



# **New Micronutrient Fertilisers for Alkaline Soils**

**Samuel Peter Stacey**

In fulfilment of the requirements for the degree of

**Doctor of Philosophy**

A thesis submitted to

**Soil and Land Systems**

**School of Earth and Environmental Sciences**

**The University of Adelaide**

**Australia**

**February, 2007**

## **Table of Contents**

|  |          |
|--|----------|
| Abstract.....  | iv       |
| Declaration.....   | vi       |
| Acknowledgements.....  | vii      |
| List of Tables .....   | viii     |
| List of Figures.....   | viii     |
| <br>   |          |
| <b>Chapter 1. Introduction and Literature Review .....</b>                     | <b>1</b> |
| Plant uptake of micronutrients.....  | 3        |
| Fate of Zn and Mn in alkaline soils .....                                      | 8        |
| Micronutrient fertilisers and their application .....                          | 11       |
| Compatibility of micronutrients and phosphates in fluid fertilisers .....      | 17       |
| Synthetic chelates in agriculture.....   | 21       |
| Chelate stability constants .....  | 24       |
| Alternative sequestering agents for trace element ions .....                   | 26       |
| 1) <i>Chelating Polymers</i> .....   | 26       |
| 2) <i>Biosurfactants</i> .....   | 29       |
| Ecotoxicological considerations .....  | 31       |
| Summary.....   | 33       |
| Aims and objectives.....   | 34       |
| References.....  | 35       |
| <br>   |          |
| <b>Chapter 2. Significant chemical properties of EDTA, PEI and rhamnolipid</b> |          |
| Introduction .....   | 48       |
| Materials and Methods .....  | 49       |
| <i>Octanol/water partition coefficients</i> .....                              | 49       |
| <i>pKa</i> .....   | 50       |
| <i>Complexing capacity and stoichiometry of complex formation</i> .....        | 51       |
| Results .....  | 54       |
| Discussion.....  | 59       |

|                  |    |
|------------------|----|
| References ..... | 62 |
|------------------|----|

### **Chapter 3. Effectiveness of polyethyleneimine (PEI), rhamnolipid and EDTA as trace element chelates on alkaline soils**

|   |    |
|---|----|
| Introduction.....   | 64 |
| Materials and Methods .....                                 | 65 |
| <i>Plant uptake of chelated Zn</i> .....                    | 65 |
| <i>Zn adsorption in alkaline and calcareous soils</i> ..... | 67 |
| <i>E<sub>e</sub> and E<sub>a</sub> Values</i> .....         | 67 |
| Results.....  | 69 |
| Discussion.....   | 78 |
| References.....   | 81 |

### **Chapter 4. Absorption kinetics of Zn from chelate-buffered solutions**

|   |    |
|---|----|
| Introduction.....   | 83 |
| Materials and methods .....   | 85 |
| <i>Pre-treatment of canola seedlings</i> .....  | 85 |
| <i>Apoplastic and symplastic uptake of <sup>65</sup>Zn from ice-cold and 20°C solutions</i> ..... | 85 |
| <i>Absorption and translocation of chelate buffered Zn</i> .....                                  | 86 |
| Results.....  | 88 |
| Discussion.....   | 93 |
| References.....   | 95 |

### **Chapter 5. The effect of chelating agents on the foliar sorption of trace element fertilisers**

|   |     |
|---|-----|
| Introduction.....   | 97  |
| Materials and Methods .....                                   | 99  |
| <i>Enzymatic isolation of citrus leaf cuticles</i> .....      | 99  |
| <i>Cuticle ultrastructure</i> .....                           | 99  |
| <i>Cuticle water partition coefficients</i> .....             | 100 |
| <i>Dialysis of fertilisers across isolated cuticles</i> ..... | 101 |

|   |     |
|---|-----|
| <i>Absorption of foliar applied Zn by cotton plants</i> ..... | 102 |
| Results.....  | 103 |
| Discussion.....   | 108 |
| References.....   | 110 |

## **Chapter 6. Conclusions and Future Directions**

|   |     |
|---|-----|
| <i>Fate of chelated trace elements in soil and their absorption by plants</i> ..... | 112 |
| <i>Foliar absorption</i> .....  | 115 |
| <i>Environmental considerations</i> .....   | 115 |
| References.....   | 116 |

## **Abstract**

Trace element deficiencies represent an ongoing limitation to agricultural productivity in Southern Australia and in many regions of the world. Trace element deficiencies are commonly encountered on alkaline and calcareous soils due to their high metal adsorption and fixation capacities. Chelating agents, such as EDTA, have been used to reduce fertiliser fixation in these soils and increase trace element transport to the rhizosphere. However, EDTA, which is the most commonly used chelating agent, can be relatively ineffective on alkaline soils and may have negative environmental implications due to its long-term persistence.

This study has identified two novel sequestering agents for use on alkaline and calcareous soils. The novel products differ significantly from EDTA in terms of their structure and functionality. For example, rhamnolipid is synthesised by *Pseudomonas* bacteria, is non-toxic, biodegradable and forms a lipophilic complex with cationic metal ions. The other chelating agent, polyethylenimine (PEI) can complex up to 4 times more metal (g Cu(II)/g ligand) than EDTA, which has important implications for chelate application rates and the cost effectiveness of chelate use.

In solution culture experiments, rhamnolipid and PEI facilitated Zn absorption into the root symplast; the kinetic rate of Zn absorption was greater than that of ZnCl<sub>2</sub> alone. On alkaline and calcareous soils the novel products were significantly ( $P \leq 0.05$ ) more effective Zn sources than EDTA or the SO<sub>4</sub><sup>2-</sup> salt. EDTA increased the concentration of Zn in soil solution. However, this did not translate to increased Zn uptake by canola plants. This was not surprising as EDTA inhibited Zn absorption by roots in the solution culture experiments.

Radioisotope experiments showed that rhamnolipid and PEI increased Zn adsorption to the soils solid phase. However, PEI increased the size of the total Zn labile pool ( $P \leq 0.05$ ) and mobilised Zn from the pool of fixed native soil Zn ( $P \leq 0.05$ ). Rhamnolipid did not significantly ( $P \geq 0.05$ ) increase the total size of the Zn labile pool in either soil, but significantly ( $P \leq 0.05$ ) increased Zn uptake by canola, probably by facilitating root absorption by the formation of lipophilic complexes with Zn.

These results showed that, on alkaline soils, chelates that increased the rate of trace element absorption into the root symplast were significantly more effective than EDTA, which was not readily absorbed by canola roots.

Experiments were also undertaken to explore the effect of chelation on the absorption of foliar applied trace element fertilisers. Perhaps not-unexpectedly, chelation reduced the absorption of foliar applied Zn. The lipophilic chelate, rhamnolipid, quadrupled Zn absorption by enzymatically excised *Citrus sinensis* cuticles but did not significantly ( $P > 0.05$ ) increase Zn absorption by live leaf tissue. Therefore, there was no discernable relationship between the  $K_{c/w}$  of fertiliser solutions and Zn permeability.

This body of work has important implications for future fertiliser development, the cost effectiveness of chelate use and the treatment of micronutrient deficiencies on alkaline soils in the world today.