

Improving micronutrient fluid fertilizers using novel chelating agents

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ABSTRACT

The low solubility of most micronutrient cations (copper (Cu), iron (Fe), manganese (Mn) and zinc (Zn)) in soils means that after addition to alkaline soil in fluid form, the metal is rapidly sorbed or precipitated. Chelates such as ethylene diaminetetraacetic acid (EDTA) and dimethylamine pentaacetic acid (DTPA) are often used to increase micronutrient solubility, but micronutrients complexed by these chelates are not readily taken up by plants. We examined a new application of two chelates, polyethylenimine (PEI) and rhamnolipid, to improve crop micronutrient nutrition through exploitation of different physical and chemical behaviours of the chelates.

PEI forms cationic complexes and rhamnolipid forms lipophilic complexes with micronutrient cations. Both PEI and rhamnolipid increased uptake of Zn by canola and wheat grown on highly alkaline soils in the field (PEI) and in glasshouse trials (rhamnolipid). The lipophilic properties of micronutrients complexed by rhamnolipid could markedly assist crop uptake of elements complexed by this chelate. This was confirmed spectroscopically using synchrotron X-ray techniques, where Zn complexed by rhamnolipid was found to move intact into canola roots. These new types of chelates, which do not form anionic micronutrient complexes, have the potential to not only increase the solubility of micronutrients in fluid fertilizers, but also to retain them in forms that are readily available to plant roots.

INTRODUCTION

Millions of hectares of arable land worldwide, particularly in arid and semi-arid regions, are deficient in plant available micronutrients and this can markedly affect human nutrition (Graham and Welch 2000). The major reason for the widespread occurrence of deficiency of micronutrients is the low availability of micronutrients to plant roots rather than their low concentration in soils. The low solubility of most micronutrient cations (copper (Cu), iron (Fe), manganese (Mn) and zinc (Zn)) in soils means that after addition to alkaline soil as the soluble form, the metal is rapidly sorbed or precipitated (Tiller *et al.* 1972; Lindsay and Norvell 1978). One method to reduce these reactions in soil is through the use of chelates. Chelates are organic compounds that bind the metal and increase water solubility (Wallace 1963). Common chelates are ethylene diaminetetraacetic acid (EDTA) and dimethylamine pentaacetic acid (DTPA) and these molecules increase micronutrient solubility through reversal of charge on the metal. The metallic cation M^{2+} becomes ML^n , where M is the micronutrient cation and L the chelate, i.e. the chelate makes the micronutrient anionic.

It is well known that EDTA and DTPA both markedly increase the solubility of micronutrient cations in soil and aid their diffusion to plant roots (Lindsay and Norvell 1978; Elgawhary *et al.* 1970a; Elgawhary *et al.* 1970b). Indeed, the high mobility of these compounds raised concerns regarding their potential use in industrial and household chemicals due to their ability to transport heavy metals in the environment (Sillanpää 1997). While these chelates have an excellent ability to retain micronutrient cations in soluble forms, the form in which the micronutrient exists in solution is, however, not readily available for uptake by plant roots. It is well known that plants absorb micronutrient cations through defined metal transporters in the plant root membrane that principally recognise the free metal cation M^{2+} (Kochian 1991). These transporters do not recognise all complexed forms of micronutrient (an exception would be Fe-phytosiderophore). Indeed, addition of EDTA or DTPA to nutrient solutions markedly depresses the uptake of micronutrients by the plant, due to complexation of the free metal cation (M^{2+}) (Halvorson and Lindsay 1977; Laurie *et al.* 1991a; Laurie *et al.* 1991b).

Thus the efficiency of chelates such as EDTA and DTPA in terms of improving crop nutrition is compromised by the poor ability of the complexed forms of micronutrients to be absorbed by plant roots. In this paper we examine new potential applications for two new chelates, polyethylenimine (PEI) and rhamnolipid, to

improve crop micronutrient nutrition through exploitation of different physical and chemical behaviours of the chelates. Full results will be reported in a subsequent publication (Stacey et al. 2008).

MATERIALS AND METHODS

The Jeneil Biosurfactant Company supplied a 25% rhamnolipid solution that contained equal proportions of R1 (504 atomic mass units (amu)) and R2 (650amu) rhamnolipids. BASF Germany supplied a highly branched 50% PEI solution with an average molecular weight of 800amu. Sub-samples of both products were digested in concentrated HNO₃ and analysed by inductively-coupled plasma optical emission spectroscopy (ICP-OES; SpectroFlame Modula, Spectro) to determine the concentrations of contaminant ions. Both products contained negligible Cu, Mn, phosphorus (P) and Zn and were used without further purification.

Octanol/water partition coefficients ($K_{o/w}$) were determined using the shake-flask method and calculated according to equation [1].

$$K_{o/w} = \frac{C_o}{C_w} \quad [1]$$

where C_o and C_w referred to the concentration of Zn in the *n*-octanol and water phase, respectively (Chiou et al. 1977). The $K_{o/w}$ indicates the ability of a chemical to diffuse easily across a biological membrane.

The efficiencies of chelated Zn fertilisers were compared using two alkaline soils from southern Australia. Soil samples were collected from field sites known to be Zn responsive at Streaky Bay, South Australia and Birchip, Victoria. Topsoils from each location were collected, oven dried, passed through a 2mm sieve and stored in sealed containers until use. Pertinent soil characteristics have been described in Table 1. The Birchip soil was an alkaline sodosol with a pH_(1:5 soil:water) of 8.8 (Table 1). The Streaky Bay soil was highly calcareous, with a CO₃ content of 39% and pH_(1:5 soil:water) = 8.7 (Table 1).

Table 1. Soil Properties

| | <i>Birchip Soil</i> | <i>Streaky Bay soil</i> |
|--------------------------------|---------------------|--------------------------|
| Description | Vertic Natixeralf | Calcixerollic xerochrept |
| pH _(1:5 soil:water) | 8.8 ± 0.01 | 8.7 ± 0.02 |
| CO ₃ (%) | 2.8 | 39 |
| Clay (%) | 40 | 25 |
| (Total mg/g soil) | | |
| Ca | 6.8 | 73.6 |
| Mg | 5.27 | 4.05 |
| Zn | 0.030 | 0.015 |
| Cu | 0.011 | 0.005 |
| Mn | 0.26 | 0.14 |

Chelated fertiliser solutions were mixed with 20g of the Birchip and Streaky Bay soils, which was banded between 100g of the unfertilised bulk soil. Total nutrient application equated to (μg/g soil) P 60, N 27, applied as fluid technical grade monoammonium phosphate, and Zn 0.6 as ZnSO₄·7H₂O (3.6 μg Zn/g soil in the fertiliser band) either as the free metal salt or chelated by EDTA, PEI or rhamnolipid. Fertiliser Zn was labelled with ⁶⁵Zn to a specific activity of 3.13 kBq/μg Zn. Each fertiliser treatment was replicated four times. Two pre-germinated canola seeds (*Brassica napus* cv. Pinnacle) were transferred to each pot. Streaky bay soil was watered with deionised water every second day to pF 2, measured using sintered glass funnels. The Birchip soil was watered to pF 2.2 due to its higher clay content and swelling properties. The soil surface was covered with polyethylene beads to reduce evaporation. The plants were grown for 21 days in a controlled environment growth chamber (10 h dark at 16°C, 14 h light at 22°C, 41% humidity) before the shoots were harvested, rinsed, dried, weighed and then digested in concentrated HNO₃. Plant digests were analysed for ⁶⁵Zn by gamma spectroscopy and for total nutrient contents by ICP-OES. Plant uptake of fertiliser Zn was calculated from the activity of ⁶⁵Zn in plant shoots and the known specific activity of the fertiliser Zn added.

To examine spectroscopically the differential uptake of Zn with EDTA and rhamnolipid, canola plants (*Brassica napus* var. Holly) were grown in a hydroponic nutrient solution that contained Ca (1 mM), N (5 mM), P₂O₅ (0.28 mM), K (1.06 mM), Mg (0.62 mM), S (0.63 mM) and Fe (17.9 μM). Plants were grown in a controlled environment growth chamber and after 2 weeks, the nutrient solution was topped up with deionised water to Zn-starve the plants. Ten days later the canola plants were transferred to pre-treatment solution for 24 hours. Canola roots were transferred to Zn

treatment solutions containing 5 μM Zn, either as ZnSO_4 or complexed with EDTA or rhamnolipid. Treatment solutions were buffered at pH 6.0 with 2 mM MES (50% as potassium salt). After 24 hours, roots were separated from canola plants and frozen in liquid N_2 . Roots were freeze cut and thin cross-sections were mounted in aluminium holders between two sheets of Kapton[®] film. The distribution of Zn in root thin sections was mapped using X-ray fluorescence at beamline 13-BM (GeoSoilEnviro Consortium of Advanced Radiation Sources) at the Advanced Photon Source, Argonne National Laboratory, Argonne, IL. Samples were carefully inserted into a freezer stage and the distribution of Zn, and its speciation, were determined using $\mu\text{-X-Ray}$ fluorescence ($\mu\text{-XRF}$) mapping, and extended X-ray absorbance fine structure spectra (EXAFS). For full details see Stacey et al. (2008).

RESULTS AND DISCUSSION

The $K_{o/w}$ values for the metals complexed by rhamnolipid were high. Normally metallic cations are hydrophilic and do not partition to the octanol phase (and hence have very low $K_{o/w}$ values). High $K_{o/w}$ values for micronutrient cations found with rhamnolipid indicated that the chelate had formed a lipophilic complex with the cation, a property likely to assist in uptake by plant roots. PEI forms cationic complexes with micronutrients, so $K_{o/w}$ values were low for this chelate (Figure 1).

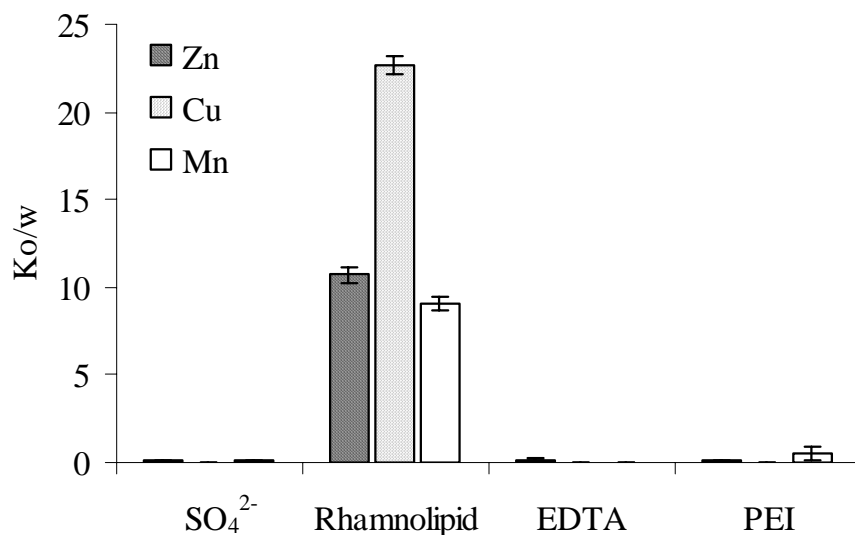


Figure 1. Octanol/water partition coefficients ($K_{o/w}$ values) for Cu, Mn and Zn with sulphate, EDTA, rhamnolipid and PEI (means \pm 1 S.E., $n=3$) (from Stacey 2007).

Canola plants were grown under Zn deficient conditions. Therefore, on Streaky Bay soil, shoot Zn concentrations were below the published critical tissue concentrations for Zn of 7-8 mg Zn/kg DM (Reuter and Robinson, 1997). Canola plants grown on Birchip soil had Zn concentrations at or above the critical Zn concentration; treatment with rhamnolipid and PEI increased shoot Zn concentration above the critical level (Figure 2). Rhamnolipid also significantly ($P \leq 0.05$) increased concentration of Zn in canola shoots grown on the highly calcareous Streaky Bay soil. EDTA did not significantly ($P > 0.05$) increase Zn uptake from either soil, compared to the ZnSO_4 control even though EDTA substantially increased the solution concentrations of Zn in both soils (data not shown).

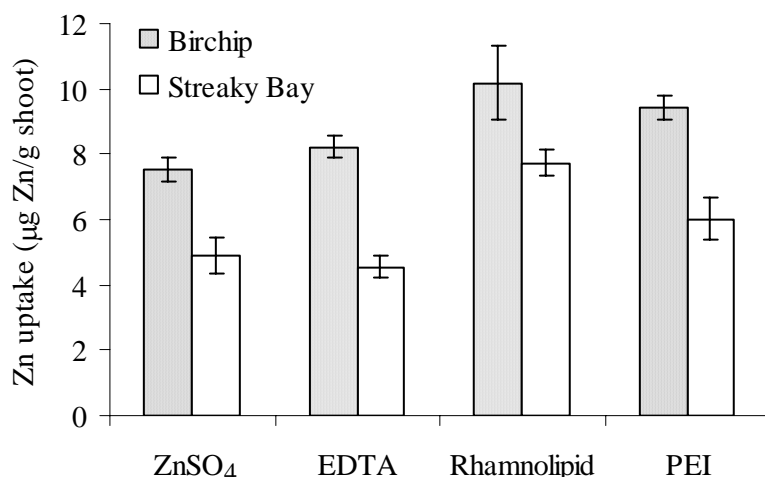


Figure 2. Uptake and translocation of Zn to canola shoots (means \pm 1 S.E.).

Examination of the roots of canola plants exposed to ZnSO₄, Zn-EDTA and Zn-rhamnolipid revealed a significantly different pattern of accumulation, and a different speciation of Zn within the plants (Figure 3). The lowest Zn μ -X-ray fluorescence signal was obtained from the Zn-EDTA treated roots (Figure 3, top), probably due to a reduction in Zn absorption by roots due to low solution Zn²⁺ activities in the presence of EDTA. The Zn signal was higher in ZnSO₄-treated roots and highest in Zn-rhamnolipid roots. EXAFS data (taken from XAS spots labelled in Figure 3) suggested that Zn was predominantly in the form of Zn-phytate-like compounds in Zn-free, ZnSO₄ and Zn-EDTA treated roots, with 70-87% of total root Zn present as Zn-phytate-like compounds in these treatments. Zinc-EDTA complexes were not detected inside root cross sections, consistent with published literature that showed Zn-EDTA complexes are not readily absorbed by intact roots via active or passive uptake pathways (Halvorson and Lindsay 1977). In roots treated with Zn-rhamnolipid, μ -EXAFS suggested that 55.3% and 87.6% of Zn was probably in the form of Zn-rhamnolipid at spots A and B respectively (Figure 3, bottom). These results suggest that Zn-rhamnolipid complexes may have been absorbed intact by roots, possibly due to the lipophilic properties of these complexes.

CONCLUSIONS

EDTA and DTPA, and other chelates that form anionic complexes with cationic micronutrients, are effective in solubilising these elements in soil, but ineffective in allowing them to be taken up by plant roots. Addition of these chelates to fluid fertilizer blends may increase the micronutrient solubility in the blend, but the resultant form of micronutrient is not one that crops can easily use. New types of chelates, which do not form anionic micronutrient complexes, have the potential to not only increase the solubility of micronutrients but also to retain them in forms that are readily available to plant roots. The fact that some of these products have lipophilic properties is an added advantage, as they appear to be able to be readily transported (intact) into the plant root.

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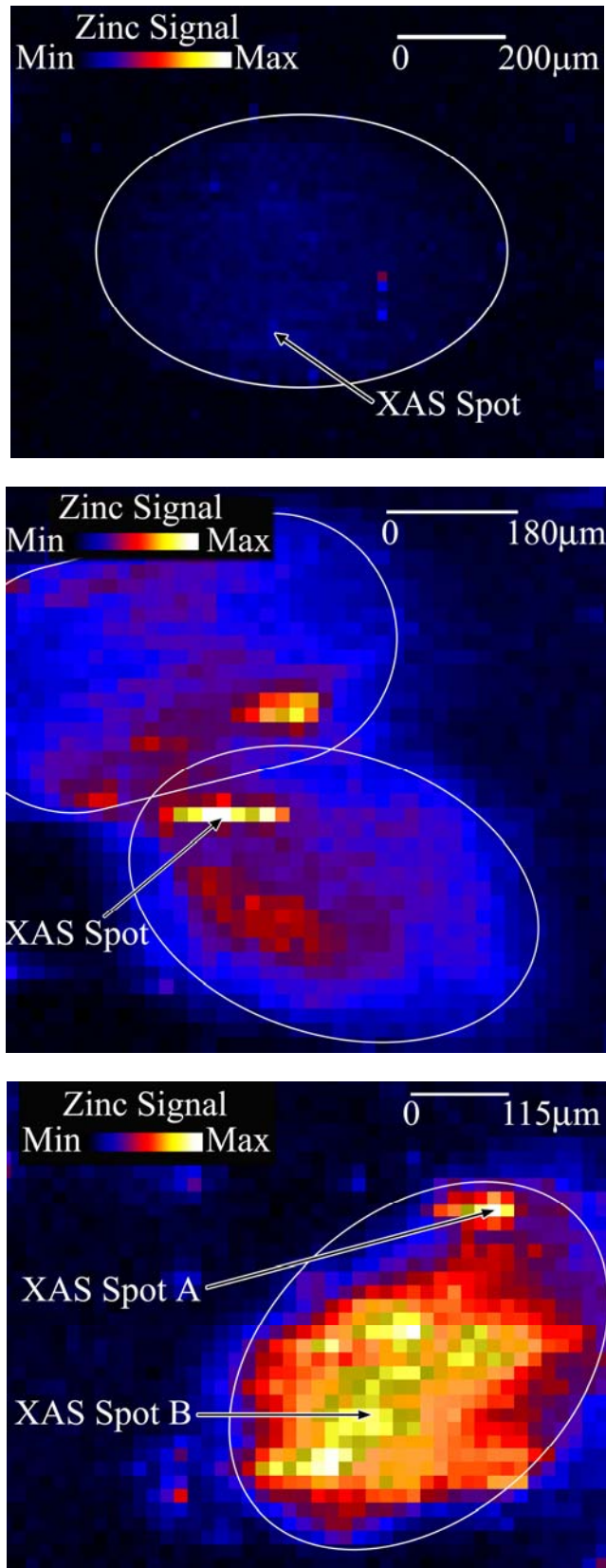


Figure 3. Zinc μ -X-ray fluorescence maps showing the distribution of Zn in a canola root treated with Zn-EDTA (top), ZnSO₄ (middle) and Zn-rhamnolipid (bottom). Speciation of Zn forms was undertaken at the XAS spots marked (from Stacey et al. 2008).

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