving trace element uptake in wheat, however, further research is required to fully ascertain whether this process could be used at a commercial scale and if the trends will apply in other cropping regions and other crops both within Australia and across the globe.

**Key words**
Boron; Copper; Iron; Manganese; Zinc; uptake; accumulation; trace element deficiencies

## Introduction
Deficiencies of essential trace elements (e.g. Se, Zn, Fe, B, Mn, Cu) affect the health of millions of people globally (Rengel *et al*., 1999). Trace element deficiencies in humans can be alleviated through the process of 'biofortification' whereby cereals and other food staples are fertilised with trace elements which are translocated to the grain and then to humans (Cakmak, 2008). Research investigating trace element biofortification in crops has focused on improvements to breeding and the effectiveness of trace element fertilisers (Rengel *et al*., 1999; White and Broadley, 2005). Far less research has explored whether the supply of macro-nutrients (e.g. N, P, K) can influence trace element uptake and sequestration in cereals (Li *et al*., 2007). This is surprising given that interactions between nutrients and trace elements are ubiquitous (Fageria, 2001). Most research on the influence of nutrients on trace element uptake in crops has focused on N (Fageria, 2001). There has been limited consideration of how interactions between N, P and K fertilisers influence trace element uptake. This is worthy of investigation, given that P and K are present in considerable concentrations in most cropping soils as a consequence of widespread fertiliser use during the second half of the 20$^{th}$ century (Neuhaus, 2012; Weaver and Wong, 2011). This research has two goals, (1) to establish if different combinations and concentrations of NPK fertilisers alter trace element uptake in wheat relative to N fertiliser alone; and (2) to determine if different residual P and K concentrations in soil alter relationships between N and trace elements in terms of uptake and sequestration in cereal grains.

## Methods
### *Glasshouse trial*
Triticum aestivum cv Mace was grown under four nutrient series - N only; N+P; N+K or N+P+K. N was applied as urea at 15, 45 and 135 mg N $kg^{-1}$ in all nutrient series. The 'N only' series received no fertiliser P or K. The N+P series received 4, 12 and 36 mg P kg-1 as triple super phosphate (TSP) in conjunction with the N rates detailed above (i.e. 15N + 4P; 45N + 12P; 135N + 36P). The N+K series received 13, 38 and 113 mg K $kg^{-1}$ as KCl in conjunction with N (i.e. 15N + 13K; 45N + 38K; 135N + 113K). The N+PK series received all N, P and K fertiliser regimes at all 3 levels described above (e.g. 15N + 4P + 13K). In addition, a reference treatment containing no added nutrients was used. Triplicate pots (n=3) were prepared for each treatment regime (n=13) in a randomised block design. Plants were grown in 1m PVC pots filled with 30cm of soil from the ex-DAFWA research station at Vasse (33.45$^{o}$S, 115.22oE) on top of yellow sand (30-100cm). The Vasse soil contained non-limiting concentrations of all trace elements as determined by DTPA extraction (Lindsay and Norvell, 1978) and $CaCl_2$ extraction (B only) followed by ICP-AES analysis (Rayment and Lyons, 2011). Concentrations of trace elements were thus consistent across all treatments and

represented indigenous trace element concentrations present in soil at seeding (i.e. no trace element fertilisers were applied) (Table 1). Plants were grown to maturity and trace element concentrations in the grain and shoots were determined using a $HNO_3$:HClO digestion (McQuaker *et al*., 1979a) followed by analysis using ICP-AES (McQuaker *et al*., 1979b).

**Field trials**

Wheat *cv* Mace was grown in three locations within Western Australia. Two trials were performed at the DAFWA field station in Wongan Hills (30.90oS, 116.71oE), one on a "high nutrient background" soil (1) and one on a "low nutrient background" soil (2). A third trial was performed at the UWA field station at Shenton Park (31.57oS, 115.48oE). Plants were grown under the same nutrient series as in the glasshouse trial. N was applied at 30, 60 or 90 kg N $ha^{-1}$ as urea. The N only series received no fertiliser P or K. The N+P series received 8, 16 or 24 kg P $ha^{-1}$ as TSP in conjunction with N (e.g. 30N + 8P). The N + K series received 25, 50 or 75 kg K $ha^{-1}$ as KCl in conjunction with relevant N rates (e.g. 30N +25K). The N + P+K series received all N, P and K fertiliser regimes described above (e.g. 30N + 8P + 25K). A reference treatment containing no added N, P or K was also used. Triplicate plots (n=3) were prepared for treatment (n=17) in a randomised block design. Plot size was 2x10m at both Wongan Hills trials and 2x2m at Shenton Park. Mace was sown at 80 kg $ha^{-1}$ in all trials. Concentrations of B, Cu, Fe, Mn and Zn were determined in soil and grain as per the glasshouse trial. Soil macronutrient and trace element concentrations across the three trials are described in Table 1.

**Table 1. Indigenous soil nutrient and trace element concentrations at seeding in field trials.**

| Trace element/ nutrient | Critical con-centrations (mg $kg^{-1}$) | Vasse soil glasshouse trial (mg $kg^{-1}$ ± SE) | Shenton Park field trial (mg $kg^{-1}$ ± SE) | Wongan Hills field trials (2) (mg $kg^{-1}$ ± SE) | |
|---|---|---|---|---|---|
| | | | | (1) 'high background nutrient' | (2) 'low background nutrient' |
| B | 0.5 | 0.50 ± 0.02 | 0.16 ± 0.01 | 0.50 ± 0.01 | 0.48 ± 0.02 |
| Cu | 0.3-0.7 | 1.52 ± 0.02 | 2.5 ± 0.6 | 0.60 ± 0.04 | 0.5 ± 0.1 |
| Fe | N/A | 142.8 ± 11.4 | 10.6 ± 0.3 | 63 ± 5 | 40 ± 6 |
| Mn | 5 | 5.6 ± 0.1 | 0.8 ± 0.1 | 10 ± 2 | 2 ± 1 |
| Zn | 0.2-0.4 | 23.3 ± 1.5 | 1.0 ± 0.1 | 0.7 ± 0.1 | 0.6 ± 0.1 |
| P | 15 | 20 ± 1 | 45 ± 3 | 33 ± 5 | 25 ± 2 |
| K | 40 | 18 ± 3 | 39 ± 3 | 175 ± 21 | 58 ± 10 |
| S | 5 | 8 ± 1 | 2 ± 1 | 48 ± 9 | 10 ± 1 |

**Results**

Grain B (P=0.001), Cu (P=0.005), Fe (P<0.001), Mn (P<0.001) and Zn (P=0.011) concentrations were influenced by different fertiliser treatments under glasshouse conditions (Figs 1-4). Different NPK fertiliser combinations and rates also influenced trace element uptake and translocation in two of the three field trials. At Shenton Park grain Cu (P<0.001); Mn (P=0.009) and Zn (P <0.001) concentrations responded to different combinations of NPK application. On the lower nutrient Wongan Hills trial (2), grain B (P=0.003) and grain Fe (P=0.011) concentrations were influenced by different NPK regimes. On the Wongan Hills trial containing high residual K and S in the soil (1) there were no statistical differences in trace element concentrations when different NPK fertiliser combinations and rates were applied.

In both glasshouse and field conditions grain B concentrations increased by up to 0.4 mg $kg^{-1}$ (22%) when N was applied in conjunction with P, particularly at high N concentrations (135 mg N $kg^{-1}$ in pots and 60-90 kg N $ha^{-1}$ in field) (Fig 1).

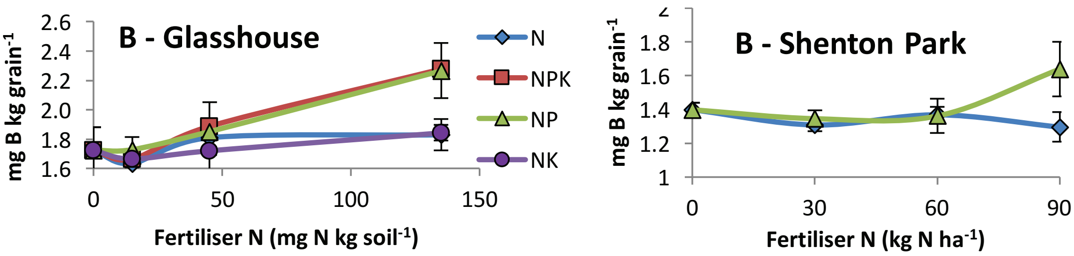


**Figure 1. Grain B concentrations in Mace when grown under different NPK fertiliser regimes under glasshouse and at Shenton Park. Error bars represent standard errors of the mean (n=3)**

Grain Cu concentrations conversely, decreased when N was added in conjunction with P and K (Fig 2), with losses in glasshouse trials between 2-4 mg $kg^{-1}$ (~20-40%) and maximum losses in field trials of approximately 1 mg $kg^{-1}$ (~30%) relative to when N was supplied alone (Fig 2).

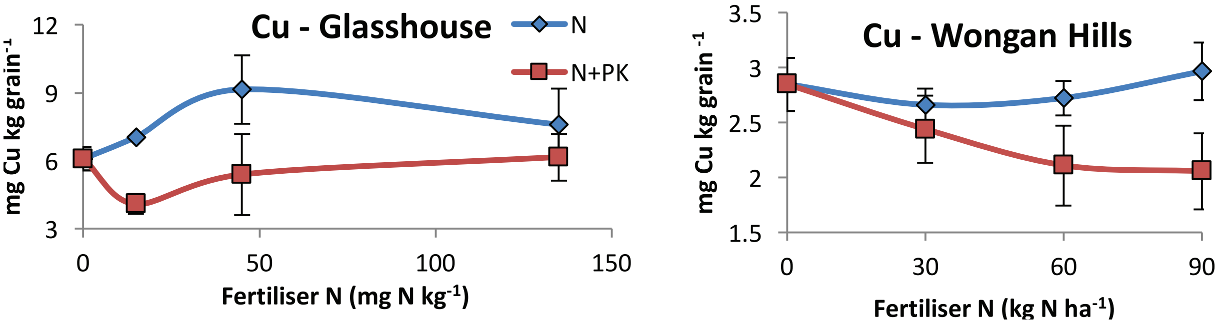


**Figure 2. Grain Cu concentrations in Mace when grown under different NPK fertiliser regimes under glasshouse and field conditions. Data from field is from the Wongan Hills 'low nutrient' site. Error bars represent standard errors of the mean (n=3)**

The addition of P reduced Fe uptake in the grain by 5-8 mg $kg^{-1}$ (≈12-25%) at all three N concentrations used in the glasshouse, however, in the field this relationship was not evident (Data not shown).

In both glasshouse and field conditions, applications of P and K fertilisers individually and in combination improved Mn uptake and sequestration (Fig 4). In the glasshouse the application of PK fertilisers improved grain Mn by up to 22 mg $kg^{-1}$ (56%); whilst in the field the application of P and K individually improved Mn concentrations by between 5-8 mg $kg^{-1}$ (19%).

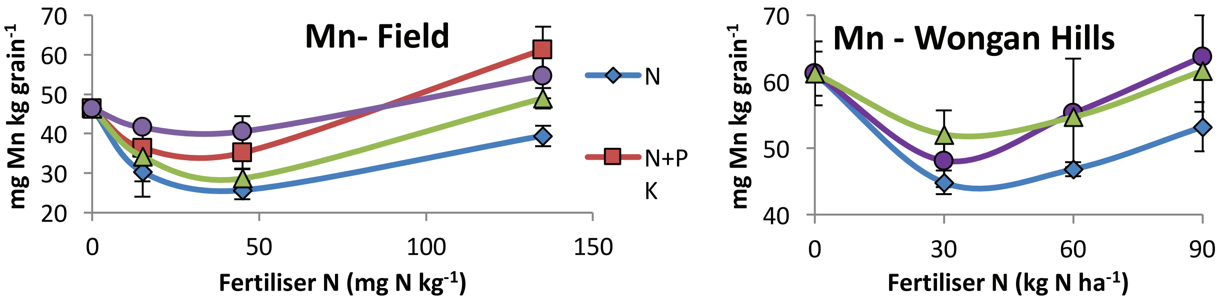


**Figure 3. Grain Mn concentrations in Mace when grown under different NPK fertiliser regimes under glasshouse and field conditions. Data from field is from the Wongan Hills 'low nutrient' site. Error bars represent standard errors of the mean (n=3).**

The application of NPK fertiliser was deleterious for grain Zn concentrations both in the glasshouse and field (≈ 5-15 mg $kg^{-1}$; 16-28% decrease) (Fig 4).

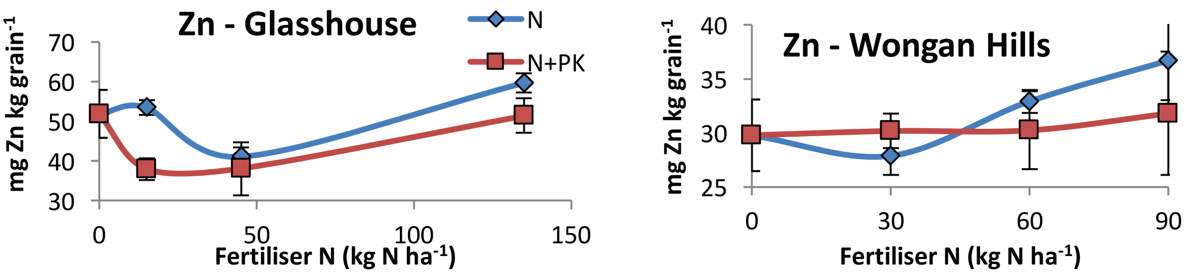


**Figure 4. Grain Zn concentrations in Mace when grown under different NPK fertiliser regimes under glasshouse and field conditions. Data from field is from the Wongan Hills 'low nutrient' site. Error bars represent standard errors of the mean (n=3).**

## Discussion and Conclusions

The uptake and accumulation of trace elements varied when different combinations and concentrations of NPK fertiliser were applied (Figs 1-4). This confirms that manipulating macronutrient fertiliser regimes can aid in trace element uptake. In all three field trials there was no effect on grain yields when P and/or K fertilisers were applied at different N concentrations (Data not shown). This is a key consideration if manipulation of P & K is to be adopted for the purposes of increasing trace element concentration (per kg grain).

The data presented here indicates that the uptake of individual trace elements was influenced by different combinations of NPK fertilisers (Figs 1-4). For example, B and Mn uptake was enhanced by the addition of composite N+P fertilisers, particularly at high concentrations (e.g. 90 kg N $ha^{-1}$ + 24 kg P $ha^{-1}$) (Figs 1 & 3). Conversely, the application of NPK fertilisers was detrimental for Cu and Zn uptake relative to 'N only' (Figs 2 & 4). Consequently, both fertiliser applications and residual NPK concentrations need to be carefully considered to optimise nutrient uptake and to avoid initiating deficiencies in some elements whilst trying to maximise the uptake of others. Furthermore, the observation of decreased Cu and Zn uptake when increased P and K concentrations are added to soil has some implications for the current state of Australian agriculture. During the latter part of the 20th century fertiliser use has increased exponentially (Angus, 2001; Cordell et al., 2013), while, at the same time, conservation farming practices such as no-till have increased the retention of P and K in many regions (Neuhaus, 2012; Weaver and Wong, 2011). Cu and Zn strongly interact with N in terms of plant uptake (Fageria, 2001), however, in these trials uptake decreased when P or K concentrations were increased (Figs 2 & 4). If such effects are widespread, continued build-up of P and K in soils has the potential to compromise Cu and Zn in grain and negatively impact human and animal nutrition.

While further studies are needed to confirm these results across differing growing environments and to explore whether manipulating trace element uptake is economically viable at a commercial scale, the results shown here indicate that increasing understanding of the interactions between macronutrients and micronutrients can lead to outcomes that are relevant for growers, fertiliser producers and crop biofortification industries. For growers, our findings provide an increased understanding of how management of NPK can maximise trace element uptake and this could become economically important if premiums are granted in the future for favourable trace element concentrations in grain. At a more basic level, application of our research findings may be a means to alleviate trace element deficiencies in crops. For fertiliser producers, the data supports continued investment in composite macronutrient/trace element fertilisers by providing information regarding nutrients and trace elements that can be combined for more efficient uptake e.g. NPK + Mn; NPK + B; N + Cu; N + Zn. For crop biofortification management, our data demonstrates that NPK nutrient management can alter the efficiency of biofortification processes.

In conclusion this research has demonstrated that trace element (B, Cu, Fe Mn and Zn) uptake and translocation in wheat can be both enhanced and compromised by the use of NPK fertilisers. Future research should endeavour to assess whether these observations are representative of wider trends both across Australia and globally.

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